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New publications in the [PARATUBERCULOSIS database](#) (1475-1483)

1475 Periasamy, S., Tripathi, B.N., Singh, N.

Mechanisms of Mycobacterium avium subsp paratuberculosis induced apoptosis and necrosis in bovine macrophages

Veterinary Microbiology, (2013) 165, 392-401

The interaction between Mycobacterium avium subsp. paratuberculosis (Map) and macrophages is a complex process to maximize the chances of their respective survival. Previous studies have shown that Map induces cell death in macrophages, but the mechanism is not known. In the present study, we investigated the mechanism by which Map induces cell death in bovine macrophages using the fluorescent and electron microscopic techniques. The macrophages infected with an equal number of Map (i.e., multiplicity of infection, MOI = 1) showed no changes of cell death, but those macrophages infected at MOI = 10 showed the morphological changes consistent with apoptosis. Strikingly, the macrophages infected by Map at MOI = 50 showed the changes of apoptosis and necrosis. The Map-induced apoptosis was a caspase-dependent mechanism at MOI = 10 while it was caspase- and nitric oxide-independent at MOI = 50. The results of the present study suggest that the mitochondrial damage following Map infection initiates the cell death processes in macrophages. (c) 2013 Elsevier B.V. All rights reserved.

1476 Dhand, N.K., Johnson, W.O., Eppleston, J., Whittington, R.J., Windsor, P.A.

Comparison of pre- and post-vaccination ovine Johne's disease prevalence using a Bayesian approach

Preventive Veterinary Medicine, (2013) 111, 81-91

This study was conducted to evaluate the effectiveness of Gudair (TM) vaccine in decreasing the prevalence of shedding of Mycobacterium avium subsp. paratuberculosis (MAP) in flocks of varying initial prevalence. Thirty-seven self-replacing Merino flocks from New South Wales and Victoria (Australia) that had been vaccinating lambs with Gudair (TM) for at least five years were enrolled in the study. These flocks had been tested prior to or at commencement of vaccination using pooled faecal culture, agar gel immunodiffusion or both tests. These pre-vaccination test results were used to estimate pre-vaccination prevalence. Post-vaccination prevalence was estimated from culture of usually 7 pools of 50 sheep collected from the enrolled flocks in 2008-2009, approximately five or more years after commencement of vaccination. A Bayesian model was developed to estimate and compare the pre- and post-vaccination prevalences for the enrolled flocks. Apparent pre- and post-vaccination prevalences for flocks were modelled as functions of the true pre- and post-vaccination prevalences, respectively, and the sensitivities and specificities of the respective diagnostic tests. Logit-normal models were specified on pre- and post-vaccination true prevalences and were then used to make inferences about the median and 90th percentile of the prevalence distributions and their differences. Priors were mostly specified based on published literature or analysis of abattoir surveillance data for this population of flocks. The analysis found a significant decline in ovine Johne's disease prevalence from a pre-vaccination median prevalence of 2.72% [95% probability interval (PI): 1.40; 6.86%] to a post-vaccination median prevalence of 0.72% (0.39; 1.27%). However 30 of the 37 flocks still contained sheep that were shedding MAP in their faeces. The results suggest that vaccination with Gudair (TM) is usually effective in reducing the prevalence of faecal shedding but the response to vaccination is variable among flocks. The Bayesian approach reported here could be implemented in similar situations to compare prevalences where information from multiple diagnostic tests with varied sensitivities and specificities is available. (c) 2013 Elsevier B.V. All rights reserved.



- 1477 Pithua, P., Aly, S.S., Haines, D.M., Champagne, J.D., Middleton, J.R., Pooch, S.E.
Efficacy of feeding a lacteal-derived colostrum replacer or pooled maternal colostrum with a low IgG concentration for prevention of failure of passive transfer in dairy calves
Javma-Journal of the American Veterinary Medical Association, (2013) 243, 277-282
Objective-To compare the efficacy of a lacteal-derived colostrum replacer (LDCR) for the prevention of failure of passive transfer of immunity (FPT) in calves with that of pooled maternal colostrum (MC). Design-Randomized field trial. Animals-568 heifer calves from 1 California dairy. Procedures-Calves were randomly allocated to 1 of 2 treatment groups and fed 2 doses (200 g of IgG) of an LDCR or 3:8 L of pooled MC. From each calf, blood samples were collected before and approximately 24 hours after treatment. Serum IgG and total protein (TP) concentrations were quantified with standard methods, and the apparent efficiency of IgG absorption was calculated. Results-At 24 hours after treatment, mean serum TP and IgG concentrations were significantly lower for calves fed pooled MC (TP, 4.77 g/dL; IgG, 750 g/L), compared with those for calves fed the LDCR (TP, 5.50 g/dL; IgG, 15.15 g/L). Calves fed the LDCR were 95% less likely to develop FPT (OR, 0.05; 95% confidence interval, 0.03 to 0.08) than were calves fed pooled MC. However, the mean IgG concentration in the pooled MC fed during the study (21.1 g/L) was substantially lower than that (64.3 g/L) determined for representative samples of pooled MC from other southwestern US dairies during a national survey. Conclusions and Clinical Relevance-Results indicated that, on this particular dairy, calves fed an LDCR were at less risk of developing FPT than were calves fed pooled MC. The LDCR evaluated was a viable alternative for the prevention of FPT in calves.
- 1478 Forde, T., De Buck, J., Elkin, B., Kutz, S., van der Meer, F., Orsel, K.
Contrasting Results of Culture-Dependent and Molecular Analyses of Mycobacterium avium subsp paratuberculosis from Wood Bison
Applied and Environmental Microbiology, (2013) 79, 4448-4454
Reduced to near extinction in the late 1800s, a number of wood bison populations (*Bison bison athabasca*) have been re-established through reintroduction initiatives. Although an invaluable tool for conservation, translocation of animals can spread infectious agents to new areas or expose animals to pathogens in their new environment. *Mycobacterium avium* subsp. paratuberculosis, a bacterium that causes chronic enteritis in ruminants, is among the pathogens of potential concern for wood bison management and conservation. In order to inform translocation decisions, our objectives were to determine the *M. avium* subsp. paratuberculosis infection status of wood bison herds in Canada and to culture and genetically characterize the infective strain(s). We tested fecal samples from bison ($n = 267$) in nine herds using direct PCR for three *M. avium* subsp. paratuberculosis-specific genetic targets with different copy numbers within the *M. avium* subsp. paratuberculosis genome. Restriction enzyme analysis (REA) and sequencing of IS1311 were performed on seven samples from five different herds. We also evaluated a panel of different culture conditions for their ability to support *M. avium* subsp. paratuberculosis growth from feces and tissues of direct-PCR-positive animals. Eighty-one fecal samples (30%) tested positive using direct IS900 PCR, with positive samples from all nine herds; of these, 75% and 21% were also positive using ISMAP02 and F57, respectively. None of the culture conditions supported the growth of *M. avium* subsp. paratuberculosis from PCR-positive samples. IS1311 REA and sequencing indicate that at least two different *M. avium* subsp. paratuberculosis strain types exist in Canadian wood bison. The presence of different *M. avium* subsp. paratuberculosis strains among wood bison herds should be considered in the planning of translocations.
- 1479 Taka, S., Liandris, E., Gazouli, M., Sotirakoglou, K., Theodoropoulos, G., Bountouri, M., Andreadou, M., Ikonomopoulos, J.
In vitro expression of the SLC11A1 gene in goat monocyte-derived macrophages challenged with Mycobacterium avium subsp paratuberculosis
Infection Genetics and Evolution, (2013) 17, 8-15
Johne's disease or paratuberculosis is a chronic, progressive intestinal disease of ruminants caused by *Mycobacterium avium* subsp. paratuberculosis (MAP). One of the genes that have been targeted with regard to resistance or sensitivity to paratuberculosis is the SLC11A1 (solute carrier family 11 member A1). Here we extend our previous work to the sequence and structure analysis of the caprine SLC11A1 gene and we assess the functional impact of the



most frequent polymorphisms of the 3' UTR region of the SLC11A1 gene to its expression in goat macrophages exposed in vitro to MAP. The role of these polymorphisms in primary immune response is also investigated with connection to gene expression of two interleukins (IL), one of which pro (IL-1 alpha), and the other anti-inflammatory (IL-10). In order to assess gene response, quantitative detection of the SLC11A1, IL-10 and IL1 alpha mRNA was performed by real time PCR before, and at 1, 3 and 24 h after exposure of primary cultures of peripheral blood monocyte-derived macrophages to MAP, collected from 54 goats of the Greek native goat breed. Sequence analysis of the 3' UTR end of the caprine SLC11A1 gene determined its full length to be 522 bases. Structure analysis confirmed the presence of two microsatellites consisted of a variable number of guanine-thymine repeats (regions A and B). The homozygous B7 genotype [B(GT_n)7/7] was associated at a statistically significant level with increased expression of the SLC11A1 and IL-1 alpha genes indicating increased in vitro responsiveness and therefore resistance of mononuclear derived macrophages to MAP infection. (c) 2013 Elsevier B.V. All rights reserved.

- 1480 Del-Pozo, J., Girling, S., McLuckie, J., Abbondati, E., Stevenson, K.
An Unusual Presentation of Mycobacterium avium spp. paratuberculosis Infection in a Captive Tundra Reindeer (*Rangifer tarandus tarandus*)
Journal of Comparative Pathology, (2013) 149, 126-131
This report describes an unusual presentation of paratuberculosis in a captive, 4-year-old female tundra reindeer (*Rangifer tarandus tarandus*). The gross and histological presentation was consistent with clinical paratuberculosis as previously reported for other ruminants, with poor body condition, subcutaneous oedema, granulomatous ileitis (multibacillary), mesenteric lymphadenitis and hepatitis. However, this animal also presented with unusual lung lesions, with necrosis and mineralization similar to that reported for *Mycobacterium bovis* in other wild and domestic ruminants. The presence of DNA of *Mycobacterium avium* subsp. paratuberculosis was confirmed by polymerase chain reaction (PCR) in intestine and lung tissue (IS900, Hsp65) and PCR tests for the detection of *Mycobacterium tuberculosis* complex and other members of the *M. avium* complex were negative. (C) 2012 Elsevier Ltd. All rights reserved.
- 1481 Zhang, J.
Transcriptome Analysis Reveals Novel Entry Mechanisms and a Central Role of SRC in Host Defense during High Multiplicity Mycobacterial Infection
Plos One, (2013) 8, Article Number: e65128 DOI: 10.1371/journal.pone.0065128
Published: JUN 18 2013-*Mycobacterium tuberculosis* (MTB) infects an estimated one-third of the global population and is one of the main causes of mortality from an infectious agent. The characteristics of macrophages challenged by MTB with a high multiplicity of infection (MOI), which mimics both clinical disseminated infection and granuloma formation, are distinct from macrophages challenged with a low MOI. To better understand the cross talk between macrophage host cells and mycobacteria, we compared the transcription patterns of mouse macrophages infected with bacille Calmette-Guerin, H37Ra and *M. smegmatis*. Attention was focused on the changes in the abundance of transcripts related to immune system function. From the results of a transcriptome profiling study with a high mycobacterial MOI, we defined a pathogen-specific host gene expression pattern. The present study suggests that two integrins, ITGA5 and ITGAV, are novel cell surface receptors mediating mycobacterium entry into macrophages challenged with high MOI. Our results indicate that SRC likely plays a central role in regulating multiple unique signaling pathways activated by MTB infection. The integrated results increase our understanding of the molecular networks behind the host innate immune response and identify important targets that might be useful for the development of tuberculosis therapy.
- 1482 Deb, R., Goswami, P.P.
Detection of Cd4/Cd8 Ratio in Mice Immunized with A Bicistronic Plasmid Construct Encoding A Ppe Gene of Mycobacterium Avium Paratuberculosis and A Cytokine Gene of Murine Gamma Interferon
Indian Journal of Animal Research, (2013) 47, 240-243
In the present study a gene encoding PPE protein of *M.a.paratuberculosis* (FJ032182) was



cloned with murine IFN-3 in a mammalian bicistronic vector pIRES 6.1 to elucidate the role of gamma interferon on the PPE gene for determination of CD4/CD8 ratio in post immunized mice. Flow cytometric analysis with mononuclear mice splenocytes on 42nd day post immunization revealed significant reduction of the CD4/CD8 ratio in the mice group immunized with pIR PPE/IFN (1.65 +/- 0.0001) compared to the pIR PPE group (2.92 +/- 0.0003). These result suggested that co-expression of murine IFN gamma in conjunction with PPE protein significantly enhanced the CM' response.

1483 Nielsen, S.S., Toft, N., Okura, H.

Dynamics of Specific Anti-Mycobacterium avium Subsp paratuberculosis Antibody Response through Age

Plos One, (2013) 8, Article Number: e63009 DOI: 10.1371/journal.pone.0063009

Published: APR 29 2013-Mycobacterium avium subsp. paratuberculosis (MAP) causes a chronic infection in cattle. MAP infected cattle with humoral immune (HI) reactions with IgG antibodies are usually those where latency of infection has ceased and their infection is progressing towards reduced milk yield, weight loss and significant bacterial excretion in feces. The proportion of detectable infections among all infected animals that will develop disease is often referred to as 'the tip of the iceberg'. The purpose of this study was to estimate this proportion. Test-records from 18,972 Danish dairy cows with MAP specific IgG antibodies on their final test-record were used to estimate age-specific sensitivities (Se). These cows were the infected ones considered to develop disease in a population with a representative age-distribution and were defined as cases. The specificity (Sp) of the test was estimated based on test-results from 166,905 cows, which had no MAP IgG antibodies in their final four test-records. The Sp, age-specific Se and maximum Se were used to estimate the probability of having HI at a given age resulting in the proportion of infected cows with HI at a given age. For cows 2 years of age, the proportion of detectable cases was 0.33, while it was 0.94 for cows 5 years of age. Thus, there was a significant shift in the tip of the iceberg with aging. This study provided a model for estimating the proportion of latent chronic infections that would progress to disease, and the results can be used to model infection dynamics.

New publications in the [CROHN'S DISEASE AND PARATUBERCULOSIS database](#) (824-829)

824 Kumar, S., Ingle, H., Prasad, D.V.R., Kumar, H.

Recognition of bacterial infection by innate immune sensors

Critical Reviews in Microbiology, (2013) 39, 229-246

Microbial challenges to the host initiate an array of defense processes through the activation of innate and adaptive immunity. Innate immunity consists of sensors or pattern-recognition receptors (PRRs) that are expressed on immune and non-immune cells and sense conserved pathogen-derived molecules or pathogen-associated molecular patterns (PAMPs) in various compartments of the host cells. Recognition of the PAMPs by PRRs triggers antimicrobial effector responses via the induction of proinflammatory cytokines and type I IFNs. Several families of PRRs, such as Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-I-like receptors (RLRs), and DNA sensors and their respective PAMPs have been well studied in innate immunity and host defense. Here, we review the recent findings on bacterial recognition by TLRs and NLRs and the signaling pathways activated by these sensors.

825 Macauley, M.S., Pfrengle, F., Rademacher, C., Nycholat, C.M., Gale, A.J., von Drygalski, A., Paulson, J.C.

Antigenic liposomes displaying CD22 ligands induce antigen-specific B cell apoptosis

Journal of Clinical Investigation, (2013) 123, 3074-3083

Antibodies confer humoral immunity but can also be harmful when they target an autoantigen, alloantigen, allergen, or biotherapeutic. New strategies are needed for antigen-specific suppression of undesired antibody responses, particularly to T cell-dependent protein antigens, because they elicit T cell help. Here we show that liposomal nanoparticles, displaying both antigen and glycan ligands of the inhibitory coreceptor CD22, induce a tolerogenic program that selectively causes apoptosis in mouse and human B cells. These SIGLEC-engaging tolerance-inducing antigenic liposomes (STALs, where SIGLEC is defined



as sialic acid-binding Ig-like lectin) induced robust antigen-specific tolerance to protein antigens in mice, preventing subsequent immune response to challenge with the same antigen. Since development of inhibitory antibodies to FVIII is a serious problem in treatment of hemophilia A patients, we investigated the potential of this approach for inducing tolerance to FVIII in a hemophilia mouse model. STALs prevented formation of inhibitory FVIII antibodies, allowing for effective administration of FVIII to hemophilia mice to prevent bleeding. These findings suggest that STALs could be used to eliminate or prevent harmful B cell-mediated immune responses.

- 826 Chen, N.H., Cheong, K.A., Kim, C.H., Noh, M., Lee, A.Y.

Glucosamine Induces Activated T Cell Apoptosis Through Reduced T Cell Receptor

Scandinavian Journal of Immunology, (2013) 78, 17-27

Glucosamine (GlcN), like N-acetylglucosamine (GlcNAc), is salvaged into the hexosamine pathway and is converted to UDP-GlcNAc. Golgi N-glycan branching enzymes produce N-glycans, using UDP-GlcNAc as a substrate, which attach to the T cell receptor (TCR) and cytotoxic T-lymphocyte antigen-4 (CTLA-4). These findings suggest that GlcN exerts the immunoregulation through TCR signalling, which could be involved not only in cytokine production but also activated T cell apoptosis. In fact, a preliminary study showed that GlcN reduced the number of CD3+ T cells of NC/Nga mice with AD-like skin lesions. Therefore, whether apoptosis of T cells would be one of the potential molecular mechanisms of GlcN-induced immunosuppression was investigated. Cultured human primary along with Jurkat T cells and purified T cells from NC/Nga mice with or without Df-induced AD-like skin lesion were used for the study. Glucosamine treatment increased the number of T cells expressing 1,6GlcNAc-branched N-glycans, with reduced ZAP-70 phosphorylation and enhanced CTLA-4 expression. Glucosamine treatment reduced the number of activated T cells from both the human primary and Jurkat cells and the dermatitis-induced mice. The expression of FasL and activated caspases, particularly caspase-3, was increased, whereas the phosphorylation of PI3K, Akt and NF- κ B was decreased by GlcN treatment. Therefore, in addition to down-regulating TCR signalling and promoting CTLA-4 expression, GlcN may also suppress T cell function by enhancing apoptosis of activated T cells, through both extrinsic and intrinsic apoptotic signalling pathways, which were regulated by the inhibition of PI3K/Akt and NF- κ B phosphorylation.

- 827 Sondermann, P., Pincetic, A., Maamary, J., Lammens, K., Ravetch, J.V.

General mechanism for modulating immunoglobulin effector function

Proceedings of the National Academy of Sciences of the United States of America, (2013) 110, 9868-9872

Immunoglobulins recognize and clear microbial pathogens and toxins through the coupling of variable region specificity to Fc-triggered cellular activation. These proinflammatory activities are regulated, thus avoiding the pathogenic sequelae of uncontrolled inflammation by modulating the composition of the Fc-linked glycan. Upon sialylation, the affinities for Fc gamma receptors are reduced, whereas those for alternative cellular receptors, such as dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN)/CD23, are increased. We demonstrate that sialylation induces significant structural alterations in the C gamma 2 domain and propose a model that explains the observed changes in ligand specificity and biological activity. By analogy to related complexes formed by IgE and its evolutionarily related Fc receptors, we conclude that this mechanism is general for the modulation of antibody-triggered immune responses, characterized by a shift between an "open" activating conformation and a "closed" anti-inflammatory state of antibody Fc fragments. This common mechanism has been targeted by pathogens to avoid host defense and offers targets for therapeutic intervention in allergic and autoimmune disorders.

- 828 Gaschnigard, J., Scurr, E., Alcais, A.

Leprosy, a pillar of human genetics of infectious diseases

Pathologie Biologie, (2013) 61, 120-128

Despite a natural reservoir of Mycobacterium leprae limited to humans and free availability of an effective antibiotic treatment, more than 200,000 people develop leprosy each year. This disease remains a major cause of disability and social stigma worldwide. The cause of this



constant incidence is currently unknown and indicates that important aspects of the complex relationship between the pathogen and its human host remain to be discovered. An important contribution of host genetics to susceptibility to leprosy has long been suggested to account for the considerable variability between individuals sustainably exposed to *M. leprae*. Given the inability to cultivate *M. leprae* in vitro and in the absence of relevant animal model, genetic epidemiology is the main strategy used to identify the genes and, consequently, the immunological pathways involved in protective immunity to *M. leprae*. Recent genome-wide studies have identified new pathophysiological pathways which importance is only beginning to be understood. In addition, the prism of human genetics placed leprosy at the crossroads of other common diseases such as Crohn's disease, asthma or myocardial infarction. Therefore, novel lights on the pathogenesis of many common diseases could eventually emerge from the detailed understanding of a disease of the shadows. (C) 2013 Elsevier Masson SAS. All rights reserved.

829 Chi, C.Y., Chu, C.C., Liu, J.P., Lin, C.H., Ho, M.W., Lo, W.J., Lin, P.C., Chen, H.J., Chou, C.H., Feng, J.Y., Fung, C.P., Sher, Y.P., Li, C.Y., Wang, J.H., Ku, C.L.

Anti-IFN-gamma autoantibodies in adults with disseminated nontuberculous mycobacterial infections are associated with HLA-DRB1*16:02 and HLA-DQB1*05:02 and the reactivation of latent varicella-zoster virus infection

Blood, (2013) 121, 1357-1366

Adult patients with disseminated nontuberculous mycobacterial (dNTM) infections usually have severe immune system defects. Recently, several studies have shown that anti-IFN-g autoantibodies may play an important role in the pathogenicity of dNTM infections. A considerable proportion of reported cases of anti-IFN-g autoantibodies show either clinical or laboratory evidence of autoimmune disease. In the present study, we identified 19 formerly healthy adults who later developed dNTM infections, of whom 17 were further investigated immunologically. High-titer anti-IFN-g autoantibodies capable of inhibiting IL-12 production in vitro were found in the plasma of all of these patients. In addition to dNTM infection, 35% and 71% of our patients also suffered from salmonellosis and herpes zoster, respectively. This observation suggests that IFN-g may be crucial in controlling salmonella infection and reactivating latent varicella-zoster virus infection in humans. 2 HLA alleles, DRB1*16:02 DQB1*05:02 (odds ratio = 8.68; 95% confidence interval, 3.47-21.90; $P = 1.1 \times 10^{-6}$); $P_c = 3.08 \times 10^{-5}$ and odds ratio = 7.16; 95% confidence interval, 3.02-17.05; $P = 1 \times 10^{-7}$); $P_c = 1.4 \times 10^{-6}$, respectively), were found in 82% (14 of 17) of our patients. In conclusion, our data suggest that anti-IFN-g autoantibodies may play a critical role in the pathogenesis of dNTM infections and reactivation of latent varicella-zoster virus infection and are associated with HLA-DRB1*16:02 and HLA-DQB1*05:02.
