



2013-09-14 Paratuberculosis databases updated (2013-09-13)

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New publications in the [PARATUBERCULOSIS database](#) (1490-1506)

1490 Eisenberg, S.W.F., Chuchaisangrat, R., Nielen, M., Koets, A.P.

Relationship between Presence of Cows with Milk Positive for Mycobacterium avium subsp paratuberculosis-Specific Antibody by Enzyme-Linked Immunosorbent Assay and Viable M. avium subsp paratuberculosis in Dust in Cattle Barns

Applied and Environmental Microbiology, (2013) 79, 5458-5464

Paratuberculosis, or Johne's disease, in cattle is caused by Mycobacterium avium subsp. paratuberculosis, which has recently been suspected to be transmitted through dust. This longitudinal study on eight commercial M. avium subsp. paratuberculosis-positive dairy farms studied the relationship between the number of cows with M. avium subsp. paratuberculosis antibody-positive milk and the presence of viable M. avium subsp. paratuberculosis in settled-dust samples, including their temporal relationship. Milk and dust samples were collected in parallel monthly for 2 years. M. avium subsp. paratuberculosis antibodies in milk were measured by enzyme-linked immunosorbent assay (ELISA) and used as a proxy for M. avium subsp. paratuberculosis shedding. Settled-dust samples were collected by using electrostatic dust collectors (EDCs) at six locations in housing for dairy cattle and young stock. The presence of viable M. avium subsp. paratuberculosis was identified by liquid culture and PCR. The results showed a positive relationship (odds ratio [OR], 1.2) between the number of cows with ELISA-positive milk and the odds of having positive EDCs in the same airspace as the adult dairy cattle. Moreover, the total number of lactating cows also showed an OR slightly above 1. This relationship remained the same for settled-dust samples collected up to 2 months before or after the time of milk sampling. The results suggest that removal of adult cows with milk positive for M. avium subsp. paratuberculosis-specific antibody by ELISA might result in a decrease in the presence of viable M. avium subsp. paratuberculosis in dust and therefore in the environment. However, this decrease is likely delayed by several weeks at least. In addition, the data support the notion that M. avium subsp. paratuberculosis exposure of young stock is reduced by separate housing.

1491 Stewart, L.D., Foddai, A., Elliott, C.T., Grant, I.R.

Development of a novel phage-mediated immunoassay for the rapid detection of viable Mycobacterium avium subsp paratuberculosis

Journal of Applied Microbiology, (2013) 115, 808-817

Aims: The objective of this study was to develop a novel screening method for detection of viable Mycobacterium avium subsp. paratuberculosis (Map) in milk and faeces, as a rapid alternative to Map culture. Methods and Results: The new method couples Map-specific peptide-mediated magnetic separation technique with an optimized phage amplification assay followed by detection of released progeny phage by ELISA in a competition assay format using polyclonal antibody produced against the D29 mycobacteriophage involved in the phage assay. Sample matrices were found not to interfere with the developed method, and the dynamic range of the assay was 3×10^2 - 6×10^8 phage/ml(-1). When low numbers of Map were present (10^2 CFU/ml(-1)), the burst size of a single host Map cell was maximal (10^3 phage per cell) resulting in a highly sensitive screening assay. Conclusion: A rapid, sensitive



immuno-based screening method suitable for the detection of viable Map in milk and faeces was developed. Significance and Impact of the Study: The novel PMS-phage-ELISA permits sensitive, qualitative detection of viable Map in milk or faeces samples within 48h, representing a substantial decrease in time to detection compared with current culture methods for Map.

1492 Toth, J.D., Aceto, H.W., Rankin, S.C., Dou, Z.

Short communication: Survey of animal-borne pathogens in the farm environment of 13 dairy operations

Journal of Dairy Science, (2013) 96, 5756-5761

A survey was conducted on 13 dairies to determine the occurrence of 5 animal-borne pathogens (*Salmonella enterica*, *Escherichia coli* 0157:117, *Campylobacter jejuni*, *Mycobacterium avium* ssp. *paratuberculosis*, and *Cryptosporidium parvum*) and their distributions across farm elements (feces, bedding, milk filters, stored manure, field soil, and stream water). Presence of *C. parvum* was measured only in feces and stored manure. All but one farm were positive for at least one pathogen species, and 5 farms were positive for 3 species. *Escherichia coli* 0157:117 was detected on 6 farms and in all farm elements, including milk filters. *Mycobacterium avium* ssp. *paratuberculosis* was detected on 10 of 13 farms and in all farm elements except for milk filters. *Salmonella enterica* and *C. jejuni* were detected at lower frequencies and were not identified in soil, stream water, or milk filters on any of the 13 farms. *Cryptosporidium parvum* was detected in feces but not in stored manure. Stored manure had the highest occurrence of pathogens (73%), followed by feces (50%), milk filters, bedding, soil, and water (range from 23 to 31%). Association of pathogen presence with farm management factors was examined by t-test; however, the small number of study farms and samples may limit the scope of inference of the associations. Pathogens had a higher prevalence in maternity pen bedding than in calf bedding, but total pathogen occurrence did not differ in calf compared with lactating cow feces or in soils with or without manure incorporation. Herd size and animal density did not appear to have a consistent effect on pathogen occurrence. The extent of pathogen prevalence and distribution on the farms indicates considerable public health risks associated with not only milk and meat consumption and direct animal contact, but also potential dissemination of the pathogens into the agroecosystem.

1493 Chiodini, R.J., Dowd, S.E., Davis, B., Galandiuk, S., Chamberlin, W.M., Kuenstner, J.T., McCallum, R.W., Zhang, J.

Crohn's Disease May Be Differentiated Into 2 Distinct Biotypes Based on the Detection of Bacterial Genomic Sequences and Virulence Genes Within Submucosal Tissues

Journal of Clinical Gastroenterology, (2013) 47, 612-620

Objective: To determine whether bacterial pathogens can be detected within the diseased submucosal tissues of patients with Crohn's disease by molecular techniques independent of cultural methods. Design: We designed a quantitative polymerase chain reaction to detect 32 virulence genes and transposons within submucosal tissues of patients with Crohn's disease and controls and compared the microbiome of the submucosa with mucosal bacterial populations. Results: Within submucosal tissues, the bacterial invasion/adherence genes *eaeA* and *invA* were detected in 43% of patients ($P=0.01$ and 0.008 vs. mucosa and controls, respectively) and the *Mycobacterium*-specific IS900 and 251F genes detected in 50% of patients ($P=0.03$ vs. mucosa and controls). These findings were mutually exclusive: invasion/adhesion genes and *Mycobacterium*-associated transposons were not detected in the same patient. Metagenomic sequencing and quantitative polymerase chain reaction results confirmed effective separation of the submucosal and mucosal microbiome and the existence of a submucosal bacterial population within diseased tissues. Conclusions: This study is the first to examine the microbial populations of submucosal tissues during intestinal disease and provide evidence of a distinct submucosal microbiome and biotypes within Crohn's disease. These data suggests that Crohn's disease may not be a single disease, but a spectrum that can be divided into distinct biotypes based on the presence of invasion/adherence genes or *Mycobacterium*-associated transposons. If corroborated by larger population studies, these findings could revolutionize the diagnosis, management, and



treatment of Crohn's disease by the identification of patient biotypes and the application of targeted chemotherapeutic treatments that go beyond supportive in nature.

1494 Paolicchi, F., Perea, J., Cseh, S., Morsella, C.

Relationship between Paratuberculosis and the microelements Copper, Zinc, Iron, Selenium and Molybdenum in Beef Cattle

Brazilian Journal of Microbiology, (2013) 44, 153-160

To study the deficiency of minerals and its relationship with Paratuberculosis, blood, serum, and fecal samples were obtained from 75 adult bovines without clinical symptoms of the disease and from two bovines with clinical symptoms of the disease, from two beef herds with a previous history of Paratuberculosis in the Province of Buenos Aires, Argentina. Serum samples were processed by ELISA and feces were cultured in Herrolds medium. Copper, zinc and iron in serum were quantified by spectrophotometry and selenium was measured by the activity of glutathione peroxidase. We also determined copper, zinc, iron and molybdenum concentrations in pastures and the concentration of sulfate in water. Mycobacterium avium subsp paratuberculosis (Map) was isolated from 17.3% of fecal samples of asymptomatic animals and from the fecal samples from the two animals with clinical symptoms. All the Map-positive animals were also ELISA-positive or suspect, and among them, 84.6% presented low or marginal values of selenium and 69.2% presented low or marginal values of copper. The two animals with clinical symptoms, and isolation of Map from feces and organs were selenium-deficient and had the lowest activity of glutathione peroxidase of all the animals from both herds. All the animals negative to Map in feces and negative to ELISA had normal values of Se, while 13.8% of animals with positive ELISA or suspect and culture negative presented low levels of Se. Half of the animals that were negative both for ELISA and culture in feces were deficient in copper but none of them presented low values of selenium. The content of molybdenum and iron in pasture was high, 2.5 ppm and 1.13 ppm in one herd and 2.5 ppm and 2.02 ppm in the other, respectively, whereas the copper: molybdenum ratio was 1.5 and 5.2, respectively. These results do not confirm an interaction between imbalances of the micronutrients and clinical Paratuberculosis, but show evidence of the relationship between selenium deficiencies in animals with Map infection and ELISA positive results.

1496 Killick, K.E., Cheallaigh, C.N., O'Farrelly, C., Hokamp, K., MacHugh, D.E., Harris, J.

Receptor-mediated recognition of mycobacterial pathogens

Cellular Microbiology, (2013) 15, 1484-1495

Mycobacteria are a genus of bacteria that range from the non-pathogenic Mycobacterium smegmatis to Mycobacterium tuberculosis, the causative agent of tuberculosis in humans. Mycobacteria primarily infect host tissues through inhalation or ingestion. They are phagocytosed by host macrophages and dendritic cells. Here, conserved pathogen-associated molecular patterns (PAMPs) on the surface of mycobacteria are recognized by phagocytic pattern recognition receptors (PRRs). Several families of PRRs have been shown to non-opsonically recognize mycobacterial PAMPs, including membrane-bound C-type lectin receptors, membrane-bound and cytosolic Toll-like receptors and cytosolic NOD-like receptors. Recently, a possible role for intracellular cytosolic PRRs in the recognition of mycobacterial pathogens has been proposed. Here, we discuss current ideas on receptor-mediated recognition of mycobacterial pathogens by macrophages and dendritic cells.

1497 Bannantine, J.P., Li, L.L., Sreevatsan, S., Kapur, V.

How does a Mycobacterium change its spots? Applying molecular tools to track diverse strains of Mycobacterium avium subspecies paratuberculosis

Letters in Applied Microbiology, (2013) 57, 165-173

Defining genetic diversity in the wake of the release of several Mycobacterium avium subsp. paratuberculosis (MAP) genome sequences has become a major emphasis in the molecular biology and epidemiology of Johne's disease research. These data can now be used to define the extent of strain diversity on the farm. However, to perform these important tasks, researchers must have a way to distinguish the many MAP isolates/strains that are present in the environment or host to enable tracking over time. Recent studies have described genetic



diversity of the *Mycobacterium avium* complex (MAC), of which MAP is a member, through pulsed-field gel electrophoresis, single sequence repeats, variable-number tandem repeats, genome rearrangements, single nucleotide polymorphisms and genomewide comparisons to identify insertions and deletions. Combinations of these methods can now provide discrimination sufficient for dependable strain tracking. These molecular epidemiology techniques are being applied to understand transmission of Johne's disease within dairy cattle herds as well as identify which strains predominate in wildlife.

1498 Pinedo, P.J., Galvao, K.N., Seabury, C.M.

Innate immune gene variation and differential susceptibility to uterine diseases in Holstein cows

Theriogenology, (2013) 80, 384-390

An immune response is mounted after binding of Toll-like receptors (TLRs) to pathogen-associated molecular patterns. The primary objective of this study was to test for the associations between bovine single-nucleotide polymorphisms (SNPs) and insertion-deletion (indel) mutations occurring in seven bovine TLR genes (TLRs 1, 2, 4, 5, 6, 9, and 10) that are known to recognize bacterial ligands and the most significant uterine diseases in dairy cows, including metritis (MET), clinical endometritis (CE), and cytologic endometritis (CYE). Custom allele-specific genotyping assays derived from multiple bovine TLR sequencing studies were utilized. Genotypes for 110 loci (SNPs and indels) that are known to be variable in domestic cattle were determined, resulting in 46 monomorphic loci, 64 loci with two alleles, and 35 loci that did not meet our inclusion criterion for minor allele frequency (≥ 0.10). The association between specific TLR genotypes and each of the uterine diseases (MET, CE, CYE) was evaluated by logistic regression with correction for confounding variables. Collectively, seven SNPs produced uncorrected P values ≤ 0.05 with respect to three different uterine diseases investigated, but none of the SNP associations endured correction for multiple testing (P values ≥ 0.05). Several confounding variables, including parity, dystocia, and ketosis before 17 DIM, remained significant after correction for multiple testing. Our analysis of these data suggest that some bovine TLR SNPs (i.e., TLRs 2, 4, 6, 9) may potentially elicit relatively small effects on uterine health in Holstein dairy cows and that some confounding variables are actually more predictive for the incidence of disease than any genetic markers evaluated herein. (C) 2013 Elsevier Inc. All rights reserved.

1499 Wagner, J., Skinner, N.A., Catto-Smith, A.G., Cameron, D.J.S., Michalski, W.P., Visvanathan, K., Kirkwood, C.D.

TLR4, IL10RA, and NOD2 mutation in paediatric Crohn's disease patients: an association with *Mycobacterium avium* subspecies paratuberculosis and TLR4 and IL10RA expression

Medical Microbiology and Immunology, (2013) 202, 267-276

Mycobacterium avium subspecies paratuberculosis (MAP) has been implicated in the pathogenesis of Crohn's disease (CD). The role of CD susceptibility genes in association with these microbes is not known. Sixty-two early onset paediatric CD patients and 46 controls with known MAP status were analysed for an association with 34 single nucleotide polymorphisms (SNPs) from 18 CD susceptibility genes. Functional studies on peripheral blood mononuclear cells (PBMCs) were conducted on 17 CD patients with known CD mutations to assess IL-6, IL-10, and TNF-alpha expression upon stimulation with MAP precipitated protein derivative (PPD) and lipopolysaccharide (LPS). In addition, surface expression of IL10R and TLR4 on resting B cells, NK cells, T cells, and monocytes was assessed. A mutation in TLR4 (rs4986790) and IL10RA (rs22291130) was significantly associated with MAP-positive CD patients compared to MAP-negative CD patients (27.6 vs. 6.1 %, $p = 0.021$, and 62.1 vs. 33.3 %, $p = 0.024$, respectively). PPD and LPS significantly increased IL-6, IL-10, and TNF-alpha production in PBMCs. IL-10 and TNF-alpha production were significantly lower in a subgroup of CD patients (5/12) with a known NOD2 mutation. Receptor for IL-10 was significantly higher expressed on NK cells (CD56low) and on NK T cells harbouring a NOD2 mutations compared to wildtype cells ($p = 0.031$ and 0.005 , respectively). TLR4 was significantly higher expressed on NK cells (CD56high) harbouring a NOD2 mutations compared to wildtype cells ($p = 0.038$).



1500 Foster, G., Stevenson, K., Reid, R.J., Barley, J.P., Baily, J.L., Harris, R.N., Dagleish, M.P.

Infection due to *Mycobacterium avium* subsp *avium* in a Free-ranging Common Seal (*Phoca vitulina*) in Scotland

Journal of Wildlife Diseases, (2013) 49, 732-734

We describe the first reported case of mycobacterial infection in a free-ranging pinniped in the Northern Hemisphere. Acid-fast bacteria were demonstrated histologically in the liver of an adult female common seal (*Phoca vitulina*), and *Mycobacterium avium* subsp. *avium* was cultured from the liver.

1501 Sikandar, A., Cheema, A.H., Adil, M., Younus, M., Zaneb, H., Zaman, M.A., Tipu, M.Y., Masood, S.

Ovine Paratuberculosis-A Histopathological Study from Pakistan

Journal of Animal and Plant Sciences, (2013) 23, 749-753

This study was conducted to elucidate the histopathological depiction of ovine paratuberculosis. Tissue samples were randomly collected from 47 sheep slaughtered at two municipal abattoirs of district Jhang, Pakistan. The tissue samples were inspected for the presence of *Mycobacterium avium* subspecies paratuberculosis (MAP) by means of acid-fast staining and gross/histopathological examination. Indirect ELISA was also performed for the confirmation of paratuberculosis. Intestinal pathological lesions were observed in 04.12% animals. While only 03.77% of mesenteric lymph nodes (MLN) were associated with gross lesions. Acid-fast staining of tissue hard pressed smears were positive for MAP in 12.76% intestinal and 10.63% MLN tissue samples. Similarly Ziehl-Neelsen (ZN) staining of the histopathological tissue sections of MAP positive smear samples reflected the occurrence of acid-fast bacilli in 100% intestinal as well as lymph nodes samples. This indicated the superiority of intestinal samples over mesenteric lymph nodes and hence tissue section could be considered to be a comparatively better preparation for the analysis of paratuberculosis with ZN staining technique. ELISA confirmed 10.63% samples positive for MAP. It was concluded from the study that infection of MAP could be precisely identified by histopathology and indirect ELISA, which tends to be unlikely if merely based upon acid-fast staining and gross examination.

1502 Sechi, P., Paolotto, P., McCrindle, C.M.E., Cenci-Goga, B.T.

Seroepidemiological study of Johne's-disease in dairy cattle in Umbria, Italy

Italian Journal of Animal Science, (2013) 12, Article Number: e31 DOI:

10.4081/ijas.2013.e31 Published: 2013-A total of 788 serum samples from dairy cattle in Umbria, Italy, were tested for the presence of antibodies to *Mycobacterium avium* subspecies paratuberculosis (Map) using a commercial enzyme-linked immunosorbent assay (ELISA) kit. The sampled animals came from 19 herds representative of the central area of the Umbria county (Perugia and Assisi districts). Using the manufacturer suggested cut-off for a positive test, 44 animals (5.6%) were positive. Using the sensitivity and specificity claimed by the manufacturer of the ELISA kit, the true prevalence in Umbria dairy cattle overall was calculated as 9.7% (99% CI, 7.0%, 12.4%).

1503 Kostoulas, P., Browne, W.J., Nielsen, S.S., Leontides, L.

Bayesian mixture models for partially verified data: Age- and stage-specific discriminatory power of an antibody ELISA for paratuberculosis

Preventive Veterinary Medicine, (2013) 111, 200-205

Bayesian mixture models can be used to discriminate between the distributions of continuous test responses for different infection stages. These models are particularly useful in case of chronic infections with a long latent period, like *Mycobacterium avium* subsp. paratuberculosis (MAP) infection, where a perfect reference test does not exist. However, their discriminatory ability diminishes with increasing overlap of the distributions and with increasing number of latent infection stages to be discriminated. We provide a method that uses partially verified data, with known infection status for some individuals, in order to minimize this loss in the discriminatory power. The distribution of the continuous antibody response against MAP has



been obtained for healthy, MAP-infected and MAP-infectious cows of different age groups. The overall power of the milk-ELISA to discriminate between healthy and MAP-infected cows was extremely poor but was high between healthy and MAP-infectious. The discriminatory ability increased with increasing age. The great overlap between the distributions of the different infection stages would have hampered our ability to discriminate between the different infection stages. Thus, the proposed method, which uses partially verified data on the true status for some individuals, is an intuitive extension to the standard non-gold standard methods, especially in the case of infections with a long latent period. (c) 2013 Published by Elsevier B.V.

1504 Kruze, J., Monti, G., Schulze, F., Mella, A., Leiva, S.

Herd-level prevalence of Map infection in dairy herds of southern Chile determined by culture of environmental fecal samples and bulk-tank milk qPCR

Preventive Veterinary Medicine, (2013) 111, 319-324

Paratuberculosis, an infectious disease of domestic and wild ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (Map), is an economically important disease in dairy herds worldwide. In Chile the disease has been reported in domestic and wildlife animals. However, accurate and updated estimations of the herd-prevalence in cattle at national or regional level are not available. The objectives of this study were to determine the herd-level prevalence of dairy herds with Map infected animals of Southern Chile, based on two diagnostic tests: culture of environmental fecal samples and bulk-tank milk qPCR. Two composite environmental fecal samples and one bulk-tank milk sample were collected during September 2010 and September 2011 from 150 dairy farms in Southern Chile. Isolation of Map from environmental fecal samples was done by culture of decontaminated samples on a commercial Herrold's Egg Yolk Medium (HEYM) with and without mycobactin J. Suspicious colonies were confirmed to be Map by conventional IS900 PCR. Map detection in bulk-tank milk samples was done by real time IS900 PCR assay. PCR-confirmed Map was isolated from 58 (19.3%) of 300 environmental fecal samples. Holding pens and manure storage lagoons were the two more frequent sites found positive for Map, representing 35% and 33% of total positive samples, respectively. However, parlor exits and cow alleyways were the two sites with the highest proportion of positive samples (40% and 32%, respectively). Herd prevalence based on environmental fecal culture was 27% (true prevalence 44%) compared to 49% (true prevalence 87%) based on bulk-tank milk real time 15900 PC. In both cases herd prevalence was higher in large herds (>200 cows). These results confirm that Map infection is wide spread in dairy herds in Southern Chile with a rough herd-level prevalence of 28-100% depending on the herd size, and that IS900 PCR on bulk-tank milk samples is more sensitive than environmental fecal culture to detect Map-infected dairy herds. (c) 2013 Elsevier B.V. All rights reserved.

1505 Antoniani, D., Rossi, E., Rinaldo, S., Bocci, P., Lolicato, M., Paiardini, A., Raffaelli, N., Cutruzzola, F., Landini, P.

The immunosuppressive drug azathioprine inhibits biosynthesis of the bacterial signal molecule cyclic-di-GMP by interfering with intracellular nucleotide pool availability

Applied Microbiology and Biotechnology, (2013) 97, 7325-7336

In Gram-negative bacteria, production of the signal molecule c-di-GMP by diguanylate cyclases (DGCs) is a key trigger for biofilm formation, which, in turn, is often required for the development of chronic bacterial infections. Thus, DGCs represent interesting targets for new chemotherapeutic drugs with anti-biofilm activity. We searched for inhibitors of the WspR protein, a *Pseudomonas aeruginosa* DGC involved in biofilm formation and production of virulence factors, using a set of microbiological assays developed in an *Escherichia coli* strain expressing the *wspR* gene. We found that azathioprine, an immunosuppressive drug used in the treatment of Crohn's disease, was able to inhibit WspR-dependent c-di-GMP biosynthesis in bacterial cells. However, in vitro enzymatic assays ruled out direct inhibition of WspR DGC activity either by azathioprine or by its metabolic derivative 2-amino-6-mercapto-purine riboside. Azathioprine is an inhibitor of 5-aminoimidazole-4-carboxamide ribotide (AICAR) transformylase, an enzyme involved in purine biosynthesis, which suggests that inhibition of c-di-GMP biosynthesis by azathioprine may be due to perturbation of intracellular nucleotide



pools. Consistent with this hypothesis, WspR activity is abolished in an *E. coli* purH mutant strain, unable to produce AICAR transformylase. Despite its effect on WspR, azathioprine failed to prevent biofilm formation by *P. aeruginosa*; however, it affected production of extracellular structures in *E. coli* clinical isolates, suggesting efficient inhibition of c-di-GMP biosynthesis in this bacterium. Our results indicate that azathioprine can prevent biofilm formation in *E. coli* through inhibition of c-di-GMP biosynthesis and suggest that such inhibition might contribute to its anti-inflammatory activity in Crohn's disease.

1506 Gupta, T., Fine-Coulson, K., Karls, R., Gauthier, D., Quinn, F.

Internalization of *Mycobacterium shottsii* and *Mycobacterium pseudoshottsii* by *Acanthamoeba polyphaga*

Canadian Journal of Microbiology, (2013) 59, 570-576

Amoebae serve as environmental hosts to a variety of mycobacteria, including *Mycobacterium avium* and *Mycobacterium marinum*. *Mycobacterium shottsii* and *Mycobacterium pseudoshottsii* are waterborne species isolated from the spleens and dermal lesions of striped bass (*Morone saxatilis*) from the Chesapeake Bay. The optimal growth temperature for these fish isolates is 25 degrees C. In the present study, amoebae were examined as a potential environmental reservoir for these fish pathogens. Several studies demonstrated that *M. avium* bacilli replicate within the trophozoite stage and reside in large numbers within the cytosol of the cyst of the free-living amoeba *Acanthamoeba polyphaga*. Results from the present study showed that *M. shottsii*, *M. pseudoshottsii*, and *M. marinum* bacilli were internalized by *A. polyphaga* trophozoites within 6 h but that intracellular viability decreased by 2 to 3 logs over 10 days. While an average of 25 *M. marinum* bacilli were identified by electron microscopy in the cytosol of the cyst, <5 *M. pseudoshottsii* and no *M. shottsii* bacilli were observed in this location. All *Mycobacterium* species examined remained viable but did not replicate after encystment and subsequent 48 h incubation in 4% HCl. This concentration of HCl will kill mycobacteria but will not enter amoebal cysts. Bacterial viability studies within stages of the amoeba life cycle indicate fewer *M. shottsii* and *M. pseudoshottsii* bacilli within the trophozoite and cyst stages relative to *M. marinum*.

New publications in the [CROHN'S DISEASE AND PARATUBERCULOSIS database](#) (831-838)

831 Liu, H.Q., Zhang, X.Y., Edfeldt, K., Nijhuis, M.O., Idborg, H., Back, M., Roy, J., Hedin, U., Jakobsson, P.J., Laman, J.D., de Kleijn, D.P., Pasterkamp, G., Hansson, G.K., Yan, Z.Q.

NOD2-Mediated Innate Immune Signaling Regulates the Eicosanoids in Atherosclerosis
Arteriosclerosis Thrombosis and Vascular Biology, (2013) 33, 2193-2201

Objective-The activity of eicosanoid pathways is critical to the inflammatory and immune responses that are associated with the progression of atherosclerosis. Yet, the signals that regulate these pathways are poorly understood. Here, we address whether the innate immune signals of nucleotide-binding oligomerization domain-containing protein (NOD) 2 affect eicosanoids metabolism in atherosclerosis. Approach and Results-Analysis of human carotid plaques revealed that NOD2 was abundantly expressed at both mRNA and protein levels by endothelial cells and macrophages. Stimulation of NOD2 in ex vivo-cultured carotid plaques by muramyl dipeptide, an extrinsic ligand of NOD2, led to release of prostaglandin E-2, upregulation of cyclooxygenase-2 and microsomal prostaglandin E synthase-1, and to downregulation of cyclooxygenase-1. NOD2 was coexpressed with cyclooxygenase-2 in lesional macrophages. NOD2-induced cyclooxygenase-2 expression in macrophages was dependent on p38 mitogen-activated protein kinase activation and was mediated by interleukin-1 beta and tumor necrosis factor-alpha. Selective lipidomic analysis of the eicosanoids released by the carotid plaques characterized the metabolites of 12-, 5-, and 15-lipoxygenase as the predominant eicosanoids that were produced by the atherosclerotic lesion in the absence of additional stimuli. Unlike the prostaglandin E-2 pathway, metabolic activity of the lipoxygenase pathways was not altered on the short-term activation of NOD2 in carotid plaques. Conclusions-These results suggest that atherosclerosis may involve enhanced NOD2-mediated innate immunity. Activation of NOD2 preferentially upregulates the prostaglandin E-2 pathway. Nevertheless, lipoxygenase pathways, such as 12-lipoxygenase,



predominate the basal synthesis and metabolism of eicosanoids in atherosclerotic plaques. These findings provide new insights into the regulation of eicosanoids in atherosclerosis.

832 Yang, S., Wang, B.W., Humphries, F., Jackson, R., Healy, M.E., Bergin, R., Aviello, G., Hall, B., McNamara, D., Darby, T., Quinlan, A., Shanahan, F., Melgar, S., Fallon, P.G., Moynagh, P.N.

Pellino3 ubiquitinates RIP2 and mediates Nod2-induced signaling and protective effects in colitis

Nature Immunology, (2013) 14, 927-+

Mutations that result in loss of function of Nod2, an intracellular receptor for bacterial peptidoglycan, are associated with Crohn's disease. Here we found that the E3 ubiquitin ligase Pellino3 was an important mediator in the Nod2 signaling pathway. Pellino3-deficient mice had less induction of cytokines after engagement of Nod2 and had exacerbated disease in various experimental models of colitis. Furthermore, expression of Pellino3 was lower in the colons of patients with Crohn's disease. Pellino3 directly bound to the kinase RIP2 and catalyzed its ubiquitination. Loss of Pellino3 led to attenuation of Nod2-induced ubiquitination of RIP2 and less activation of the transcription factor NF-kappa B and mitogen-activated protein kinases (MAPKs). Our findings identify RIP2 as a substrate for Pellino3 and Pellino3 as an important mediator in the Nod2 pathway and regulator of intestinal inflammation.

833 Trinath, J., Hegde, P., Sharma, M., Maddur, M.S., Rabin, M., Vallat, J.M., Magy, L., Balaji, K.N., Kaveri, S.V., Bayry, J.

Intravenous immunoglobulin expands regulatory T cells via induction of cyclooxygenase-2-dependent prostaglandin E2 in human dendritic cells

Blood, (2013) 122, 1419-1427

CD4(+)CD25(+)FoxP3(+) regulatory T cells (Tregs) play a critical role in the maintenance of immune tolerance. Intravenous immunoglobulin (IVIg), a therapeutic preparation of normal pooled human IgG, expands Tregs in various experimental models and in patients. However, the cellular and molecular mechanisms by which IVIg expands Tregs are relatively unknown. As Treg expansion in the periphery requires signaling by antigen-presenting cells such as dendritic cells (DCs) and IVIg has been demonstrated to modulate DC functions, we hypothesized that IVIg induces distinct signaling events in DCs that subsequently mediate Treg expansion. We demonstrate that IVIg expands Tregs via induction of cyclooxygenase (COX)-2-dependent prostaglandin E2 (PGE(2)) in human DCs. However, costimulatory molecules of DCs such as programmed death ligands, OX40 ligand, and inducible T-cell costimulator ligands were not implicated. Inhibition of PGE(2) synthesis by COX-2 inhibitors prevented IVIg-mediated Treg expansion in vitro and significantly diminished IVIg-mediated Treg expansion in vivo and protection from disease in experimental autoimmune encephalomyelitis model. IVIg-mediated COX-2 expression, PGE(2) production, and Treg expansion were mediated in part via interaction of IVIg and F(ab'')(2) fragments of IVIg with DC-specific intercellular adhesion molecule-3-grabbing nonintegrin. Our results thus uncover novel cellular and molecular mechanism by which IVIg expands Tregs.

834 Mosenson, J.A., Eby, J.M., Hernandez, C., Le Poole, I.C.

A central role for inducible heat-shock protein 70 in autoimmune vitiligo

Experimental Dermatology, (2013) 22, 566-569

Inducible heat-shock protein 70 (HSP70i) is a protein regulated by stress that protects cells from undergoing apoptosis. Such proteins are marvellously well conserved throughout evolution, which has placed them in the spotlight for helping to understand the intriguing relationship between infection and immunity. In the presence of stress proteins, dendritic cells (DCs) will sense this alarm signal and respond by recruiting immune cells of different plumage to fit the occasion. In times of stress, melanocytes will secrete antigen-bound HSP70i to act as an alarm signal in activating DCs that comes equipped with an address of origin to drive the autoimmune response in vitiligo. Here we pose that if the autoimmune response is funnelled through HSP70i, then blocking the stress protein from activating DCs can lend new treatment opportunities for vitiligo.



- 835 Yu, H., Lee, D.Y.W., Nanjundiah, S.M., Venkatesha, S.H., Berman, B.M., Moudgil, K.D.

Microarray Analysis Reveals the Molecular Basis of Antiarthritic Activity of Huo-Luo-Xiao-Ling Dan

Evidence-Based Complementary and Alternative Medicine, (2013) none, Article Number: 524746 DOI: 10.1155/2013/524746 Published: 2013-Rheumatoid arthritis (RA) is a chronic inflammatory disease of autoimmune origin. Huo-luo-xiao-ling dan (HLXL) is an herbal mixture that has been used in traditional Chinese medicine over several decades to treat chronic inflammatory diseases including RA. However, the mechanism of the anti-arthritic action of this herbal remedy is poorly understood at the molecular level. In this study, we determined by microarray analysis the effects of HLXL on the global gene expression profile of the draining lymph node cells (LNC) in the rat adjuvant arthritis (AA) model of human RA. In LNC restimulated in vitro with the disease-related antigen mycobacterial heat-shock protein 65 (Bhsp65), 84 differentially expressed genes (DEG) (64 upregulated and 20 downregulated) versus 120 DEG (94 upregulated and 26 downregulated) were identified in HLXL-treated versus vehicle (Water)-treated rats, respectively, and 62 DEG (45 upregulated and 17 downregulated) were shared between the two groups. The most affected pathways in response to HLXL treatment included immune response, inflammation, cellular proliferation and apoptosis, and metabolic processes, many of which are directly relevant to arthritis pathogenesis. These results would advance our understanding of the mechanisms underlying the anti-arthritic activity of HLXL.

- 836 Singh, H., Ray, S., Kaur, M., Gupta, V., Kumar, H., Talapatra, P., Mathur, R., Arya, S., Ghangas, N.

Exacerbation of latent lupus: is the culprit acid-fast bacilli or antitubercular therapy?

Clinical Rheumatology, (2013) 32, 1233-1236

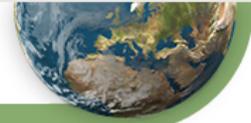
Typical as well as atypical presentations of systemic lupus erythematosus are being increasingly recognized due to improved diagnostic methods. In a tuberculosis-endemic country like India, it was traditionally believed that the occurrence of tuberculosis in lupus was due to the chronic immunosuppression caused by lupus or because of the use of steroids or isoniazid-induced lupus. Increasingly several patients with no recorded predisposition to lupus with a history of treatment for tuberculosis are coming with evidence of systemic lupus erythematosus rather than a drug-limited story. Whether the development of an autoimmune state is a mere conjecture or the presence of acid-fast bacilli in the body for a prolonged duration causes complex antigenic interactions leading to an antigenic response needs to be looked into. We present a report of three such patients and review the pathogenetic interactions that could possibly explain the role of mycobacterial antigens as a putative antigen in the pathogenesis of lupus.

- 837 Banerjee, R., Balaji, M., Sasikala, M., Anuradha, S., Rao, G.V. , Reddy, D.N.

Granulomas of Intestinal Tuberculosis and Crohn's Disease Can Be Differentiated by CD73 Cell Surface Marker Expression: A Pilot Study

Digestive Diseases and Sciences, (2013) 58, 2301-2307

Intestinal tuberculosis (ITB) and Crohn's disease are similar granulomatous disorders. Granulomas are present in both and difficult to differentiate on histopathology alone. A recent study demonstrated recruitment of mesenchymal cells (MSCs) at the periphery of granulomas in lymph node tuberculosis which suppressed T cell responses. We hypothesized that granulomas of ITB would also recruit MSCs to evade host immune response. The purpose of this study was to demonstrate MSC markers in granulomas of ITB and evaluate whether distribution of MSC markers could differentiate between granulomas of Crohn's and ITB. We initially retrospectively enrolled 17 patients with confirmed ITB (8) or Crohn's (9) with granulomas on histopathology. Tissues were evaluated by immunofluorescence for MSC markers CD29, CD90, CD73 and absence of haematopoietic markers CD31, CD34, CD45 and CD14. Double-staining was done to confirm presence of MSCs. Subsequently, 23 postoperative specimens of Crohn's (18) and ITB (5) were analyzed for validation. Overall, 27 Crohn's and 13 ITB cases were assessed. CD29 and CD90 positive cells were noted around both ITB and Crohn's granulomas. MSC marker CD73 was expressed around the granulomas



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of ITB alone and was completely absent in the Crohn's. The subsequent assessment of granulomas in postoperative specimens of Crohn's and ITB also showed similar results. Granulomas of ITB and Crohn's disease can be differentiated by CD73 MSC surface marker expression. The differential CD73 expression around ITB granuloma indicates that Mycobacterium tuberculosis evades host immunity by recruiting MSCs with CD73 expression. MSCs with increased CD73 expression could be the future for therapeutic intervention in Crohn's.

838 Zhang, W., Hui, K.Y., Gusev, A., Warner, N., Ng, S.M.E., Ferguson, J., Choi, M., Burberry, A., Abraham, C., Mayer, L., Desnick, R.J., Cardinale, C.J., Hakonarson, H., Waterman, M., Chowers, Y., Karban, A., Brant, S.R., Silverberg, M.S., Gregersen, P.K., Katz, S., Lifton, R.P., Zhao, H., Nunez, G., Pe'er, I., Peter, I., Cho, J.H.

Extended haplotype association study in Crohn's disease identifies a novel, Ashkenazi Jewish-specific missense mutation in the NF-kappa B pathway gene, HEATR3

Genes and Immunity, (2013) 14, 310-316

The Ashkenazi Jewish population has a several-fold higher prevalence of Crohn's disease (CD) compared with non-Jewish European ancestry populations and has a unique genetic history. Haplotype association is critical to CD etiology in this population, most notably at NOD2, in which three causal, uncommon and conditionally independent NOD2 variants reside on a shared background haplotype. We present an analysis of extended haplotypes that showed significantly greater association to CD in the Ashkenazi Jewish population compared with a non-Jewish population (145 haplotypes and no haplotypes with P-value <10⁻³, respectively). Two haplotype regions, one each on chromosomes 16 and 21, conferred increased disease risk within established CD loci. We performed exome sequencing of 55 Ashkenazi Jewish individuals and follow-up genotyping focused on variants in these two regions. We observed Ashkenazi Jewish-specific nominal association at R755C in TRPM2 on chromosome 21. Within the chromosome 16 region, R642S of HEATR3 and rs9922362 of BRD7 showed genome-wide significance. Expression studies of HEATR3 demonstrated a positive role in NOD2-mediated NF-kappa B signaling. The BRD7 signal showed conditional dependence with only the downstream rare CD-causal variants in NOD2, but not with the background haplotype; this elaborates NOD2 as a key illustration of synthetic association.
