

NEWSLETTER

Teleost fish are the most primitive bony vertebrates... *that contain immunoglobulins*



Dr. J. Oriol Sunyer is an associate professor of immunology in the Department of Pathobiology. Dr. Sunyer received a B.A. in Biology from the Universitat Autònoma de Barcelona (Spain) in 1991. In 1993 he moved to the University of Pennsylvania School of Medicine (SOM) where he performed most of the experimental work for his Ph.D. thesis. In 1995 he received his Ph.D. in Physiology and Immunology from the Universitat Autònoma de Barcelona, followed by postdoctoral training at the University of Pennsylvania (SOM). In 1999, he joined the Department of Pathobiology at Penn Vet as an assistant professor. The overarching research goals of Dr. Sunyer focus

on the evolution of key innate and adaptive immune processes of vertebrates using teleost fish and mice as animal models.

Complement and inflammation in fish--In mammals, activation and cleavage of C₃ and C₅ molecules results in the generation of C_{3a} and C_{5a} anaphylatoxins respectively. These anaphylatoxins play crucial roles in inflammation and exert their functions mainly through C_{3a} and C_{5a} receptors (C_{3a}R and C_{5a}R). Until recently, virtually nothing was known about the structure, function and evolution of either C_{3a} and C_{5a} molecules or their receptors in non-mammalian species. Dr. Sunyer's

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What comes first?

Thinking, writing grants, funding--research investigators are perpetually faced with a conundrum. "How do I divide my time... generating preliminary data, reading, writing, reviewing, teaching, giving talks?" Nevertheless, research is an important mandate in the division of academic activity. To aid researchers in searching for funding opportunities (NIH and Foundations); new search strategies for PubMed; brushing up on PowerPoint animation; creating a bibliographic database; there are workshops close at hand:

[Penn Libraries Workshops](#)



Dr. Sunyer continued from page 1

group was the first to structurally and functionally characterize C3a, C5a and their respective receptors in teleost fish (1, 2).

During the last 10 years, Dr. Sunyer's group has generated a panel of more than 15 different antibodies that recognize a number of rainbow trout complement molecules. Using these antibodies, and in collaboration with Dr. David Nelson (University of Rhode Island), Dr. Sunyer's group is currently funded by the US Department of Agriculture (USDA) to study the harmful effects of proteases from *Vibrio Anguillarum*, (a fish pathogen) on the functional activity of key components of the rainbow trout complement system.

Discovery of phagocytic B cells in fish and mammals--Until recently, dogma dictated that normal cells of lymphoid origin (i.e., B cells, T cells) were unable to perform phagocytosis. Contrary to this belief, recent studies in Dr. Sunyer's laboratory have shown that large subsets

of trout B cells are capable of efficient phagocytosis (3). In contrast T-cells and thrombocytes were devoid of such capacity. After particle internalization, trout B cells initiated degradative pathways through phagolysosome formation. Significantly, phagocytic B cells could effectively kill internalized bacteria. These studies were the first to identify and characterize phagocytic activity in normal B cells from any animal species. These findings were reported in *Nature Immunology* (3) and made the cover of the 2006

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Dr. Sunyer in his laboratory with postdoctoral researcher, Dr. Y Zhang. Photo of a research Rainbow Trout.

THE VERTEBRATE ANIMAL SECTION IN YOUR NIH APPLICATION.....NIH News

Proper completion of the Vertebrate Animal Section (VAS) of your NIH proposals is now factored into the overall impact score. Applications lacking a VAS or that do not address all five points may be deferred, or, alternatively, the application's impact/priority score may be negatively affected.

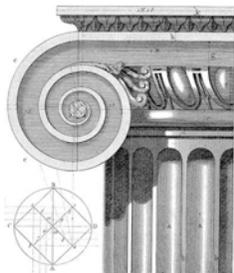
Detailed description of proposed use of animals--including species, strains, ages, sex, and number to be used

Method of euthanasia and reasons for its selection

Information on veterinary care of animals

Justification for use of animals, choice of species, numbers to be used

Description of procedures for minimizing discomfort



PI's and program directors are encouraged to review the VAS worksheet and the required elements. Reviewers evaluate the VAS, NIH verifies any concerns, and applicants are given the opportunity to resolve issues prior to an award.

<http://grants.nih.gov/grants/olaw/VASchecklist.pdf>

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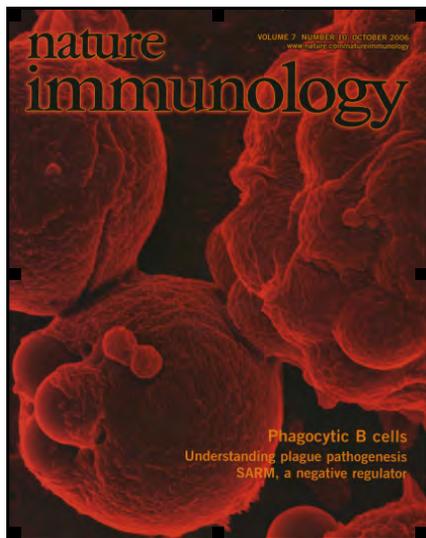


Fig. 1. B lymphocytes from fish have potent phagocytic and microbicidal capabilities.

October issue (Fig. 1). Recent reports have confirmed Dr. Sunyer's findings in other fish, demonstrating the presence of phagocytic B cells in both Atlantic salmon and cod. In addition, phagocytic B cells have also been recently described in reptilians. One of the objectives of Dr. Sunyer's group is to assess the role of phagocytic B cells in antigen presentation processes in fish. To this end, his group recently cloned and functionally characterized CD80/86 and IL-2 molecules in rainbow trout to evaluate co-stimulatory molecules and cytokines involved in fish antigen presentation pathways (4). This year Dr. Sunyer has received a 3 year National Science Foundation (NSF) award to study further the roles of fish B cells in phagocytosis and inflammation in systemic and mucosal compartments of teleost fish.

The conservation of phagocytic function in B cells from several classes of vertebrates including fish, amphibians and reptilians,

has prompted Dr. Sunyer's group to evaluate the phagocytic capacity of primary murine B cell subsets. Studies recently submitted by his group reveals the presence of a previously unrecognized subset of murine B cells with phagocytic and microbicidal capacities. Moreover these phagocytic murine B cells are able to efficiently present phagocytosed antigen to CD4⁺ T cells. This positions murine phagocytic B cells at the crossroads that link innate with adaptive immune processes and provides an additional layer of complexity in the immune system of these species.

A specialized mucosal immunoglobulin unearthed in Teleost fish: Implications for fish and mammalian mucosal immune responses

In mammals, IgA is the main immunoglobulin involved in mucosal immunity. Teleost fish are the most primitive bony vertebrates that contain immunoglobulins, and in contrast to mammals and birds, they were thought to be devoid of IgA or a functional equivalent. As such, there was no evidence of immunoglobulin specialization in teleost mucosal and systemic areas and therefore, fish IgM was regarded as the only functional antibody in both compartments. In 2005, a previously unknown immunoglobulin isotype, IgT, was discovered in teleost fish. However, nothing was known about the protein structure of IgT, its production by putative B cells, and more importantly, its function was an enigma. However, this year Dr. Sunyer's group reported the

structural and functional characterization of IgT, identifying this immunoglobulin as the first specialized mucosal immunoglobulin to be described in a non-tetrapod species (5). In the same study, Dr. Sunyer's group provided direct evidence for the existence of a novel fish B cell lineage uniquely expressing surface IgT. This B cell lineage represented the predominant B cell subset in the fish gut. More critically, in response to an intestinal parasite, fish showed a compartmentalized response of IgT and IgM immunoglobulins into gut mucosal and systemic areas respectively. Supporting the role of IgT in mucosal immunity, Dr. Sunyer's study found that a majority of trout intestinal bacteria were coated with IgT. These findings have recently been published in the 2010 September issue of Nature Immunology (5) and are highlighted on its cover (Fig. 2). The aforementioned findings challenge the present paradigm that specialization of immunoglobulin isotypes in systemic and mucosal compartments arose during the evolution of tetrapods. From an applied perspective, the new capability of measuring not only IgM but also IgT responses will greatly facilitate the evaluation and understanding of fish immune responses as well as the protective effects of fish vaccines. Thus, the design of future fish vaccines is likely to be more effective, stimulating not only systemic but also mucosal immunity. In the words of Dr. Roger Beachy, the director of the National Institute of Food and Agriculture, "Dr. Sunyer's work will change

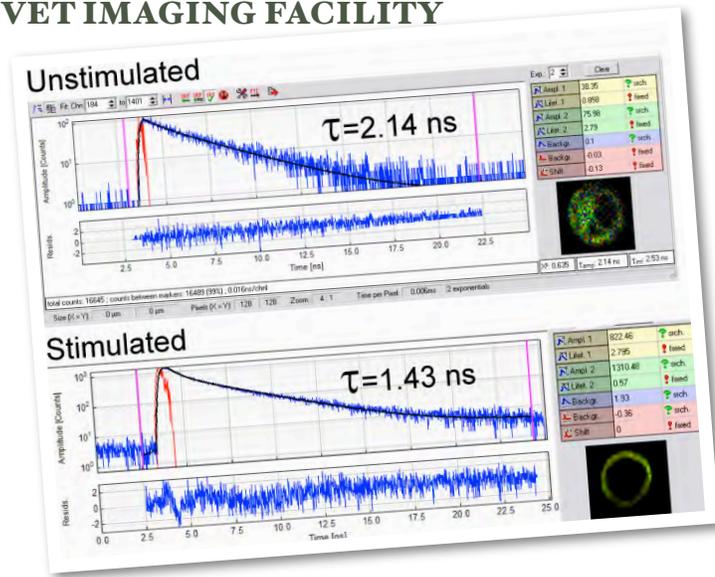
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A GRANT to UPGRADE the PENN VET IMAGING FACILITY

A grant was recently awarded to Dr. **Bruce Freedman**, associate professor in the Department of Pathobiology in the amount of \$496,332 under the shared instrumentation grant program (SIO) of the National Center for Research Resources (NCRR) to enhance and upgrade the existing multi-photon spectral imaging microscope in the Penn Vet Imaging Facility. NCRR is part of the National Institutes of Health (NIH) and provides laboratory scientists and clinical researchers with the tools and training they need to understand, detect, treat and prevent a wide range of diseases. Support from NCRR enables discoveries made at a molecular and cellular level to move to animal-based studies, and then to patient-oriented clinical research.

The **Penn Vet Imaging facility** was established in 2008 in recognition of the need for state of the art instrumentation and expertise to provide multi-photon and molecular imaging capability and expertise to a wide research community at Penn. Since its inception, the facility has cultivated a large user base of over 30 active labs and has developed a wealth of collective expertise on a range of projects that involve real-time imaging of pathogen infection and immune responses, Förster resonance energy transfer (FRET) based approaches to track the interactions and activity of signaling molecules, *in-situ* photo-activation of molecules for studies of immune cell signaling, fluorescence recovery after photo-bleaching (FRAP) to look at protein trafficking in cells, and live imaging of calcium and protein movements within cells.

The initial success of the facility simultaneously revealed the need for improved capacity to perform deep tissue, live cell, and quantitative FRET-based imaging. Dr. Freedman worked closely with Dr. Lingli Zhang, technical director of the Penn Vet Imaging Facility and more than a dozen laboratories from The Schools of Veterinary Medicine, Medicine, and Arts and Sciences to secure funding for these core instrument enhancements. The newly awarded grant will be used to purchase a range of components for the existing Leica DM6000 SP5



Subcellular Protein Interactions Measured using Fluorescence Lifetime Imaging Microscopy (FLIM). An increase in the rate of fluorescence decay (τ , bottom panel, nanosecond timescale) occurs when two fluorescent proteins physically interact. Stimulation of B lymphocytes induces relocation of the ER resident protein STIM1 toward the plasma membrane calcium channel ORAI1 (see pseudocolor images). Changes in the rate of decay of the donor fluorophore (here CFP-conjugated STIM1) was used to obtain quantitative measurements of the interaction between these two proteins. This direct interaction between STIM1 and ORAI1 is critical for initiating calcium signals that regulate the immune response of B cells. One of the applications for the new FLIM system will be to define the mechanism by which calcium signals, and thereby immune responses of lymphocytes, are regulated by antigen.

multi-photon spectral imaging microscope. These include a resonant scanner and additional detectors to significantly increase the temporal resolution, spatial coverage and detection sensitivity of the microscope; an environment control and manipulation system allowing long term real time imaging of whole animals, living tissues, and cells; a 405 nm laser system for UV excitation, photo-activation and FRET imaging; and a **fluorescence lifetime imaging (FLIM)** system. Recently, FLIM has emerged as a robust and highly quantitative approach to probe molecular interactions in cells that would be impossible or difficult to measure with intensity based imaging. As there are currently no open-access FLIM systems at the University of Pennsylvania, it is anticipated that this new capability will stimulate the development of a wide range of novel projects on campus. Visit the facility on 3 Hill Pavilion and the online site: [Penn Vet Imaging Facility](#) or call (215) 746-0471.

ANGELA FUSELLO & ALEXANDRA O'KEEFE RECEIVE RESEARCH AWARDS



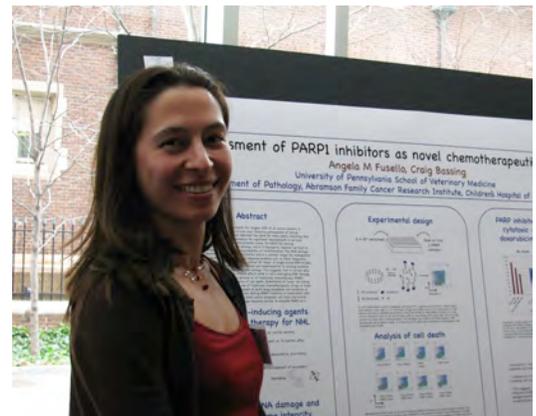
Alexandra O'Keefe

Last year **Angela Fusello** V¹² and **Alexandra O'Keefe** V¹¹ were the Penn Vet recipients of summer student fellowships from the Morris Animal Foundation. The goal of the Veterinary Scholars Summer Research Program is to increase the number of veterinarians involved in biomedical and clinical research. The program provides an opportunity for veterinary students and undergraduate students interested in veterinary medicine to explore non-practice careers by engaging in a mentored research project and through informal and formal interactions with scientists. Angela Fusello assessed PARP₁ inhibition as a chemotherapeutic

strategy for canine lymphoma (Mentor Dr. Craig Bassing) and Alexandra studied immune-mediated hemolytic anemia in Cocker spaniels and other breeds by flow cytometry (Mentor Dr. Urs Giger).

During the Morris Animal Foundation's annual conference held this past June in Denver, they both received an award for their outstanding research presentations.

Angela Fusello



RESEARCH CENTER NEWS

The Center for Animal Transgenesis and Germ Cell Research has completed its reorganization.

The mission of the center is to advance our understanding of the fundamental processes of reproduction as it relates to fertility control and treatment of infertility. This announcement is to appoint **Dr. Jeremy Wang** as Director and **Dr. Dirk Vanderwall** as Associate Director. Dr. Wang,

Associate Professor of Animal Biology, is interested in basic research on regulation of meiosis, piRNA biogenesis, and genetic causes of male infertility. Dr. Vanderwall, Associate Professor of Clinical Studies (NBC) and Chief, Section of Reproduction, is engaged in clinical research on equine embryos and oocytes. This school-wide research center brings together faculty members engaged in both basic and clinical research on reproductive biology. For details, refer to the [center website](#). Intellectually, the center interacts

with both Penn Institute of Regenerative Medicine (IRM) and Center for Research on Reproduction and Women's Health (CRRWH). The center co-sponsors the weekly seminar series on reproduction with CRRWH. To become a faculty member of the center, please contact Pat Bodek bodekp@vet.upenn.edu or the membership link at the website.



NEW FACULTY AT PENN VET

The Department of Pathobiology has welcomed a new member to their faculty. She is **Carolina Lopez, PhD**. Formerly with the Mount Sinai Medical Center in New York, she is interested in studying the events leading to the development of efficient anti-viral immunity. She and her laboratory staff are settling in on the 3rd Floor of Hill Pavilion.

SOME RECENT AWARDS

Ralph Meyer

Michelson Grant in Reproductive Biology for the development of a non-surgical sterilant/technology for use in both male and female cats and dogs. \$570,000
3/1/2010--2/28/2013

Narayan Avadhani

ARRA NIH Supplement
Role of mitochondria targeted CYP2E1 and HO-1 in alcohol mediated tissue injury
\$50,080 9/5/2010--7/31/2011

Bruce Freedman

NCRR Shared Instrumentation Grant
FLIM system, resonant scanner, and UV laser for 2 photon microscope. \$496,332
8/1/10-7/31/11

Silke Jennrich

American Heart Association
Postdoctoral Fellowship,
Lung exit of T cells during influenza virus infection \$88,000
7/1/10-6/30/12

Oriol Sunyer

NIH - R01 - Specific Contribution of a New IG Isotype (IGT) in Teleost Fish Immune Responses \$808,292
9/1/10-7/31/14

Oriol Sunyer

NSF- Role of teleost fish B cell subsets in innate and adaptive immunity against pathogens \$460,586
9/1/10-8/31/13

Gustavo Aguirre

Retinal Remodeling in Canine Models of LCA/Early Onset Retinal Degeneration Foundation Fighting Blindness
\$58,143 7/1/10-6/30/11

John Lewis

MBF Therapeutics
Treatment of Feline Oral Squamous Cell Carcinoma (SCC) w/ 2-DFMO and MQT 1569. \$15,940 8/25/10-8/31/11

Nicola Mason

AKC Canine Health Foundation
Identification and characterization of a canine derived Single Chain Antibody that binds & neutralizes canine VEGF (2010)
\$70,907

Nicola Mason

ADVAXIS, Inc.
The use of recombinant Listeria monocytogenes expressing Her-2/neu to stimulate anti-tumor immunity in dogs with appendicular osteosarcoma.
\$413,000 7/19/10-7/18/12

Nicola Mason

Theralogics, Inc.
Investigations of NBD peptide in the Treatment of Canine Non-Hodgkin's Lymphoma
\$193,140 8/1/10-7/31/11

Beena John

NIH R21 AI090234, Real time imaging of tolerance induction by mucosal dendritic cells.
\$275,000
9/15/10-8/31/11

Charles Vite

Ara Parseghian Medical Research Foundation. Evaluating Treatment Strategies for Feline Niemann-Pick C Disease
\$90,000 7/1/2010-6/30/2011

Special Honors

Dr. **Tracy Bale, PhD** was selected to receive The Endocrine Society's **2011 Richard E. Weitzman Memorial Award**. This award is given to a young investigator in recognition of meritorious accomplishments in the field of endocrinology.

Dr. **Mo Salman, BVMS, MPVM, PhD**, of Colorado State University, is the 2010 recipient of the prestigious **Penn Vet World Leadership in Animal Health Award**. **Brittany Gross V13**, and **Nikkita Patel, VMD/PhD** are the 2010 recipients of the Penn Vet Student Inspiration Awards.

Dr. **Leszek Kubin, PhD**, Research Professor of Physiology in the Department of Animal Biology was nominated to serve on the NIH Sleep Disorders Research Advisory Board charged with designing a 5-year plan of research on sleep and circadian disorders and will serve as a liaison between NIH institutes, professional organizations and patients.

Recent papers



Crystal structure of the conserved herpesvirus fusion regulator complex gH-gL.
TK Chowdary, TM Cairns, D Atanasu, G Cohen, RJ Eisenberg, & EE Heldwein.
2010 *Nature Structure & Mol. Biol.* 17 (7): 882-889.



IgT, a primitive immunoglobulin class specialized in mucosal immunity. YA Zhang, I Salinas, J Li, D Parra, S Bjork, Z Xu, S LaPatra, J Bartholomew & JO Sunyer (2010) *Nature Immunology* 11(9): 827-835.



The early generation of a heterogeneous CD4+ T cell response to *Leishmania major*. SL Colpitts and Phillip Scott (2010) *J Immunol.* 185(4):2416-23.



Vudathala D, Cummings M., Murphy L.: Analysis of multiple anticoagulant rodenticides in animal blood and liver using principles of QuEChERS method. *J. of Analytical Toxicology* 34 (5):273-279, 2010



Neely, R.J., Brose, M., Gray, C., McCorkell, K.A., Leibowitz, J.M., Ma, C. Rothstein, J.L. & May, M.J. (2010) The Ret/PTC-3 oncogene activates classical NF- κ B by stabilizing NIK. *Oncogene* (In Press). NIHMSID: 225887



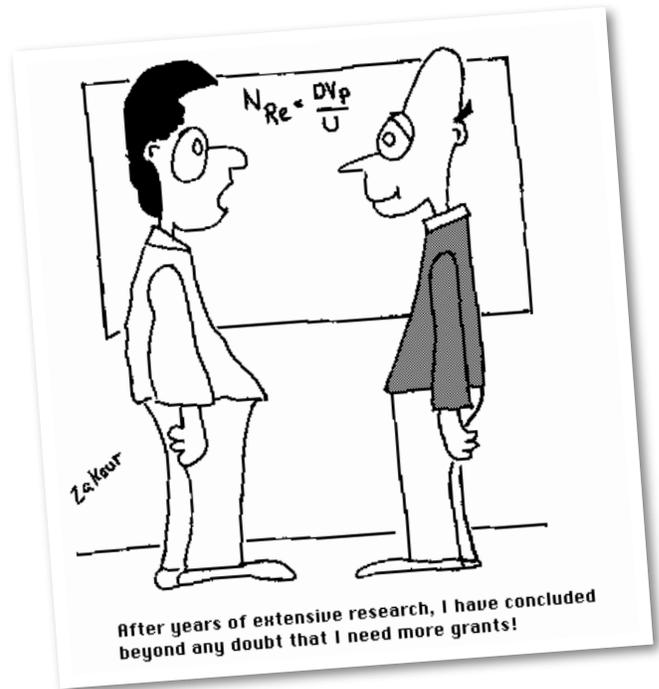
Zhang, F., S. Saha, S. Shabalina, and A. Kashina (2010) Differential Arginyl-ation of Actin Isoforms is Regulated by Coding-Sequence-Dependent Degradation. *Science* Sept 17; 329: 1534-1537 (also featured in Nature News)

[more publications on page 8](#)

SEARCHING FOR FUNDING OPPORTUNITIES

SPIN is part of the SPIN Plus system, a comprehensive service that provides Penn investigators with the most current information on available funding opportunities (both national and international including foundations) for projects. SPIN Plus is not just limited to scientific opportunities; there are listings for other disciplines including education and social work among others. Visit the website:

<http://project.pennera.upenn.edu/SPIN/> and logon with your Penn key and password. Look at the column on the left and select find funding. You may select either SPIN (funding opportunity database) or SMARTS (funding opportunity email alert service). Also, you may find help at the AAAS **GRANTSNET** site: <http://sciencecareers.sciencemag.org/funding> This site is helpful to early career faculty and postdoctoral fellows. And, to search foundations go to: <http://foundationcenter.org/pnd/rfp/>



PUBMED PLUS -- VETERINARY



The **Atwood Library** homepage now links to a new version of PubMed Plus -- **PubMed Plus -- Veterinary**. Besides the PubMed Plus links to Penn full-text articles, the Veterinary version includes optional limits to Systematic Reviews, Veterinary-related

articles, articles reviewed in the Faculty of 1000 and articles with Penn-identified first authors.

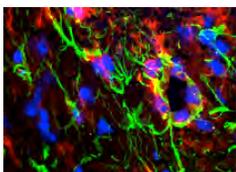
Articles reviewed in Faculty of 1000 -- Faculty of 1000 is a Penn-subscribed resource that systematically highlights and reviews the most interesting papers published in the biological sciences, based on the recommendations of over 2300 selected leading researchers. Next month's Library feature will be a synopsis of Penn Vet research that has been reviewed in Faculty of 1000. Links to the reviews are available in the Abstract view.

Veterinary-related articles
This is a quick limit to articles

matching PubMed's Veterinary Medicine/Animal Health complex search strategy, which includes related subject headings and veterinary journals as well as key title words to match articles in OldMEDLINE and unindexed new citations. The search strategy is detailed at http://www.nlm.nih.gov/services/veterinarymed_details.html.

Make your own filters

These are just a sampling of possible PubMed filters. If you would like your own customized version of PubMed, please contact Margaret Lindem, the Veterinary Librarian, at 215-898-8874. mlindem@pobox.upenn.edu.



WHAT IS A TRM? A *Tangible Research Material* is technology that may or may not be patentable. It is a unique research product such as biological (e.g. cell lines, micro-organisms) or chemical substances, electronic materials (e.g. chips or circuits), software or other tangible products of research. TRMs may be licensed to a commercial entity to generate income to support research activities or as net revenue income. CTT HAS A HELP DESK: CTTINFO@CTTUPENN.EDU

how we look at disease prevention in fish, and his breakthrough will have a profound impact on the future of the aquaculture industry. I am proud that the USDA supports such innovative research".

Significantly, Dr. Sunyer's findings show that fish IgT and human IgA systems appear to utilize similar mechanisms to maintain healthy intestines. This has recently been recognized by the National Institute of Health (NIH), as this year Dr. Sunyer has received a 5 year ROI award from the NIH to study further the role of IgT and IgT⁺ B cells in fish mucosal immunity.

Dr. Sunyer's office and laboratory are located in 413 Rosenthal Building.



Figure 2 Mucosal antibodies in fish

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- Boshra, H., Wang, T., Hove-Madsen, Hansen, J., Li, J., Matlapudi, A., Secombes, C., L. Tort, J. O. Sunyer. 2005. Characterization of a C₃a receptor in rainbow trout and *Xenopus*: the first identification of C₃a receptors in nonmammalian species. *Journal of Immunology*. 175:2427-37.
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- Jens J, Snella E, Kan SH, Ellinwood NM. Early versus late treatment of spinal cord compression with long-term intrathecal enzyme replacement therapy in canine mucopolysaccharidosis type I. *Mol Genet. Metab* Jul 22. 2010 Epub ahead of print, PMID: 20655780
- Kellett-Gregory LM, Mittleman Boller E, **Brown DC, Silverstein DC**. Ionized calcium concentrations in cats with septic peritonitis: 55 cases (1990-2008). *J Vet Emerg Crit Care*. 2010; 20(4):398-405.
- Quinn KP, Dong L, **Golder FJ**, Winkelstein BA. Neuronal hyperexcitability in the dorsal horn after painful facet joint injury. *Pain*. 2010. [Epub ahead of print] PubMed PMID: 20739124.
- Ziegler, S and **Artis, D.** (2010). Sensing the outside world: TSLP regulates innate and adaptive immune responses at barrier surfaces. *Nature Immunology* 11:289-93. [PMID:20300138]

More recent Penn Vet faculty publications.....

- Michelle Y. Powers, DVM, DACVS; Georga T. Karbe, Med vet; Thomas P. Gregor, BS; Pamela McKelvie, VMD; William T. N. Culp, VMD, DACVS; Hilary H. Fordyce, VMD; **Gail K. Smith**, VMD, PhD (2010) Evaluation of the relationship between Orthopedic Foundation for Animals' hip joint scores and PennHIP distraction index values in dogs. *JAVMA* 237 (5): 532-541.
- Kasinathan RS, Goronga T, Messerli SM, Webb TR, **Greenberg RM.** (2010) Modulation of a *Schistosoma mansoni* multidrug transporter by the antischistosomal drug praziquantel. *FASEB J.* (1):128-35.
- Dickson PI, Hanson S, McEntee MF, **Vite CH**, Vogler CA, Mlikotic A, Chen AH, Ponder KP, **Haskins ME**, Tippin BL, Le SQ, Passage MB, Guerra C, Dierenfeld A,

The Penn Vet Research Newsletter is distributed quarterly: suggestions, requests, comments, new publications and story ideas should be sent directly to:

resnews@vet.upenn.edu

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Associate Dean for Research

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