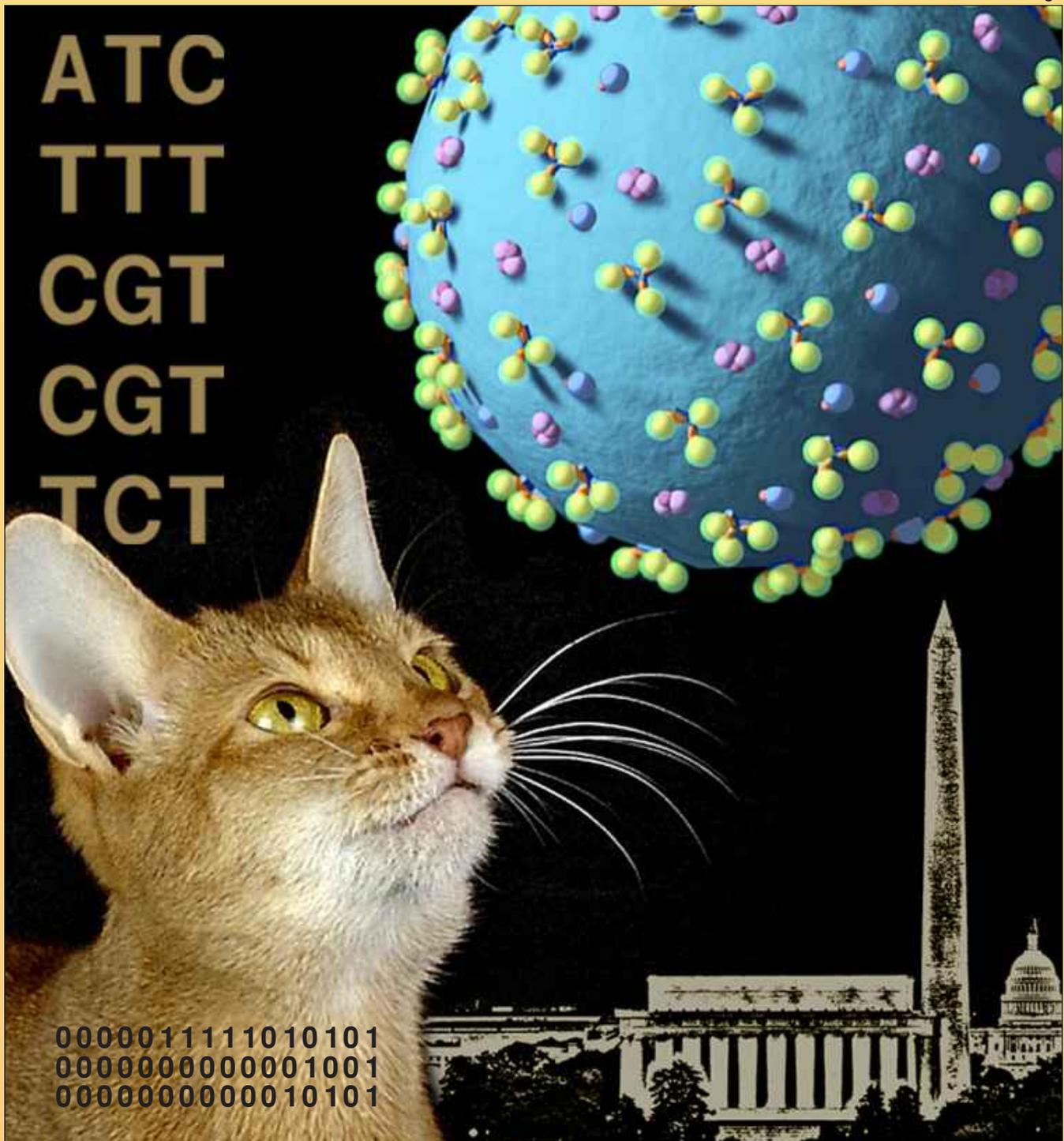


# 8<sup>th</sup> International Feline Retrovirus Research Symposium

## Cat Genomics and Infectious Diseases in the 21<sup>st</sup> Century



Cinnamon the Cat. Photo courtesy of Dr. Kristina Narfstrom, University of Missouri-Columbia

Washington, DC • October 8–11, 2006

Conference Organizers:

- Dr. Mauro Bendinelli, University of Pisa
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- Dr. Mauro Pistello, University of Pisa
- Dr. Alfred L. Roca, Laboratory of Genomic Diversity, SAIC-Frederick, Inc.
- Dr. Mary Tompkins, North Carolina State University
- Dr. Susan Vande Woude, Colorado State University
- Dr. Brian Willett, University of Glasgow
- Dr. Janet K. Yamamoto, University of Florida
- Dr. Naoya Yuhki, Laboratory of Genomic Diversity, NCI

# 8<sup>th</sup> International Feline Retrovirus Research Symposium 2006 Program & Agenda

**Unless Otherwise Noted, All Podium Presentations are in the Ballroom**

## Sunday, October 8, 2006

3:00 – 5:45	p.m.	Registration	Ballroom Foyer
5:45 – 6:00	p.m.	Stephen O'Brien	Welcome
6:00 – 7:15	p.m.	Albert Osterhaus	1 Keynote Speaker: <i>Newly Emerging Viral Infections</i>
7:15 – 9:15	p.m.	Opening Reception <b>Sponsor: Schering Plough Animal Health</b>	Washington Room

## Monday, October 9, 2006

7:00 – 8:30	a.m.	Poster Preparation Posters available for viewing until 3:40 pm break on Tuesday. Official poster session will be from 6-7 pm Monday night	Washington Room
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8:00 – 12:00	a.m.	Registration	Ballroom Foyer
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### **Session 1 Introduction & Overview (Moderator: Mary Tompkins)**

8:30 – 8:40	a.m.	Stephen O'Brien	Introduction
8:40 – 9:10	a.m.	Mary Tompkins	2 Plenary: <i>Immune Dysfunction In FIV Infection: An Overview</i>
9:10 – 9:40	a.m.	Brian Willett	3 Plenary: <i>Chemokines And Co-Stimulatory Molecules: Unravelling The Mechanism Of Infection With FIV</i>
9:40 – 10:10	a.m.	Sue VandeWoude	4 Plenary: <i>Mechanisms of Feline Lentivirus Interference and Control</i>
10:10 – 10:40	a.m.		Break
10:40 – 11:10	a.m.	Peter Rottier	5 Plenary: <i>Genetically Modified Feline Coronaviruses as Vaccines for Protection against Feline Infectious Peritonitis (FIP)</i>
11:10 – 11:40	a.m.	Hans Lutz	6 Plenary: <i>Diagnosis of Feline Leukemia Virus (FeLV) Infection: Review and Outlook</i>
11:40 – 12:10	p.m.	Oswald Jarrett	7 Plenary: <i>How FeLV Changed The World</i>
12:10 – 1:30	p.m.	Complimentary Lunch	Ballroom, General Session Room

### **Session 2 Emerging Pathogens (Moderator: Colin Parrish)**

1:30 – 2:00	p.m.	Colin Parrish	8 Plenary: <i>Variation In Transferrin Receptor Binding Determines Host Susceptibility To Feline And Canine Parvoviruses</i>
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2:00 – 2:20	p.m.	James Fox	9 Plenary: <i>Helicobacter spp. Influences the Development of Primary Gastric Lymphoma in Cats: A Testable Hypothesis</i>
2:20 – 2:40	p.m.	Edward Breitschwerdt	10 Plenary: <i>Bartonellosis: Of Cats, Dogs and Veterinary Professionals</i>
2:40 – 3:00	p.m.	Craig Packer	11 Plenary: <i>The Ecology of Disease Outcomes in the Serengeti</i>
3:00 – 3:20	p.m.	Christiaan Winterbach	12 Plenary: <i>Sero-Prevalence Of Feline Immunodeficiency Virus In African Lions – Is It Host Density Dependent?</i>
3:20 – 3:50	p.m.	Break	

### **Session 3 FIV Virology (Moderator: Eric Poeschla)**

3:50 – 4:10	p.m.	Eric Poeschla	13 Plenary: <i>An Essential Co-Factor For HIV And FIV Integration</i>
4:10 – 4:30	p.m.	Edward Hoover	14 Plenary: <i>In Vivo CXCR4 Expression, Lymphoid Cell Phenotype, And Feline Immunodeficiency Virus Infection</i>
4:30 – 4:45	p.m.	Benjamin Luttge	15 <i>Mechanisms Of FIV Release From Infected Cells</i>
4:45 – 5:00	p.m.	Benjamin Wang	16 <i>Pathogenic Determinants Of Feline Immunodeficiency Virus Accessory Gene ORF-A</i>
5:00 – 5:15	p.m.	Mauro Pistello	17 <i>Development of a Self-Inactivating Bicistronic Lentiviral Vector for Heterologous Primary Cells Transduction</i>
5:15 – 5:30	p.m.	Eric Poeschla	18 <i>Species-Specific Post-Entry Restriction Of FIV</i>
5:30 – 5:45	p.m.	Barnabe Assogba	19 <i>APOBEC In FIV Infection And Latency</i>

### **POSTER SESSION**

6:00 – 7:00	p.m.	Poster Session	Washington Room (posters will be up until Tuesday 3:40 p.m.)
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### **FREE EVENING**

## **Tuesday, October 10, 2006**

### **Session 4 Feline Immunology (Moderator: Naoya Yuhki)**

8:00 – 8:30	a.m.	Naoya Yuhki	20 Plenary: <i>Evaluation Of 2 X Cat Whole Genome Shotgun Sequence Assembly Based On Complete Annotation Of BAC Based MHC Sequence And SNP Analysis Of Two MHC Haplotypes</i>
8:30 – 9:00	a.m.	Mauro Bendinelli	21 Plenary: <i>The Tryptophan-Rich Motif (TrpM) in the Membrane- Proximal External Region (MPER) of the FIV TM as a Model for Fusion Inhibitors and Immunogens</i>
9:00 – 9:15	a.m.	Angela Mexas	22 <i>Effect Of CD4+CD25+ Regulatory T Cells On CD4+ T Helper Cell Immune Responses During The Acute Phase Of FIV Infection</i>

9:15 – 9:30 a.m. Jonathan Fogle *23 Ex Vivo Depletion Of CD4+CD25+ Regulatory T Cells Leads To Enhanced CD8+ T Cell IFN- $\omega$ ; Production During Acute FIV Infection*

9:30 – 9:45 a.m. Tracy Lehman *24 The Effects Of Chronic Feline Immunodeficiency Virus Infection On Bone-Marrow Derived Dendritic Cell Cytokine Production In Response To Toll-Like Receptor And CD40 Ligation*

9:45 – 10:15 a.m. Break

## **Session 5 FIV Vaccines & Therapy (Moderator: Janet Yamamoto)**

10:15 – 10:45 a.m. Janet Yamamoto *25 Plenary: Probing the Mechanisms of Dual-subtype FIV Vaccine Protection*

10:45 – 11:05 a.m. Mauro Pistello *26 Plenary: Should Accessory Proteins Be Structural Components of Lentiviral Vaccines? Lessons Learned From the Accessory ORF-A Protein of FIV*

11:05 – 11:20 a.m. Eiji Sato *27 Sequence Analysis of MHC-I Genes Confirms MHC-I-restriction in Dual-subtype FIV Vaccine Protection*

11:20 – 11:35 a.m. Francesca Bonci *28 A New Fluorimetric Assay For Monitoring Cytotoxic T-Lymphocyte Activity Elicited By A Prime Boost Vaccination Against FIV Env*

11:35 – 11:50 a.m. S. Rochelle Smithberg *29 Preservation Of Anti-FIV-P24 Effector Cells During Depletion Of Treg Cells From Feline Lymphoid Tissues*

11:50 – 12:05 p.m. Katrin Hartmann *30 Influence Of Feline Interferon- $\gamma$ ; On The Survival Time Of Cats Infected With Feline Infectious Peritonitis*

12:05 – 12:20 a.m. Kristina Howard *49 Difference In Mucosal FIV Pathogenesis Using Decreased Doses Of Viral Inoculum*

12:20 – 1:30 p.m. Complimentary Lunch Ballroom, General Session Room

## **Session 6 Feline Genomics & Viral Evolution (Moderator: Stephen J. O'Brien)**

1:30 – 2:15 p.m. Stephen O'Brien *31 Plenary: The Annotation and Promises of the Feline Genome Sequence*

2:15 – 2:35 p.m. Joan Pontius *32 Plenary: Use of Online Resources for the Annotation of Retroviral Interactions in the *Felis catus* Genome*

2:35 – 3:05 p.m. Alfred Roca *33 Plenary: Endogenous Retroviruses in the Domestic Cat Genome*

3:05 – 3:25 p.m. Jill Pecon-Slaterry *34 Plenary: Genetic Signatures Of FIV Evolution In Felidae*

3:25 – 3:40 p.m. Warren Johnson *35 Mitochondrial Introgressions Into The Nuclear Genome Of The Domestic Cat*

3:40 – 4:10 p.m. Break Poster Presenters: Please take down posters in Washington Room to prepare for banquet.

## **Session 7                    The Cat as a Model of Infectious Disease (Moderator: John Elder)**

4:10 – 4:40	p.m.	John Elder	36 Plenary: <i>FIV As A Model For Development Of Interventions Against HIV</i>
4:40 – 4:55	p.m.	Allison German	37 <i>Feline Foamy Virus Infection Associated With Subclinical Pathology In Cats</i>
4:55 – 5:10	p.m.	Surender Kumar	38 <i>Effect Of Alloantigen Exposure On MLR And Immune Phenotype</i>
5:10 – 5:25	p.m.	Nicola Fletcher	39 <i>Development Of A Feline Specific In Vitro Blood-Brain Barrier And Its Applications In The Study Of Feline Immunodeficiency Virus Infection</i>
5:25 – 6:00	p.m.	Round Table Discussion: <i>Integration Of Genomics And Infectious Disease In The Cat Model: Ongoing And Future Perspectives.</i> Stephen O'Brien (chair), John Elder, Hans Lutz, Sue VandeWoude, Mauro Pistello, Brian Willett	
6:00 – 7:00	p.m.	Cocktails	Sky Terrace <b>Sponsor: Kyoritsu Seiyaku Corporation</b>
7:00 – 11:00	p.m.	Banquet William Hardy, Jr.	Washington Room 40 <i>Lessons From Time Spent in Cat Houses: An Historical Overview of the Feline Leukemia Virus and Other Pathogens</i> <b>Sponsor: Office Of Rare Diseases, National Institutes of Health</b> <b>Sponsor: Fort Dodge Animal Health</b> <b>Sponsor: Pfizer Animal Health</b>

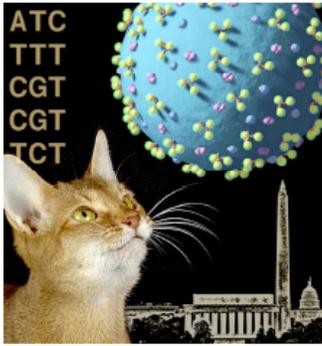
## **Wednesday October 11, 2006**

### **Session 8                    Retroviral Infection of Exotic Cat Species (Moderator: Mary Poss)**

8:00 – 8:30	a.m.	Mary Poss	41 Plenary: <i>Molecular Biology And Evolution Of Feline Lentiviruses</i>
8:30 – 8:50	a.m.	Melody Roelke	42 Plenary: <i>Wild African Lions And Florida Pumas Infected With FIV Reveal Distortions In Their T-Lymphocyte Profiles With Significant CD4 Cell Depletion: Clinical &amp; Pathological Consequences</i>
8:50 – 9:05	a.m.	Mark Cunningham	43 <i>Epizootiology and Management of Feline Leukemia Virus in Free-Ranging Florida Panthers—Research Update</i>
9:05 – 9:25	a.m.	Meredith Brown	44 Plenary: <i>Genetic Characterization Of Feline Leukemia Virus (FeLV) In The Free-Ranging Florida Panther (Felis concolor coryi) Population</i>
9:25 – 9:45	a.m.	Jennifer Troyer	45 Plenary: <i>FIV Cross-Species Transmission: An Evolutionary Prospective</i>

### **Session 9                    FeLV Virology & Pathogenesis (Moderator: Takayuki Miyazawa)**

9:45 – 10:15	a.m.	Takayuki Miyazawa	46 Plenary: <i>A Soluble Envelope Protein Of Endogenous Retrovirus Present In Serum Of Domestic Cats Mediates Infection Of A Pathogenic Variant Of Feline Leukemia Virus</i>
10:15 – 10:45	a.m.	Break	
10:45- 11:15	a.m.	Laura Levy	47 Plenary: <i>Selection and Pathogenesis of an Unusual FeLV Isolate Predominant in a Natural Cohort</i>
11:15 – 11:35	a.m.	Regina Hofmann-Lehmann	48 Plenary: <i>How Molecular Methods Change Our Views Of FeLV Infection And Vaccination</i>
11:35 – 11:50	a.m.	Andrea Torres	50 <i>Insight Into FeLV:Host Relationships Using Real-Time DNA And RNA qPCR</i>
11:50 – 12:05	p.m.	Regina Hofmann-Lehmann	51 <i>Cellular Segregation Of Feline Leukemia Virus In Leucocyte Subsets Of Long-Term Experimentally Infected Cats</i>
12:05 – 12:20	p.m.	Ravi Tandon	52 <i>Copy Number Polymorphisms Of Endogenous Feline Leukemia Virus-Like Sequences</i>
12:20 – 12:35	p.m.	Julie Levy	53 <i>Long-Term Outcome Of Cats With Natural FeLV And FIV Infection</i>
12:35 – 12:50	p.m.	Yasuhito Fujino	54 <i>Identification Of A Common Proviral Integration Site, Flit-1, In Feline Leukemia Virus-Induced Thymic Lymphomas</i>
12:50 – 1:05	p.m.	Stephen O'Brien	Concluding Remarks



# 8th International Feline Retrovirus Research Symposium

Washington, DC October 8–11, 2006

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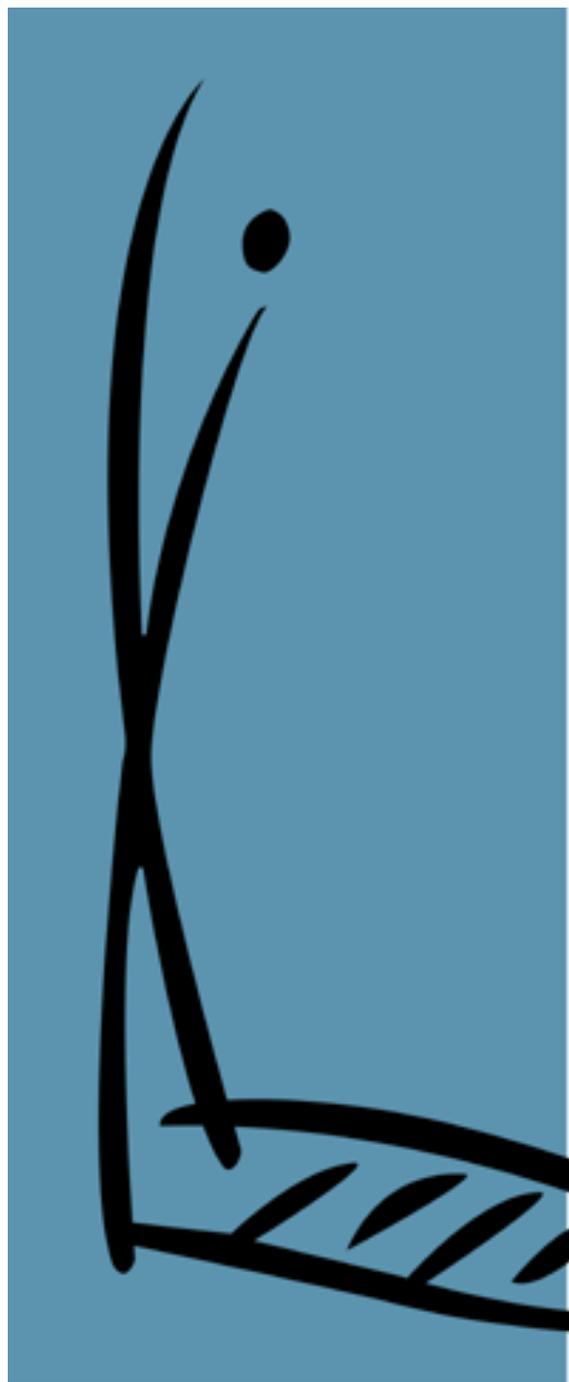
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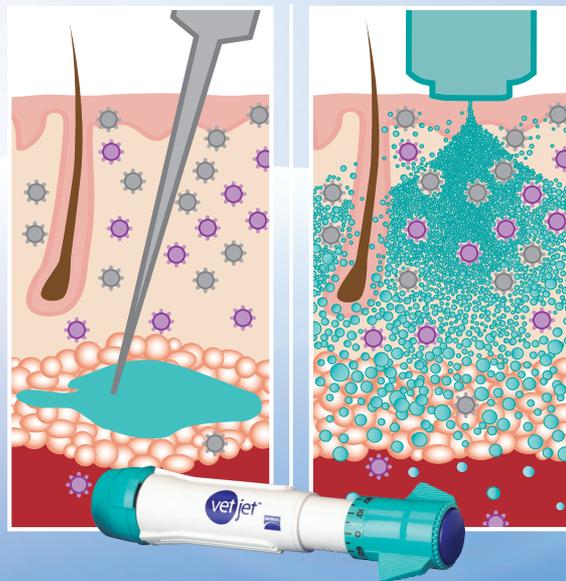


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# Prevalence of Retroviruses in Feline Oral Disease Cases

Jan Bellows, DVM, DAVIDC, DABVP<sup>1</sup>, Jessica L. Lachtara<sup>2</sup>

1. Hometown Animal Hospital and Dental Clinic, Weston, FL USA  
2. IDEXX Laboratories, Inc., Westbrook, ME USA

## Purpose

A study was conducted to determine the prevalence of feline immunodeficiency virus and feline leukemia virus in feline oral disease patients.

## Methodology

- Private veterinary practitioners were recruited to test feline patients that presented with oral disease.
- The IDEXX SNAP<sup>®</sup> FIV/FelV Combo Test was used to test this cat population from January through April 2006.
- Results were returned by fax for analysis.
- Prevalence was calculated as the percentage of positive test results reported out of the population of cats with oral disease tested.

## Results

- A total of 8,982 orally diseased cats were screened. Presenting cases included cats with plaque, calculus, gingivitis, periodontal disease, feline odontoclastic resorption lesions, gingivostomatitis and other oral diseases as defined by the practitioner.
- Of 8,982 cats tested, 1,276 were retrovirus-positive, a 14.2% prevalence (Figure 1).
- Cats with gingivitis and stomatitis exhibited 14.3% and 23.9% prevalence of retroviral infection, respectively (Figure 2).

## Discussion

Recent studies reported the prevalence of FeLV nationwide as 3.3%, and the prevalence of FIV as 3.4%.<sup>1</sup> In this study, the higher prevalence of retroviral infection in orally diseased cats indicates that almost one of every seven cats with oral disease has a retroviral infection. This study suggests screening cats with oral disease for retroviral infection to help understand and treat the multifactorial underlying causes.

FIV- and FeLV-infected cats often present vague, subtle signs of illness, but once a cat has been identified with one of these diseases, the veterinarian and the pet owner can work together to deliver proper care for the cat. The potential correlation in cats between oral disease and immune systems that are suppressed by FIV or FeLV gives veterinarians valuable new information in their efforts to fight these contagious viruses.

Figure 1

### Orally Diseased Cats

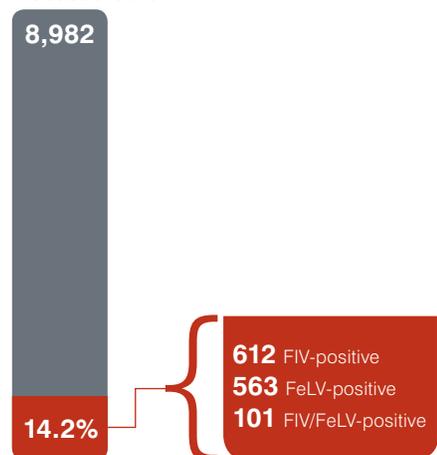


Figure 2

### Cats with gingivitis



### Cats with stomatitis



1,276 Number of retrovirus-positive cats 1,113 467



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1. Levy JK, Crawford PC, Brien JL. Prevalence of FIV and FeLV in the United States. Proceedings from: Seventh International Feline Retrovirus Research Symposium; September 11–15, 2004; Pisa, Italy.

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**ABSTRACTS--PODIUM PRESENTATIONS**

## 1. Presenting Author: Osterhaus

Newly Emerging Viral Infections

Albert D.M.E. Osterhaus

Department of Virology, Erasmus MC, Rotterdam, The Netherlands

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In the past century, pandemic outbreaks of influenza and AIDS have cost the lives of tens of millions of people. These events were all caused by multiple introductions of animal viruses – influenza A viruses and SIV of birds and non-human primates respectively – into the human population. Besides these introductions causing major pandemics in humans, a large number of other virus infections have spilled over from animal reservoirs to humans or other susceptible species, resulting in considerable morbidity and mortality as “virgin soil” epidemics. The most recent examples in humans are the introduction of SARS coronavirus and influenza A viruses (H5N1 and H7N7) from the animal world, which caused global concern about their potential to be at the origin of new pandemics. Over the last decades there seems to be a dramatic increase in the emergence or re-emergence of virus threats in humans and animals worldwide. A long list of exotic names like Ebola, Lassa, Rift-Valley, Crimea-Congo, Hendra, Nipah and West-Nile is the illustration of names of just some of the places associated with the origin of viruses that crossed the species boundary to humans, with dramatic consequences in the last ten years alone. Similarly, recent mass mortalities among wild aquatic and terrestrial mammals caused by previously known and newly discovered morbiliviruses, as well as outbreaks of hog cholera, foot-and-mouth disease and fowl plague among domestic animals, highlight this trend.

Although improved detection and surveillance techniques, as well as increased media attention may have contributed to our perception of an increase in the incidence of outbreaks of virus infections, it is becoming more and more clear that major changes in our modern society increasingly create new opportunities for virus infections to emerge: a complex mix of changes in social environments, medical and agricultural technologies and ecosystems continues to create new niches for viruses to cross species boundaries and to rapidly adapt to new species. In combating this global threat, we should make optimal use of the new tools provided by the unprecedented advances made in the research areas of virology, molecular biology, immunology, epidemiology, genomics and bioinformatics. Serious investment in these areas in the future will not only be highly cost-effective but will also save many lives of humans and animals. In addition, better collaboration and coordination between all the stakeholders is urgently needed, to establish early warning systems and effective preparedness plans.

## 2. Presenting Author: Mary Tompkins

Immune Dysfunction In FIV Infection: An Overview.

Mary B. Tompkins. Dept. of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh NC 27606.

Our ability to study the feline immune response has been driven by the use of the cat as a model for human retrovirus infections. As human AIDS became a major problem, funding for the development of reagents to identify specific feline cell types became more available, resulting in a number of monoclonal antibodies identifying lymphocyte subsets. After the isolation of FIV, there was an explosive development of reagents and methods allowing for analysis of many aspects of feline immune function. Over the past 15 years, numerous laboratories have identified mechanisms of feline retrovirus-induced immune suppression, including lymphocyte subset alterations, cytokine dysregulation, and activation of Treg cells. Additionally, chronic low level antigenemia appears to result in T cell hyperactivation, which is manifested as a progressive loss of CD62L, an upregulation of activation markers such as CD49d, and CD44, and of the co-stimulatory molecules B7.1, B7.2, and CTLA-4. Thus infection with HIV/FIV results in profound immune dysregulation manifested by both immune suppression and immune activation.

3. Presenting Author: Brian J. Willett

CHEMOKINES AND CO-STIMULATORY MOLECULES: UNRAVELLING THE MECHANISM OF INFECTION WITH FIV.

Brian J. Willett\*, Elizabeth L. McMonagle & Margaret J. Hosie.

Retrovirus Research Laboratory, Division of Veterinary Infection and Immunity, Institute for Comparative Medicine, University of Glasgow, Glasgow G61 1QH, UK,

The feline homologue of CD134 (fCD134) is the primary binding receptor for feline immunodeficiency virus (FIV), targeting the virus preferentially to activated CD4+ helper T cells. However, with disease progression, the cell tropism of FIV broadens such that B cells and monocyte/macrophages become significant reservoirs of proviral DNA, suggesting that receptor utilisation may alter with disease progression. Previously, we showed that strains of FIV differ in their utilisation of CD134; the prototypic strain PPR requires a minimal determinant in CRD1 of fCD134 to confer near optimal receptor function while strains such as GL8 and CPGammer require additional determinants in the CD134 CRD2. Here, we map this determinant to a loop in CRD2 governing the interaction between the receptor and its ligand; substitution of amino acids S78N,S79Y,K80E restored full viral receptor activity to the CDR2 of human CD134 in the context of feline CD134 with tyrosine-79 appearing to be the critical residue for restoration of receptor function. Mutation of this region of CD134 determined species-specificity of CD134L binding, consistent with these regions forming a point of contact between CD134 and CD134L and raising the possibility that infection with primary pathogenic strains of FIV may be sensitive to modulation by CD134L. Revealing the biological significance of the differential utilization of CD134 by FIVs may inform the design of novel strategies for vaccination and therapy and further strengthen the comparative value of FIV infection of the domestic cat as a non-primate model for HIV and AIDS.

#### 4. Presenting Author: Sue VandeWoude

##### Mechanisms of Feline Lentivirus Interference and Control

Sue VandeWoude\* (1), Julie Terwee (1), Jennifer Troyer (2), and Mary Poss (3). (1) Department of Microbiology, Immunology, Pathology, Colorado State University, Fort Collins, CO, 80523, USA; (2) Laboratory of Genomic Diversity, National Cancer Institute-Frederick, Frederick, MD 21782, USA; (3) Division of Biological Sciences, University of Montana, Missoula, MT, 59812, USA.

Whereas the incidence FIV in domestic cats is approximately 1%, and infection results in a progressive immunodeficiency syndrome, nondomestic cat FIVs often occur with seroprevalence rates above 30% that increase with age. Lack of concurrent clinical disease suggests that these viruses are at most only marginally pathogenic for native hosts. Our studies have demonstrated that puma-origin FIVs can infect domestic cats and cat cells. Puma FIV (FIV-pco) infection of domestic cats demonstrates distinct differences from naturally occurring or experimental FIV infection. Namely, FIV-pco infection of domestic cats: 1. Causes productive infection via IV or oronasal exposure, resulting in seroconversion and lymphadenopathy; 2. Does not induce clinical disease or changes in circulating CD4<sup>+</sup> levels; 3. Results in initial rise in viral load followed by diminution below detectable limits in a majority of animals; 4. Is associated with adaptive immune responses that follow, rather than proceed, viral control; 5. Distinct from domestic cat FIV, is distributed in gastro-intestinal vs. lymphoid compartments, correlating with divergent receptor use; 6. Results in cytokine profile changes distinct from typical FIV infection; and, 7. Consistent with cytidine deaminase activation, demonstrates high rates of G to A substitution errors following in vivo replication. Further, infection with FIV-Pco blunts subsequent challenge with virulent domestic cat FIV both in vitro and in vivo. This feline model of parameters underlying successful cross-species lentiviral transmission provides a useful tool to study mechanisms of innate and adaptive immune correlates of protection against primary lentiviral infection and secondary challenge with virulent isolates.

Supported by NIH-AI52055 and AI-49765

5. Presenting Author: Peter J.M. Rottier

Genetically Modified Feline Coronaviruses as Vaccines for Protection against Feline Infectious Peritonitis (FIP).

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Feline coronaviruses are common pathogens of cats. Two serotypes (I and II) have been recognized, each one of them additionally occurring in two markedly different pathotypes. The most common pathotype, enteric FCoV (FECV) causes mild, often unapparent enteritis, the other one, feline infectious peritonitis virus (FIPV), a devastating, highly lethal systemic infection. It has become clear that FIPVs arise from FECVs by mutation in infected cats, but the mutations responsible for the virulence shift have not yet been determined. As there are no adequate therapeutic options to treat cats suffering from FIP, effective vaccines are urgently needed. In this presentation I will report about our studies aimed to generate live virus vaccines by genetically attenuating virulent FIPV.

6. Presenting Author: Hans Lutz

## **Diagnosis of Feline Leukemia Virus (FeLV) Infection. Review and Outlook**

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FeLV is a gammaretrovirus of cats that causes immunodeficiency, anemia and lymphomas. It exists in an endogenous and an exogenous form and only the latter is of clinical importance. Diagnosis of FeLV infection is based on the detection of exogenous virus or viral components; detection of antibodies specific for FeLV are hardly used in the clinical setting.

Shortly after the discovery of FeLV, detection of FeLV proteins in tissues of FeLV infected cats was accomplished by Hardy and coworkers, using immunodiffusion techniques. Indirect immunofluorescence assay (IFA) followed that allowed the specific detection of gag proteins within blood leukocytes and platelets. The ELISA tests based on monoclonal antibodies that came into use in the eighties had the advantage that they could be performed under field conditions. They were found to be highly sensitive and were the basis of further insight into the pathogenesis of the infection. During the nineties, ELISA methods were complemented by immunochromatography procedures. Recently, PCR and RT-PCR procedures have become available. These procedures are especially useful for further characterization of the pathogenesis. By detection of provirus, it became clear that around 10% of all cats in the field are provirus-positive but negative for FeLV antigen in blood, suggesting that they have overcome antigenemia, but still carry viral genes. RT-PCR allows the detection of viral RNA in saliva and feces; in pooled samples, even 1 positive cat in 30 non-infected cats can be readily detected. With increasing degree of automation, PCR procedures will become widely used also in clinics.

7. Presenting Author: Oswald Jarrett

## HOW FeLV CHANGED THE WORLD

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FeLV research has had a significant impact on feline welfare, comparative biology and human retrovirology. Since its discovery, the prevalence of FeLV has declined dramatically until the infection is now rare in some areas. The benefits to cat health have been equally striking, as the outcome of persistent infection is almost always fatal. This success is due to the application of diagnostic tests to identify and separate infected from non-infected cats; and vaccination. Because FeLV has evolved in groups of cats in close contact, but is poorly transmitted in free-ranging cats, these measures have reduced the incidence of infection in the whole community. Continuing vigorous application of these measures should eradicate the infection from even larger populations of cats.

Various by-products of FeLV research that have been valuable in comparative medicine include: the discovery of several oncogenes, including *sis* and *kit*, that are involved in signal transduction; examples of ways in which genes may collaborate in leukaemogenesis; and, through the study of FeLV-C, which causes pure red cell aplasia, the identification of the human haem transporter that is essential for erythroid differentiation.

FeLV research also strongly influenced the discovery of human retroviruses. As an example of a horizontally transmitted, naturally occurring virus causing leukaemia, FeLV provided crucial support for the establishment of the Special Virus Cancer Program. Subsequently, the search for viruses in T-cell tumours, driven by the knowledge that this is the predominant form caused by FeLV, led to the discovery of HTLV, and subsequently HIV.

8. Presenting Author: Colin Parrish

Variation in transferrin receptor binding determines host susceptibility to feline and canine parvoviruses.

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The host ranges of viruses are specific properties that are determined by the interactions between viral and host components, and variation in viruses to allow them to infect and spread among new hosts is quite uncommon but can lead to disease epidemics. Feline parvovirus (FPV) is a well established pathogen of cats. Canine parvovirus (CPV) is a host range variant of FPV which first emerged around 1978 and then spread worldwide, and within 2 years that original CPV strain was replaced by an antigenic and host range variant. The different virus strains show distinct host ranges for the cat and dog, and those were primarily due to differences in the capsid protein of the virus, which also altered the antigenic structure of the capsid.

The key host determinant of cell infection and host range is the transferrin receptor (TfR), and capsid interactions with the TfR appear to have been refined by selection of the viruses. Although all of the viruses can infect feline cells by binding the feline TfR, the canine host range and canine cell infection by the various CPV strains results from canine TfR binding. The affinities of the interactions of the capsids with the TfRs are quite variable, with higher affinities being seen for the binding to the feline TfR, and only very low affinity binding to the canine TfR.

The feline and canine TfRs differ by 11% of their protein sequences, but residues within the receptor apical domain have the greatest influence on viral binding. The most important difference was a mutation that introduced a new glycosylation site into the canine TfR, and when that site was changed the mutant receptor bound to all of the viruses including FPV.

An interesting aspect of the evolution of the viruses is that several of the naturally variant viruses contain antigenic changes, but most of the variations that affect antibody binding also affect TfR binding, and the selection is therefore most likely for the TfR binding rather than immune selection.

Less well understood aspects of the virus-host interactions include the factors that allow FPV to infect canine thymocytes *in vivo*, but not other cells in the dog. Others are the determinants of the restricted ability of the first CPV strains to infect the cat, while the more recently emerged strains were able to infect cats and cause mild disease. Variation in the TfR expression, glycosylation and binding may also be involved in these different natural host ranges.

9. Presenting Author: James G. Fox

*Helicobacter* spp. Influences the Development of Primary Gastric Lymphoma in Cats: a Testable Hypothesis

J.G. Fox,<sup>1\*</sup> R.P. Marini,<sup>1</sup> H. White,<sup>1</sup> S. Schelling<sup>2</sup>

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Gastric mucosa-associated lymphoid tissue (MALT) lymphoma in humans is associated with *H. pylori* and more recently '*H. heilmannii*' infections. Alimentary lymphoma is the most common form of lymphoma in domestic cats. Numerous studies have shown that these tumors are predominantly of B cell origin and are predominantly negative for feline leukemia virus by antigen tests. Gastric colonization by *Helicobacter* spp in cats is very common but is of unknown significance. To ascertain whether *Helicobacter* infection in cats was associated with primary gastric lymphoma, tissues collected by gastric endoscopic biopsy or surgical resection from 72 cats (25 with gastric lymphoma, 23 with gastritis, and 24 with normal gastric mucosa) were evaluated histologically in hematoxylin and eosin-and Warthin Starry-stained sections. Lymphomas were of two major morphologic types, lymphocytic and lymphoblastic. Ninety-two percent of tissue samples from cats with gastric lymphoma were positive for argyrophilic organisms; the percentage positivity for cats with gastritis and for normal cats were 78 and 54 % respectively. These findings raise the intriguing possibility that MALT gastric lymphoma of the cat may be associated with *Helicobacter* spp. infection. Such a causal relationship has been established in the *Helicobacter felis* and *H. heilmannii* infected mouse-model and the *H. mustelae* model of ferrets. Recently, eradication of *Helicobacter heilmannii* in humans with MALT-lymphoma effected regression of the tumors. These preliminary findings have broad implications for the treatment and understanding of gastric lymphoma in cats.

## 10. Presenting Author: Ed Breitschwerdt DVM

### Bartonellosis: Of Cats, Dogs and Veterinary Professionals

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The genus *Bartonella* is currently comprised of at least 20 species or subspecies of vector-transmitted, fastidious, gram-negative bacteria that are highly adapted to one or more mammalian reservoir hosts. *Bartonella* organisms usually cause a long-lasting intraerythrocytic bacteremia. Domestic and wild cats can serve as a reservoir for *B. henselae*, whereas domestic and wild canines appear to be the reservoir for *B. vinsonii* subspecies *berkhoffii*. In a seemingly healthy, well-adapted host species (particularly true of cats, rodents and ruminants), chronic *Bartonella* bacteremia can be readily detected by blood culture or PCR amplification of organism specific DNA from various study populations.

Immunosuppression is an important factor that dictates the pattern of disease expression in cats, dogs and people infected with a “host adapted” versus a “non-host adapted” *Bartonella* spp. When using comparable diagnostic modalities, isolation or PCR detection of non-host adapted *Bartonella* species is much more difficult than the detection of host-adapted species. Although incompletely studied, concurrent infection with FIV and *B. henselae* appears to cause a significant increase in lymphadenopathy and gingivitis in cats. In people, HIV-induced immunosuppression facilitates the development of *Bartonella* associated vasoproliferative lesions (bacillary angiomatosis, peliosis hepatitis). In children, an incomplete immune response can cause granulomatous inflammation (lymphadenitis, hepatitis and splenitis), following cat scratches or bites (Cat Scratch Disease).

With the advent of more sensitive diagnostic techniques, a spectrum of “non-host adapted” *Bartonella* spp. are being found in sick dogs and people. Due to the ability of *Bartonella* spp. to induce chronic bacteremia in healthy individuals, implicating disease causation for host adapted *Bartonella* spp. can be challenging, particularly in pet cats. During the past decade an increasing number of *Bartonella* spp. have been implicated as a cause of human (7 species) and canine (4 species) endocarditis. The dog appears to provide an excellent comparative medical model for human bartonellosis. *Bartonella vinsonii* (*berkhoffii*), first isolated from a dog with endocarditis in our laboratory, was subsequently described as a cause of human endocarditis. Antinuclear antibodies have been reported in dogs naturally infected with *B. vinsonii* (*berkhoffii*) and anti-neutrophil cytoplasmic antibodies in people exposed to *B. henselae*. *Bartonella henselae* causes peliosis hepatitis, a unique pathological lesion in HIV infected individuals and we have amplified and sequenced *B. henselae* from the liver of a dog with peliosis hepatitis and from dogs with granulomatous hepatitis or lymphadenitis. *Bartonella* species that infect cats or dogs via vector transmission can subsequently be transmitted to people by a scratch or bite. Recently, we have generated data supporting persistent infection with *B. henselae* and *B. vinsonii* *berkhoffii* in immunocompetent veterinary professionals.

## 11. Presenting Author: Craig Packer

The Ecology of Disease Outcomes in the Serengeti

Craig Packer

University of Minnesota

Disease surveys show that lions in the Serengeti and Ngorongoro Crater, Tanzania, are continuously exposed to anthrax, Babesia, bovine TB, FHV, FIV, and trypanosomiasis, and these lions also suffer from discrete outbreaks of calicivirus, CDV, coronavirus, parvovirus, and Rift Valley Fever at 4-10 yr intervals. None of these diseases appear to pose a significant threat to the host populations except in combination with each other. CDV only appears to inflict significant morbidity and mortality when the lions are simultaneously exposed to exceptionally high levels of tick-borne piroplasms, and this co-infection is driven by periodic droughts in the region. In contrast, co-infection with two or more species of trypanosomes appears to confer highly effective cross-immunity, particularly against the causative agent of sleeping sickness, *Trypanosoma brucei rhodesiense*. However, co-infection with FIV has not yet been found to increase the pathogenicity of any other disease. Although we lack a control group of FIV- adult lions, we cannot find any evidence that FIV poses a substantial threat to these populations.

12. Presenting Author: Christiaan W. Winterbach

Sero-prevalence of Feline immunodeficiency virus in African lions – is it host density dependent?

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Tau Consultants (Pty) Ltd, Maun, Botswana,<sup>1</sup> and Tswane University of Technology, Pretoria, South Africa,<sup>2</sup> and WILDCRU, Oxford,<sup>3</sup> and IRSP Program, SAIC-Frederick, Frederick,<sup>4</sup> and Laboratory of Genomic Diversity, National Cancer Institute-Frederick, Frederick, Maryland 21702,<sup>5</sup>

## ABSTRACT

Feline immunodeficiency virus FIV infects a wide variety of host species, and it is confirmed to be endemic in free-ranging populations of nine Felidae and one Hyaenidae species. This includes the lion (*Panthera leo*) in Africa, where FIV is widely distributed in multiple populations at diverse FIV-prevalence levels.

We found a correlation ( $R^2 = 0.732$ ,  $n=9$ ) between sero-prevalence of FIV and lion population density, indicating that the prevalence of FIV in lion populations may be density dependant. In populations with a high sero-prevalence of FIV most individuals are FIV positive before they reach sexual maturity, and most FIV transmission should result from other types of contact between individuals. We investigated indirect measures of potential contact between individuals and between prides to determine if these indices support the FIV density dependence hypothesis. High density lion populations from the Okavango Delta (Botswana) and the Kruger National Park in South Africa are compared with low density populations from the Makgadikgadi Pans National Park and the Kgalagadi Transfrontier Park in Botswana. Average group size and pride size were used as indicators of contact between individuals within the pride. The potential contact between prides was assessed from home range size, the home range overlap between neighbouring prides and the average and maximum distances lions moved.

13. Presenting Author: Eric Poeschla

## An essential co-factor for HIV and FIV integration

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The transcriptional coactivator LEDGF/p75 (p75) can bind to lentiviral (HIV-1, FIV) integrase proteins, shield them from proteasomal degradation, and tether them to chromatin. p75 knockdown changes the genomic pattern of integration by reducing the viral bias for active genes. However, severely p75-depleted cells that revealed these phenotypes have supported undiminished or marginally-reduced overall levels of lentiviral replication and integration, causing skepticism that p75 has a more fundamental role in the viral life cycle. Here we solve this problem by identifying and targeting a fractionally minute yet virologically potent chromatin-bound p75 residuum refractory to previous depletion methods. Eradication of this spatially opportune reservoir in human CD4+ T cells by intensified and specifically rescued RNAi demonstrates that p75 is a required and potent lentiviral integration co-factor. For both HIV-1 and FIV, virological activity requires both linkages of the integrase-to-chromatin tether. Combining endogenous knockdown with a dominant-negative p75 fragment caused a five hundred-fold reduction in HIV-1 susceptibility. The functional super-abundance of p75 may help to explain evolution of lentiviral dependence on this single protein.

14. Presenting Author: Edward A. Hoover

**Abstract:** IN VIVO CXCR4 EXPRESSION, LYMPHOID CELL PHENOTYPE, AND FELINE IMMUNODEFICIENCY VIRUS INFECTION

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Primary isolates of feline immunodeficiency virus (FIV) appear to require binding to CD134 in conjunction with CXCR4(X4) to infect IL-2 dependent T-cell derived cells in culture. However, much less is known about the role of X4 for infection of cells in vivo. To investigate the correlation between X4 expression and FIV infection in cells and tissues of cats acutely or chronically infected with FIV-C-Pgmr, we used high speed fluorescence activated cell sorting combined with realtime PCR to analyze cell phenotypes from lymph node, thymus, bone marrow and blood for FIV infection and X4 expression. X4 expression was greatest in lymph node cells, both in frequency and in mean fluorescence intensity. Assuming a minimum of one proviral copy per cell, the maximum mean proportion of X4+ cells bearing provirus was ~50% overall. Conversely, ~50% of the FIV DNA burden was associated with X4-cell subsets. Overall, the thymus had the highest FIV DNA burden in unsorted cells and X4+ cells. Proviral loads were higher in T cells than in B cells regardless of X4 status or tissue. The bone marrow contained the highest frequency of FIV-infected X4- cells. In acute infection, viral DNA levels were highest in thymus, whereas in chronic infection viral burden was greatest in bone marrow cells. Taken together these results demonstrate both a positive association of X4 expression and FIV infection in vivo and also demonstrate that substantial apparently X4-independent infection also occurs in other target cell populations.

## 15. Presenting Author: Benjamin G. Luttge

### Mechanisms of FIV Release From Infected Cells.

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Retroviruses use short peptide motifs called “late domains” to usurp cellular endosomal sorting machinery to promote virus release. For example, it has been shown that ProThrAlaPro (PTAP) in HIV-1 Gag interacts with TSG101, an essential component of ESCRT I. HIV-1 release can become severely restricted upon exogenous expression of the N-terminal fragment of TSG101 (TSG-5') containing the PTAP binding site. We proposed that FIV utilizes a similar motif (PSAP) to bind TSG101 and facilitate virus release. Expression of TSG-5' in HeLa cells inhibited FIV release, and stable TSG-5' expression in CrFK cells inhibited FIV replication. Defects in virus budding were confirmed by EM analysis. Mutagenesis of the PSAP motif also resulted in inhibited FIV release and inefficient virus replication. FIV release was also sensitive to expression of other dominant negative forms of ESCRT-related components, such as VPS4 and AIP1/Alix, in a manner similar to other retroviruses. Endogenous AIP1/Alix, rather than TSG101, has been shown to facilitate the release of the non-primate lentivirus EIAV in HeLa cells and may also be partially utilized by HIV. Mutagenesis of a potential AIP1/Alix binding site motif (LxxL) in FIV Gag did not affect FIV release in our studies, but may affect virus replication in feline T-cells. Our data suggest that FIV relies on the PSAP motif as a likely TSG101 binding site to exploit the cellular endosomal sorting machinery as a platform for virus release in HeLa cells, and that this mechanism of virus release is conserved in feline cells.

16. Presenting Author: Ben Wang

### Pathogenic Determinants of Feline Immunodeficiency Virus Accessory Gene Orf-A

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FIV accessory gene *orf-A* encodes a 77 amino acid protein which is critical during virus replication. Our previous studies have shown Orf-A to be important in late steps of the FIV life cycle involved in virion formation and in early steps involved in virus infectivity and have mapped critical Orf-A domains needed for these steps in replication. In an ongoing localization study, expression plasmids for wild-type (WT) and various mutant GFP-Orf-A fusion proteins were transfected into mammalian and feline cell lines to examine subcellular distribution of Orf-A. Fluorescence and confocal microscopy studies revealed that FIV-pPPR Orf-A expressed either as an N-terminal or C-terminal GFP fusion, localized to the nucleus when assayed 24 hr post-transfection. Similar findings were observed for a GFP fusion protein encoding Orf-A derived from the pathogenic NCSU FIV molecular clone pJSY3. Furthermore, studies testing point mutants involving a stretch of lysines and arginines spanning residues 44-53, confirmed this sequence to be critical for nuclear localization. However, assessment of additional GFP-Orf-A deletion mutants revealed N-terminus to also be critical for Orf-A nuclear localization, indicative of a bipartite NLS. Studies utilizing these mutant Orf-A expression plasmids to map determinants for cell cycle arrest and apoptosis are ongoing and will test for correlation of Orf-A subcellular localization with cytotoxic properties expressed by Orf-A .

17. Presenting Author: Mauro Pistello

## Development of a Self-Inactivating Bicistronic Lentiviral Vector for Heterologous Primary Cells Transduction

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Lentiviral vectors (LVs) are efficient tools for gene transfer in dividing and non-dividing target cells. Compared to other vectors, LVs offer several advantages including stable transduction, transportation of relatively large heterologous genes (transgenes), and high versatility.

If appropriately engineered, FIV has been shown to vehiculate transgenes in heterologous cells at comparable efficiency to HIV vectors whose inherent safety problems have hitherto prevented their *in vivo* use.

We have previously produced and validated a self-inactivating, broad-host-range, monocistronic FIV-LV. From this prototype we have constructed a bicistronic vector (FIV-LV-BC) co-delivering two transgenes expressed under the same cytomegalovirus promoter and the internal ribosome entry site of encephalomyocarditis virus cloned between the transgene cassettes.

Stability and efficiency of vehiculation of the bicistronic vector were evaluated with the FIV-LV-BC carrying the glycoprotein B1 of Herpes Simplex type-1 (gB1) and the Green Fluorescent Protein (GFP). The FIV-LV-BC and transduction protocol were initially assessed for efficiency of transduction in feline, human and murine cell lines and subsequently optimized for murine dendritic cells (DCs) transduction. All tests were performed with control FIV-LVs carrying gB1 or GFP alone.

The results indicated that both vectors deliver the transgenes at comparable level in heterologous cell lines and *ex-vivo* murine DCs. FIV-LV-BC construct might help to design effective and powerful gene therapy protocols and vaccination strategies.

18. Presenting Author: Eric Poeschla

**Species-specific post-entry restriction of FIV**

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We have been analyzing species-specific post-entry restrictions of FIV. Rhesus and human TRIM5 $\alpha$  proteins were found to restrict pseudotyped FIV and stable RNAi knockdown of endogenous TRIM5 $\alpha$  markedly increased FIV infectivity. While nonprimate cell lines varied in susceptibility, normalized FIV and HIV-1 vectors varied concordantly. In human and monkey cells, FIV and HIV-1 restrictions were discordant in some but not all cell types, with the greatest relative deficit for FIV observed in human T cells. Restrictions were saturable in some cells but not others. Further analyses of human restriction of FIV in different cell types and the role of cyclophilin A will be presented.

19. Presenting Author: Barnabe Assogba

APOBEC in FIV infection and latency

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The innate immune response is critical to enteric disease resistance and the induction of mucosal adaptive immunity. Our laboratory recently identified that mucosal administration of low dose (10<sup>2</sup> and 10<sup>3</sup>) cell-associated feline immunodeficiency virus (FIV) promotes viral latency in feline peripheral blood mononuclear cells (PBMC) and tissue lymphocytes. In this study, our objective is to determine whether the expression of feline apolipoprotein B mRNA-editing enzyme catalytic-polypeptide 3G (fAPOBEC3G) in these cats contributes to viral latency. To this end, we isolated total RNA and collected protein lysate from feline cell lines, PBMC and tissue lymphocytes of each cat and developed reverse transcriptase RT-PCR and western blot assays to quantify fAPOBEC3G in cats exposed to high versus low dose cell-associated FIV. fAPOBEC3G was significantly expressed in uninfected feline T cell lines (ECD4+ and MYA-1 cells), Crandell feline kidney and primary bone marrow derived macrophages and variably expressed in primary feline PBMC. However, following FIV-infection, protein expression decreased or was completely abolished in feline primary and cells lines. Interestingly, lytic FIV-infected correlates with a decrease in fAPOBEC3G at the early stage of the post-infection whereas stable expression was observed in latently FIV-infected cats. This is the first report that the expression of fAPOBEC3G is abrogated in lytic but not in latent FIV infection. Dissecting these pathways in an animal model of latency will further our understanding of the host defense mechanisms against HIV-1 infection.

## 20. Presenting Author: Naoya Yuhki

Evaluation of 2 x Cat Whole Genome Shotgun Sequence Assembly based on Complete Annotation of BAC based MHC Sequence and SNP Analysis of two MHC Haplotypes.

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The Major Histocompatibility Complex encodes many immunoregulatory molecules that control both adaptive and innate immune systems by presenting a peptide to TCR (T-cell receptor) and by interacting NKR (NK receptor). Gene annotation of Major Histocompatibility Complex (MHC) Regions in the domestic cat was completed and identified 202 possible coding regions by GENSCAN program. The feline MHC is located on a pericentromeric region of a long arm of chromosome B2 and was split into two regions by the break on the distal class I region and translocated to a subtelomeric region of the same B2 chromosome by a chromosome inversion. The first region spans 2.976 Mbp sequence, which encodes six classical class II antigens (three DRA and three DRB antigens), nine antigen processing molecules (DOA/DOB, DMA/DMB, TAPASIN, and LMP2/LMP7, TAP1/TAP2), twelve class I antigens (MHC\_IA to MHC\_IL), two class I related (MIC) molecules. The second region spans 0.362 Mbp sequence encoding no class I genes (in human HLA at least eleven class I genes in this HLA-A corresponding region) and only framework genes, including three olfactory receptor genes were found. Three major feline endogenous retrovirus groups: FeLV-subtype A-like, ECE-RD114-like and, Porcine Leukemia virus-like sequences were found within a 100 Kbp interval in the middle of class I region.

The 2 x Cat Whole Genome Shotgun (WGS) Sequence Assembly was completed from DNA molecules isolated from an Abyssinian female cat, Cinnamon. This sequence assembly was aligned based on approx, 1800 markers and conserved sequence blocks and canine genome sequences. To evaluate this assembly/alignment, sequence contigs from 2x cat WGS were aligned with three MHC models: BAC-based cat MHC sequence and canine WGS sequence assembly (canFam2), and human WGS sequence assembly (hg 17). In the comparison of canine genome vs. cat BAC based alignment, 486 versus 1339 WGS contigs were correctly aligned in the MHC (36.3 %), while 389 contigs were correctly aligned in the human MHC model (29.0 %). For coding sequence, 54 % of coding genes (with more than 50% of total exons) were found in the cat 2x WGS contigs based on the canine MHC model and this number has been increased to 69 % and 85 % in gene rich MHC class II and class III region, respectively. Only 32 % of coding genes were correctly aligned in MHC class I region based on the canine MHC model, suggesting that (i) class I region close to heterochromatin regions (pericentromeric and subtelomeric) has different gene contents in each species and/or (ii) species-specific gene turnover in class I region is taking place.

Cinnamon MHC locates on a homozygous region based on SNP count (2 in 10 Kbp). Therefore we attempted to analyze SNP level in two MHC haplotypes (Cinnamon and Gus (BAC) MHCs). 11,654 SNPs were found in 3.34 Mbp cat MHC (0.0034 SNP per bp), which is comparable to the human MHC SNP count and rate: 16,013 SNPs in 4.75 Mbp (0.00337 SNP per bp), but higher coding SNPs and nonsynonymous substitutions.

21. Presenting Author: Mauro Bendinelli

The Tryptophan-Rich Motif (TrpM) in the Membrane-Proximal External Region (MPER) of the FIV TM as a Model for Fusion Inhibitors and Immunogens

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The TrpM in the MPER of the TM glycoprotein is essential for cell entry of FIV as well as HIV-1 and SIV. Moreover, the TrpM of HIV-1 is contained entirely or partly in several entry inhibitors, including T200 (enfuvirtide) which is extensively used in AIDS therapy, and in the epitopes recognized by the few monoclonal antibodies that possess broad neutralizing activity. Recently, we focused on the corresponding region of FIV in an attempt to design fusion inhibitors and protective immunogens.

With regard to fusion inhibitors, we found that a synthetic octapeptide reproducing solely the TrpM effectively inhibited all the FIV isolates tested in vitro. Furthermore, the retroinverso analogue of this peptide exhibited satisfactory pharmacokinetic properties and exerted substantial antiviral activity in vivo. Finally, extensive structure-activity studies allowed the development of small modified peptides which are as stable as the retroinverso one but much less costly to produce.

With regard to MPER-derived immunogens, we found that the TrpM expresses at least one potential B epitope as determined by immunizing cats with a phage-conjugated 20-mer peptide, and yet FIV-infected cats fail to react with it serologically. To investigate whether the FIV MPER could be highly membrane-context dependent, we synthesized a lipoylated 20-mer peptide reproducing this region and explored its conformational behavior in a membrane mimicking environment. Given its satisfactory structural features, this lipoylated peptide is currently being studied for the ability to elicit neutralizing antibodies when adsorbed onto membrane-like micelles and injected into cats.

22. Presenting Author: Angela Mexas

Effect Of CD4+CD25+ Regulatory T Cells On CD4+ Thelper Cell Immune Responses During The Acute Phase Of FIV Infection

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HIV-induced AIDS may be mediated by the activation of immunosuppressive CD4+CD25+ T regulatory cells (Tregs). Tregs have been shown to regulate CD4+ and CD8+ immune responses to HIV and FIV antigens in vitro. We tested the hypothesis that Tregs become infected and activated during the acute stage of FIV infection leading to the suppression of CD4+ Thelper cell responses. Cats were experimentally infected with FIV-NCSU-1 and blood and lymph node biopsies were collected at 1 week intervals following inoculation. Real-Time PCR was used to determine plasma viremia and relative number of FIV copies in CD4+CD25+ and CD4+CD25- T cell subsets. Flow cytometry was used to assess the absolute numbers of each cell type and the expression of TGFβ1 on CD4+CD25+ and CD4+CD25- T cells at each time point. Treg suppression of IL-2 production in CD4+ Thelper cells was assessed by ELISPOT assays. Our results show that peak viremia levels correlate with maximal infectivity in lymph node CD4+CD25+ and CD4+CD25- populations. FIV-gag-mRNA levels are higher in CD4+CD25+ T cells than CD4+CD25- lymph node T cells. Activation of FOXP3 and increased expression of TGFβ1 in CD4+CD25+ cells correlates with peak plasma viremia and FIV-gag-mRNA levels in CD4+CD25+ T cells. Our findings support the hypothesis that early activation of immunosuppressor function of Treg cells may limit an effective anti-FIV response contributing to the establishment of the chronic infection and the immunodeficiency caused by this virus.

23. Presenting Author: Jonathan E. Fogle, DVM

Ex vivo depletion of CD4+CD25+ regulatory T cells leads to enhanced CD8+ T cell IFN- $\gamma$  production during acute FIV infection.

Jonathan E. Fogle, Angela M. Mexas, Mary B. Tompkins, Wayne A. Tompkins. North Carolina State University, College of Veterinary Medicine, Raleigh, NC, 27606 USA.

We reported previously that CD4+CD25+ Treg cells in long-term, asymptomatic, FIV-infected cats are constitutively activated and suppress T cell immune responses. As T cell immune dysfunction in HIV and FIV infection becomes evident early after infection, we tested the hypothesis that CD4+CD25+ Treg cells become activated during the acute stage of FIV infection and suppress CD8+ responses to immune stimulation. SPF cats were infected with NCSU1 FIV. Plasma viremia, as well as PBMC and LN lymphocyte phenotype was assessed at regular intervals from day 0 through day 140. A popliteal lymph node was surgically excised at each of these intervals. Approximately  $6 \times 10^5$  cells of the following was obtained by FACS: unfractionated lymph node, lymph node depleted of CD4+CD25+ cells, CD8+ cells and CD4+ cells. Each group was either unstimulated or ConA stimulated overnight and assayed, via a feline specific ELISpot, for IFN-gamma production. Peripheral blood evaluation demonstrated that the CD8+ nadir at 14 days corresponded with peak plasma viremia and was followed by an increase in CD8+ number to greater than pre-infection values by day 35. Depletion of CD4+CD25+ lymphocytes significantly enhanced the production of IFN-gamma. The majority of IFN-gamma production was typically by the CD8+ population, but the CD4+ population also produced a considerable amount. The same observations were not evident in uninfected cats evaluated in an identical manner. Ex vivo analysis of these populations demonstrates the profound effect T regulatory cells have on the antiviral immune response during the acute stage of FIV infection.

24. Presenting Author: Tracy L. Lehman

The Effects of Chronic Feline Immunodeficiency Virus Infection on Bone-Marrow Derived Dendritic Cell Cytokine Production in Response to Toll-Like Receptor and CD40 Ligation

Tracy L. Lehman\* (1), Kevin P. O'Halloran (1), Samantha A. Fallon (1), Jennifer A. Campbell (1), Lindsey M. Habermann (1), Shila Nordone (2), Gregg A. Dean (2), Edward A. Hoover (1), Paul R. Avery (1). (1) Colorado State University, Fort Collins, CO, 80523 USA, (2) North Carolina State University, Raleigh, NC, 27606 USA.

Dendritic cells (DCs) are potent antigen presenting cells (APCs) and play a crucial role in pathogen recognition and initiation of primary T cell responses within the regional lymph nodes. Impaired APC function is thought to be central to lentivirus-associated immunodeficiency. It is unclear whether the initial steps in pathogen recognition, the amplification of T cell responses, or both are impaired during lentiviral infection. We evaluated cytokine production in response to Toll-like receptor (TLR) or CD40 ligation in bone marrow derived DCs (BM-DCs) from naïve and chronically FIV-infected cats. Real-time PCR quantification of cytokine message from purified BM-DCs stimulated with ligands to TLR 2, 3, 4, 7, and 9 was performed. TLR 4 ligation in BM-DCs from infected cats resulted in a significant decrease in the IL12/IL10 ratio compared to naïve BM-DCs. Stimulation of TLR 9 and TLR2 showed a similar trend. Stimulation of TLR 7 alternately resulted in an increase in the IL12/IL10 ratio in BM-DCs from infected cats. No difference was noted in response to TLR3 ligation. Preliminary data from BM-DCs co-cultured with 3T3 cells expressing feline CD40L shows a trend to higher IL-12 expression in the infected animals. Our data suggests that the initial steps in recognition of certain pathogens are altered in BM-DCs from FIV-infected cats, but the ability to amplify an appropriate cell-mediated T cell response remains intact in these cells. Experiments to determine whether FIV infection alters TLR expression on BM-DCs and which intracellular signaling pathways are involved in the altered TLR responses are ongoing.

25. Presenting Author: JK Yamamoto

Probing the Mechanisms of Dual-subtype FIV Vaccine Protection.

Janet Yamamoto\*, Ruiyu Pu, and Eiji Sato. Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Gainesville, FL 32608.

The commercial dual-subtype FIV vaccine, consisting of inactivated subtype-A (FIV<sub>Pet</sub>) and -D (FIV<sub>Shi</sub>) viruses, protected domestic cats against homologous-subtype strains and two subtype-B strains, Florida FIV<sub>FC1</sub> and Japanese FIV<sub>Aom2</sub>. Hence, the mechanisms of dual-subtype vaccine protection were investigated using passive- and adoptive-transfer systems. Passive transfer of vaccine antibodies to naïve cats one day before challenge (15 CID<sub>50</sub>) protected 100% of the cats against FIV<sub>Pet</sub> but not against FIV<sub>FC1</sub>. Passive protection against FIV<sub>Pet</sub> correlated with the presence of high virus neutralizing antibody (VNA) titers to FIV<sub>Pet</sub>. Little-to-no VNA titer was detected against FIV<sub>FC1</sub>. Adoptive-transfer Studies 1 and 2 were performed with MHC-matched inbred cats from F2 and F3/F4 generations, respectively. In Study 1, 3 of 4 adoptive-transfer recipients of T-cell-enriched population from vaccinated/MHC-matched siblings were protected against FIV<sub>Pet</sub> (25 CID<sub>50</sub>) given one day after the adoptive transfer. Whereas, all four recipients of either B cells from vaccinated/MHC-matched siblings or PBMC from unvaccinated/unrelated cats were infected. In Study 2, 4 of 4 recipients of T-cell-enriched population, 2 of 3 recipients of CD8<sup>+</sup> T-cell-enriched population, and 2 of 3 recipients of CD4<sup>+</sup> T-cell-enriched population from vaccinated/MHC-matched siblings were protected against FIV<sub>Pet</sub>. However, all four recipients of either PBS or cells (T cells or PBMC) from unvaccinated cats were infected. High levels of FIV-specific IFN- $\gamma$  and perforin production were observed in the T cells from vaccinated donors. Results from current and ongoing studies suggest that MHC-restricted CD8<sup>+</sup> T-cell and CD4<sup>+</sup> T-cell immunity are important in vaccine protection against homologous-subtype strains and against subtype-B strains.

26. Presenting Author: Mauro Pistello

Should Accessory Proteins Be Structural Components of Lentiviral Vaccines? Lessons Learned From the Accessory ORF-A Protein of FIV

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The FIV regulatory protein Rev and accessory proteins Vif and ORF-A are essential for efficient viral replication and full-blown pathogenesis. Expressed at very low level during viral replication, they are nevertheless processed for recognition by cytotoxic T-lymphocytes (CTLs) and trigger cellular immune responses in FIV-infected cats. Recent advances in the methods used for characterization of FIV cell-mediated immune responses should allow for a much more comprehensive assessment of the role of accessory proteins specific CTLs in controlling FIV infection.

The observation that the accessory ORF-A protein of FIV is continuously expressed during viral replication and targeted by cellular immune responses in natural FIV infection, prompted us to investigate the protective potential of this protein. To this aim cats were immunized with three different strategies (protein alone in alum adjuvant, DNA alone, or DNA prime-protein boost) and generated clearly detectable immune responses. Upon challenge with ex vivo homologous FIV, ORF-A immunized cats showed distinct enhancement of acute-phase infection possibly due to an increased expression of the FIV receptor CD134. However, at subsequent sampling points plasma viremia was reduced and CD4<sup>+</sup> T-lymphocytes in the circulation declined more slowly in ORF-A immunized than in control animals.

These findings support the contention that a multi-component vaccine, with the inclusion of both accessory and structural proteins, can improve the host's ability to control lentivirus replication and slow down disease progression but also draw attention to the fact that even simple immunogens that eventually contribute to protective activity can transiently exacerbate subsequent lentiviral infections.

27. Presenting Author: Eiji Sato

### Sequence Analysis of MHC-I Genes Confirms MHC-I-restriction in Dual-subtype FIV Vaccine Protection

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Our understanding about the mechanisms of protective immunity conferred by dual-subtype FIV vaccine may be useful in developing HIV vaccine. Dual-subtype FIV vaccine protects cats against heterologous subtype-B FIV strains mainly by FIV-specific T-cell immunity. Adoptive-transfer (A-T) studies of washed blood cells from dual-subtype FIV vaccinated cats protected naïve MHC-II-matched cats, but not MHC-II-unrelated cats, against FIV challenge (Pu, R, et al., 1999). In order to evaluate the importance of MHC-restricted immunity, cats were inbred and matched according to MHC-II compatibility-based mixed leukocyte reaction (MLR) analysis. As the next step, the role of MHC-I-restricted T-cell immunity in vaccine protection was evaluated by comparing the MHC-I sequences at the peptide-binding groove of the A-T donors and recipients. MHC-I of the inbred cats was sequenced by RT-PCR method. MHC-restriction corresponds to the ability of the MHC to bind and to present the viral peptides to effector T cells. Eleven of 14 A-T recipients of enriched T cells from vaccinated/MHC-II-matched siblings were protected against FIV challenge, whereas all eight control cats were infected. Protected A-T recipients had at least one MHC-I sequence identical to one of the corresponding A-T donor's MHC-I sequences at the peptide-binding groove. In contrast, the MHC-I sequences from the unprotected A-T recipients were significantly different in the peptide-binding groove sequences from the corresponding A-T donors. Current results demonstrate the importance of both MHC-I- and MHC-II-restricted T-cell immunity in FIV vaccine protection. Moreover, our findings suggest the importance of the candidate HIV vaccines to induce MHC-restricted T-cell immunity against HIV.

28. Presenting Author: Francesca Bonci

A new fluorimetric assay for monitoring cytotoxic T-lymphocyte activity elicited by a prime-boost vaccination against FIV Env

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Cytotoxic T lymphocytes (CTLs) play a crucial role in the immunological control of immunodeficiency virus infections. Accurate measures of CTL responses are therefore of critical importance for studying pathogenesis and evaluating vaccines and immunotherapies against lentiviruses. To the aim of measuring the CTL responses against FIV Env, we have developed a flow cytometry-based CTL (F-CTL) assay which makes use of autologous fibroblasts transformed with SV40 and transduced with a bicistronic FIV vector expressing FIV Env and the reporter GFP. Transduced fibroblasts are then used as both stimulatory and target cells when incubated at different effector:target ratios with PBMC from FIV vaccinated or infected animals. As internal control for the assay, PBMC are restimulated in presence of mock-transduced fibroblasts. Specific lysis is measured by FACS analysis of target fluorescent fibroblasts as percent reduction in number of GFP-expressing cells.

This method is currently used to measure the CTL responses elicited by a prime-boost vaccine strategy for FIV in which cats were primed with a plasmid co-expressing Env and feline GM-CSF and boosted with autologous lymphocytes expressing Env and feline IL-15. GM-CSF was chosen as an adjuvant for priming due to its recognized ability to induce dendritic cell proliferation, and the immunostimulatory cytokine IL-15 as it promotes the activation of several immune effectors, such as macrophages and CTL. F-CTL assay has proven sensitive, reproducible and more informative than the standard <sup>51</sup>Cr-release assays. In addition, given the versatility of FIV vectors, it might be easily adapted to analyze different antigen-specific CTLs.

29. Presenting Author: S. Rochelle Smithberg

Preservation of anti-FIV-p24 effector cells during depletion of Treg cells from feline lymphoid tissues

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Evidence suggests that antigen-specific regulatory T (Treg) cells can be expanded from naïve CD4<sup>+</sup> T cells through interaction with activated dendritic cells. CD4<sup>+</sup>CD25<sup>+</sup> Treg cells in FIV-infected cats are activated and may play a role in suppressing critical effector CD4<sup>+</sup> and CD8<sup>+</sup> T cell functions during viral infection, whereas Treg cells from uninfected cats maintain an inactive phenotype. Whether Treg cell activation in FIV immunopathogenesis is beneficial or detrimental to the infected host remains to be determined. We propose *in vivo* depletion of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells during FIV infection to resolve this question.

We have developed a strategy to deplete feline Treg cells from circulation with an anti-CD25 monoclonal antibody. However, higher percentages of Treg cells reside in tissues compared to circulation, and the extent of Treg cell depletion in tissues is unknown. In the present study we assess the kinetics of Treg cell depletion and rebound in various compartments. Treg cells are identified by cell surface markers, FoxP3 expression, and *ex vivo* suppressor function.

To address the concern that CD25<sup>+</sup> Treg cell depletion strategies may also deplete activated CD25<sup>+</sup> effector cells, we vaccinated cats with FIV-p24-GST recombinant protein prior to depletion. We follow anti-FIV-p24-GST effector cell activity during CD25<sup>+</sup> cell depletion in the peripheral blood and tissues by measuring IFN $\gamma$  production and T cell proliferation in response to antigen stimulation. We aim to validate the CD25<sup>+</sup> cell depletion strategy as a means to study effector cell activity in the absence of Treg cells.

30. Presenting Author: Katrin Hartmann

Influence of feline interferon- $\omega$  on the survival time of cats infected with feline infectious peritonitis

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Feline interferon- $\omega$  was recently licensed for use in veterinary medicine in some European countries and Japan. In a Japanese study (not controlled and only including a small number of cats with FIP not confirmed), a 2 year survival time was described in cats treated with feline interferon- $\omega$  and glucocorticoids. Aim of this study, therefore, was to evaluate efficacy of interferon- $\omega$  in cats with confirmed FIP in a controlled trial. In this placebo-controlled double-blind trial, 37 cats with FIP were treated randomly with feline interferon- $\omega$  (106 IU/kg s.c. every 48 hours 7 days, subsequently once every week) or placebo. In all cats, FIP was confirmed by histology or immunostaining of FCoV antigen in effusion/tissue macrophages. All cats received glucocorticoids, either as dexamethasone (in case of effusion) or prednisolone. There was no statistically significant difference in the survival time of cats treated with interferon- $\omega$  versus placebo. Cats survived for a period of 3 to 200 days (mean 18 days). There was only one long-term survivor (> 3 months) that appeared to be in the interferon- $\omega$  group. This cat had been presented with effusion that totally disappeared; it did not show signs until euthanized 200 days after treatment initiation due to recurrence of FIP. Although there was no difference in the mean survival time, few cats may benefit from treatment including interferon- $\omega$ .

31. Presenting Author: Stephen J. O'Brien

The Domestic Cat Genome Sequence- Annotation and Comparative Inference

Stephen J O'Brien for  
The Feline Genome Annotation Consortium

The "light" coverage genome sequence (2x coverage) of an Abyssinian domestic cat named Cinnamon was completed late in 2005 by Agencourt Bioscience Corp, Beverly MA. An international consortium of genome scientists has collaborated to annotate the genome of the cat and specific aspects of that annotation are being featured at this Conference. In my presentation, I will outline the strategy for sequence, assembly, creating the genetic map, and identifying genes. We have taken a strongly comparative approach in mapping and in gene annotation drawing heavily from gene lists, and Conserved Sequence Blocks (CSBs) identified in the six mammal species with available 7x coverage genome sequence (human, chimp, mouse, rat, dog, and cow). The results reveal nearly 20,000 feline genes plus 132,493 CSBs used to build the gene map, depending upon the framework RH map of 1794 ordered Type 1 markers. Annotation highlights include the identification of evolutionary breakpoints that derive from historic translocations and inversions in distinct mammal lineages, characterization of genomic repeats including STRs, SINEs, endogenous retroviral sequences, nuclear mitochondrial (NUMT)fossil sequences, micro RNAs and GC content distinctions. Polymorphic SNPs and indels were characterized in the context of the long stretches of chromosome homozygosity found in Cinnamon derived from a history of domesticated inbreeding. In addition, patterns of gene evolution among pairs of sequenced species within primate, carnivores and rodents were employed to detect selective accelerations in gene divergence and adaptation. In all the comparative insight derived for the cat "light" sequence not only greatly facilitates gene discovery for hereditary disease models of the cat, but also sheds new light on the tempo and mode of gene/genome evolution in mammals.

32. Presenting Author: Joan Pontius

Use of Online Resources for the Annotation of Retroviral Interactions in the *Felis catus* Genome

List of Authors

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3. Stephen J. O'Brien, Laboratory of Genomic Diversity, NCI Frederick, Frederick MD 21702

Online resources at the National Center for Biotechnology Informatics (NCBI) have been used to annotate the regions of the *Felis catus* genome that may be relevant to Feline retrovirus research.

The Taxonomy Database at NCBI includes genomic sequence for over 50 retroviridae genomes, including those of FIV, FeLV, RD114 and Type C retrovirus. NCBI's Gene References Into Function Database (GeneRIF) includes over 800 human genes involved with HIV interactions. By using BLAST to find similarity between these annotated sequences and those of the newly assembled cat genome, orthologs are proposed for 700 of the HIV interaction genes in cat. Over 40 potential regions of FeLV integration into the cat genome have been annotated, as well as more than ten regions for both RD114 and Type C retrovirus, whereas none were detected for FIV.

Access to NCBI's resources as well as to the Laboratory of Genomic Diversity's annotation of *Felis catus* will be discussed.

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33. Presenting Author: Alfred L. Roca

Endogenous Retroviruses in the Domestic Cat Genome.

Alfred L. Roca\* (1), Natalia Volfovsky (2), Joan Pontius (1), Robert Stephens (2), Naoya Yuhki (3) and Stephen J. O'Brien (3).

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Domestic cat (*Felis catus*) genome traces with homology to endogenous retroviruses were identified and analyzed. For endogenous feline leukemia viruses (enFeLV), 145 traces matched the proviral long terminal repeats (LTRs) while almost two hundred matched proviral genomic regions between the LTRs. Phylogenetic analyses of LTR and gag regions both indicated that proviral sequences fell into two groups, suggesting at least two separate invasions of the genome by enFeLVs. For the endogenous retrovirus RD-114, 66 traces matched the partial sequence available for the provirus; these showed little variation. We also detected novel feline endogenous retro-element distantly related to various mammalian retroviruses. One for which a full BAC sequence was available displayed disrupted open reading frames that suggested an ancient origin. Our results indicate that genomic sequencing will allow the identification of new retro-elements and the study of retroviral integration and evolution within genomes. (Funded by NCI Contract #N01-CO-12400.)

34. Presenting Author: Jill Pecon-Slattery

### Genetic signatures of FIV evolution in Felidae

Jill Pecon-Slattery(1)\*, Jennifer Troyer(2), Carrie McCracken(2), Melody Roelke(2), Warren Johnson(1), Stephen J. O'Brien(1)

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Feline immunodeficiency virus (FIV) infection in domestic cat (*Felis catus*) has proven to be a powerful tool for understanding the genetic, virologic, and pathogenic consequences of lentiviral infection. Serological screening, coupled with sequence data, suggest that FIV is species-specific, has infected at least 31 different species, and is distributed world-wide. Some studies suggest FIV may be less pathogenic in some of these exotic cat species, and genetic changes linked with more benign form of the virus may be ascertained by comparative genomic analyses. We compare full-length sequence from lion, puma and domestic cat and define distinct differences in genome structure and genetic change. Further, we determine patterns of evolution and selection within the pol-RT region among 76 strains of FIV from 7 different species. Our results suggest FIV in felids has evolved in a similar manner as HIV/SIV in primates with a rich history of co-evolution between host and pathogen.

35. Presenting Author: Warren Johnson

Mitochondrial introgressions into the nuclear genome of the domestic cat

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Translocation of mtDNA into the nuclear genome, also referred to as numt, was first reported in the domestic cat (*Felis catus*) by Lopez et al. (1994). It consisted of a translocation of 7.9 kb of the mitochondrial genome that inserted into the nuclear genome of an ancestral of the domestic cat around 1.8 million years ago. Such pioneer work was followed by several studies reporting the incidence of numts in dozens of animal and plant species. However, a broad view of the extent of mtDNA transfer among eukaryotes has been achieved only recently, with the public release of several whole genome sequence projects. More than a decade after the initial characterization of the Lopez et al. – numt, the recent release of the domestic cat nuclear genome sequences (2X coverage) provides the resource to obtain more comprehensive insights into the extent of mtDNA transfer in the cat and its significance for the study of genome evolution. In this study, we attempt to reconstruct the evolutionary dynamics of numt integration over time in the domestic cat genome and we compared our results with others retrieved from several vertebrate species.

Lopez, J.V., Yuhki, N., Masuda, R., Modi, W., O'Brien, S.J., 1994. Numt, a recent transfer and tandem amplification of mitochondrial DNA to the nuclear genome of the domestic cat. *J. Mol. Evol.* 39, 174-190.

36. Presenting Author: John H. Elder

**FIV as a model for Development of Interventions against HIV.** J.H. Elder<sup>1</sup>, Y-C. Lin<sup>1</sup>, S. de Rozières<sup>1</sup>, K. Tam<sup>1</sup>, M. Sundstrum<sup>1</sup>, C. K. Grant<sup>2</sup> and A. de Parseval<sup>1</sup>.

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Studies in our laboratory have centered on the molecular characterization of FIV and in using that information to draw relevant parallels to HIV-1. The ultimate goal is to develop broad-based inhibitors that will be useful as treatment modalities in both lentivirus systems. In one line of research, we have used FIV protease (PR) as a mutational system to study the molecular basis of substrate/inhibitor specificity for lentivirus PRs. Previous efforts have resulted in development of a broad-based inhibitor, TL-3, that shows efficacy against FIV, SIV, and HIV in addition to several drug resistant HIVs *in vitro* and *ex vivo*. *In vivo* studies against FIV showed resolution of FIV-PPR-induced CNS effects, as well as evidence of lowered pathogenesis of FIV-C. Further work using chimeric FIV/HIV PRs has now demonstrated that discrete substitutions in the active site of FIV PR with structurally equivalent residues of HIV-1 PR dramatically alter the specificity of the mutant PRs in *in vitro* analyses to increase HIV character. Chimeric mutant PRs encoding 4 to 12 HIV-1 equivalent substitutions were prepared and Gag-Pol polyprotein cleavage was then assessed in pseudovirions from transduced cells. As with *in vitro* analyses, inhibitor specificities of the mutants showed increased HIV-1 PR character when analyzed against the natural substrate. In addition, the mutant PRs still processed the FIV polyprotein, but the apparent order of processing was altered relative to that observed with wild type FIV PR. The findings likely explain the failure to produce infectious FIVs bearing these mutations and suggests that altering substrate cleavage order may be as effective as blocking PR activity in inhibiting virus replication.

Additional studies have centered around understanding virus envelope/receptor interactions and in mapping CD134-dependent neutralizing epitopes. The findings again draw parallels to infection by HIV in that as with CD4 enhancement of chemokine receptor binding by the human lentivirus, CD134 binding plays a similar role in facilitating entry of FIV into the target cell via CXCR4. As with CD4-induced neutralizing epitopes on HIV SU, FIV SU also has neutralizing epitopes which become exposed only after CD134 binding. Thus, FIV may also serve to develop ways to intervene with receptor interactions that will translate to HIV.

37. Presenting Author: Allison C German

Feline foamy virus infection associated with subclinical pathology in cats

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Feline foamy virus (FFV) is a widespread retrovirus in cats. It is thought to be apathogenic, making it an interesting candidate for a gene therapy vector. However, there have been reports of association of FFV with chronic progressive arthritis and a cofactor effect with feline immunodeficiency virus. This study investigated the relationship between FFV infection and signs of disease.

Eight specific pathogen free cats were infected with FFV. The cats were examined twice weekly and blood and pharyngeal samples were taken. Haematology, biochemistry and quantitative polymerase chain reaction (qPCR) were performed. Tissue samples were collected throughout the 6 month period.

FFV was detected by qPCR in the blood within the first two weeks of infection and viraemia persisted throughout the study. All cats were normal on clinical examination, except one cat with an unrelated gingivitis. No cat developed pyrexia. The biochemical profile and blood cell counts remained within normal limits except for one cat with a persistent eosinophilia. Initial fluctuations in white cell counts settled within three weeks. All tissue samples contained FFV DNA; lymphoreticular tissues, salivary gland and lung had the highest viral loads. Although there were no gross pathological lesions on post mortem examination, a mild glomerulonephritis and a moderate interstitial pneumonia were observed in all cats.

We conclude that within a 6 month period of infection, although cats appeared clinically normal, histopathological changes were observed in the lungs and kidneys. Further investigation of the significance of these changes is warranted before FFV is developed as a vector for gene delivery.

38. Presenting Author: Surender Kumar

EFFECT OF ALLOANTIGEN EXPOSURE ON MLR AND IMMUNE PHENOTYPE

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We hypothesized that mucosal exposure to alloantigens may induce an allogeneic mucosal immune responses that could alter the susceptibility to sexual HIV-1 infection. To examine the role of alloantigen exposure in lentiviral susceptibility, the cervicovaginal mucosa of cats was exposed once weekly for 12 weeks to 5,000 allogeneic peripheral blood mononuclear cells (PBMC) from four heterologous sources (alloantigen), 5,000 autologous PBMC (self antigen) or media (control). Blood samples were obtained every two weeks during the exposure period. Mixed lymphocyte reaction (MLR) and flow phenotyping were used to identify alloantigen activation of the immune system. We found that vaginal exposure to alloantigen enhanced allogeneic MLR response but blunted the proliferative response to subcellular antigen. Furthermore, there was an expansion of the circulating naïve lymphocyte pool with possible expansion of circulating T-regulatory lymphocytes. To determine whether these changes influence the susceptibility to mucosal FIV infection, cats were challenged by atraumatic vaginal exposure to either cell-associated or cell-free FIV one week following the final antigen exposure. This is first study to evaluate the role of vaginal alloantigen exposure on lentiviral infection. We have identified that vaginal alloantigen exposure induces distinct patterns of systemic allo-immune responses that appear to be immunoregulatory in nature. This is important because allogeneic immune responses could be initiated in people following sexual exposure to allogeneic cellular material. If induction of non-specific immune responses can modulate the threshold for infection, this may provide a mechanism for topical intervention against sexually transmitted HIV-1.

39. Presenting Author: Nicola Fletcher

**The Development of a Feline-Specific *in vitro* Blood-Brain Barrier and its Applications in the Study of Feline Immunodeficiency Virus Infection**

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Feline Immunodeficiency Virus (FIV) infection of cats is an established model of HIV-1 infection in man. Like HIV, FIV is neurotropic, and central nervous system infection occurs early in infection. In association with early infection, there is prominent leukocyte trafficking across the blood-brain barrier. However, the precise mechanisms of FIV and HIV entry into the brain remain to be elucidated. In order to study the interaction of FIV with the blood-brain barrier, we have developed an *in vitro* model of the feline BBB using primary cultures of feline brain capillary endothelial cells (FBEC) and feline astrocytes grown as a co-culture on opposite sides of Transwell filters. FBEC were characterised by uptake of DiI-Ac-LDL, and immunocytochemistry to identify von Willebrand Factor. Feline astrocytes were characterised by immunocytochemistry to identify GFAP. Functionality of the BBB model was assessed by measurement of tight junction formation between FBECs, using transmission electron microscopy (TEM), transendothelial electrical resistance measurements and paracellular permeability assays. The presence of tight junction proteins in the FBEC cultures was confirmed by Western blots. The ability of FIV<sub>GL-8</sub> to infect cells of the feline BBB was assessed by immunocytochemistry, TEM and measurement of reverse transcriptase activity in BBB cells exposed to FIV. FBECs exposed to FIV developed cytoplasmic vacuoles, and virus particles were present in these vacuoles when viewed using TEM. Feline astrocytes were positive for FIV antigen by immunofluorescence. No productive infection was detected in either cell type by quantitative ELISA. The ability of cell-free FIV to migrate across the BBB model, and to disrupt the tight junctions between FBEC, was measured by quantitative ELISA and FD-4 permeability assays respectively. Cell-free FIV did not affect tight junction integrity up to two days post infection. The transmigration of a feline CD4+ T lymphocyte cell line (Mya-1 cells) across the *in vitro* BBB model was measured in the presence or absence of FIV<sub>GL-8</sub> and/or recombinant feline TNF- $\alpha$ . No differences appeared to exist between infected or uninfected Mya-1 cells in terms of their ability to transmigrate across the BBB model. A FD-4 permeability assay was then carried out to measure the integrity of the *in vitro* BBB tight junctions following Mya-1 transmigration. When TNF- $\alpha$  was added to the BBB model, a statistically significant increase in transmigration of Mya-1 cells was observed. When FIV-infected Mya-1 cells were added to both compartments of the BBB model, there was a further significant increase in the transmigration of the Mya-1 cells across the BBB model. Transmigration of Mya-1 cells across the *in vitro* BBB was accompanied by a moderate increase in BBB permeability. This BBB model system provides an ideal opportunity to characterise the interaction of FIV with the feline blood-brain barrier, thus providing information relevant to HIV-induced neurological disease.

40. Presenting Author: William Hardy

**Lessons From Time Spent in Cat Houses: An Historical Overview of the Feline Leukemia Virus and Other Pathogens.**

**William D. Hardy\*, Jr., V.M.D. and Evelyn E. Zuckerman, B.S. National Veterinary Laboratory, Inc. Franklin Lakes, NJ 07417 USA**

In addition to their 9 lives, cats are unique for their abundant pathogenic microorganisms consisting of 3 Genera of *Retroviruses*: *Gammaretroviruses* (formerly *Oncoretroviruses*), *Lentiretroviruses*, and *Spumaretroviruses*, and 6 Species of zoonotic bacteria of the Genus *Bartonella*: *henselae*, *clarridgeiae*, *koehlerae*, *elizabethae*, *bovis*, and *quintana*. Persistent infections with these pathogens account for the numerous chronic inflammatory and degenerative conditions that occur frequently in pet cats. During the last 35 years, the cat has been an important animal model of naturally occurring diseases. In this regard, the observation of the horizontal infectious spread of retroviruses was first documented with the feline leukemia virus (FeLV) in pet cats in the 1970s. Shortly thereafter, it was found that FeLV caused more immunosuppressive and degenerative diseases than neoplasia (“leukemia”) in cats. These observations suggested the possible retroviral etiology of the human immunodeficiency syndrome (AIDS) that was emerging in the early 1980s. Currently, the study of *Bartonella* infection in cats and humans is elucidating new insights into the mechanisms of induction of chronic inflammatory diseases. Thirty years after the discovery of FeLV- and FIV-induced immunosuppressive diseases, it seems apparent that chronic *Bartonella* infections were probably responsible for many of the secondary “immunosuppressive” inflammatory diseases observed in cats. We have learned much in cat houses during the past 35 years!

41. Presenting Author: Mary Poss

Molecular biology and evolution of feline lentiviruses

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Feline immunodeficiency virus (FIV) is a lentivirus that has been identified in many members of the family Felidae but domestic cats are the only FIV host in which infection results in disease. We studied FIVpco infection of cougars (*Puma concolor*) as a model for asymptomatic lentivirus infections to understand the mechanisms of host-virus coexistence. Several natural cougar populations were evaluated to determine if there are any consequences of FIVpco infection on cougar fecundity, survival, or susceptibility to other infections. We have sequenced full length viral genomes and conducted a detailed analysis of viral molecular evolution on these sequences and on genome fragments of serially sampled animals to determine the evolutionary forces experienced by this virus in cougars. In addition, we have evaluated the molecular genetics of FIVpco in a new host, domestic cats, to determine the evolutionary consequences to a host-adapted virus associated with cross-species infection. Our results indicate that there are no significant differences in survival, fecundity or susceptibility to other infections between FIVpco-infected and uninfected cougars. The molecular evolution of FIVpco is characterized by a slower evolutionary rate and an absence of positive selection, but also by proviral and plasma viral loads comparable to those of epidemic lentiviruses such as HIV-1 or FIVfca. Evolutionary and recombination rates and selection profiles change significantly when FIVpco replicates in a new host.

42. Presenting Author: Melody Roelke

Wild African Lions and Florida pumas infected with FIV Reveal distortions in their T-Lymphocyte Profiles with significant CD4 cell Depletion: Clinical & Pathological Consequences

Melody E. Roelke<sup>\*1</sup>, Jill Pecon-Slatery<sup>2</sup>, Christiaan Winterbach<sup>3</sup>, Hanlie Winterbach<sup>3</sup>, Meredith. Brown<sup>2</sup>, Mark Cunningham<sup>4</sup>, C. Dahlem Smith<sup>1</sup>, Craig Packer<sup>5</sup>, Sue VandeWoude<sup>6</sup> and Stephen J. O'Brien<sup>2</sup>

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### Abstract

Feline immunodeficiency virus (FIV) is a lentivirus related to human immunodeficiency virus (HIV) that causes feline AIDS in domestic cat (*Felis catus*). Serological surveys indicate that at least 25 other species of cat possess antibodies that cross-react with domestic cat FIV. Most infected nondomestic cat species are without major symptoms of disease. Long-term studies of FIV genome variation and pathogenesis reveal patterns consistent with co-adaptation of virus and host in free-ranging African lion (*Panthera leo*) and puma (*Puma concolor*) populations. This presentation examines correlates of immunodeficiency in both wild and captive lions and pumas by quantifying CD5+, CD4+ and CD8+ T-cells subsets. Free-ranging FIV-Ple infected lions show Immunofluorescent Flow Cytometry (IFC) profiles marked by a dramatic decline in CD4+ subsets, a reduction of the CD4+ / CD8+ ratio, reduction of CD8+ $\beta^{\text{high}}$  cells, and expansion of the CD8+ $\beta^{\text{low}}$  subset relative to uninfected lions. An overall significant depletion in CD5+ T cells in seropositive lions is linked with a compensatory increase in total CD5- lymphocytes resulting in no change in total lymphocytes. The effects of FIV-Pco infection in pumas are less profound but still significant with IFC profiles altered in 50% of the seropositive individuals examined. FIV infected puma display a more generalized response of lymphopenia expressed as a significant decline in total lymphocytes, CD5+T-cells, and CD5- lymphocytes as well as a significant reduction in CD4+ T-cells. Like lions, seropositive pumas have a significant decline in CD8+ $\beta^{\text{high}}$  cells but differ but not showing compensatory expansion of CD8+ $\beta^{\text{low}}$  cells relative to controls. The results observed with FIV-infected lion and puma parallels human (HIV) and Asian monkey (SIV) CD4+ diminution, and suggests there may be unrecognized immunological consequences of FIV infection in these two species of large cats. Field clinical and pathological observations will be discussed in the context of FIV infection.

43. Presenting Author: Mark Cunningham

Epizootiology and Management of Feline Leukemia Virus in Free-Ranging Florida Panthers – Research Update

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Feline leukemia virus (FeLV) has been reported only rarely in non-domestic felids and was not detected in Florida panthers (*Puma concolor coryi*) during almost 20 yr of routine surveillance. The finding of 2 FeLV antigen-positive panthers during the 2002-2003 capture season led to an epizootiologic investigation of this disease in the population. Between 1990 and 2005, the prevalence of positive antibody tests increased significantly and antibody-positive panthers were concentrated in the northern portion of panther range. Five of 71 (7%) study animals sampled between July 2002 and June 2005, were antigenemic and had home ranges in the northern portion of panther range. All antigenemic panthers were positive by viral culture and PCR, and 3 were IFA positive at capture. Clinical signs and clinical pathology at capture (n = 4) included lymphadenopathy, anemia, and lymphopenia. All infected panthers died; causes of death were septicemia (n = 2), intraspecific aggression (n = 2), and unknown (n = 1). Average time from diagnosis to death was 9.25 (SD ±10.3) wks. Based on ELISA (antibody and antigen), IFA/IHC, PCR, culture, and clinical findings, panthers developed transient, latent, or persistent infections following exposure. Management currently includes vaccination and test-removal. Between August 2003 and June 2006, 46 free-ranging panthers have received at least 1 inoculation (Fel-O-Vax Lvk®); 18 were boosted. Currently, 40% of the population has received at least 1 inoculation. No new cases have been diagnosed since July 2004 indicating the epizootic may be over. However, the potential for reintroduction of the virus remains.

44. Presenting Author: Meredith Brown

Genetic characterization of feline leukemia virus (FeLV) in the free-ranging Florida panther (*Felis concolor coryi*) population

Meredith Brown<sup>1\*</sup>, Mark Cunningham<sup>2</sup>, Alfred L. Roca<sup>3</sup>, Jennifer Troyer<sup>3</sup>, Warren Johnson<sup>1</sup>, and Stephen J. O'Brien<sup>1</sup>

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An outbreak of feline leukemia virus (FeLV) was recently detected in the free-ranging Florida panther (*Felis concolor coryi*) population. From November of 2002 until August of 2005, five panthers tested antigen positive for FeLV and died. All positive panthers were geographically clustered from the Okaloacoochee Slough State Forest (OKS) region. In most cases, FeLV-related disease involving lymphadenopathy, anemia, septicemia, and weight loss was documented. This report presents the genetic characterization of proviral FeLV in free-ranging Florida panthers collected during clinical examination and necropsy. The FeLV isolated from the free-ranging panther population is subtype A likely introduced into the Florida panther population 3-4 years ago from a single infection by a domestic cat (*Felis catus*) strain. Texas heritage did not protect infected individuals from developing disease associated with FeLV. FIV was associated with FeLV cases but was not necessary for disease to appear in the FeLV-infected panthers. The Florida panther strain, designated PCO-FeLV, is most similar in sequence to a virulent subtype A 945-FeLV that causes non-T cell disease in the domestic cat. (Funded by NCI Contract #N01-CO-12400.)

45. Presenting Author: Jennifer Troyer

FIV cross-species transmission: an evolutionary perspective Jennifer Troyer\*(1), Sue VandeWoude (2), Samuel Franklin (2), Jill Slattery (3), Melody Roelke (1), Kevin Crooks (4), Agostinho Antunes (3), Warren Johnson (3) and S. J. O'Brien (3).

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Like the primate lentiviruses, feline immunodeficiency viruses (FIVs) are transmitted via direct contact (e.g. fighting, sexual contact, and mother-offspring transmission). This alone likely poses a behavioral barrier to cross-species transmission in the wild. Recent studies have identified several host intracellular anti-viral proteins that contribute to species-specificity of primate lentiviruses, suggesting that adaptive mechanisms have evolved to further limit spread of lentiviruses between species. Consistent with these observations, phylogenetic evidence supports the prediction that transmission between cat species is a rare event in free-ranging species, though it has been noted to occur occasionally in captive settings. In contrast to these findings, we have recently documented that puma and bobcats in Southern California share an FIV strain. We are investigating the evolution of both viral strains and host intracellular restriction proteins to elucidate the process of host adaptation. Further, we are using viral and host genetics to track patterns of migration and barriers to transmission within the African lion. These studies illustrate the utility of FIV as a model to discover the variables necessary for establishment and control of lentiviral infections.

46. Presenting Author: Takayuki Miyazawa

A soluble envelope protein of endogenous retrovirus present in serum of domestic cats mediates infection of a pathogenic variant of feline leukemia virus

Takayuki Shojima(1), Daisuke Fukui(2), and Takayuki Miyazawa\*(1). (1) Institute for Virus Research, Kyoto University, Kyoto 606-8507, Japan. (2) Asahikawa Municipal Asahiyama Zoological Park and Wildlife Conservation Center, Asahikawa, Hokkaido 070-8205, Japan.

T-lymphotropic feline leukemia virus (FeLV-T), a highly pathogenic variant of FeLV, induces severe immunosuppression in cats. FeLV-T is fusion-defective because in its PHQ motif, a gammaretroviral consensus motif in the N-terminal of an envelope protein, histidine is replaced with aspartate. Infection by FeLV-T requires FeLIX, a truncated envelope protein encoded by an endogenous FeLV, for transactivation of infectivity and Pit1 for binding FeLIX. Although Pit1 is present in most tissues in cats, the expression of FeLIX is limited to certain cells in lymphoid organs. Therefore, the host cell range of FeLV-T was thought to be restricted to cells expressing FeLIX. However, because FeLIX is a soluble factor and expressed constitutively in lymphoid organs, we presumed it to be present in blood and evaluated its activities in sera of various mammalian species using a pseudotype assay.

We demonstrated that cat serum has FeLIX activity at a functional level, suggesting that FeLIX is present in cats and FeLV-T may be able to infect cells expressing Pit1 regardless of the expression of FeLIX *in vivo*. In addition, FeLIX activities in sera were detected only in domestic cats and not in other feline species tested.

In this study, we proved that a large amount of truncated envelope protein of endogenous retrovirus is circulating in the blood to facilitate the infection of a pathogenic exogenous retrovirus. Our results have important implications for overcoming species barriers and the risks of xenotransplantation.

47. Presenting Author: Laura S. Levy

Selection and Pathogenesis of an Unusual FeLV Isolate Predominant in a Natural Cohort.

Laura S. Levy.

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Genetic variation among FeLV isolates was examined in a cohort of naturally infected cats with thymic lymphoma of T-cell origin, non-T-cell multicentric lymphoma, myeloproliferative disorder or anemia. The predominant isolate, designated FeLV-945, was identified exclusively in disorders of non-T-cell origin. The FeLV-945 LTR was shown to contain a unique 21-bp repeat element, triplicated in tandem downstream of enhancer. The 21-bp triplication was shown to act as a transcriptional enhancer and to confer a replicative advantage through the assembly of a distinctive transcription factor complex. Oncogene utilization during tumor induction by FeLV-945 was studied using a recombinant Moloney murine leukemia virus containing the FeLV-945 LTR. This approach identified novel loci of common proviral integration in tumors, including the regulatory subunit of PI-3Kgamma. Mutational changes identified in FeLV-945 SU were shown not to alter receptor usage as measured by host range and superinfection interference, but to significantly increase the efficiency of receptor binding. To determine whether the unique sequence elements of FeLV-945 influence the course of infection and disease in vivo, recombinant viruses were constructed in which the FeLV-945 LTR alone, or the FeLV-945 SU gene and LTR were substituted into the prototype isolate FeLV-A/61E. Longitudinal studies of infected animals showed that substitution of the FeLV-945 LTR into FeLV-A/61E resulted in a significantly more rapid disease onset, but did not alter the tumorigenic spectrum. In contrast, substitution of both the FeLV-945 LTR and SU gene changed the disease outcome entirely. Together, these observations indicate that the distinctive LTR and SU gene of FeLV-945 mediate a rapid pathogenesis with distinctive clinical features and oncogenic mechanism.

48. Presenting Author: Regina Hofmann-Lehmann

How molecular methods change our views of FeLV infection and vaccination.

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FeLV was discovered 40 years ago and vaccines have been commercially available for almost two decades. So far, most FeLV pathogenesis and vaccine studies were conducted assaying parameters, such as virus isolation and antigen detection. Recently, sensitive FeLV-specific molecular methods were developed. Using real-time polymerase chain reaction assays, we found that cats believed to be immune to FeLV infection turned provirus-positive after virus exposure. Moreover, efficacious FeLV vaccines – known to protect cats from antigenemia and FeLV-associated disease – were found unable to prevent provirus-integration and minimal viral replication. Remarkably, no difference was found in initial proviral and viral plasma RNA loads (week 1) between cats with different infection outcome. Subsequently, cats protected from persistent antigenemia had lower provirus and RNA loads than persistently infected cats. Analyzing the proviral and viral RNA loads in different leukocyte subsets, mainly CD4+ and B lymphocytes were found to be infected in protected cats, while in cats with persistent viremia also granulocytes and monocytes were provirus positive. The latter cell populations were also the main locus of virus replication in persistently infected cats. So far, the biological relevance of proviral integration in aviremic cats is unclear. FeLV provirus persisted for several years; antigenemia reoccurred in some of these cats and led to FeLV-associated disease. It must be assumed that aviremic provirus-positive cats play a role as FeLV carriers and, following reactivation, may act as an infection source. The use of sensitive molecular methods will contribute to a more in-depth understanding of the FeLV pathogenesis.

49. Presenting Author: Kristina E. Howard

## Differences in Mucosal FIV Pathogenesis Using Decreasing Doses of Viral Inoculum

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Evidence suggests that initial viral burden during HIV and FIV infection may influence the course of infection. It is also known that mucosal leukocyte populations experience significant damage early in FIV, SIV and HIV infection. It is unknown however; the role initial virus dose may play in the severity of mucosal FIV pathogenesis. The goals of this study were to 1) determine an *in vivo* titrated infectious dose of vaginally administered FIV, and 2) assess the effects of different doses on immune populations, particularly in the mucosal sites as compared to systemic lymphoid tissues.

Three groups of specific pathogen free cats were vaginally inoculated with both cell-associated and cell-free FIV. A high, medium and low dose were used to achieve 100%, 50% and 0% infection rates based on plasma viremia and proviral load. Serum, plasma and PBMC samples were collected at weeks 0, 2, 4, and 6. Study animals were euthanized 8 weeks post-infection to assess PBMC, multiple lymph nodes, thymus, bone marrow, spleen, and small intestinal intraepithelial lymphocytes (IEL) and lamina propria lymphocytes (LPL).

In IEL we found decreased numbers of CD8+ T cells, decreased expression of CD5+bright as well as CD103, and a large increase in CD3+CD4-CD8- T cells. LPL typically had inverted CD4:CD8 ratios, with overall decreases in CD4+ and CD8+ T cells as well as B cells. Our data suggest that regardless of virus dose, more significant alterations are found in sites such as IEL, LPL, and spleen as compared to PBMC or peripheral lymph nodes.

50. Presenting Author: Andrea N. Torres

### Insight into FeLV:host relationships using real-time DNA and RNA qPCR

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(2) Department of Pathobiological Sciences, University of Wisconsin – Madison, Madison, WI 53706, USA

We previously defined four categories of FeLV infection, designated as abortive, regressive, latent, and progressive. To determine if detectable viral DNA is transcriptionally active in the absence of antigenemia, we developed and validated a real-time viral RNA qPCR assay. This assay proved to be highly sensitive, specific, reproducible, and allowed reliable quantitation. We then applied this methodology, together with real-time DNA qPCR and p27 gag antigen capture ELISA, to examine vaccinated and unvaccinated cats challenged with FeLV. Two commercially available whole inactivated virus (WIV) FeLV vaccines provided substantial protection against FeLV challenge. In nearly every recipient of these vaccines, neither viral DNA, RNA, nor antigen could be detected in blood. Our inability to detect the virus using different assays appears to represent virtual 'sterilizing immunity' in the protected vaccinates. These results lend support to the tenet that successful immunity to retroviral infection can be obtained with WIV immunoprophylaxis. In addition, we found that circulating viral RNA and DNA levels were highly correlated. This indicates that the vast majority of viral DNA is transcriptionally active, even in the absence of antigenemia. The real-time qPCR assays are more sensitive than the most commonly used FeLV diagnostic assay, the gag antigen capture ELISA. Finally, the four host:virus relationships previously defined by viral DNA and antigen detection, were reinforced when viral RNA levels were also assessed. To determine whether detectable viral RNA represents infectious virus, plasma viral infectivity assays are being performed. Real-time DNA and RNA qPCR assays will permit greater depth in understanding of FeLV:host relationships.

51. Presenting Author: Regina Hofmann-Lehmann

Cellular segregation of feline leukemia virus in leucocyte subsets of long-term experimentally infected cats.

Andrea C. Pepin, Ravi Tandon, Valentino Cattori\*, Barbara Riond, Hans Lutz, and Regina Hofmann-Lehmann. Clinical Laboratory, Vetsuisse Faculty, University of Zurich, CH-8057 Zurich, Switzerland

Cats exposed to feline leukemia virus (FeLV) may develop different outcomes of the infection: persistent and transient viremia, atypical infection as well as immunity. During the acute phase of the infection, proviral and viral burdens in the peripheral blood of persistently and transiently infected cats are not significantly different. Thus, we hypothesized that not the overall viral load in the peripheral blood, but rather the loads in specific leucocyte subsets influence the infection outcome. The aim of this study was to establish a method to sort feline white blood cells and to determine the proviral and viral RNA loads in specific leucocyte subsets of chronically FeLV-infected p27-positive and p27-negative cats. Peripheral blood mononuclear cells were purified over a percoll gradient, stained, and sorted by flow cytometry. Granulocytes were sorted from defibrinated blood. DNA and RNA were extracted and FeLV provirus and viral RNA quantified by TaqMan® real-time PCR and RT-PCR, respectively. In p27-positive and p27-negative cats, specific leucocyte subsets showed differentially high proviral loads: in p27-negative cats, mainly CD4<sup>+</sup> and B lymphocytes were provirus-positive, whereas in p27-positive cats all leucocyte subsets were highly positive. In p27-positive cats, monocytes and granulocytes beared the highest viral RNA loads, whereas only one p27-negative cat was positive for viral RNA. The herein described methods are an important prerequisite to measure proviral and viral loads in leucocytes of FeLV-infected cats, and thus to gain a deeper knowledge of the course and the pathogenesis of the FeLV infection.

52. Presenting Author: Ravi Tandon

Copy number polymorphism of endogenous feline leukemia virus-like sequences

Ravi Tandon\*, Valentino Cattori, Barbara Willi, Marina L. Meli, Hans Lutz, Regina Hofmann-Lehmann

Clinical Laboratory, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland. In the cat genome, endogenous feline leukemia virus (enFeLV) exists as multiple, nearly full length proviral sequences. Even though no infectious virus is produced from enFeLV sequences, transcription and translation have been demonstrated in tissues of healthy cats and in feline cell lines. To test the hypothesis that the enFeLV loads play a role in exogenous FeLV-A infection and pathogenesis, we designed three real-time PCR assays to quantify U3 and env enFeLV loads (two within U3 amplifying different sequences; one within env). Applying these assays, we investigated the loads in blood samples derived from Swiss privately owned domestic cats, specific pathogen-free (SPF) cats and European wildcats (*Felis silvestris silvestris*). Significant differences in enFeLV loads were observed between privately owned cats and SPF cats as well as among SPF cats originating from different catteries and among domestic cats of different breeds. Within privately owned cats, FeLV infected cats had higher loads than uninfected cats. In addition, higher enFeLV loads were found in wildcats compared to domestic cats. The assays described herein are important prerequisites to quantify enFeLV loads and thus to investigate the influence of enFeLV loads on the course of FeLV infection.

53. Presenting Author: Julie Levy

### Long-term outcome of cats with natural FeLV and FIV infection

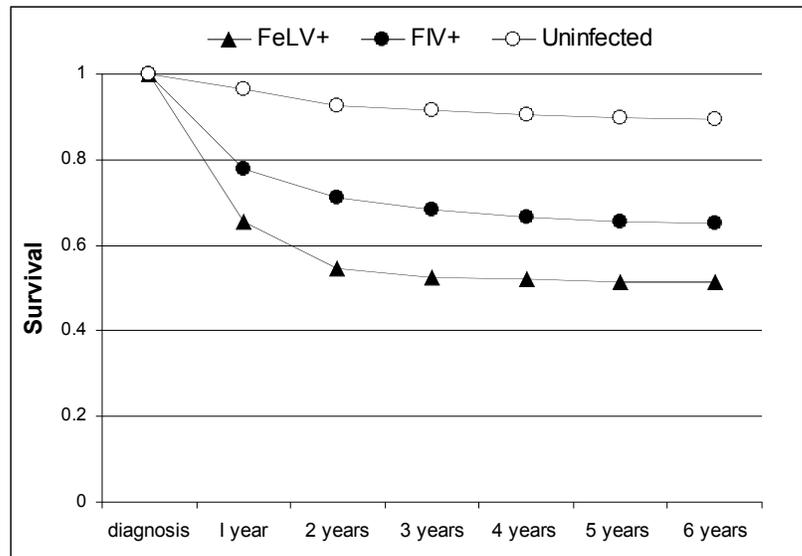
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Cats in the USA were recently reported to have an infection prevalence of 2.3% for FeLV and 2.5% for FIV, suggesting that 4-8 million cats are infected at any given time. Despite the frequency of these infections, prognosis for survival is poorly understood. The purpose of this study was to determine long-term survival of cats with natural FeLV or FIV infections.

Computerized records from a national veterinary practice with >500 clinics (Banfield, The Pet Hospital) were searched for cats that were tested for FeLV/FIV in the year 2000 and for which outcome information was available in 2006.

A total of 67,963 cats met the inclusion criteria with 6 years of follow-up. Of these, 990 (1.5%) were diagnosed with FeLV, and 1110 (1.6%) were diagnosed with FIV. Survival was significantly greater each year for cats free of retroviral infection than for infected

cats and for cats infected with FIV compared to cats infected with FeLV ( $P < 0.0001$ ). Most deaths in retroviral-infected cats occurred in the first year after diagnosis. The 6-year survival rate was 90% for uninfected cats, 65% for FIV-infected cats, and 51% in FeLV-infected cats. Although long-term survival in retrovirus-infected cats was lower than in uninfected cats, a majority of infected cats were still alive 6 years later. The high rate of death in the first year after diagnosis was likely due to disease conditions that



prompted the veterinary visit and subsequent diagnosis of FeLV or FIV or to euthanasia of healthy retrovirus-infected cats for purposes of infection control.

54. Presenting Author: Yasuhito Fujino

## Identification of a common proviral integration site, *flit-1*, in feline leukemia virus-induced thymic lymphomas

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Development of lymphomas by feline leukemia virus (FeLV) infection is mediated, at least in part, by somatically acquired insertional mutagenesis in which the integrated provirus activates a proto-oncogene or disrupts a tumor suppressor gene. In this study, a new proviral integration site termed *flit-1* was identified from an experimentally induced thymic lymphoma in a cat by screening its genomic DNA library. Of 25 FeLV-positive thymic lymphomas in cats, 5 were shown to have a proviral insertion within the *flit-1* locus by Southern blotting analysis. One, in which *flit-1* was originally identified, of the five thymic lymphomas harbored an FeLV proviral insertion adjacent to *c-myc* gene, whereas the other four lymphomas contained no proviral insertion in the region.

Extensive sequence analysis of the flanking regions of *flit-1* and database searching have shown that the *flit-1* locus did not contain any coding region but it was conserved on human chromosome 12 and mouse chromosome 15. The human and murine homologs of *flit-1* were shown to be positioned approximately 30-kb upstream to activin A receptor type II-like 1 (*ACVRL1*) gene which encodes a cell-surface receptor for transforming growth factor (TGF)-beta superfamily. Expression of *ACVRL1* mRNA was detected in two feline lymphoma tissues with *flit-1* rearrangement, but not in the lymphoma tissues without *flit-1* rearrangement and normal lymphoid tissues. Therefore, *flit-1* appears to represent a novel common integration region of FeLV provirus which may influence the expression of one or more cellular genes in thymic lymphomagenesis.

**ABSTRACTS--POSTERS**

Poster 1. Presenting Author: Tom Beck

Evolution of the APOBEC Gene Family in the Domestic Cat (*Felis catus*)

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In recent years a variety of host restriction genes have been identified in humans and mammals that modulate retrovirus infectivity, replication, assembly and/or cross-species transmission. One of these host encoded genes, APOBEC3 (Apolipoprotein B mRNA-editing enzyme catalytic) is capable of terminally editing feline foamy virus in the absence virally-encoded Bet protein, but not in its presence similar to the interplay of APOBEC3 and the HIV encoded protein Vif. The editing capacity of APOBEC3 appears to be species specific and limits cross-species transmission of retroviruses. To identify and characterize APOBEC genes in the feline genome, we first identified APOBEC related sequences in the scaffolds of the partial (2x) genome sequence of the domestic cat and compared these phylogenetically to their human and dog counterparts. Interestingly, this analysis revealed several APOBEC3 related genes in the domestic cat, however, none of which were identical to the published cat APBEC3 cDNA. After organizing a fosmid database for targeted sequencing of the selected fosmid clones used in the 2X genome sequence and verifying its accuracy by PCR and end-sequencing, we isolated a set of fosmids containing APOBEC3 genes. The results of nucleotide sequencing and phylogenetic analysis will be presented.

Poster 2. Presenting Author: Valentino Cattori or Regina Hofmann-Lehmann

Early phase feline leukemia virus shedding kinetics in experimentally infected cats

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Feline Leukemia Virus (FeLV) infection in felids results mostly from oronasal exposure to saliva and nasal secretions, especially through mutual grooming and sharing of food dishes and water bowls. Most recently, our group demonstrated that the detection of FeLV RNA in saliva may be a useful indicator of viremia, and that the detection of salivary viral RNA by RT-PCR could become a reliable tool for the diagnosis of FeLV infection. The presented study compares the kinetics of viral shedding in plasma, saliva, feces and urine during the early stages of the infection in experimentally infected specific pathogen-free cats. We assessed p27 antigen release, the presence of infectious particles and quantified the viral RNA up to 15 weeks post-infection. The results obtained show that RNA load kinetics were in agreement with the persistence of the infection not only in plasma, but also in saliva, feces and urine as well. Viral RNA loads were linearly associated with p27 antigen loads in plasma, saliva and feces, while no p27 was detectable in urine during the first 15 weeks after infection. In addition to saliva, infectious virus could be isolated from the urine and feces of persistently infected cats as early as three to six weeks post-infection, indicating additional potential ways of transmission of the virus.

Poster 3. Presenting Author: Keesjan Cornelisse

**Simultaneous administration of a multivalent modified live vaccine against FHV, FCV and FPLV mixed with a FeLV vaccine is safe and enhances the immune responses to the FHV, FCV fractions.**

**Keesjan Cornelisse, Carrie Passmore, Ann Robinson, Theo Kanellos. Pfizer Animal Health.**

**Objective:** Felocell™CVR is a freeze-dried pellet containing live attenuated virus strains for the immunisation of cats against FPLV, FCV and FHV. Leukocell™-2 is an aqueous suspension containing inactivated, adjuvanted sub-unit FeLV vaccine for the immunisation of cats against persistent viraemia and lymphoid tumours as well disease associated with FeLV infection. Extensive *in vitro* and *in vivo* experiments were undertaken to ensure antigen compatibility, immunogenicity, and acceptable safety after combining these two vaccines.

**Method and Results:** *In-vitro* evaluation showed no viricidal effects on the Felocell CVR modified live viruses, even 2 hours after mixing with Leukocell-2. *In vivo* results showed that combining these products in the same syringe prior to administration did not alter their safety profiles as assessed by repeat dose studies. After administration of the combination, the immunogenicity of the FeLV and FPLV components were similar to that following administration of the separate vaccines as assessed by serology. Administration of the mixed vaccines enhanced significantly the onset of immunity and the overall antibody responses to the FCV and FHV fractions (9.97 and 6.72 fold increase of the geometric mean, respectively).

**Conclusion:** The mixing of Felocell and Leukocell-2 to allow simultaneous administration was associated with a good safety profile and enhanced immune responses to the FHV, FCV fractions.

Poster 4. Presenting Author: Cynda Crawford

Diagnostic assays to differentiate FIV-infected from FIV-vaccinated cats.

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Current serological tests cannot distinguish between cats that are FIV-vaccinated, FIV-infected, or both. Commercial PCR assays vary significantly in diagnostic accuracy. Virus culture is highly reliable, but is time-consuming and expensive. The purpose of this study was to validate a streamlined virus culture assay and an improved PCR assay for diagnosis of FIV infection.

Blood was collected from uninfected, unvaccinated cats (n=25), uninfected cats vaccinated with Fel-O-Vax (n=25), and FIV-infected cats (n=58). The FIV-infected cats included both experimental (n=4) and natural infections (n=54). PBMC and CD8-depleted PBMC were cultured alone and with IL-2 dependent and independent CD4 T cells and tested for p24 at 4, 7, 10, and 14 days. Conventional nested PCR using “universal” primers designed from highly conserved regions in the LTR-gag region of FIV subtypes A, B, and C was performed on DNA extracted from whole blood. PCR products were sequenced to confirm the presence of FIV and virus subtype.

PBMC cultured with IL-2 dependent CD4 T cells correctly identified all infected cats when culture supernatants were tested on both days 4 and 14. PCR combined with product sequencing yielded high sensitivity (84%) and specificity (100%) and determined that cats were infected with subtype A, B, C, both A and C, and A/B recombinant viruses.

A streamlined culture assay was developed but still required IL-2 and 2 weeks incubation for 100% accuracy. The PCR assay was highly accurate, but depended on sequencing for best results. Both assays conformed with the DIVA principle for distinguishing infected from vaccinated cats.

Poster 5. Presenting Author: Charlene Edinboro

Prevalence of feline leukemia and immunodeficiency viruses in feral cats on the San Francisco Peninsula

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The spay/neuter clinic of a large humane shelter in San Mateo County, a mixed suburban and rural county of 700,000 people south of San Francisco, provides discounted or free services for feral cats. Cats receiving free services are euthanized if they test positive for feline leukemia virus (FeLV) or feline immunodeficiency virus (FIV). A recent objection to this policy by some colony caretakers provided the impetus for this study to determine the prevalence of these diseases among feral cats presented to the clinic.

Results indicated that in the years 2001 to 2003, 28 of 1769 (1.6%) cats tested positive for FeLV and 85 (4.8%) tested positive for FIV. Sex information was not recorded for all cats; however, for those with this information, FeLV-positive results among male cats ranged from 0.6% to 3.3% in the 3 years. Females had similar results. FIV-positive results ranged from 7.9 to 8.2% for males and 1.7% to 2.6% for females ( $p < 0.01$  for each year). These proportions did not decrease over the 3-year study period, and the prevalences differed compared with feral cat spay/neuter clinics in North Carolina and Florida (higher for FIV, lower for FeLV).

Examination of the geographical distribution of feral cats presented to the shelter and of these diseases in the county indicated that feral cats from certain cities were more likely to be positive for one of these infectious diseases. Efforts at disease reduction among feral cats can be focused in communities where FeLV and FIV prevalences are highest.

Poster 6. Presenting Author: Audrey Gutierrez

Vaccine Studies of Domestic Cats Primed with a DNA Vaccine and Boosted with a Whole Inactivated Virus.

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The wide sequence diversity observed for FIV and other lentiviruses impedes development of a vaccine capable of providing cross clade immunity. Amino acid sequence diversity within env genes of various FIV isolates provides the basis of the currently characterized 5 FIV subtypes, A-E. To address the issue of cross clade immunity, a multivalent DNA vaccine strategy has been proposed. This strategy was based on a FIVpPPRΔVif (subtype A) DNA based vaccine, previously shown to induce protection against a homologous challenge. We hypothesized that domestic cats primed with FIVpPPRΔVif and boosted with a whole killed virus (WKV) FIV-B (subtype B), will induce cross-protective immune responses against different FIV subtypes. Two groups of cats (n=6) received priming immunizations with FIVΔVif DNA while one group was boosted with FIVΔVif and the second group boosted with a WKV FIV-B vaccine. A third group was primed and boosted with a WKV FIV-B vaccine and a fourth group served as an unvaccinated control. All groups were challenged with wild-type FIV-B. Groups that incorporated FIVΔVif in the inoculation, as well as the unvaccinated group did not demonstrate protection against challenge, although quantitative assay of virus loads are still in progress. Analyses of virus-specific cellular and humoral immune responses are in progress and may identify differential responses between DNA and WKV-vaccinated animals.

Poster 7. Presenting Author: Katia Haipek

Evaluation of CD4+ and CD8+ T-Lymphocytes count in cats with gingivitis and naturally-infected with feline immunodeficiency virus (FIV).

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Chronic and intractable gingivitis in FIV-infected cats is a relatively common clinical problem in veterinary practice. The role of FIV in the etiology of persistent stomatitis is still undetermined. Oral manifestations often found in HIV-infected people are frequently the first clinical sign of the infection and can be considered as an indicator of the progression of the HIV infection. The purpose of this study was to evaluate the CD4+ and CD8+ T-lymphocytes count and CD4+:CD8+ ratio in a colony of cats with chronic gingivitis. To achieve these goals, a colony of twenty domestic shorthair cats was used. All cats had some degree of gingival inflammation with scores ranging from 1 through 4. Ten cats were FIV-positive and ten were FIV-negative. As a control, twenty cats without gingivitis were used (ten cats were FIV-positive and ten were FIV-negative). CD4+ and CD8+ T-lymphocytes counts were performed by means of flow cytometry in all forty cats and results compared. The results showed that cats with gingivitis and FIV-infected had a lower CD4+ T cells count than cats with gingivitis but not FIV-infected. There was no difference in CD8+ T lymphocytes count among the cats with gingivitis infected or not with the FIV. The CD4+:CD8+ ratio was lower in cats with gingivitis and FIV-infected. One can conclude that FIV infection induces immunological disorders in cats with gingival inflammation.

Poster 8. Presenting author: Lawana M. Hartsell

**Conversion of feline CD4<sup>+</sup>25<sup>-</sup> T helper cells to a regulatory T cell phenotype *in vitro* is contingent upon TGF- $\beta$ /TGF- $\beta$ RII signaling**

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CD4<sup>+</sup>25<sup>+</sup> Treg cells play a major role in maintaining peripheral self tolerance, as well as regulating immune responses to pathogens. Evidence suggests that Treg cells are chronically activated and support a productive virus infection in FIV-infected cats. How Treg cells maintain stable numbers in the peripheral immune compartment despite being targets of virus infection is not known. Therefore, we developed an *in vitro* ConA/TGF- $\beta$  stimulation model to study Treg suppressor function and peripheral homeostasis during a productive FIV infection. Feline CD4<sup>+</sup>25<sup>-</sup> T cells were FACS purified and incubated with soluble TGF- $\beta$  and ConA for 2-5 days in order to determine if they could be converted to a Treg phenotype. An increase in the surface expression of CD25 and TGF- $\beta$  was observed, as well as an increase in the mRNA levels of FoxP3 and TGF- $\beta$ , suggesting that the Th cells were converted to a Treg phenotype. This was confirmed by their ability to suppress the proliferation of ConA-activated CD4<sup>+</sup>25<sup>-</sup> cells *in vitro*. Treatment of ConA/TGF- $\beta$  stimulated CD4<sup>+</sup>25<sup>-</sup> T cells with anti-TGF- $\beta$  or anti-TGF- $\beta$ RII abrogated conversion to a Treg phenotype suggesting that recruitment of Treg cells from the CD4<sup>+</sup>25<sup>-</sup> T cell pool is mediated by TGF- $\beta$ /TGF- $\beta$ RII signaling.

Poster 9. Presenting Author: Kathleen Hayes

Methamphetamine Increased FIV Infection of Astrocytes by Alteration of Receptor and Co-Receptor Expression and Promoting Proliferation.

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Methamphetamine (METH) usage has been linked with the increased risk of HIV-1 infection and progression to HIV-associated dementia. FIV-infected cats treated with METH had altered AEVP, and fluctuations in brain glutamate as measured by HMRS. In previous studies we discovered that METH enhanced infection of primary feline astrocytes and the astrocyte cell line G355 when co-cultured with FIV-MD-infected T-cells. Our recent work has measured the effect of METH on growth properties and virus receptors, CXCR4 and OX40 expression levels by uninfected and FIV-infected T-cells and by G355 cells. In the 24 hr observation period for optimal effects, METH increase proliferation of G335 but not T-cell. Glutamine synthetase expression by G355 cells was reduced after co-culture with FIV infected T-cells, and reduced even further by the addition of METH. METH treatment caused an increase CXCR4 and OX40 mRNA expression by uninfected lymphocytes but not by FIV-infected T-cells, which already have greatly reduced OX40 and CXCR4 messenger expression. The mRNA levels continued to rise throughout the 24 hr observation period. However the mRNA increase did not translate into increased surface expression of either OX40 or CXCR4 by T-cells. METH did increase surface expression of CXCR4 by G355 cells during the same observation window. Interestingly the maximum mRNA expression for CXCR4 and OX40 was 3 hr post METH exposure after which expression declined to control levels. In these cells, CXCR4 protein was significantly increased only at the 24 hr observation point. In summary, METH stimulates astrocytes to modulate their expression of key entry proteins and increase their rate of proliferation. Both phenomena are supportive of increased transmission of FIV to astrocytes.

Poster 10. Presenting Author: Jess Hayward

### Kiwi Cat FIV

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Although Feline Immunodeficiency Virus (FIV) is fairly well researched worldwide, only a single serological survey from the late 1980's has looked at FIV in the domestic cat (*Felis catus*) of New Zealand (NZ). Here we present results from a molecular study of FIV in naturally-infected individuals of feral and domestic NZ cats. An FIV prevalence of 21.5 % (n=334) was established from feral cat populations. Sexually-mature male cats had the greatest prevalence of 51 % (n=70).

Nested Polymerase Chain Reaction was used to amplify the envelope V3-V6 region from lymph nodes of feral cats and blood samples of domestic cats. Phylogenetic analyses established the presence of two previously-described subtypes, A and C, in NZ infected cats. In comparison, only subtype A has been documented in Australia.

In addition, twelve outlying sequences on the phylogenetic tree were tested for recombination and five of these were found to be putative intersubtype recombinants. However, we did not detect any evidence of dual infection in individuals.

Furthermore, a group of seventeen env sequences that were distinct from all known FIV subtypes was identified. These may represent a novel New Zealand-specific subtype emerging from subtype A.

Poster 11. Presenting Author: Robin Hopwood-Courville

**TGF- $\beta$  *in vitro*-converted CD4<sup>+</sup>CD25<sup>-</sup> Treg like cells upregulate p21<sup>cip1</sup> and bcl-2**

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CD4<sup>+</sup>CD25<sup>+</sup> Treg cells are anergic, arrested in the G<sub>0</sub>/G<sub>1</sub> phase of the cell cycle yet programmed for cell survival. Signaling mechanisms involved in Treg cell cycle arrest and survival have not been reported. We previously reported that Treg cells from chronically FIV-infected cats are functionally activated *in vivo* and express high levels of surface TGF- $\beta$ . In many cell types, TGF- $\beta$  causes a G<sub>0</sub>/G<sub>1</sub> cell-cycle arrest by inhibiting cyclin-dependent kinase (Cdk) activity via activation of cyclin-dependent kinase inhibitors (CdkIs) and has varying effects on apoptotic regulatory proteins. Our laboratory has demonstrated that CD4<sup>+</sup>CD25<sup>-</sup> cells stimulated with TGF- $\beta$  and Con A acquire the characteristics of naturally occurring CD4<sup>+</sup>CD25<sup>+</sup> Treg cells, suggesting a role for TGF- $\beta$  in peripheral Treg homeostasis. The objective of this study was to determine the effect TGF- $\beta$  and Con A treatment has on the CdkI, p21<sup>cip1</sup> and the apoptotic regulatory proteins, bcl-2 and bax. Bead purified CD4<sup>+</sup>CD25<sup>-</sup> T cells from control and FIV-infected cats were stimulated with Con A or TGF- $\beta$   $\pm$  Con A for 4 days in the presence of rhIL-2. Cellular protein was separated on SDS-PAGE gels, transferred and immunoblotted for p21<sup>cip1</sup>, bcl-2 and bax. TGF- $\beta$  *in vitro*-converted CD4<sup>+</sup>CD25<sup>-</sup> T cells from both control and FIV-infected cats express greater levels of p21<sup>cip1</sup> than unstimulated control cells. Converted cells expressed increased levels of the anti-apoptotic protein, bcl-2, with no change in the pro-apoptotic protein, bax. In conclusion, TGF- $\beta$  may regulate anergy and resistance to activation induced cell death in Treg cells and play a role in peripheral homeostasis.

Poster 12: Presenting Author: Kristina E. Howard

Development of a Flow Cytometric Intracellular Cytokine Assay for Feline Leukocytes.

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Identification of the specific lymphocyte subsets and their cytokine production profile during HIV and FIV infection may help delineate mechanisms of pathologic changes to the immune system. Studies of HIV-infected humans routinely assess intracellular cytokine production of multiple cytokines in combination with cell-surface markers such as CD4 and CD8. Those routinely analyzed include IFN- $\gamma$ , IL-2 and TNF- $\alpha$ , and MIP-1 $\beta$ . To date, intracellular cytokine staining has been described for IFN- $\gamma$  and TNF- $\alpha$  in the cat. Given the paucity of monoclonal antibodies (mAb) commercially available for cytokine assessment in the cat, it has been difficult in the FIV/cat model to demonstrate comparable cytokine analysis. To this end, we have developed an intracellular cytokine assay that utilizes five-color FACS analysis to measure intracellular IFN- $\gamma$ , IL-2 and TNF- $\alpha$  in conjunction with CD4<sup>+</sup> and CD8<sup>+</sup> T cells.

Initially, numerous mAb reported or developed to be reactive to either IFN-  $\gamma$  or IL-2 were tested while refining intracellular staining techniques. Monoclonal antibodies identified to stain positively for their respective cytokine were optimized to be used in a multi-cytokine/cell-surface antigen FACS panel. Using both specific pathogen free and healthy random-source cats, we determined the kinetics of cytokine production and cytokine response to alternate mitogens/peptides. These data will demonstrate the development and use of this panel and will show the distribution and frequency of cytokine-producing leukocytes isolated from various tissues in response to acute FIV infection.

Poster 13. Presenting Author: Gila Kahila Bar-Gal

**Molecular subtyping of Feline Immunodeficiency virus from domestic cats in Israel.**

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Knowledge of the geographical distribution of feline immunodeficiency virus (FIV) subtypes is important for understanding different disease courses and for vaccine use. Although FIV is present in both domestic and feral cat populations in Israel its subtypes were unknown. Our study characterized the subtype of FIV and its genetic diversity among Israeli domestic cats using molecular methods. The study was carried out on 25 blood samples collected from FIV antibody positive cats from different locations in Israel. Following DNA extraction, polymerase chain reaction (PCR) was performed to amplify two regions of gag gene (300 bp) and envelope (env) gene (280bp). Genotypes were assessed by direct sequencing of PCR products and comparison with previously reported FIV sequences. Phylogenetic analysis allowed classification of the Israeli sequences into the appropriate subtype. Most of the samples grouped close or within subtype B. These results support the possibility of using the commercial FIV vaccine, which was found to be efficient against genetically diverse FIV including heterologous strains classified as subtype B.

Poster 14. Presenting Author: Dieter Klein

Poster 1. FACTORS INVOLVED IN INFECTION OF NON-DIVIDING CELLS BY FELINE IMMUNODEFICIENCY VIRUS

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In contrast to oncoretroviruses, lentiviruses have the ability to infect non-dividing cells. This characteristic is presumably based on unique factors assembled in the lentiviral preintegration complex that can be actively transported across an intact nuclear membrane. In human immunodeficiency virus type 1 (HIV-1) several factors involved in this essential step (e.g. matrix, integrase, cDNA flap and Vpr) have been described. However, their relative contribution to this process still remains to be elucidated. Much less is known about the factors involved in other lentiviruses such as feline immunodeficiency Virus (FIV). Particularly, the roles of the central DNA-flap, a cis-acting DNA-structure, and the OrfA, a small protein that is probably exerting a HIV-1 Vpr-like function, have been investigated. Using a replication deficient, pseudotyped FIV-vector system, the effect of several candidate factors has been studied. In FACS analysis, the presence of either or both of these factors conferred no improvement in transduction of quiescent HiB5 cells or aphidicolin arrested cell lines relative to a FIV-vector lacking both the cDNA-flap and the OrfA. However, preliminary experiments demonstrated that both factors enhance the amount of 2-LTR circles, suggesting that these factors increase the nuclear entry of the preintegration complex. Further experiments using a series of quantitative real-time PCR assays will concentrate on the impact of the above modifications in more detail.

Poster 15. Presenting Author: Susan M. Lankford

## Cloning of Feline FoxP3 and Quantitative Assessment of Treg Distribution During Acute FIV

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FoxP3, a member of the forkhead family of transcription factors, has been shown to be important for the development and function of CD4<sup>+</sup>CD25<sup>+</sup> T regulatory (Treg) cells and is expressed predominantly in this subset of cells. Studies in our lab involve the immunosuppressive activity of Treg cells in the cat during feline immunodeficiency virus (FIV) immunopathogenesis. Consequently, better tools for analysis of FoxP3 expression would enable more complete characterization of Treg cells. We have cloned feline FoxP3 cDNA and report here, for the first time, the feline FoxP3 sequence. The cDNA codes for a protein 430 amino acids in length that is 96% similar to canine, 91% similar to human, and 86% similar to mouse FoxP3. The intron/exon boundaries were estimated by aligning the cDNA sequence to feline genomic DNA sequences and the splice sites were found to be similar to those in other species. An exon 2 splice variant was detected in the cat, similar to that reported in human. We have developed a quantitative feline FoxP3 real time PCR assay that we have used in combination with flow cytometry, to determine the frequency and distribution of Treg in acutely FIV infected cats. In addition, recombinant expressed feline FoxP3 protein will enable us to screen available FoxP3 antibodies for cross-reactivity.

Poster 16. Presenting Author: Alora S. LaVoy

### Sequencing and Expression of Feline CD45 Isoforms

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CD45, leukocyte common antigen, is a tyrosine phosphatase receptor found on all leukocytes. It has multiple isoforms based on the inclusion of exons A, B, or C in the final spliced protein. Some isoforms include CD45R, CD45RA, CD45RB and CD45RO which typically identify B-lymphocytes, naïve T cells, subsets of B cells and T cells, and effector/memory T cells, respectively. The ability to identify these isoforms is paramount to determine the presence of effector/memory T cells responding to infections such as FIV.

Epitopes of CD45 common to all leukocytes are useful as pan-leukocyte markers in flow cytometry; however, monoclonal antibodies (mAb) reported to react with feline CD45 are either no longer available or have not been conclusively demonstrated to recognize specific CD45 isoforms. Antibodies directed against CD45 in other species such as canine and human, have not been found to cross-react with feline CD45. Thus, we have cloned and sequenced full-length feline CD45 cDNA and created mammalian expression plasmids containing the extracellular and transmembrane domains of CD45RA and CD45RO splice variants. 293T cells transfected with these plasmids will be used to screen mAbs developed against feline antigens and those that may cross-react from other species to further expand our ability to identify CD45 isoforms in felines. If existing mAb do not recognize these CD45 isoforms, the expression plasmids will be transfected into autologous mouse cells and injected into mice for the generation of new hybridomas.

Poster 17. Presenting Author: Alfred Legendre

Acute changes in cats infected with 8 different North American field strains of Feline Immunodeficiency Virus.

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Blood was obtained from 8 Feline Immunodeficiency Virus (FIV) infected cats from different regions. Two adult Specific Pathogen Free (SPF) cats were injected intravenously with ½ or 1 ml of blood from each field sample. Cats were evaluated for 12 weeks. The clade of each field strain was determined by Dr. Janet Yamamoto's laboratory. The California, Canada, Virginia and Washington State samples were clade A. The Florida, New York, Tennessee and Texas samples were clade B. The blood samples were evaluated by PCR using three different sets of primers. The Virginia and Florida samples were negative on all tests. The other samples were positive on some and negative on others.

One week after injection of ½ or 1 ml of blood, 11 of the 16 cats were positive for FIV antibodies using an ELISA test. This was attributed to passive transfer of antibodies. These antibodies disappeared in some of the cats and an active serologic response was noted 4 to 6 weeks after infection in most cats. The cats infected with the Virginia strain did not develop antibodies during the 12 week after blood injection even though FIV antigen was detected 6 weeks after injection. All cats became viremic after blood injection as determined by an ELISA test developed by Dr. Vahlenkamp. The amount of virus detected in the plasma differed in the two cats infected with the same blood sample. All cats remained clinically normal. There was a gradual decrease in the packed cell volume (PCV) in all cats and the cats infected with the Canada sample had the lowest PCVs. Nine of the 16 cats had one or more episodes of mild neutropenia occurring from 5 to 12 weeks after infection. The CD4+/CD8+ ratio of all cats was significantly lower at 6, 7 and 9 weeks after infection compared to pre-infection levels. The differences between the different field strains appeared to be minor.

Poster 18. Presenting Author: Julie Levy

Prevalence of FeLV and FIV in cats with abscesses or bite wounds

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Bites from infected cats are a highly efficient method for transmission for FIV and FeLV due to high levels of virus in the saliva. The purpose of this study was to determine the seroprevalence of FIV/FeLV at the time of treatment of a cutaneous abscess or bite wound in a prospective clinical trial.

Veterinarians were asked use an ELISA that simultaneously tests for FeLV p27 antigen and FIV p24/p15 antibodies when cats presented for treatment of cutaneous abscesses or bite wounds. Each cat's age, sex, access to outdoors, and previous cutaneous wound history were recorded. Risk factors were compared using the chi square test.

A total of 958 cats were enrolled in the study. Most were males (66%), adults (91%), and had outdoor access (94%). A majority of the cats (60%) had no previous history of wounds. Seroprevalence was 13% for FIV and 9% for FeLV. Risk of FIV was significantly higher in adults (13%) vs. juveniles (5%), males (17%) vs. females (5%), and cats with a previous history of cutaneous wounds (19%) vs. those without (9%). Risk of FeLV was significantly higher in males (11%) vs. females (5%) and in cats with previous cutaneous wounds (13%) vs. those without (6%).

Overall, 19% of cats with abscesses or bite wounds were positive for FIV and/or FeLV, which is substantially higher than the rate of 3% reported from a national survey of veterinary clinics in the US. It is recommended to test all cats at the time of treatment of wounds, rather than delaying testing for two months as currently recommended, since a delay is associated with a low rate of testing compliance.

Poster 19. Presenting Author: Saapiroon Maksaereekul

**The Effects of Cytokine Adjuvants in a *Vif*-deleted FIV Proviral Vaccine**  
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Studies in our laboratory have focused on a *vif*-deleted FIV-pPPR provirus (FIV $\Delta$ *vif*) as a FIV DNA vaccine. We proposed to enhance the efficacy of this proviral DNA vaccine by using granulocyte macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor-alpha (TNF- $\alpha$ ) as cytokine adjuvants. As a vaccine adjuvant, GM-CSF may enhance CD4 T cell proliferation and antibody response, whereas TNF- $\alpha$  will potentially induce an inflammatory response resulting in increased CD8 T cell cytotoxic activity. Accordingly, combination of these two cytokines may elicit a broader immune response to generate effector cells crucial for protection. To examine adjuvant effects of GM-CSF and TNF- $\alpha$  on this FIV DNA vaccine, we designed a vaccine study consisting of the following experimental and control groups (n=6 per group). Groups 1 and 2 were immunized with FIV $\Delta$ *vif* provirus and feline GM-CSF and feline TNF- $\alpha$  expression plasmids, or with FIV $\Delta$ *vif* plasmid alone, respectively. Control groups (3 and 4) were vaccinated with GM-CSF and TNF- $\alpha$  expression plasmids, or with vector plasmid alone. Cats were immunized with 600  $\mu$ g of each plasmid intramuscularly and boosted with the same plasmids at 32 weeks after priming immunization. Vaccinated cats were challenged with biological isolate FIV-PPR at 10 weeks post-boost and challenge studies are ongoing. Analysis of viral load and humoral and cellular immune responses are in progress and will determine the efficacy of these cytokines as adjuvants with a highly attenuated proviral DNA vaccine, as well as possibly identify correlates of protection against a wild type moderately pathogenic FIV challenge.

Poster 20. Presenting Author: Rick B. Meeker

Lentivirus-mediated neuropathogenesis: Role of virus trafficking through the cerebrospinal fluid.

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Feline immunodeficiency virus (FIV) gains rapid access to the cerebrospinal fluid (CSF) and central nervous system (CNS) where, like HIV, it is thought to establish a protected viral reservoir, evolve uniquely and induce neuropathogenesis. To better understand the functional significance of the early virus penetration into the CSF, we injected FIV into the lateral cerebral ventricle and followed infection and markers of neuropathogenesis over time. FIV injected into the ventricle exited the CSF compartment rapidly and efficiently, giving rise to high levels of plasma FIV RNA followed by high CSF viral RNA, significant proviral DNA in the brain. Although little overt CNS pathology was seen at 32-56 weeks post-inoculation several CSF samples collected at various times were toxic to primary cultures of cortical/hippocampal neurons. The toxicity did not correlate with CSF viral loads or diversification of envelope and was consistent with a destabilization of intracellular calcium, presumably due to deficits in calcium export across the plasma membrane. In contrast to the cell-free virus, FIV-infected macrophages provoked an acute inflammatory response and suppressed the CD4+:CD8+ T cell ratio but did not produce a detectable viremia. The neurotoxic response after early penetration of virus into the CSF appears to be independent of the CSF viral load, unrelated to envelope diversification and is associated with minimal pathology.

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Poster 21. Presenting Author: Christopher S. Petty

## **Regulation of Treg Cell Homeostasis During Chronic FIV Infection via TGF- $\beta$ /TGF- $\beta$ RII Signaling**

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Suppression of CD4<sup>+</sup>CD25<sup>-</sup> T helper cell proliferation by CD4<sup>+</sup>CD25<sup>+</sup> Treg cells is crucial for both maintenance of peripheral tolerance to self-antigens and control of immune responses to foreign antigen. A number of studies have demonstrated that CD4<sup>+</sup>CD25<sup>+</sup> Treg cells are activated by a variety of pathogens, and we have reported that they are chronically activated and constitutively immunosuppressive, yet maintain stable homeostasis in asymptomatic FIV-infected cats. Studies have suggested that peripheral Treg homeostasis and suppressor function are dependent on signals transduced by TGF- $\beta$ . The potential role of TGF- $\beta$ /TGF- $\beta$  RII signaling in maintaining Treg cell homeostasis and suppressor function was studied within the context of chronic, asymptomatic FIV infection. Given that Treg cells express cell surface TGF- $\beta$  when activated, we asked whether Treg cells from FIV-infected cats that are constitutively immunosuppressive, display cell surface TGF- $\beta$ , and if they could recruit additional Treg cells from the T helper pool. Our results show that freshly isolated FACS purified Treg cells from chronically FIV-infected cats but not control cats display TGF- $\beta$  on their surface. Further we show that unstimulated Treg cells from FIV-infected cats, but not control cats, convert CD4<sup>+</sup>CD25<sup>-</sup> T cells to anergic, immunosuppressive CD4<sup>+</sup>CD25<sup>+</sup>TGF- $\beta$ <sup>+</sup>FoxP3<sup>+</sup> Treg cells. Treg conversion of Th cells is dependent on TGF- $\beta$  signaling, as either anti-TGF- $\beta$ 1 or anti-TGF- $\beta$ RII abrogated Th cell conversion. These data suggest that Treg homeostasis may be maintained in FIV-infected cats by Treg-mediated recruitment from the Th pool.

Poster 22. Presenting Author: Hervé Poulet

Impact of the FIV challenge dose on the immune response during the chronic phase of infection

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Three groups of five kittens each were experimentally infected with three different doses of FIV. A fourth group was kept as non-infected controls. Viral loads and immune parameters were followed up to 31 months after infection. For viremia, the three infected groups could be distinguished only at 1-week post infection where viral loads were positively correlated to the challenge dose. However, viral loads at peak and at set-point were similar in the three infected groups. Analysis of the T cell CD4, CD8 and CD8 $\beta^{\text{lo}}$ CD62L $^{-}$  subsets showed no major differences between the 3 challenge doses. In contrast, the 3 groups of cats could be distinguished based on their FIV-specific T cell responses. During the acute phase of infection, the frequency of FIV env and gag-specific IFN $\gamma^+$  and TNF $\alpha^+$  T cell responses was maximal at 9 weeks post-infection, the strength of which tended to positively correlate with the FIV challenge dose. In addition, pools of overlapping FIV env and gag peptides, encompassing the whole gene sequences, were used to better compare the FIV-specific chronic T cell responses of the 3 groups of cats. This analysis revealed that, overall, the group of cats challenged with FIV intermediate dose presented a higher frequency of T cells responding to both env and gag peptides. Moreover, this group consistently responded to a larger set of gag protein regions compared to the 2 other groups.

The present study strongly indicates that the priming of the FIV-specific T cell responses influences both the frequency and the diversity of the resulting FIV-specific chronic T cell responses. A better understanding of the fine equilibrium between the very early viral replication and FIV-specific immune response will be critical for the development of efficacious vaccines.

Poster 23. Presenting Author: Fatemeh Rouhollah

Abstract: Investigate immune responses with recombinant fusion protein gp41-p24(HIV-1)

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Abstract

We evaluated prime-boost immunization of mice (BALB/c) with fusion protein recombinant gp41-p24(HIV-1) in MPL+TDM adjuvant. Two immunologic part of HIV-1 gp41 and p24 sequences which can be translated to 34 and 245 amino acids length peptides were chose. The gp41-p24 was selected for \_expression in E.coli K12BL21 (DE3) and purification with immobilized metal ion affinity chromatography (IMAC) on Ni-NTA column, ion exchange chromatography column and gel filtration was done using superdex 75-column XK 35/600 prepacked column. Recombinant gp41-p24 fusion protein was 32KDa MW. so refolded protein with dilution method an appropriate volume of HEPES buffer was added gradually to the protein for diminishing urea from 8 to 1M. consequently the protein was dialyzed for complete removing of urea 1M. The specificity and sensitivity refolded protein were evaluated by ELISA and western blot assays, and investigation secondary structure by circular dichroism (CD) assay. This protein has two disulfide bridges in its structure. Immunization of mice with gp41-p24 in MPL adjuvant (Female BALB/C mice with 6-8 weeks). we had two experimental groups and 3 control group of mice in this study. Immunization with method prime-boost enhance humeral and cellular immune responses. induced immune responses, measured by LTT, ELISA and flow cytometry assays. for example , significantly enhanced )and antibody response after boosting , and enhanced Th1 cytokines (IL-2 and IFN Th2 (IL-4 and IL-10) and lymphocyte proliferation and detection CD4+ and CD8+ T-cell. In future this finding may be relevant for design and testing of HIV candidate vaccines. .

Key word: gp41-p24, MPL+TDM, E.coli K12BL21 (DE3), IMAC, CD, LTT, ELISA

Poster 24. Presenting Author: Wendy S. Sprague

### Susceptibility of Dendritic Cells to Feline Immunodeficiency Virus Infection

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Feline immunodeficiency virus (FIV) interacts with dendritic cells (DC) during initiation of infections. We studied the susceptibility of monocyte-derived feline DC to FIV infection *in vitro* and the potential transfer of infection from DC to CD4<sup>+</sup> T cells. We detected FIV uptake into membrane bound vesicles in FIV-pulsed and washed DC within 2 hours of inoculation. While only low concentrations of FIV DNA were found in virus-exposed isolated DC significantly higher DNA levels were detected when resting CD4<sup>+</sup> T cells were added to the DC cultures. Viral replication intensified several log-fold, however, when CD4<sup>+</sup> T blasts were added. Likewise, FIV p27 capsid antigen was not detected in DC cultured alone, or those cultured with resting CD4<sup>+</sup> T cells, yet was readily detected in media when CD4<sup>+</sup> T blasts were added. To determine whether transfer of FIV infection required productively infected DC (vs. virus bound to but not internalized by DC), virus-exposed DC were cultured for 2 days to allow for degradation of un-internalized virus, then CD4<sup>+</sup> T blasts were added. Infection of T cells was un-impaired, indicating that T cell infection is likely mediated by *de novo* viral replication in DC and transferred during normal DC/T cell interactions. These results demonstrate that feline DC support limited FIV infection, which nevertheless is sufficient to efficiently transfer infection to susceptible T cells and trigger the major burst of viral replication. These DC/virus interactions are similar to those believed to occur in HIV and SIV infections.

Poster 25. Presenting Author: Jennifer L. Troyer

## CROSS SPECIES TRANSMISSION OF LENTIVIRUS BETWEEN BOBCATS AND PUMAS IN SOUTHERN CALIFORNIA AND FLORIDA

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Lentiviruses similar to feline immunodeficiency virus (FIV) have been documented in over 20 felid species. Genetic analysis of viruses isolated from species with high seroprevalence has demonstrated that each species harbors its own unique strain of lentivirus. We evaluated the prevalence and characteristics of lentivirus infection in bobcats (*Lynx rufus*) and pumas (*Felis concolor*) in three geographic locations in the Los Angeles metropolitan area and in one bobcat from southern Florida. Seroprevalence of infection determined by puma lentivirus (PLV) immunoblot ranged from 27-100% of study animals depending on the species and study site. Genetic sequencing and phylogenetic analysis of proviral fragments of the *pol* gene identified two unique viruses. One strain, previously identified as PLV clade-B, has been reported in pumas throughout much of western North America and South America and was detected in 7 of the 12 infected study pumas. PLV clade-A, previously reported only from pumas in southern Florida and southern California, was isolated from 5 of the infected pumas and all infected bobcats (17) from which sequence could be obtained. Phylogenetic analysis of the same proviral genomic region from a bobcat previously captured in southern Florida identified it as a PLV clade-A virus. Further, PLV clade-A virus present within each study site shared greater sequence homology with virus infecting other individuals in that location, regardless of whether the host was a bobcat or puma, than with virus infecting conspecific hosts in one of the other study sites. These data are strongly suggestive that cross species transmission of lentivirus occurred between bobcats and pumas in southern California and also probably occurred in southern Florida. This is the first report of cross-species lentivirus transmission not involving captive or domesticated felids. We hypothesize that the proximate cause of this transmission is puma predation on bobcats with transmission of a bobcat lentivirus, PLV clade-A, to pumas.



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