

AAVI Newsletter August 2007



President:	Joan Lunney,
President Elect:	Chris Davies
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Message from AAVI President, Joan Lunney

This has been a busy year for the American Association of Veterinary Immunologists (AAVI). Your board has been planning our society's future. Go to our updated AAVI website www.theaavi.org to identify opportunities. Thanks to Eileen Thacker and Chris Minion for the upkeep of our website.

Thanks to Will Goff and the members of our elected Nominations committee for soliciting such a strong selection of AAVI Nominees. As an AAVI member you should have already received an email notice of the 2007 AAVI ballot for new officers. See below for the ballot. Your votes are due to AAVI Secretary Treasurer, Eileen Thacker ethacker@iastate.edu by August 17th.

The major content of this Newsletter involves our awards. AAVI was awarded a \$10,000 grant from USDA CSREES for AAVI Junior Scientist Travel Stipends for the 8th International Veterinary Immunology Symposium (8IVIS) in Ouro Preto, Brazil. As a result our Student Awards Committee, chaired by Carol Chitko-McKown, requested applications. The abstracts from the 7 awardees are presented in this Newsletter. I thank Past President Lorraine Sordillo, and AAVI Secretary Treasurer, Eileen Thacker, for their efforts in writing our successful grant. [Special thanks to Eileen for assuring that AAVI could submit through the grants.gov website.] During the CRWAD meetings we will again have student competitions and the instructions and judging criteria are also presented in this Newsletter.

Past President Lorraine Sordillo worked with AAVI Vice-President Jim Harp and member Will Goff to update and revise our society's By-Laws. At our AAVI Business meeting we will vote on acceptance of these updated By-Laws. Please take

the time to read them at our website

www.theaavi.org.

Ron Schultz, and our 2007 Fund Raising Committee, and other AAVI members, have helped to attract solid company support for AAVI, renewing support for AAVI from animal health and vaccine companies. Their 2007 committee is expanding this effort and redefining our commercial supporters, by identifying new contacts among molecular biology and immune reagent suppliers. Please send them your suggestions for new companies or contacts.

The AAVI is dedicated to the development, promotion, and dissemination of knowledge in veterinary immunology. As such we work with several organizations to promote research and meetings about veterinary immunology. For our 2007 joint AAVI-ACVM Symposium at the CRWAD meetings this Dec. Jim Harp and Chris Chase have organized a very informative series of talks on "Nutritional Immunology". At the 2007 CRWAD meetings AAVI will recognize our 2007 Distinguished Veterinary Immunologist, John Butler (see article below). At the American Association of Immunologists (AAI) in May in Miami AAVI cosponsored with AAI's Veterinary Immunology Committee (VIC) a session on "Comparative Models of Disease." Plans are in discussion with Bill Golde, Chair of AAI VIC, for a 2008 session; President-Elect Chris Davies will represent AAVI for this symposium. Following the 2008 AAI meetings in San Diego, on April 9-11, 2008, Bill Golde has organized a "Comparative Models of Immune Response" meeting to be held at Lake Arrowhead Conference Center, operated by University of California, Lake Arrowhead, CA (see article below).

Please take the next step. I actively encourage you, our members, to identify an AAVI committee

on which to serve. Tell us what new initiatives we should be pursuing. Encourage fellow faculty members and young scientists and trainees to join AAVI. Share this Newsletter with your colleagues and administrators to keep them informed of progress in veterinary immunology. The list of committees and their chairs is at the end of this Newsletter and on the AAVI website www.theaavi.org.

I look forward to seeing many of you at the 8th International Veterinary Immunology Symposium in Brazil next week. Alternately I hope to see you all in Chicago for the CRWAD meetings Dec. 2-4, 2007, for our AAVI-ACVM symposium on Sunday and for our AAVI Business meeting at lunch on Monday Dec. 3.

I hope you each enjoy the remainder of your summer.

Message from AAVI Secretary Treasurer, Eileen Thacker

Greetings:

I want to remind you that dues for 2007 are past due for this year, but I am always willing to accept dues. Thank you for those who have paid already this year. We have had quite a few new members this year, which is great. I would encourage each of you to enlist new members in our organization to keep it strong and vital.

The webpage www.theaavi.org is up and running and we are trying to keep it up to date and current. Many of you have contacted me with new information and we are trying to get it added as soon as we can.

I will be sending out information about the upcoming business meeting and luncheon to be held Monday Dec. 3 during CRWAD. It will be 11:30-1:00 PM in the Illinois Room and followed by the presentation of the AAVI DVI award lecture at 1:30PM. I am looking at the menus provided and the cost for lunch is expected to be \$40-45.

Unfortunately, we don't have time to go out of the hotel and return in time for the DVI presentation, so we will continue at this point to have the business meeting and luncheon in the hotel. It is important that you let me know if you are coming. The hotel was very accommodating last year and provided additional tables, food, etc for people that showed up without a reservation. While I will be sending a

message out, let me know if you know at this time you will be attending the luncheon.

I look forward to seeing many of you in Chicago in December!!!

Thanks,
Eileen Thacker
AAVI Secretary-Treasurer
2118 Vet Med Bldg
Iowa State University
Ames, IA 50011
ethacker@iastate.edu

Dr. John E. Butler is the 2007 Distinguished Veterinary Immunologist

Submitted by Eileen Thacker, AAVI Secy/Treas.

The American Association of Veterinary Immunologists (AAVI) is pleased to announce that their 2007 Distinguished Veterinary Immunologist Awardee is Dr. John E. Butler, Professor in the Department of Microbiology at the University of Iowa. In fact, Dr. Butler has the distinct honor of being both the US AAVI awardee and the international DVI awardee this year!

The DVI Award is presented every year by the AAVI to an individual whose contribution to veterinary immunology is widely acknowledged as significant and important to the understanding of the immunology of domestic and/or wild animals.

Dr Butler has had a long and productive career. His primary research focus has been the comparative immunology and biology of mammalian antibodies, a field where he has made seminal contributions to our understanding of cattle and swine immunoglobulin gene structure and diversity. Dr Butler has been a consistent advocate for the importance of immunology in domestic animals, originating the Comparative Immunoglobulin Workshop (CIgW) and now maintaining the CIgW website, www.medicine.uiowa.edu/cigw/. He has served as a mentor for numerous veterinary immunologists internationally as well as graduate and undergraduate student researchers.

The AAVI DVI award will be presented to Dr Butler at the annual Conference of Research Workers in Animal Diseases (CRWAD) meeting to be held December 2-4, 2007 in Chicago, IL.

AAVI Constitution and By-Laws Update
*Submitted by Lorraine Sordillo, Chairperson,
Constitution/By-Laws Committee*

The Constitution of the AAVI should be updated annually according to the by-laws of our organization. The Constitution/By-Laws Committee should review the constitution each year and propose changes suggested by the Board or AAVI Membership as outlined in Article VII (Amendments). The Constitution/By-Laws Committee should consist of three members, but this standing committee has not been active for the last several years. As a consequence, the current version of the Constitution does not accurately reflect some of the necessary operational changes that have taken place over recent years. A committee of three was appointed by the President to review the Board minutes from 2002-2006 and make recommend changes to the Board. The Committee consisted of Lorraine Sordillo (chairperson), Jim Harp, and Will Goff. Major proposed modifications include the following:

1. modification of the President-Elect duties under Article III, Section 10
2. modification of the Vice-President duties under Article III, Section 11
3. deletion of the Outreach/Education Committee to be replaced by the Student Awards Committee
4. description of the composition and function of the Student Awards Committee
5. description of the composition of the Constitution/By-Laws Committee
6. inclusion of the Fund Raising Committee as a standing committee

Proposed changes to the Constitution were presented to the Board for consideration in July 2007 and the changes were deemed worthy by unanimous consent.

AAVI/ACVM Symposium 2007

Submitted by James A. Harp, AAVI Vice President

The 2007 AAVI-ACVM joint symposium will be held Sunday, December 2 from 1:30 PM to 5:00 PM at the Marriott hotel in Chicago Illinois just prior to the 2007 Conference of Research Workers in Animal Diseases (CRWAD) meeting. This year's topic is "Nutritional Immunology" and the program of speakers is listed below.

The symposium is open to all registrants at CRWAD; you do NOT have to be a member of AAVI or ACVM to attend. Information on registration for the CRWAD meeting can be found at: www.cvmb.colostate.edu/microbiology/crwad/index.htm

Please plan to arrive early for the CRWAD meeting so you can attend the AAVI-ACVM symposium!

AAVI/ACVM Symposium 2007
Nutritional Immunology

Sunday December 2, 2007, 1:30-5:00 PM
Chicago, Illinois

Co-chairs:

James A. Harp, USDA/ARS, Ames, IA
*Christopher C. L. Chase, South Dakota State
University*

- 1:30PM Joseph Urban, USDA/ARS, Beltsville
Probing the role of micronutrients in immunity to parasitic infection in mouse and swine disease models
- 2:15PM Simin Meydani, Tufts University
Nutrition, immune response & infectious diseases in the aged
- 3:00PM Break
- 3:30PM Kirk Klasing, University of California, Davis
General mechanisms by which nutrition impacts immunity and resistance to infectious diseases
- 4:15PM Margherita Cantorna, Penn State University
Vitamin D, Immunoregulation and the risk of autoimmunity
- 5:00PM Adjourn

AAVI Elections 2007

Submitted by Will Goff, Chair, AAVI Nominating Committee

As AAVI members, you have the right and responsibility to determine who will lead the association in the near future. You should have received a ballot with instructions for returning it electronically to the Secretary/Treasurer. If you have not already done so, please make sure to do so before August 17th.

If, for some reason, you are an active member and are reading this newsletter and have not received a ballot, it appears here. Please copy and paste it as an attachment to a message to the AAVI Secretary/Treasurer, Eileen Thacker, ethacker@iastate.edu

Thanks to our elected nominating committee [Chair Will Goff, Mark Estes, Jim Roth, Phil Elzer, Pat Shewen and Chris Chase] for recruiting this excellent panel of nominees. Thanks to all members who agreed to be considered for service to the association.

AAVI Election Ballot 2007

Vice President: (vote for one)

Doug Bannerman - USDA-ARS, Beltsville
Hyun Lillehoj - USDA-ARS, Beltsville

Secretary/Treasurer: (vote for one)

Gina Pighetti - University of Tennessee
Carol Chitko-McKown - USDA-ARS, Clay Center

Board Member: (vote for one)

Stephen Kania - University of Tennessee
Krishna Murthy - Southwest Foundation for Biomedical Research, San Antonio
Shayan Sharif- University of Guelph

Nominating Committee: (vote for two)

Patricia Allen - USDA-ARS, Beltsville
David Hurley - University of Georgia
Antonio Garmendia - University of Connecticut
Isis Mullarky - Virginia Tech University
Randy Sacco - USDA-ARS, Ames

Comparative Models of Immune Response

April 9-11, 2008

Lake Arrowhead Conference Center, operated by University of California, Lake Arrowhead, CA
Contributed by Bill Golde, USDA ARS

The annual meeting of the American Association of Immunologists (AAI) in 2008 is in conjunction with Experimental Biology in San Diego from April 5-9. With the sponsorship of Western University for Health Sciences, School of Veterinary Medicine and the USDA Office of Technology Transfer, we have organized a Comparative Models of Immune Response (CMIR) meeting. This meeting is designed to bring together investigators studying human immune responses, rodent model systems and immune responses in livestock species to develop new ideas and approaches through the understanding of comparative immunology. The CMIR will be held April 9-11, 2008, the two days immediately following the AAI meeting. The heightened awareness of livestock immunology in the human biologics field will not only advance human vaccine and biologics development, but by broadening the interest and funding of research in large animal and avian species, animal health will benefit greatly. The meeting is at a remote, all inclusive venue that can accommodate up to 200 participants. Registration is \$375 and includes the meeting, room for 2 nights and all meals. More details can be obtained at the web site, www.westernu.edu/cmire. All sessions will have invited speakers and speakers chosen from the abstracts. Posters will be up for viewing the entire meeting.

Scientific Program

Wed. April 9 Plenary Session I. Innate Responses and Immune Evasion
Keynote address

Thurs. April 10 Plenary Session II. Biodefense: Immune Responses to High Impact Pathogens

Plenary Session III. Comparative Immunogenetics of Immune Responses

Plenary Session IV. Comparative Models of Allergy, Nutrition, and Neoplasia

Social Hour and Posters

Friday, April 11 Plenary Session V. Platforms for Vaccine Development

Confirmed Speakers:

Chuck Czuprynski, U. Wisconsin; Tom Phillips, Western U.; David Woodland, Trudeau Inst.; John Butler, U. Iowa; Wendy Brown, Washington State U.; Bill Golde, USDA Mark Estes, UTMB Galveston; Lynette Corbeil, UCSD

**AAVI Graduate Student Competition at
CRWAD 2007**

*Submitted by Carol Chitko-McKown, AAVI Awards
Committee Chairperson*

Once again, the American Association of Veterinary Immunologists (AAVI) will be sponsoring a competition for the best oral and poster presentations by graduate students at the annual CRWAD meeting, December 2-4, 2007 in Chicago.

Awards and Submission Requirements: The first and second place presenters in both categories will each receive a cash award. Membership in the AAVI by the student and/or mentor is not required, but is requested. ***This year we are requesting that when you submit your abstract noting that you will be entering the AAVI competition, that you also request inclusion in the immunology section of the meeting.*** Although in the past presentations have been given in other sections, it makes it difficult on the judging team to bolt between different sections during the meeting – we’d really appreciate your help in this matter!

Judging Criteria: The forms that will be used to judge both the oral and the poster presentations are shown on the next page. When preparing your presentation, please refer to them to insure that all important information is included in your presentation.

Judges Needed: We will also need individuals (in permanent career positions) to help judge the competition; six if at all possible. If you would be willing to serve your association in this capacity, please email me at carol.chitkomckown@ars.usda.gov and indicate if you have a preference for judging poster or oral presentations.

We look forward to learning about the exciting research being performed by our up-and-coming young scientists! See you in Chicago!

**AAVI Student Oral Presentation
Judging Criteria – 2007**

<p>Impact of research - 10 points The student clearly presented the importance of research problem and/or disease conditions being addressed.</p>	
<p>Abstract - 25 points ∞ The abstract was well written. ∞ The abstract clearly reflected the research presented. ∞ The abstract clearly defined conclusion.</p>	
<p>Actual data - 40 points ∞ The experimental design, procedures and methods were clearly stated. ∞ The quality and quantity of the experiments was appropriate. ∞ Appropriate controls and statistical methods were utilized. ∞ Conclusions were reflective of the data presented.</p>	
<p>Presentation – 25 points ∞ The presentation was well organized and clearly delivered. ○ There was adequate/ordered introduction, results and conclusions. ○ All the figures and tables were clearly explained. ∞ The slide presentation was of high quality ○ Figures and tables easy for the audience to interpret. ○ Slides were easily read at the back of the large presentation rooms. ∞ The amount of information presented was appropriate ○ Too few or too many slides? ○ Too much text? ∞ Student used good public speaking technique. ∞ Presentation was delivered in the allocated time. ○ Generally 10 – 12 minutes. ○ There was adequate time for questions. ∞ Responses to questions demonstrated the student’s ability to defend the work.</p>	
<p>TOTAL</p>	

**AAVI Student Poster Presentation
Judging Criteria – 2007**

<p>Impact of research - 10 points</p> <ul style="list-style-type: none"> ∞ The student clearly presented the importance of research problem and/or disease conditions being addressed 	
<p>Abstract - 25 points</p> <ul style="list-style-type: none"> ∞ The abstract was well written ∞ The abstract clearly reflected the research presented ∞ The abstract clearly defined conclusion 	
<p>Actual data - 40 points</p> <ul style="list-style-type: none"> ∞ The experimental design, procedures and methods were clearly stated. ∞ The quality and quantity of the experiments was appropriate ∞ Appropriate controls and statistical methods were utilized. ∞ Conclusions were reflective of the data presented 	
<p>Presentation – 25 points</p> <ul style="list-style-type: none"> ∞ The presentation was well organized. <ul style="list-style-type: none"> ○ There was adequate/ordered introduction, results and conclusion that the reader could understand the poster in the absence of the presenter ○ If asked, the student made a short presentation utilizing the poster. ∞ The poster presentation was of high quality. <ul style="list-style-type: none"> ○ The reader in the absence of the student could interpret figures and tables. ○ The reader from 3 feet away easily read poster. ○ Appealing presentation. ∞ The amount of information presented was appropriate. <ul style="list-style-type: none"> ○ Too few or too many panels? ○ Too much text? ∞ Responses to questions demonstrated the student’s ability to defend the work 	
TOTAL	

**AAVI Junior Scientist Travel Stipends
8th International Veterinary Immunology Symposium
in Ouro Preto, Brazil**

Submitted by Carol Chitko-McKown, AAVI Awards Committee Chairperson

The American Association of Veterinary Immunologists (AAVI) awarded seven, \$1,425 travel awards to defray the costs of attending the 8th International Veterinary Immunology Symposium (8IVIS) in Ouro Preto, Brazil, August 16 – 19, 2007. These awards were funded by the United States Department of Agriculture, Cooperative State Research, Education and Extension Service.

Four postdoctoral fellows, nine Ph.D. students, and one undergraduate student submitted copies of their 8IVIS abstracts along with a letter of support from their advisor stating that the applicants will be involved in the study of veterinary immunology at the time of the meeting. The applications were submitted from 11 colleges from four different countries. Either the applicant or the mentor was required to be an active member of AAVI. A panel of judges selected the winners based upon the quality and originality of the abstracts.

The winners of the 2007 Junior Scientist Travel Awards are Dr. Rosane Oliveira, a postdoctoral fellow with Dr. Harris Lewin at the University of Illinois, Dr. Reginaldo Bastos, a postdoctoral fellow with Dr. Will Goff at Washington State University/USDA-ARS, Dr. Sandra Sommer a postdoctoral fellow with Dr. Paul Coussens at Michigan State University, Ms. Robin Cissell, a Ph.D. student with Dr. Steve Kania at the University of Tennessee, Sudarvili Shanthalingam a Ph.D. student with Dr. S. Srikumaran at Washington State University, Mr. Kuldeep Chattha a Ph.D. student with Dr. Patricia Shewen at the University of Guelph, and Ms. Kathryn MacKinnon a Ph.D. student with Dr. David Notter at Virginia Tech.

As an indication of the high quality research being performed by this generation of young veterinary immunologists, at least one of the abstracts has been selected to be given as an oral presentation. The winning abstracts are included below. Congratulations and best wishes to all!!!

KNOCK-DOWN OF BOVINE LEUKEMIA VIRUS TAX BY RNAi AND ITS EFFECTS ON HOST GENE EXPRESSION

Rosane Oliveira¹, Allison Sommers¹, Robin E. Everts¹, Harris A. Lewin^{1,2}

¹Department of Animal Sciences and ²Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Bovine Leukemia Virus (BLV) tax protein is a transcription transactivator of host cell genes that modulates cell growth and proliferation. By interfering with the transcription of host cell genes, tax expression is believed to be an essential first step in the dysregulation of homeostasis leading to the transformation of B-lymphocytes. To better understand the role of BLV tax in driving lymphoproliferation and cell transformation in the host, BLV tax gene expression in a BLV-infected bovine lymphoblastoid cell line (BL3*) was knocked down by RNA interference (RNAi). Gene expression profiling was then performed using a 13,257-element cattle oligonucleotide microarray. A tax-specific small-interfering RNA (siRNA) and control scrambled siRNA were used for transfections. Transfection efficiency was analyzed by flow cytometry of cells transfected with a GFP-construct, and tax mRNA levels were assayed by quantitative PCR. Six biological replicates of the knock-down were performed with both BL3* and BL3^o (uninfected parental cell line). Each treatment comprised 5 or 6 technical replicates to provide enough RNA for the microarrays (total of 132 transfections). The average transfection and knockdown efficiencies were 92.9% and 72.25%, respectively. RNA isolated from each treatment was pooled, and four independent transcriptome comparisons were carried out using microarray analysis (total of 46 slides). The different comparisons allowed the identification of genes specifically knocked down by tax-siRNA, as well as cell line-specific and off-target effects. In BL3* cells treated with tax-siRNA, 186 genes were found to be differentially expressed after removal of off-target effects (*t*-test, false discovery rate, *P*-value<0.2). The genes affected by tax knock-down were mined for affected pathways and functions using Ingenuity Pathway Analysis. Among the canonical pathways significantly affected by the BLV tax knockdown were ERK/MAPK signaling, oxidative phosphorylation and glutathione metabolism. Analysis of the distribution of genes according to Gene Ontology processes revealed that critical pathways in cell growth (e.g. cell signaling, cell cycle and cell death/apoptosis) are affected directly or indirectly by BLV tax and/or other virally-encoded genes.

On the basis of these results, a model for BLV-induced lymphoproliferation and transformation is proposed. We postulate that persistent lymphocytosis in BLV-infected cattle is caused by tax-induced dysregulation of a self-renewing population of pre-B lymphocytes.

Transformation leading to lymphosarcoma is a rare event that is caused by secondary mutations in the infected B cell precursor. Our results demonstrate the power of RNAi coupled with microarray analysis for dissecting the genetic and cellular processes leading to cell transformation by retroviruses.

INTERACTION OF NATURAL KILLER CELLS, MONOCYTES AND DENDRITIC CELL POPULATIONS IN CATTLE

Reginaldo G. Bastos^{1,2}, Carl Johnson¹, Wendy C. Brown², Will L. Goff¹

¹ Animal Disease Research Unit, USDA-ARS, Washington State University, Pullman, Washington, USA.

² Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington, USA.

Interactions between innate immune cell populations, such as natural killer (NK) cells with monocytes/macrophages and dendritic cells (DC) have been of recent interest. We are investigating interactions of these cells from cattle and our hypothesis is that microbial-exposed DC or monocyte interaction with NK cells result in the production of IFN- γ thus contributing to the innate response to initial disease. A NKp46⁺ population was obtained by incubating total spleen or peripheral blood mononuclear cells with recombinant human IL-15 for 2 weeks followed by depletion of B-lymphocytes, T-lymphocytes, $\alpha\alpha$ -T-lymphocytes and monocytes. The NKp46⁺ population was identified as CD3⁻, TcR1⁻ CD2^{+/-} and CD8⁺ and was able to produce IFN- γ in response to exogenous IL-12/IL-18. CD13⁺ DC and CD172a⁺ monocytes were obtained by positive selection from total spleen or peripheral blood mononuclear cells for determining the role of soluble factors and cell-to-cell contact in the interaction with NK cells. CD13⁺ DC or CD172a⁺ monocytes were incubated for 24 hr with heat-killed *Mycobacterium bovis* BCG (HK-BCG) either in the presence or absence of exogenous IL-18 or recombinant bovine CD40L. NKp46⁺ cells were then added and co-cultured for 24 hr in trans-well and cell-to-cell formats. The supernatants were then checked by ELISA for the presence of IFN- γ . CD172a⁺ cells exposed to HK-BCG- induced IFN- γ production from

NKp46⁺ cells. The interaction was CD40L independent but this T-cell signal significantly enhanced the interaction ($P < 0.05$). Moreover, the production of IFN- γ was completely suppressed by the presence of the p38 MAPK inhibitor SB203580, demonstrating the involvement of innate NF- κ B pathway in the interaction. In contrast, BCG-exposed CD13⁺ DC obtained from the spleen failed to induce IFN- γ production from NKp46⁺ cells regardless of cell-to-cell contact or addition of CD40L or in the presence of IL-18. We and others have recently demonstrated that CD13⁺ DC represent an immature phenotype. Therefore, we incubated CD13⁺ DC for 72 hours in the presence of IL-4, GM-CSF and Flt3 ligand prior to interaction with BCG and NK cells. Despite maturation, splenic DC maintained unique characteristics compared to monocytes. In conclusion, we provide evidence of interaction of innate immune cell populations that may have importance early during an infection and in driving the acquired immune response in cattle.

MYCOBACTERIUM PARATUBERCULOSIS SUPPRESSES CD40 SIGNALING INDUCED IL-12P40 AND iNOS GENE EXPRESSION IN BOVINE MONOCYTE-DERIVED MACROPHAGES.

Sandra Sommer, Charles B. Pudrith, Chris Colvin and Paul M. Coussens.

Dept. of Animal Science, Molecular Pathogenesis, Michigan State University, East Lansing, MI, USA
Mycobacterium avium spp. *paratuberculosis* (MAP), the causative agent of Johne's disease, is a facultative intracellular pathogen, residing in subepithelial macrophages. Clearance of MAP critically depends upon an appropriate pro-inflammatory and cytotoxic Th-1 immune response leading to activation and/or lysis of persistently infected macrophages to promote bacterial killing. Work in vivo has shown, that the appropriate Th-1 immune response occurring early in MAP infection is lost, followed by an ineffective, antibody-mediated Th-2 response. Our overall hypothesis is that once MAP persists within naïve macrophages, it reduces the ability of infected macrophages to react to normal T cell signaling, failing to be activated and destroy MAP, and failing to properly signal T cells to respond. To test this hypothesis, we investigated the effect of MAP infection on CD40 signaling, a main pathway used by T cells to activate macrophages. Our recent studies demonstrate that a short-lived response of bovine monocyte derived macrophages (MDM) to MAP infection *in vitro* is apparently followed by a block in the ability of infected

cells to respond normally to subsequent external activation. We have demonstrated by using Q-RT-PCR that normal MDM respond to CD40 ligand (CD40L) stimulation by up-regulation of immune response genes, including those encoding IL-6, TNF α , iNOS, and IL-12p40. Consistent with these results, western blot analyses indicated that CD40L stimulation causes a rapid, but short-lived activation of JNK, ERK1/2 and p38 MAPK. Studies with specific inhibitors revealed that the CD40L-mediated increase in IL-6 and IL-8 gene expression is dependent upon activation of ERK1/2 and JNK, while increases in IL-12p40 and iNOS gene expression are dependent upon activation of p38. Once infected with MAP, however, MDM cells fail to up-regulate the expression of iNOS and IL-12p40 encoding genes in response to CD40L, whereas the expression of the other tested genes, such as IL-8 and TNF α is not repressed. Using flow cytometric analysis we determined, that failure of infected macrophages to respond to CD40L was not due to down-regulation of CD40 on the cell surface of MAP-infected MDM. Western blot analysis also revealed that interference with CD40L-mediated increases in gene expression does not appear to be due to prevention of p38, ERK1/2, or JNK activation, suggesting the block is downstream of these kinases. Continuing studies are underway to uncover the mechanism responsible for MAP interference with CD40 signaling in infected macrophages.

DETECTION OF ANTIBODIES TO OVINE HERPESVIRUS-2 INTERLEUKIN-10 HOMOLOGUE IN SHEEP-ASSOCIATED MALIGNANT CATARRHAL FEVER

Cissell, Robin L¹, Shahira Abdel Wahab², Robert L. Donnell³, and Stephen A. Kania²

¹Comparative and Experimental Medicine Program, ² Department of Comparative Medicine, and ³Department of Pathobiology, University of Tennessee Veterinary Teaching Hospital, Knoxville, TN, USA.

Interleukin-10 (IL-10) interferes with monocyte and macrophage activation of Th1 helper lymphocyte production of nitric oxide and synthesis of various inflammatory mediators. An IL-10 homologue (vIL-10) is produced by several herpes viruses and is hypothesized to help the virus down regulate, and thus evade, host immune responses. The gammaherpes rhadinovirus *Ovine herpesvirus-2* (OvHV-2) encodes an IL-10 like molecule highly homologous to mammalian IL-10. This virus causes sheep-associated malignant catarrhal fever (MCF), the most common form of MCF in the United

States. MCF is a lymphoproliferative and inflammatory syndrome which has delayed clinical presentation hypothesized to be influenced by the production of vIL-10. For this study, the gene encoding vIL-10, Ov2.5 ORF, was amplified by PCR, cloned, sequenced, and a predicted 30 amino acid segment from the amino terminus synthesized. This synthetic peptide was used to develop a novel direct enzyme-linked immunosorbant assay (ELISA) to detect isotype-specific antibodies to OvHV-2 vIL-10. Work to date indicates that lambs do not have detectable levels of maternally derived antibody to vIL-10 during the first few weeks of age. Ewes, which are refractory to clinical infection, generally have high levels of antibody to vIL-10. A weak correlation exists between the vIL-10 ELISA and a commercially available competitive-inhibition ELISA (CI-ELISA). We believe the vIL-10 ELISA will refine the ability to identify ruminants exposed to sheep-associated MCF, provide an important tool for determining the role of vIL-10 in disease pathogenesis, and may contribute toward the development of a new vaccine strategy for the control of malignant catarrhal fever.

TRANFECTION OF PORCINE SIALOADHESIN INTO A MURINE MACROPHAGE CELL-LINE RENDERS IT PERMISSIVE FOR PRRSV REPLICATION

Sudarvili Shanthalingam¹, Weiguo Liu¹, Kevin Snekvik¹, Asit Pattnaik², Fernando A. Osorio² and Subramaniam Srikurmaran¹

¹*Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA and*

²*Department of Veterinary and Biomedical Sciences, University of Nebraska, Lincoln, NE USA.*

Sialoadhesin is a macrophage-specific adhesion molecule. It is the prototypic member of the sialic acid binding immunoglobulin-like lectin (siglec) family, and hence referred to as siglec-1, and also as CD169. It binds sialylated ligands on other hematopoietic cells, predominantly neutrophils, but also monocytes, natural killer cells, B-cells and cytotoxic T lymphocytes. It is a non-phagocytic receptor, but may aid in phagocytosis. Other functions of sialoadhesin are not clear. Earlier studies have identified sialoadhesin as the cell-surface protein on porcine alveolar macrophages (PAMs) that mediates internalization of porcine reproductive and respiratory syndrome virus (PRRSV). In these studies, transfection of porcine sialoadhesin into a porcine kidney cell-line (PK-15) rendered it susceptible to PRRSV infection. However, the internalized virus did not replicate. We hypothesized that replication of PRRSV requires

porcine sialoadhesin as well as additional macrophage-specific factors. The objective of this study was to clone the cDNA encoding porcine sialoadhesin, transfect into a murine macrophage cell-line, and determine the permissiveness of the transfectant cell-line for PRRSV replication.

Total RNA from PAMs was extracted and cDNA was made by RT-PCR. The PCR-amplified cDNA for sialoadhesin was cloned into a mammalian expression vector and sequenced. Four independent clones were sequenced. Comparison of our sequence data (Genbank accession no. DO176853) with that published previously (Genbank accession no. NM_214346) revealed 15 amino acid difference, which likely represents polymorphism. The cDNA for sialoadhesin was transfected into a mouse macrophage cell-line. The transfectants were labeled with a monoclonal antibody to sialoadhesin, and subjected to fluorescence-activated cell sorting. We obtained 51 single cell clones which expressed porcine sialoadhesin on the cell surface. Of these, 17 clones continued to express porcine sialoadhesin on their surface, to varying degrees. Three of these clones stably expressing sialoadhesin were found to be susceptible to PRRSV infection. The clone AA9 was tested further to determine its ability to support PRRSV replication. AA9 cells supported the replication of PRRSV. The titer of PRRSV obtainable in AA9 cells is between 2×10^5 and 2×10^6 TCID₅₀/ml. This cell-line should be valuable for large scale propagation of PRRSV for vaccine production.

VARIATION IN EXPRESSION OF FC GAMMA RECEPTOR IIB AND CD21 ON BOVINE LYMPHOCYTES WITH AGE

Kuldeep Chattha, M.A. Firth, D.C. Hodgins, Patricia Shewen

University of Guelph, Dept. of Vet. Pathobiology, Guelph, Ontario, Canada

It is difficult to induce active immune responses in neonates, partly due to the limited functional ability of neonate's immune system and partly due to inhibition mediated by maternal antibodies. Fc gamma receptor II B (CD32) is a major receptor responsible for suppression of antibody responses, through interaction with immune complexes of maternal antibodies (primarily IgG) and antigens, by virtue of its intracellular immunoreceptor tyrosine-based inhibitory motif (ITIM). CD21 (CR2), a receptor for complement component C3d, is expressed by B lymphocytes and binding results in lowering of the threshold for B cell activation. Thus it plays an important role in enhancing antibody responses to complement-

associated antigens. CD21 (activating component), B cell receptor (membrane IgM) and CD32 (inhibitory component) form an intricate triad for determining the threshold of activation for B cells. Because cellular distribution of CD21 and CD32 of cattle has not been well documented, this study aimed to determine the variation in expression of CD21 and CD32 on bovine lymphocytes with age, particularly emphasizing differences between neonates and adults.

Blood was collected from 20 healthy Holstein calves, 1 to 90 days of age, and 8 healthy adults. The percentage, absolute number and mean fluorescence intensity of CD21 and CD32 positive cells was determined using fluorochrome labeled monoclonal antibodies and flow cytometry. The percentage and absolute number of CD21 and CD32 positive lymphocytes increased with age from birth until about 60 days of age, with CD21 showing a greater percentage increase compared to CD32. The proportion of CD32 positive lymphocytes expressing CD21 also increased with age. In both calves and adults, the number of cells expressing CD32 was greater than those expressing CD21. Mean fluorescence intensity for CD32 was significantly higher for adults ($P < 0.01$), indicating a greater number of CD32 receptors per lymphocyte compared to neonates. RT-PCR using RNA extracted from PBMCs of neonatal calves suggested production of a soluble version of Fc gamma RIIB (sCD32), lacking transmembrane region, which might contribute to lower membrane CD32 expression in neonates.

Lower expression of CD21 in neonates combined with reduced levels of C3d in serum may be a limiting factor for activation of neonatal B cells. An age related increase in the percentage and absolute number of CD21 and CD32 positive lymphocytes, along with reduction in the level of maternal antibodies might help explain the ability of older calves to produce effective antibody responses compared to neonates.

EFFECTS OF *HAEMONCHUS CONTORTUS* ON THE HUMORAL AND CELLULAR IMMUNE RESPONSE OF PARASITE RESISTANT HAIR SHEEP

Kathryn M MacKinnon, Isis K. Mullarky and David R. Notter, Virginia Tech, Blacksburg Virginia, USA

Among sheep producers, the parasitic nematode *Haemonchus contortus* is a major animal health concern. Caribbean hair sheep are known to be more resistant to this abomasal parasite than conventional wool sheep. Our objective was to determine differences in gene expression associated with parasite resistance between

resistant hair and susceptible wool lambs. To address this objective, 12 hair and 12 wool lambs were each infected with 10,000 *H. contortus* larvae and 14 animals of each breed were left as uninfected controls. Fecal egg counts (FEC) were measured at day 0, 16, 21 and 27 in all animals to assess worm burden. Susceptible wool lambs had higher FEC compared to resistant hair lambs throughout the study. Total RNA was extracted from abomasum and abomasal lymph node tissues. After reverse transcription, gene expression of Th1 and Th2 cytokines, cytokine receptors and antibodies were evaluated by real-time RT-PCR. Gene expression patterns were relatively consistent between abomasum and lymph node tissues, indicating potential coordination of local and humoral immune responses during infection. Compared to uninfected control animals, infected sheep had decreased expression of IFN- γ in lymph node tissue and increased expression of IL-5, IL-13 and IgE in both tissues. However, even though Th2-type mechanisms seemed to be in place by this time, there was no difference in expression level of the cross-regulatory cytokine IL-4. At 3 days post-infection, resistant hair sheep had greater ($P < 0.01$) expression of IgE in lymph node tissue when compared to wool lambs. By 27 days post-infection, resistant hair lambs had lower ($P < 0.10$) expression of IFN- γ , and higher ($P < 0.10$) expression of IgE and Th2 cytokine IL-13 in both tissues as compared to susceptible wool lambs. The mean level of expression of IL-4 receptor α did not differ between breeds, tissues and days, but individual expression levels of this receptor were highly correlated (-0.98) with FEC at 27 days post-infection. These results suggest that gastrointestinal nematode infection in resistant as compared to susceptible sheep elicits a modified Th2-type immune response, characterized by decreased IFN- γ , steady IL-4 expression and increased IL-13 and IgE. Differential regulation of Th2 cytokines between breeds may be partially responsible for differences in parasite resistance.

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Paul Coussens, Michigan State University coussens@msu.edu

Cynthia Baldwin, University of Massachusetts cbaldwin@vasci.umass.edu