

2013-05-01-038 Paratuberculosis databases updated (2013-04-26)

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New publications in the PARATUBERCULOSIS database (1410-1417)

1410 Stewart, L.D., McNair, J., McCallan, L., Gordon, A., Grant, I.R.

Improved Detection of Mycobacterium bovis Infection in Bovine Lymph Node Tissue Using Immunomagnetic Separation (IMS)-Based Methods

Plos One, (2013) 8, Article Number: e58374 DOI: 10.1371/journal.pone.0058374
Published: MAR 4 2013-Immunomagnetic separation (IMS) can selectively isolate and concentrate Mycobacterium bovis cells from lymph node tissue to facilitate subsequent detection by PCR (IMS-PCR) or culture (IMS-MGIT). This study describes application of these novel IMS-based methods to test for M. bovis in a survey of 280 bovine lymph nodes (206 visibly lesioned (VL), 74 non-visibly lesioned (NVL)) collected at slaughter as part of the Northern Ireland bovine TB eradication programme. Their performance was evaluated relative to culture. Overall, 174 (62.1%) lymph node samples tested positive by culture, 162 (57.8%) by IMS-PCR (targeting IS6110), and 191 (68.2%) by IMS-MGIT culture. Twelve (6.9%) of the 174 culture positive lymph node samples were not detected by either of the IMS-based methods. However, an additional 79 M. bovis positive lymph node samples (27 (13.1%) VL and 52 (70.3%) NVL) were detected by the IMS-based methods and not by culture. When low numbers of viable M. bovis are present in lymph nodes (e. g. in NVLs of skin test reactor cattle) decontamination prior to culture may adversely affect viability, leading to false negative culture results. In contrast, IMS specifically captures whole M. bovis cells (live, dead or potentially dormant) which are not subject to any deleterious treatment before detection by PCR or MGIT culture. During this study only 2.7% of NVL lymph nodes tested culture positive, whereas 70.3% of the same samples tested M. bovis positive by the IMS-based tests. Results clearly demonstrate that not only are the IMS-based methods more rapid but they have greater detection sensitivity than the culture approach currently used for the detection of M. bovis infection in cattle. Adoption of the IMS-based methods for lymph node testing would have the potential to improve M. bovis detection in clinical samples .

1411 Frolkis, A., Dieleman, L.A., Barkema, H.W., Panaccione, R., Ghosh, S., Fedorak, R.N., Madsen, K., Kaplan, G.G.

Environment and the inflammatory bowel diseases

Canadian Journal of Gastroenterology, (2013) 27, E18-E24
Inflammatory bowel diseases (IBD), which consists of Crohn disease and ulcerative colitis, are chronic inflammatory conditions of the gastrointestinal tract. In genetically susceptible individuals, the interaction between environmental factors and normal intestinal commensal flora is believed to lead to an inappropriate immune response that results in chronic inflammation. The incidence of IBD have increased in the past century in developed and developing countries. The purpose of the present review is to summarize the current knowledge of the association between environmental risk factors and IBD. A number of environmental risk factors were investigated including smoking, hygiene, microorganisms, oral contraceptives, antibiotics, diet, breast-feeding, geographical factors, pollution and stress. Inconsistent findings among the studies highlight the complex pathogenesis of IBD. Additional studies are necessary to identify and elucidate the role of environmental factors in IBD etiology.



1412 Weigoldt, M., Meens, J., Bange, F.C., Pich, A., Gerlach, G.F., Goethe, R.

Metabolic adaptation of *Mycobacterium avium* subsp *paratuberculosis* to the gut environment

Microbiology-Sgm, (2013) 159, 380-391

Knowledge on the proteome level about the adaptation of pathogenic mycobacteria to the environment in their natural hosts is limited. *Mycobacterium avium* subsp. *paratuberculosis* (MAP) causes Johne's disease, a chronic and incurable granulomatous enteritis of ruminants, and has been suggested to be a putative aetiological agent of Crohn's disease in humans. Using a comprehensive LC-MS-MS and 2D difference gel electrophoresis (DIGE) approach, we compared the protein profiles of clinical strains of MAP prepared from the gastrointestinal tract of diseased cows with the protein profiles of the same strains after they were grown in vitro. LC-MS-MS analyses revealed that the principal enzymes for the central carbon metabolic pathways, including glycolysis, gluconeogenesis, the tricarboxylic acid cycle and the pentose phosphate pathway, were present under both conditions. Moreover, a broad spectrum of enzymes for beta-oxidation of lipids, nine of which have been shown to be necessary for mycobacterial growth on cholesterol, were detected in vivo and in vitro. Using 2D-DIGE we found increased levels of several key enzymes that indicated adaptation of MAP to the host. Among these, FadE5, FadE25 and AdhB indicated that cholesterol is used as a carbon source in the bovine intestinal mucosa; the respiratory enzymes AtpA, NuoG and SdhA suggested increased respiration during infection. Furthermore higher levels of the pentose phosphate pathway enzymes Gnd2, Zwf and Tat as well as of KatG, SodA and GroEL indicated a vigorous stress response of MAP in vivo. In conclusion, our results provide novel insights into the metabolic adaptation of a pathogenic mycobacterium in its natural host.

1413 Bull, T.J., Schock, A., Sharp, J.M., Greene, M., McKendrick, I.J., Sales, J., Linedale, R., Stevenson, K.

Genomic variations associated with attenuation in *Mycobacterium avium* subsp *paratuberculosis* vaccine strains

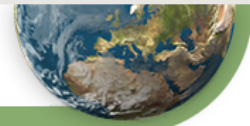
Bmc Microbiology, (2013) 13, Article Number: 11 DOI: 10.1186/1471-2180-13-11

Published: JAN 22 2013-Background: *Mycobacterium avium* subspecies *paratuberculosis* (MAP) whole cell vaccines have been widely used tools in the control of Johne's disease in animals despite being unable to provide complete protection. Current vaccine strains derive from stocks created many decades ago; however their genotypes, underlying mechanisms and relative degree of their attenuation are largely unknown. Results: Using mouse virulence studies we confirm that MAP vaccine strains 316 F, II and 2e have diverse but clearly attenuated survival and persistence characteristics compared with wild type strains. Using a pan genomic microarray we characterise the genomic variations in a panel of vaccine strains sourced from stocks spanning over 40 years of maintenance. We describe multiple genomic variations specific for individual vaccine stocks in both deletion (26-32 Kbp) and tandem duplicated (11-40 Kbp) large variable genomic islands and insertion sequence copy numbers. We show individual differences suitable for diagnostic differentiation between vaccine and wild type genotypes and provide evidence for functionality of some of the deleted MAP-specific genes and their possible relation to attenuation. Conclusions: This study shows how culture environments have influenced MAP genome diversity resulting in large tandem genomic duplications, deletions and transposable element activity. In combination with classical selective systematic subculture this has led to fixation of specific MAP genomic alterations in some vaccine strain lineages which link the resulting attenuated phenotypes with deficiencies in high reactive oxygen species handling.

1414 Ng, S.C., Bernstein, C.N., Vatn, M.H., Lakatos, P.L., Loftus Jr, E.V., Tysk, C., O'Morain, C., Moun, B., Colombel, J.F.

Geographical variability and environmental risk factors in inflammatory bowel disease Gut, (2013) 62, 630-649

The changing epidemiology of inflammatory bowel disease (IBD) across time and geography suggests that environmental factors play a major role in modifying disease expression.



Disease emergence in developing nations suggests that epidemiological evolution is related to westernisation of lifestyle and industrialisation. The strongest environmental associations identified are cigarette smoking and appendectomy, although neither alone explains the variation in incidence of IBD worldwide. Urbanisation of societies, associated with changes in diet, antibiotic use, hygiene status, microbial exposures and pollution have been implicated as potential environmental risk factors for IBD. Changes in socioeconomic status might occur differently in different geographical areas and populations and, consequently, it is important to consider the heterogeneity of risk factors applicable to the individual patient. Environmental risk factors of individual, familial, community-based, country-based and regionally based origin may all contribute to the pathogenesis of IBD. The geographical variation of IBD provides clues for researchers to investigate possible environmental aetiological factors. The present review aims to provide an update of the literature exploring geographical variability in IBD and to explore the environmental risk factors that may account for this variability. PDF WILL NOT BE AVAILABLE.

1415 Gimenez, G., Belaunzaran, M.L., Poncini, C.V., Blanco, F.C., Echaide, I., Zamorano, P.I., Lammel, E.M., Cappa, S.M.G., Isola, E.L.D.

Babesia bovis: lipids from virulent S2P and attenuated R1A strains trigger differential signalling and inflammatory responses in bovine macrophages

Parasitology, (2013) 140, 530-540

The intra-erythrocytic protozoan *Babesia bovis* is an economically important pathogen that causes an acute and often fatal infection in adult cattle. Babesiosis limitation depends on the early activation of macrophages, essential cells of the host innate immunity, which can generate an inflammatory response mediated by cytokines and nitric oxide (NO). Herein, we demonstrate in bovine macrophages that lipids from *B. bovis* attenuated R1A strain (LA) produced a stronger NO release, an early TNF alpha mRNA induction and 2-fold higher IL-12p35 mRNA levels compared to the lipids of virulent S2P strain (L-V). Neither L-A nor LV induced anti-inflammatory IL-10. Regarding signalling pathways, we here report that L-A induced a significant phosphorylation of p38 and extracellular signal-regulated kinases 1 and 2 (ERK1/2) whereas L-V only induced a reduced activation of ERK1/2. Besides, NF-kappa B was activated by L-A and L-V, but L-A produced an early degradation of the inhibitor I kappa B. Interestingly, L-V and the majority of its lipid fractions, exerted a significant inhibition of concanavalin A-induced peripheral blood mononuclear cell proliferation with respect to L-A and its corresponding lipid fractions. In addition, we determined that animals infected with R1A developed a higher increase in IgM anti-phosphatidylcholine than those inoculated with S2P. Collectively, S2P lipids generated a decreased inflammatory response contributing to the evasion of innate immunity. Moreover, since R1A lipids induced a pro-inflammatory profile, we propose these molecules as good candidates for immunoprophylactic strategies against babesiosis.

1416 Zhang, W.X., Zhang, G.W., Peng, J., Zhang, J.L., Yang, Y., Lai, S.J.

A synonymous mutation in NOD2 gene was significantly associated with non-specific digestive disorder in rabbit

Gene, (2013) 516, 193-197

Nucleotide-binding oligomerization domain containing 2 (NOD2) plays a pivotal role in the host innate and adaptive immunity by recognizing the pathogenic agents. Therefore, its genetic polymorphisms and association with susceptibility to infectious diseases have been widely reported in human and farm animals. In the present study, we investigated the genetic polymorphisms in 3171 bp coding region of NOD2 gene and association with non-specific digestive disorder (NSDD) in rabbit. A total of four coding single-nucleotide polymorphisms (cSNPs) were detected. Among them, c.2961C>T was further genotyped for case (n=176) and control (n=130) based on association analysis, which revealed that C allele carried the potential protective role for susceptibility to NSDD with the odds ratio (OR) values of 0.52 (95% confidence interval (CI) 0.37-0.73, P<0.01). Under the dominant inheritance model, CC genotype was associated with decreased susceptibility to NSDD (OR = 0.38, 95% CI 0.24-0.60, P<0.01). Along with the aggravation of NSDD, we observed higher mRNA expression of NOD2 gene (P<0.05). However, the mRNA expression pattern of CC genotype would be



interacted by the different status of NSDD, which only showed the significantly increased level in severe NSDD group ($P < 0.05$). These results revealed by genetic association and gene expression analysis suggested that the NOD2 gene was associated with the susceptibility to NSDD in rabbit. However, the causative mutations linked to c.2961C>T and corresponding functional depiction should be further explored by performing exhaustive genetic studies. (C) 2013 Elsevier B.V. All rights reserved.

1417 Munster, P., Fechner, K., Volkel, I., von Buchholz, A., Czerny, C.P.

Distribution of *Mycobacterium avium* ssp. *paratuberculosis* in a German zoological garden determined by IS900 semi-nested and quantitative real-time PCR

Veterinary Microbiology, (2013) 163, 116-123

Little data concerning the distribution of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) in zoological gardens is available. The presence of MAP in captured wildlife might provide further information on non-ruminant hosts and expand the list of animals susceptible to MAP being potential sources of MAP transmission. Therefore, a German zoological garden with recent history of clinical paratuberculosis in Barbary sheep (*Ammodramus leucurus*) and an alpaca (*Lama pacos*) was selected to estimate the distribution of MAP infections in 21 mammalian and avian species. Pooled faecal samples from individual animals of each species were tested for the presence of MAP. A previously developed 15900 semi-nested PCR (snPCR) assay, amplifying a 587 bp and a 278 bp fragment, was used for the detection of MAP-DNA. Based on this snPCR, in 14 out of the 21 pooled faecal samples MAP-DNA was detected. MAP positive snPCR results were observed in ruminants and camelids as well as in non-ruminants such as equines, primates, rodents, and birds. Moreover, a quantitative real-time PCR demonstrated that the concentration of MAP-DNA was within the range of $2.2 \times 10(3)$ - $9.6 \times 10(6)$ MAP-DNA equivalents per gram faeces. The highest amount was shed by primates such as Black-and-white ruffed lemurs (*Varecia variegata*) and Cottontop tamarins (*Saguinus oedipus*). This is the first survey investigating the presence of MAP in a German zoo, which includes non-ruminants. The results of the present study confirm the wide host range of MAP and demonstrate that MAP occurs more frequently in zoo animals than expected. In order to restrict further spread of MAP in European zoos, additional investigations regarding the existing transmission pathways of MAP in zoos are recommended. (C) 2013 Elsevier B.V. All rights reserved.

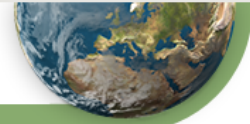
New publications in the [CROHN'S DISEASE AND PARATUBERCULOSIS database](#) (795-800)

795 Jones, S.A., Mills, K.H.G., Harris, J.

Autophagy and inflammatory diseases

Immunology and Cell Biology, (2013) 91, 250-258

Autophagy is a cellular mechanism for the sequestration and degradation of intracellular pathogens and compromised organelles, particularly damaged mitochondria. Autophagy also clears other cellular components, such as inflammasomes and cytokines, thus providing an important means of regulating inflammation. Defects in autophagy have been found by genetic association studies to confer susceptibility to several autoimmune and inflammatory disorders, particularly inflammatory bowel disease. Thus, the manipulation of autophagy in disease situations is of growing interest for therapeutic targeting; however, the involvement of autophagy in cellular homeostasis, in normal immune function and in inflammation is manifold. An appreciation of the intricacies of the contributions of this process to inflammation, and how these are altered by various immune and environmental stimuli, is essential for the understanding and interpretation of studies of inflammation and the design of therapeutics exploiting the manipulation of autophagy. This review focuses on the known roles of autophagy in the induction and maintenance of inflammation and on its role in the aetiology and regulation of inflammatory and autoimmune disorders. Immunology and Cell Biology (2013) 91, 250-258; doi:10.1038/icb.2012.82; published online 15 January 2013.



796 Kidder, D., Richards, H.E., Ziltener, H.J., Garden, O.A., Crocker, P.R.

Sialoadhesin Ligand Expression Identifies a Subset of CD4(+) Foxp3(-) T Cells with a Distinct Activation and Glycosylation Profile

Journal of Immunology, (2013) 190, 2593-2602

Sialoadhesin (Sn) is a sialic acid-binding Ig-like lectin expressed selectively on macrophage subsets. In a model of experimental autoimmune encephalomyelitis, Sn interacted with sialylated ligands expressed selectively on CD4(+) Foxp3(+) regulatory T cells (Tregs) and inhibited their proliferation. In this study, we examined the induction of Sn ligands (SnL) on all splenic CD4(+) T cells following in vitro activation. Most CD4(+) Tregs strongly upregulated SnL, whereas only a small subset of similar to 20% CD4(+) Foxp3(-) T cells (effector T cells [Teffs]) upregulated SnL. SnL+ Teffs displayed higher levels of activation markers CD25 and CD69, exhibited increased proliferation, and produced higher amounts of IL-2 and IFN-gamma than corresponding SnL- Teffs. Coculture of activated Teffs with Sn+ macrophages or Sn+ Chinese hamster ovary cells resulted in increased cell death, suggesting a regulatory role for Sn-SnL interactions. The key importance of alpha 2,3-sialylation in SnL expression was demonstrated by increased binding of alpha 2,3-linkage-specific Maackia amurensis lectin, increased expression of alpha 2,3-sialyltransferase ST3GalVI, and loss of SnL following treatment with an alpha 2,3-linkage-specific sialidase. The induction of SnL on activated CD4(+) T cells was dependent on N-glycan rather than O-glycan biosynthesis and independent of the mucin-like molecules CD43 and P-selectin glycoprotein ligand-1, previously implicated in Sn interactions. Induction of ligands on CD4(+) Foxp3(-) Teffs was also observed in vivo using the New Zealand Black 3 New Zealand White F1 murine model of spontaneous lupus and SnL levels on Teffs correlated strongly with the degree of proteinuria. Collectively, these data indicate that SnL is a novel marker of activated CD4(+) Teffs that are implicated in the pathogenesis of autoimmune diseases. The Journal of Immunology, 2013, 190: 2593-2602.

797 Jamontt, J., Petit, S., Clark, N., Parkinson, S.J., Smith, P.

Nucleotide-Binding Oligomerization Domain 2 Signaling Promotes Hyperresponsive Macrophages and Colitis in IL-10-Deficient Mice

Journal of Immunology, (2013) 190, 2948-2958

IL-10 contributes to the maintenance of intestinal homeostasis via the regulation of inflammatory responses to enteric bacteria. Loss of IL-10 signaling results in spontaneous colitis in mice and early onset enterocolitis in humans. Nucleotide-binding oligomerization domain (NOD) 2 is an intracellular receptor of bacterial peptidoglycan products, and, although NOD2 mutations are associated with Crohn's disease, the precise role of NOD2 in the development of intestinal inflammation remains undefined. To determine the role of NOD2 in the development of colitis on the clinically relevant genetic background of IL-10-deficient signaling, we generated mice lacking IL-10 and NOD2 (IL-10(-/-)NOD2(-/-)). Loss of NOD2 in IL-10(-/-) mice resulted in significant amelioration of chronic colitis, indicating that NOD2 signaling promotes the development of intestinal inflammation in IL-10(-/-) mice. Contrary to previous reports investigating immune function in NOD2(-/-) mice, T cell proliferative capacity and IL-2 production were not impaired, and immune polarization toward type 1 immunity was not affected. However, loss of NOD2 in IL-10-deficient macrophages reduced IL-6, TNF-alpha, and IL-12p40 production in response to bacterial stimulation. Further analysis of the intrinsic macrophage response before the onset of inflammation revealed that, in the absence of IL-10, synergistic signaling between various TLRs and NOD2 resulted in hyperresponsive, proinflammatory macrophages, thus providing the appropriate immune environment for the development of colitis. Data presented in this study demonstrate that NOD2 signaling contributes to intestinal inflammation that arises through loss of IL-10 and provides mechanistic insight into the development of colitis in inflammatory bowel disease patients with impaired IL-10 signaling. The Journal of Immunology, 2013, 190: 2948-2958.

798 Park, S., Ha, S.D., Coleman, M., Meshkibaf, S., Kim, S.O.

p62/SQSTM1 Enhances NOD2-Mediated Signaling and Cytokine Production through Stabilizing NOD2 Oligomerization

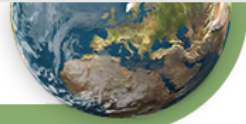


Plos One, (2013) 8, Article Number: e57138 DOI: 10.1371/journal.pone.0057138
Published: FEB 20 2013-NOD2 is a cytosolic pattern-recognition receptor that senses muramyl dipeptide of peptidoglycan that constitutes the bacterial cell wall, and plays an important role in maintaining immunological homeostasis in the intestine. To date, multiple molecules have shown to be involved in regulating NOD2 signaling cascades. p62 (sequestosome-1; SQSTM1) is a multifaceted scaffolding protein involved in trafficking molecules to autophagy, and regulating signal cascades activated by Toll-like receptors, inflammasomes and several cytokine receptors. Here, we show that p62 positively regulates NOD2-induced NF-kappa B activation and p38 MAPK, and subsequent production of cytokines IL-1 beta and TNF-alpha. p62 associated with the nucleotide binding domain of NOD2 through a bi-directional interaction mediated by either TRAF6-binding or ubiquitin-associated domains. NOD2 formed a large complex with p62 in an electron-dense area of the cytoplasm, which increased its signaling cascade likely through preventing its degradation. This study for the first time demonstrates a novel role of p62 in enhancing NOD2 signaling effects.

- 799 Wang, N., Huang, C., Hasegawa, M., Inohara, N., Fujimoto, Y., Fukase, K.
Glycan Sequence-Dependent Nod2 Activation Investigated by Using a Chemically Synthesized Bacterial Peptidoglycan Fragment Library
Chembiochem, (2013) 14, 482 -488
Nucleotide oligomerization domain-containing protein 2 (Nod2), an innate immune receptor, recognizes bacterial cell-wall peptidoglycan (PGN), the minimum ligand of which is muramyl dipeptide (MDP). Enzymatic digestion of PGN appears to be important for Nod2 recognition. PGN is degraded by muramidase or glucosamidase through a process that produces two types of glycan sequence; glycans containing GlcNAc(14)MurNAc or MurNAc(14)GlcNAc. In this report, a range of disaccharide or tetrasaccharide fragments of each sequence were chemically synthesized, and their activities in stimulating human Nod2 (hNod2) were investigated. The results reveal that hNod2 recognitions is dependent on the glycan sequence, as demonstrated by comparing the activities of glycans with the same peptide moieties. (MurNAc(14)GlcNAc)₂-containing structures exhibited stronger activity than those containing (GlcNAc(14)MurNAc)₂. The results suggest that differences in the enzymatic degradation process affect the host's immunomodulation process.
- 800 Vasseur, F., Sendid, B., Broly, F., Gower-Rousseau, C., Sarazin, A., Standaert-Vitse, A., Colombel, J.F., Poulain, D., Jouault, T.

The CARD8 p.C10X mutation associates with a low anti-glycans antibody response in patients with Crohn's disease

Bmc Medical Genetics, (2013) 14, Background: Crohn's disease (CD) is associated with elevated anti-glycans antibody response in 60% of CD patients, and 25% of healthy first-degree relatives (HFDRs), suggesting a genetic influence for this humoral response. In mice, anti-glucan antibody response depends on the NLRP3 inflammasome. Here, we explored the effect of mutated CARD8, a component of the inflammasome, on anti-glycans antibody response in human. Methods: The association between p.C10X mutation (rs2043211) of the CARD8 gene and the levels of anti-glycans antibody response was examined in 39 CD families. The family-based QTDT association test was used to test for the genetic association between CARD8 p.C10X mutation and anti-glycan antibodies in the pedigrees. The difference in antibody responses determined by ELISA was tested in a subgroup of CD probands (one per family) and in a subgroup of HFDRs using the Wilcoxon Kruskal Wallis non-parametric test. Results: The QTDT familial transmission tests showed that the p.C10X mutation of CARD8 was significantly associated with lower levels of antibody to mannans and glucans but not chitin ($p=0.024$, $p=0.0028$ and $p=0.577$, for ASCA, ALCA and ACCA, respectively). These associations were independent of NOD2 and NOD1 genetic backgrounds. The p.C10X mutation significantly associated or displayed a trend toward lower ASCA and ALCA levels ($p=0.038$ and $p=0.08$, respectively) only in the subgroup of CD probands. Such associations were not significant for ACCA levels in both subgroups of CD probands and of HFDRs. Conclusion: Our results show that ASCA and ALCA but not ACCA levels are under the influence of CARD8 genotype. Alteration of CARD8, a component of inflammasome, is



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associated with lower levels of antibodies directed to mannans and glucans at least in CD patients.
