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New publications in the PARATUBERCULOSIS database (1402-1408)

1402 Rosu, V., Bandino, E., Cossu, A.
Unraveling the transcriptional regulatory networks associated to mycobacterial cell wall defective form induction by glycine and lysozyme treatment
Microbiological Research, (2013) 168, 153-164
It is known that a combined glycine/lysozyme treatment is able to induce in vitro the mycobacterial conversion from the bacillary to the cell wall defective forms. These forms also naturally occur in vivo as a response to various antimicrobial factors such as lysozyme released by phagocytic cells. Although they have been successfully isolated from patients with several chronic diseases, their role in pathogenesis is still unknown, mainly due to the difficulties in handling the in vivo isolated variants. Moreover, nothing is known about the transcriptional peculiarities that may exist in comparison to the vegetative phase. Hence, in this study, we simulated in vitro the induction of the mycobacterial cell wall defective state by using a glycine and lysozyme-based treatment in order to identify the gene expression profiles of both pathogenic and non-pathogenic mycobacteria. DNA-microarray results showed that in contrast to the non-pathogenic Mycobacterium smegmatis species, glycine and lysozyme treated forms of Mycobacterium tuberculosis and Mycobacterium avium subspecies paratuberculosis regulated a repertoire of genes usually expressed in vivo during adaptation and persistence within host environments. Results suggest that the cell wall defective state may represent an important stage in the life-cycle of pathogenic mycobacteria that potentially coordinates persistence. (C) 2012 Published by Elsevier GmbH.

1403 Scope, A., Spergser, J., Vobornik, A., Gumpenberger, M., Hooijberg, E.H., Reifinger, M.
Atypical mycobacteriosis in a ural owl (Strix uralensis, PALLAS 1771) from the Austrian reintroduction project
Wiener Tierarztliche Monatsschrift, (2013) 100, 85-92
A ural owl (Strix uralensis) was presented with reduced activity, emaciation and respiratory distress. The bird was severely emaciated and had an enlarged vent with a soft to fluctuant consistency. The differential blood count revealed a severe relative lymphopenia and nnonocytosis as well as a moderate leucocytosis. Radiographs showed increased opacity of the entire coelomic cavity. Ultrasonography revealed a mass with an echogenicity similar to that of liver parenchyma, with multiple cystic inclusions in the abdomen. At necropsy, the soft mass, initially suspected to be a cystic neoplasm of the liver, filled almost the entire body cavity. Histopathology of the structure identified a large blood clot demarcated by a broad band of inflammatory cells and giant cell granulomas from compressed liver tissue. Further granulomas were found in the spleen. Acid fast rods in the necrotic areas of the granulomas were visualized after Ziehl-Neelsen staining. Culture and PCR of liver and spleen demonstrated the presence of M. avium subsp. silvaticum in all samples tested. In all published cases of mycobacteriosis in Strigiformes, classical tubercles were reported. In the present case, radiographs and computed tomography, as well as gross necropsy, initially indicated a cystic neoplasia. The typical changes associated with nnycobacteriosis were only seen in histopathology and were confirmed by microbiology and PCR. M. avium subsp. silvaticum is a pathogen that may occur in immunocompetent hosts, although infection has not yet been reported in humans. Nevertheless, the possibility that the agent has a zoonotic potential cannot be excluded. PDF WILL NOT BE AVAILABLE.

1404 Okura, H., Nielsen, S.S., Toft, N.
Modeling the Effect of Direct and Indirect Contamination of On-Farm Bulk Tank Milk with Mycobacterium avium subsp paratuberculosis
Foodborne Pathogens and Disease , (2013) 10, 270- 277
Mycobacterium avium subsp. paratuberculosis (MAP) in milk of bovine origin is suspected of being implicated in Crohn's disease in humans. Milk can be contaminated via direct excretion of MAP in milk or indirectly via fecal contamination of the milk. This study aimed at estimating the level of MAP in farm bulk tank milk and simulating the effect of direct and indirect contamination with MAP. The effect of discarding milk from test-positive cows at different prevalences was assessed. The concentration of MAP in milk was estimated using a simulation model, while taking direct and indirect contamination with MAP into account. Direct MAP contamination of milk was related to infection stages, while indirect contamination was associated with within-herd prevalence and distribution of cows in different stages of infection. Discarding of milk based on diagnostic test results was included as a control option. Median MAP load in farm bulk tank milk at within-herd infection prevalences from 7.5% to 60% were estimated to be 0.54-7.53 CFU/mL milk. Maximum concentration at the prevalence of 60% could be 1186 CFU/mL caused by shedding of high amounts of MAP in feces. At the

prevalence of 15%, discarding milk from test positive cows would result in discarding 11% of milk and reduce the MAP level by 80%. Due to poor sensitivity of the diagnostic test, removing test-positive cows would not further reduce the already low concentration of MAP and it would not guarantee the milk as MAP-free. The model was relatively simple yet capable of capturing true infection status and associated contributions from milk and feces. Further knowledge on distribution of fecal excretion from infected cows is required because very few "super-shedders" might play a major role.

1405 Cossu, D., Masala, S., Cocco, E., Paccagnini, D., Tranquilli, S. , Frau, J., Marrosu, M.G., Sechi, L.A.
Association of Mycobacterium avium subsp paratuberculosis and SLC11A1 polymorphisms in Sardinian multiple sclerosis patients
Journal of Infection in Developing Countries, (2013) 7, 203-207
Introduction: Recent findings propose that Mycobacterium avium subsp. paratuberculosis (MAP) infection could act as risk factor in favoring multiple sclerosis (MS) progression. SLC11A1 is a gene associated with mycobacterial survival in the host and it may be involved in the induction and maintenance of autoimmune disease. Methodology: In this preliminary study, 100 MS patients and 100 healthy controls (HCs) from Sardinia were enrolled. Eight single nucleotide polymorphisms (SNPs) in the SLC11A gene were searched by PCR RFLP-genotyping. IS900 specie specific PCR was undertaken to search for MAP presence. Indirect ELISA was performed to asses if MS patients displayed a stronger humoral response against MAP2694 protein compared to the HCs. Results: Only rs2276631 SNP was associated with MS. MAP DNA was detected in 23 out of 100 MS patients (23%) and in 7 out of 100 HCs (7%). A strong humoral response against MAP2694 protein was detected in 36% of MS patients and only in 3% of HCs. A correlation between ELISA sero-positivity and the rs2276631 SNP was also found. Conclusion: Our preliminary results suggest that the Sardinian population might be prone to develop autoimmune disease due to polymorphisms in immunomodulating the SLC11A1 gene, which is important in the immune response against intracellular bacteria such as MAP.

1406 Arsenault, R.J., Li, Y., Maattanen, P., Scruten, E., Doig, K., Potter, A., Griebel, P., Kusalik, A., Napper, S.
Altered Toll-Like Receptor 9 Signaling in Mycobacterium avium subsp paratuberculosis-Infected Bovine Monocytes Reveals Potential Therapeutic Targets
Infection and Immunity, (2013) 81, 226-237
Mycobacterium avium subsp. paratuberculosis is the causative agent of Johne's disease in cattle. The complex, multifaceted interaction of M. avium subsp. paratuberculosis with its host includes dampening the ability of infected cells to respond to stimuli that promote M. avium subsp. paratuberculosis clearance. By disrupting host defenses, M. avium subsp. paratuberculosis creates an intracellular environment that favors the establishment and maintenance of infection. Toll-like receptors (TLRs) are important sensors that initiate innate immune responses to microbial challenge and are also immunotherapeutic targets. For example, TLR9 contributes to host defense against M. avium subsp. paratuberculosis, and its agonists (CpG oligodeoxynucleotides [ODNs]) are under investigation for treatment of Johne's disease and other infections. Here we demonstrate that M. avium subsp. paratuberculosis infection changes the responsiveness of bovine monocytes to TLR9 stimulation. M. avium subsp. paratuberculosis inhibits classical TLR9-mediated responses despite a 10-fold increase in TLR9 expression and maintained uptake of CpG ODNs. Other TLR9-mediated responses, such as oxidative burst, which occur through noncanonical signaling, remain functional. Kinome analysis verifies that classic TLR9 signaling is blocked by M. avium subsp. paratuberculosis infection and that signaling instead proceeds through a Pyk2-mediated mechanism. Pyk2-mediated signaling does not hinder infection, as CpG ODNs fail to promote M. avium subsp. paratuberculosis clearance. Indeed, Pyk2 signaling appears to be an important aspect of M. avium subsp. paratuberculosis infection, as Pyk2 inhibitors significantly reduce the number of intracellular M. avium subsp. paratuberculosis bacteria. The actions of M. avium subsp. paratuberculosis on TLR9 signaling may represent a strategy to generate a host environment which is better suited for infection, revealing potential new targets for therapeutic intervention.

1407 Carta, T., Alvarez, J., de la Lastra, J.M.P., Gortazar, C.
Wildlife and paratuberculosis: A review
Research in Veterinary Science, (2013) 94, 191-197
Paratuberculosis (PTB) is an infectious granulomatous enteritis caused by Mycobacterium avium paratuberculosis (MAP) causing significant economic losses in livestock. However, PTB in free-living and captive wildlife has not been as extensively studied as in livestock. We reviewed the existing literature references on MAP to (i) determine the potential impact of MAP infection in wildlife species; (ii) analyze whether wildlife reservoirs are relevant regarding MAP control in domestic ruminants; (iii) assess the importance of MAP as the cause of potential interferences with tuberculosis diagnosis in wildlife. The :mean MAP prevalence reported in wildlife was 2.41% (95% confidence interval 1.76-3.06). Although MAP should be considered an important disease in farmed cervids, its impact on free-ranging species is questionable. MAP reservoirs may exist locally but their significance for PTB control in livestock is quite limited. The most critical aspect derived of MAP infection in wildlife is the interference with tuberculosis diagnosis. (C) 2012 Elsevier Ltd. All rights reserved.

1408 Ponnusamy, D., Periasamy, S., Tripathi, B.N., Pal, A.
Mycobacterium avium subsp paratuberculosis invades through M cells and enterocytes across ileal and jejunal mucosa of lambs
Research in Veterinary Science, (2013) 94, 306-312

Mechanism of Mycobacterium avium subsp. paratuberculosis (Map) invasion through intestinal mucosa is not completely understood. In the present study, we developed an in vivo multiple-intestinal loop model in lambs to investigate (i) the type of cells involved in the bacterial uptake across the intestinal mucosa, (ii) the efficiency of bacterial uptake in different segments of the small intestine and (iii) the ability of different strains of Map to invade the various segments of the small intestine. Four loops on ileum and four loops each on Peyer's patch and non-Peyer's patch areas of jejunum were constructed by surgical procedure. The caprine, bovine, and vaccine strains of Map were used for infection. Map-infected intestinal loop tissues were collected at 1, 3, 6, 12, and 24 h post-infection and processed for electron microscopy, histology, bacterial culture and bacterial counting. All these parameters revealed that Map invaded through M cells and the enterocytes and bacterial translocation across M cells was greater than the enterocytes. Bacterial invasion was greater in ileal loops when compared to jejunal loops. Within the jejunal loops, bacterial uptake was higher in Peyer's patch areas than that of non-Peyer's patch areas. The caprine and bovine strains of Map showed greater ability for invasion into the small intestinal mucosa than that of the vaccine strain. Published by Elsevier Ltd.

1409 Munster, P., Volkel, I., Wemheuer, W., Schwarz, D., Doring, S. , Czerny, C.P.

A Longitudinal Study to Characterize the Distribution Patterns of Mycobacterium avium ssp paratuberculosis in Semen, Blood and Faeces of a Naturally Infected Bull by IS 900 Semi-Nested and Quantitative Real-Time PCR

Transboundary and Emerging Diseases, (2013) 60, 175-187

Johne's disease is caused by Mycobacterium avium ssp. paratuberculosis (MAP) and has been recognized as an important bacterial infection in ruminants. Although MAP has been detected in semen and within the reproductive organs of bulls, the bacterial distribution and shedding patterns are currently not well characterized. Our investigation was performed to detect and quantify MAP in faeces, semen and blood samples repeatedly drawn from a naturally infected but asymptomatic 18-month-old German Simmental breeding bull candidate over a period of 3years (June 2007November 2010). Qualitative and quantitative polymerase chain reaction (PCR) techniques were used to correlate the presence and matrix-specific amounts of MAP. In total, 65 sampling dates were selected. Mycobacterium avium ssp. paratuberculosis was detected intermittently in all matrices with MAP-free intervals of up to 18weeks by an IS900 semi-nested PCR. The number of MAP-positive results from semen and blood samples was higher than from faecal samples. A quantitative polymerase chain reaction detected the highest MAP contents in faeces (103106MAP/g), while lower amounts were found in semen and blood samples (102105MAP/ml). Although no significant agreement was calculated between the presence of MAP in faeces and blood, a statistically significant positive correlation between its occurrence in semen and blood was determined ($r=0.38$, $P<0.05$, $n=29$). The present study contributes to a more detailed understanding of MAP distribution patterns in faeces, semen and blood of a subclinically infected breeding bull candidate. It highlights the possible role of breeding bulls as a source of MAP transmission and indicates the need for further monitoring and hygienic measures to prevent the spread of the infection via semen.

New publications in the CROHN'S DISEASE AND PARATUBERCULOSIS database (790-794)

790 Mancino, A., Habbeddine, M., Johnson, E., Luron, L., Bebien, M., Memet, S., Fong, C., Bajenoff, M., Wu, X.F., Karin, M., Caamano, J., Chi, H.B., Seed, M., Lawrence, T.

I kappa B kinase alpha (IKK alpha) activity is required for functional maturation of dendritic cells and acquired immunity to infection

Embo Journal, (2013) 32, 816-828

Dendritic cells (DC) are required for priming antigen-specific T cells and acquired immunity to many important human pathogens, including Mycobacteriuim tuberculosis (TB) and influenza. However, inappropriate priming of auto-reactive T cells is linked with autoimmune disease. Understanding the molecular mechanisms that regulate the priming and activation of naive T cells is critical for development of new improved vaccines and understanding the pathogenesis of autoimmune diseases. The serine/threonine kinase IKK alpha (CHUK) has previously been shown to have anti-inflammatory activity and inhibit innate immunity. Here, we show that IKK alpha is required in DC for priming antigen-specific Tcells and acquired immunity to the human pathogen Listeria monocytogenes. We describe a new role for IKK alpha in regulation of IRF3 activity and the functional maturation of DC. This presents a unique role for IKK alpha in dampening inflammation while simultaneously promoting adaptive immunity that could have important implications for the development of new vaccine adjuvants and treatment of autoimmune diseases. The EMBO Journal (2013) 32, 816-828. doi:10.1038/emboj.2013.28; Published online 19 February 2013.

791 Li, C.F., Zhou, R.W., Mkhikian, H., Newton, B.L., Yu, Z.X., Demetriou, M.

Hypomorphic MGAT5 polymorphisms promote multiple sclerosis cooperatively with MGAT1 and interleukin-2 and 7 receptor variants

Journal of Neuroimmunology, (2013) 256, 71-76

Deficiency of the Golgi N-glycan branching enzyme Mgat5 in mice promotes T cell hyperactivity, endocytosis of CTLA-4 and autoimmunity, including a spontaneous multiple sclerosis (MS)-like disease. Multiple genetic and environmental MS risk factors lower N-glycan branching in T cells. These include variants in interleukin-2 receptor-alpha (IL2RA), interteukin-7 receptor-alpha (IL7RA), and MGAT1, a Golgi branching enzyme upstream of MGAT5, as well as vitamin D3 deficiency and Golgi substrate metabolism. Here we describe linked intronic variants of MGAT5 that are associated with reduced N-glycan branching, CTLA-4 surface

expression and MS (p = 5.79 x 10(-9), n = 7,741), the latter additive with the MGATI, IL2RA and IL7RA MS risk variants (p = 1.76 x 10-9, OR = 0.67 1.83, n = 3,518). (C) 2013 Elsevier B.V. All rights reserved.

792 Adler, J., Rahal, K., Swanson, S.D., Schmiedlin-Ren, P., Rittershaus, A.C., Reingold, L.J., Brudi, J.S., Shealy, D., Cai, A., McKenna, B.J., Zimmermann, E.M.

Anti-Tumor Necrosis Factor alpha Prevents Bowel Fibrosis Assessed by Messenger RNA, Histology, and Magnetization Transfer MRI in Rats With Crohn's Disease

Inflammatory Bowel Diseases, (2013) 19, 683-690

Objective: Treatment of Crohn's disease (CD) with anti-tumor necrosis factor alpha (TNF alpha) decreases intestinal inflammation, but the effect on fibrosis remains unclear. We hypothesized that treatment with rat-specific anti-TNF alpha will decrease the development of intestinal fibrosis in a rat model of CD. We further hypothesized that magnetization transfer magnetic resonance imaging (MT-MRI) will be sensitive in detecting these differences in collagen content. Methods: Rats were injected in the distal ileum and cecum with peptidoglycan-polysaccharide (PG-PS) or human serum albumin (control) at laparotomy and then received intraperitoneal injections of rat-specific anti-TNF alpha or vehicle daily for 21 days after laparotomy. Rats underwent MT-MRI abdominal imaging on day 19 or 20. MT ratio was calculated in the cecal wall. Cecal tissue histologic inflammation was scored. Cecal tissue procollagen, cytokine, and growth factor messenger RNAs were measured by quantitative real-time PCR. Results: PG-PS-injected rats treated with anti-TNF alpha had less histologic inflammation, and cecal tissue expressed lower levels of proinflammatory cytokine messenger RNAs than vehicle-treated PG-PS-injected rats (IL-1 beta: 5.59 +/- 1.53 versus 10.41 +/- 1.78, P = 0.02; IL-6: 23.23 +/- 9.33 versus 45.89 +/- 11.79, P = 0.07). PG-PS-injected rats treated with anti-TNF alpha developed less intestinal fibrosis than vehicle-treated PG-PS-injected rats by tissue procollagen I (2.87 +/- 0.66 versus 9.28 +/- 1.11; P = 0.00002), procollagen III (2.25 +/- 0.35 versus 7.28 +/- 0.76; P = 0.0000009), and MT-MRI (MT ratio: 17.79 +/- 1.61 versus 27.95 +/- 1.75; P = 0.0001). Insulin-like growth factor I (2.52 +/- 0.44 versus 5.14 +/- 0.60; P = 0.0007) and transforming growth factor beta 1 (2.34 +/- 0.29 versus 3.45 +/- 0.29; P = 0.006) were also decreased in anti-TNF alpha-treated PG-PS-injected rats. Conclusions: Anti-TNF alpha prevents the development of bowel wall inflammation and fibrosis in the PG-PS rat model of CD. MT-MRI measurably demonstrates this decrease in intestinal fibrosis. (Inflamm Bowel Dis 2013;19:683-690).

793 Heul, A.M.V., Fowler, C.A., Ramaswamy, S., Piper, R.C.

Ubiquitin Regulates Caspase Recruitment Domain-mediated Signaling by Nucleotide-binding Oligomerization Domain-containing Proteins NOD1 and NOD2

Journal of Biological Chemistry, (2013) 288, 6890-6902

NOD1 and NOD2 (nucleotide-binding oligomerization domain-containing proteins) are intracellular pattern recognition receptors that activate inflammation and autophagy. These pathways rely on the caspase recruitment domains (CARDs) within the receptors, which serve as protein interaction platforms that coordinately regulate immune signaling. We show that NOD1 CARD binds ubiquitin (Ub), in addition to directly binding its downstream targets receptor-interacting protein kinase 2 (RIP2) and autophagy-related protein 16-1 (ATG16L1). NMR spectroscopy and structure-guided mutagenesis identified a small hydrophobic surface of NOD1 CARD that binds Ub. In vitro, Ub competes with RIP2 for association with NOD1 CARD. In vivo, we found that the ligand-stimulated activity of NOD1 with a mutant CARD lacking Ub binding but retaining ATG16L1 and RIP2 binding is increased relative to wild-type NOD1. Likewise, point mutations in the tandem NOD2 CARDs at positions analogous to the surface residues defining the Ub interface on NOD1 resulted in loss of Ub binding and increased ligand-stimulated NOD2 signaling. These data suggest that Ub binding provides a negative feedback loop upon NOD-dependent activation of RIP2.

794 Hedl, M., Abraham, C.

NLRP1 and NLRP3 inflammasomes are essential for distinct outcomes of decreased cytokines but enhanced bacterial killing upon chronic Nod2 stimulation

American Journal of Physiology-Gastrointestinal and Liver Physiology, (2013) 304, G583-G596

Upon chronic microbial exposure and pattern-recognition receptor (PRR) stimulation, myeloid-derived cells undergo a distinct transcriptional program relative to acute PRR stimulation, with proinflammatory pathways being downregulated. However, other host-response pathways might be differentially regulated, and this concept has been relatively unexplored. Understanding mechanisms regulating chronic microbial exposure outcomes is important for conditions of ongoing infection or at mucosal surfaces, such as the intestine. The intracellular PRR nucleotide oligomerization domain 2 (Nod2) confers the highest genetic risk toward developing Crohn's disease (CD). We previously identified mechanisms mediating downregulation of proinflammatory pathways upon chronic Nod2 stimulation; here we sought to define how chronic Nod2 stimulation regulates bacterial killing. We find that, despite downregulating cytokine secretion upon restimulation through PRR and live bacteria, chronic Nod2 stimulation of human monocyte-derived macrophages enhances bacterial killing; this dual regulation is absent in CD Nod2-risk carriers. We show that chronic Nod2-mediated reprogramming of human monocyte-derived macrophages to a state of enhanced bacterial killing requires upregulated reactive oxygen/nitrogen species pathway function through increased p67phox/p47phox/nitric oxide synthase-2 expression; selectively knocking down each of these genes reverses the enhanced bacterial killing. Importantly, we find that, during chronic Nod2 stimulation, NLRP3/NLRP1 inflammasome-mediated caspase-1 activation with subsequent IL-1 secretion is essential for the subsequent bifurcation to downregulated proinflammatory cytokines and upregulated bacterial killing. Therefore, we identify mechanisms mediating the distinct inflammatory and microbicidal outcomes upon chronic stimulation of the CD-associated protein Nod2.