Beth Callan is a professor of medicine (clinician-educator track) in the Department of Clinical Studies-Philadelphia and medical director of the Penn Animal Blood Bank. Beth received a B.S. in Chemistry from Chestnut Hill College in 1984 and a V.M.D. from Penn Vet in 1988. She then completed a rotating internship, residency in internal medicine, and fellowship in hematology/transfusion medicine, all at Penn Vet, and has since remained affiliated.

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The 2015 Zoetis Award for Veterinary Research Excellence was awarded to Dr. P Jeremy Wang, Department of Biomedical Sciences, at the Annual Faculty Research Retreat on June 19th. Dr. Wang has a publication appearing in EMBO entitled “TEX11 is mutated in infertile men with azoospermia and regulates genome-wide recombination rates in mouse”, 2105, EMBO Mol Med. 2015 Jul 1. pii: e201404967, Epub ahead of print.
Beth's current clinical research interests are centered on red blood cell (RBC) storage and gene therapy for canine hemophilia.

**Duration of RBC storage: Is old blood bad?**

The shelf-life of stored RBCs is typically 42 and 35 days for human and canine RBCs, respectively. Given that blood is a precious and limited resource, both human and veterinary blood banks typically dispense the oldest RBC units first to reduce wastage. However, accumulating evidence suggests that transfusion of RBCs stored for 14 days or longer increases rates of infection, thrombosis, morbidity, and mortality in hospitalized human patients. During storage *in vitro*, RBCs undergo cumulative biochemical and biomechanical changes that reduce their recovery *in vivo*. After transfusion, such storage-damaged RBCs are quickly cleared from the circulation by reticuloendothelial macrophages and the consequent catabolism of hemoglobin leads to a rapid release of iron. If iron is returned to plasma at a pace that exceeds the rate of transferrin-mediated iron uptake, circulating non-transferrin-bound iron (NTBI) levels increase. As NTBI participates in redox reactions that promote oxidative damage, cytotoxicity, and endothelial activation, determining the time frame in which stored RBCs can be safely utilized for transfusions is critical for optimal outcome.

In collaboration with Dr. Eldad Hod from Columbia University, Dr. Callan investigated the effect of duration of RBC storage on transfusion-associated inflammation in healthy dogs receiving autologous RBC units stored for 7 (fresh) and 28 (old) days. Administration of old, but not fresh, RBC units was associated with a pro-inflammatory cytokine response, exemplified by monocyte chemoattractant protein-1, and accompanied by increased neutrophil counts and decreased platelet counts (Figure 1).1 In addition, old RBC transfusions were associated with decreased post-transfusion RBC recovery, increased serum bilirubin (evidence of extravascular hemolysis), and increased NTBI at two and six hours post-transfusion. Together with the finding that transfusion with old RBC units was a negative risk factor for survival in dogs with immune-mediated hemolytic anemia (IMHA),2 Drs. Callan, Hod, and Thawley (Penn Vet), are conducting a randomized, blinded clinical trial in which client-owned dogs with primary IMHA receive fresh (stored <7 days) or old (stored 21-28 days) RBCs. *continued on page 3*
Figure 1 – Transfusion of old, but not fresh, PRBCs results in increases in blood level of (A) monocyte chemotactant protein-1 and (B) neutrophil count and (C) a decrease in platelet count (median ± interquartile range).

If transfusion of “older” RBCs to dogs with IMHA is associated with an increased inflammatory response, increased morbidity and/or mortality, it will support changing current transfusion practices to include the use of “fresh” RBCs to transfuse anemic canine patients. As such, this study will have a significant impact on canine health and veterinary blood banks. However, as discarding older RBCs could lead to a shortage of RBC units, statistically significant findings are clearly needed to warrant such a change.

**Gene therapy: Hope for dogs with hemophilia**

Hemophilia A (HA) and hemophilia B (HB) are hereditary bleeding disorders caused by a deficiency in the blood coagulation factor VIII (FVIII) and factor IX (FIX), respectively. These coagulopathies occur in both humans and dogs and in their most severe form result in spontaneous bleeding, most commonly into joints, hematoma formation and potentially life-threatening hemorrhage following trauma or surgery. Humans with severe HA or HB require prophylactic administration of recombinant human FVIII or FIX protein several times weekly to control bleeding. Research dogs with HA and HB have been used as models to evaluate novel therapies for human HA and HB. Gene transfer using an adeno-associated viral (AAV) vector to deliver the genes for canine FVIII (cFVIII) and FIX to dogs has been successfully performed in more than 70 research dogs and such studies reveal that a 2-3% increase in FVIII and FIX activity reduces bleeding episodes by >90%. Long-term follow-up (> 9 years) has documented the efficacy and safety of gene therapy using AAV vectors to ameliorate bleeding tendencies in research dogs.

In collaboration with Drs. Katherine High and Valder Arruda from Children’s Hospital of Philadelphia (CHOP) and Dr. Mark Haskins (Penn Vet), Dr. Callan has adopted this technique to treat two privately owned dogs with severe HA (plasma FVIII activity < 1%) using an AAV8-cFVIII vector administered as a continued on page 6
ANNUAL FACULTY RESEARCH RETREAT at PENN VET

On June 19, 2015, 115 faculty, residents, postdocs, students and staff gathered for the Annual Faculty Research Retreat at New Bolton Center. Phillip Scott, Vice Dean for Research and Academic Resources convened the conference. James M Wilson, M.D., Ph.D., delivered the Robert R. Marshak Lecture entitled “Gene Therapy for Animal and Human Health”. Penn Vet’s research resources were presented in talks by Tom Parsons, V.M.D., Ph.D., Swine Teaching & Research Center—A Pig’s Eye View of Animal Welfare; William Beltran, D.V.M., Ph.D., Targeting Gene Therapy for X-linked Retinitis Pigmentosa to Patient-relevant Stages of Disease; J.Oriol Sunyer, Ph.D., Fish Immunology —Exploring the Evolution of Immunity; Michael Povelones, Ph.D., Insectary: Exploring Mosquito -Pathogen Interactions; Charles Vite, D.V.M., Ph.D., Referral Center for Animal Models of Genetic Disease; Christopher Lengner, Ph.D., Center for Animal Transgenesis and Germ Cell Research—Implementation of CRISPR/Cas9-Mediated Genome Editing; Dorothy C. Brown, MSCE, D.V.M., Veterinary Clinical Investigations Center—Making the Impossible Studies Possible; Thomas Schaer, V.M.D., Preclinical Research Services-Challenges and Opportunities.

Four Penn Vet Cores were also presented: Comparative Pathology (Amy Durham, VMD, DACVP); Penn Vet Imaging (Bruce Freedman, PhD); Center for Host-Microbial Interactions Bioinformatics (Dan Beiting, PhD); and the Non-Invasive Whole Animal Imaging Facility (Ellen Puré, PhD).
Visiting Khorana Scholar, Shreya Nahata, attends the program at New Bolton Center.

Dr. Oriol Sunyer gave his talk on the Penn Vet fish immunology facility and the evolution of immunity.

The Allam House at New Bolton Center—a beautiful setting for the annual faculty research retreat.

Dr. Chris Lengner spoke about his research and the Center for Animal Transgenesis and Germ Cell Research.

Participants enjoy posters and lunch.
Dr. Callan continued from page 3

single peripheral venous injection. Both dogs had experienced 11 to 12 bleeding episodes that required transfusion support during the year prior to treatment (Figure 2). Following gene therapy there was a 5-fold increase in circulating FVIII, with sustained expression for at least 18 months post-treatment, and no signs of adverse events. The dramatic reduction in bleeding episodes in the two privately owned dogs has been life-changing (and potentially life-saving) for them.

Based on these promising results, the Penn Vet/CHOP collaborative group is undertaking a pilot study (funded in part by the Institute for Translational Medicine and Therapeutics) to evaluate a new variant of the AAV8-cFVIII vector in treating privately owned dogs with HA with severe bleeding tendencies. Like humans, dogs with hemophilia may develop antibodies that inhibit FVIII or FIX function following repeated plasma transfusions, making it more challenging to control bleeding. Notably, our ability to treat privately owned (outbred, as opposed to inbred research colony dogs) hemophilia dogs with different FVIII mutations provides a unique opportunity to gain further insights into the risk of immune responses in gene and protein therapy. Finally, FVIII and FIX promote coagulation by converting FX to factor Xa (FXa) to promote thrombin generation and fibrin clot formation. We are currently determining if infusion of a zymogen-like FXa variant that has a longer half-life and is less active than wild-type FXa (to prevent excessive coagulation) may be helpful in controlling bleeding in dogs with hemophilia, even in the presence of inhibitory antibodies. Dr. Callan predicts that such studies will greatly benefit canine hemophilia patients and are of direct translational importance to human patients as well.

Support for Dr. Callan’s research comes from the AKC Canine Health Foundation, the Companion Animal Research Fund (Department of Clinical Studies PHL), and the Institute for Translational Medicine and Therapeutics of the Perelman School of Medicine and the School of Veterinary Medicine at the University of Pennsylvania.

References:


RECENT AWARDS
(Direct Costs)

Montserrat Anguera
University Research Foundation
Faulty X-chromosome silencing as significant contributor for female-bias in autoimmunity
$47,000  8/1/15 to 7/31/16

Christopher Lengner
University Research Foundation
Identifying markers for the prospective isolation of reserve intestinal stem cells
$50,000  8/1/15 to 7/31/16

Olena Jacenko
University Research Foundation
Could a young niche rejuvenate hematopoiesis
$50,000  8/1/15 to 7/31/16

Kendra Bence
Howard Hughes Medical Institute: Chronobiology Program—Investigating a novel link between phosphatase regulation of central GLP-1 activity and the circadian control of feeding
$30,000  5/1/15-4/30/16

Jeremy Wang
NIH/U01 HD084007
Targeting the piRNA pathway and meiotic recombination for male contraception
$1,400,000  5/01/2015-3/31/20

Oriol Sunyer
NIH R01 GM085207
Primordially conserved principles governing mucosal immune responses to pathogens and microbiota
$862,045  2/15/15—11/30/18

Leszek Kubin
NIH R01 HL047600
Premotor control of upper airway and REM sleep atonia
$1,000,000  7/15/15—4/30/19

Carolina Lopez
NIH R21 AI109472
IL-10 producing neutrophils during respiratory virus infection
$275,000  7/15/15-7/14/17

Jorge Alvarez
McCabe Foundation Pilot
Fund: The role of the Hhpathway in regulating CNS immunity. $19,323
7/1/2015—6/30/2016

Jorge Alvarez
Multiple Sclerosis Society of Canada Role of Non-Conventional CNS Barriers During Homeostasis and Neuro-inflammation.
$278,000  1/1/15-12/31/17

Michael Povelones
McCabe Foundation Pilot
$19,323  7/1/15–6/30/16

Lisa Murphy
USDA—Maintenance of Membership Laboratory Requirements
$50,000  4/1/15-3/31/16

Ron Harty
Fox Chase Chemical Diversity Center Development of small molecule therapeutics against RNA viruses
$84,374  3/1/15-2/29/16

Igor Brodsky
Burroughs Wellcome Fund Investigators in the Pathogenesis of Infectious Disease Award—Defining the role of caspase-8 in the regulation of anti-microbial host defense.
$500,000  7/1/15-6/30/20

James Marx
ASLAP Foundation Summer Fellowship Program
$5,000  5/1/15-9/30/15

Raymond Sweeney
Immune Solutions Ltd Evaluation of an oral vaccine for Johne’s Disease in calves
$97,760  5/1/2015-4/30/2016

Publications


Igor E. Brodsky, PhD is one of five early-career researchers at the University of Pennsylvania who has received funding from the Burroughs Wellcome Fund (BWF) for their excellence in biomedical research, in topics including heart disease, sleep, and infectious diseases. Dr. Brodsky is an assistant professor in the Department of Pathobiology, School of Veterinary Medicine. He will focus on the activity of an enzyme called caspase-8, which plays a key role in how the immune system defends against invading microbes. His research could help identify therapeutic targets to either boost or tone down the immune system’s response to infection or inflammation. (see awards page 7)

HONORS

In May, Pathobiology post-doctoral researcher, Dr. Ana Misic spoke at the 4th Annual American Society For Microbiology conference on Methicillin-resistant Staphylococi in Animals: Veterinary and Public Health Implications. She spoke on antimicrobial resistance in zoonotic bacteria and foodborne pathogens. Her talk was entitled: The Finished Genome and Methylome for the Veterinary Pathogen, Staphylococcus schleiferi.

In April, Ciara Gimblet, (graduate student in the Scott Lab) gave an oral presentation at the Woods Hole Immunoparasitology Meeting, where she won an award for the Best Presentation! The title of her talk was: “Leishmania major induces transmissible alterations in the skin microbiome”.

VMD/PhD student Jonathan Madara for winning the Perelman School of Medicine 2015 “Art in Science” competition. He won in the graduate student level for his image of “Ebola Virus-like Particle Budding”. Jonathan’s mentor is Dr. Bruce Freedman and his collaborators were Dr. Ron Harty & Dr. Gordon Ruthel (PennVet Imaging Core)

Ralph Brinster, VMD, PhD, received an honorary doctor of laws degree in May from the University of Calgary. The highest academic honor is bestowed on individuals whose notable achievements and community service merit recognition. Ralph Brinster, one of the world’s most accomplished veterinary scientists, received the honors at the Cumming School of Medicine and the faculties of law, veterinary medicine and graduate studies. A Richard King Mellon Professor of Reproductive Physiology at Penn Vet, he continues to perform cutting-edge research and is developing novel techniques for the culture and genetic modification of germline stem cells to restore fertility in male children undergoing cancer treatment.

Dr. Meryl Littman spoke at the NY State Spring Veterinary Conference on tickborne diseases on May 15-17 2015. She also spoke on Lyme disease at the ACVIM 33rd National Medical Forum, Indianapolis, in June 2015.

The Penn Vet Research Newsletter is Distributed Quarterly

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