The MAP Gap

Bridging The Space Between The Science And You

Human Paratuberculosis Foundation Quarterly Newsletter Fall 2021

Human Para News

While the global COVID pandemic continues to provide innovations in medical research in certain sectors, MAP research projects are also beginning again. At long last, our researchers have finally been given clearance to proceed with **WGS testing** of the 40 MAP isolates selected from the **MAP/Crohn's Testing Study.** Testing will be completed by the end of the summer, and we hope to have the analysis and final results published in early 2022. A big thanks to our dedicated MAP researchers for their patience and perseverance! Stay tuned for updates.

Our **third MAP study** with Dr. Nicole Parrish of Johns Hopkins (investigating Antibiotic Susceptibility of Different MAP Strains) is well underway. The project is expected to be completed in January 2022, with results published later that year.

Human Para is in the planning stages for our next research project. More details to come! We are also hoping to host or participate in another conference when travel and large gatherings are permitted.

If you haven't visited us recently, we invite you to check out **HumanPara.org**. There are many excellent resources for patients and doctors, and we are constantly adding updated articles and features! You can also find real time information on our updated **Facebook page**❖

INSIDE THIS ISSUE

Research Corner

Below please find the abstracts for the most relevant research publications on MAP and mycobacteria, including hyperlinks to the full article. While this is not an exhaustive list, we thought these articles were the most relevant to our community this quarter.

A Mycobacterium species for Crohn's disease? (June 2021)

In ruminants Mycobacterium avium subspecies paratuberculosis (MAP) is the causative organism of a chronic granulomatous inflammatory bowel disease called Johne's disease (JD). Some researchers have hypothesised that MAP is also associated with Crohn's disease (CD), an inflammatory bowel disease in humans that shares some histological features of JD. Despite numerous attempts to demonstrate causality by researchers, direct microbiological evidence of MAP involvement in CD remains elusive. Importantly, it has not been possible to reliably and reproducibly demonstrate mycobacteria in the tissue of CD patients. Past attempts to visualise mycobacteria in tissue may have been hampered by the use of stains optimised for Mycobacterium tuberculosis complex (MTB) and the lack of reliable bacteriological culture media for both non-tuberculous mycobacteria (NTM) and cell-wall-deficient mycobacteria (CWDM). Here we describe a Ziehl-Neelsen (ZN) staining method for the demonstration of CWDM in

resected tissue from patients with Crohn's disease, revealing the association of CWDM in situ with host tissue reactions, and posit this as a cause of the tissue inflammation. Using the ZN stain described we demonstrated the presence of CWDM in 18 out of 18 excised tissue samples from patients diagnosed as having Crohn's disease, and in zero samples out of 15 non-inflammatory bowel disease controls.
Aitken JM et al. Pathology. 2021 Jun 19:S0031-3025(21) 00234-8.❖

Warm, Sweetened Milk at the Twilight of Immunity- Alzheimer's Disease -Inflammaging, Insulin Resistance, *M. paratuberculosis* and Immunosenescence (Aug. 2021)

This article prosecutes a case against the zoonotic pathogen *Mycobacterium avium* ss. *paratuberculosis* (MAP) as a precipitant of Alzheimer's disease (AD). Like the other major neurodegenerative diseases AD is, at its core, a proteinopathy. Aggregated extracellular amyloid protein plaques and intracellular tau protein tangles are the recognized protein pathologies of AD. Autophagy is the cellular housekeeping process that manages protein quality control and recycling, cellular metabolism, and pathogen elimination. Impaired autophagy and cerebral insulin resistance are invariant features of AD. With a backdrop of age-related low-grade inflammation (inflammaging) and heightened immune risk (immunosenescence), infection with MAP subverts glucose metabolism and further exhausts an already exhausted autophagic capacity. Increasingly, a variety of agents have been found to favorably impact AD; they are agents that promote autophagy and reduce insulin resistance. The potpourri of these therapeutic agents: mTOR inhibitors, SIRT1 activators and vaccines are seemingly random until one recognizes that all these agents also suppress intracellular mycobacterial infection. The zoonotic mycobacterial MAP causes a common fatal enteritis in ruminant animals. Humans are exposed to MAP from contaminated food products and from the environment. The enteritis in animals is called paratuberculosis or Johne's disease; in humans, it is the putative cause of Crohn's disease. Beyond Crohn's, MAP is associated with an increasing number of inflammatory and autoimmune diseases: sarcoidosis, Blau syndrome, autoimmune diabetes, autoimmune thyroiditis, multiple sclerosis, and rheumatoid arthritis. Moreover, MAP has been associated with Parkinson's disease. India is one county that has extensively studied the human bio-load of MAP; 30% of more than 28,000 tested individuals were found to harbor, or to have harbored, MAP. This article asserts an unfolding realization that MAP infection of humans 1) is widespread in its presence, 2) is wide-ranging in its zoonosis and 3) provides a plausible link connecting MAP to AD.
Dow CT. Front. Immunol. 12:714179.❖

The Diagnosis of Mycobacterium avium Infections Using Serodiagnosis With Novel Lipid Antigens (April 2021)

Mycobacterium avium subspecies Paratuberculosis (MAP) is an endemic pathogen in ruminants, present in a high proportion of herds worldwide. Its presence within cattle herds creates an economic burden on both farmers and the wider economy; due to lost milk production and premature culling. Currently there is a lack of sensitive and rapid detection techniques, as culture can take months to give results and traditional PCR cannot distinguish viable from non-viable cells. Tests utilising defined synthetic mycolic acids and their sugar esters have already shown promise at diagnosing tuberculosis. This will form the basis of work described here, translating those procedures to the detection of MAP using both ELISA and a flow through device. MAP has also been shown to survive pasteurisation, thus making it into the food chain, and has been proposed as an aetiological agent for the development of Crohn's disease.
• First, a study of strongly positive experimentally infected cattle samples against negative serum from a herd with no history of MAP resulted in a single antigen sensitivity/specificity of 100/100.
• Second, a study of 40 negative and 40 positive, naturally infected cattle samples from Canada resulted in a sensitivity/specificity of 85/75. Combined with the first study and utilising all 5 common antigens for diagnosis resulted in a sensitivity/specificity of 84/93
• Initial testing and translation of flow through procedures from M. tb to MAP with a pooled cattle sample resulted in defined red spots, with the control remaining clear.
• MAP specific antigens tested against human Crohn's samples as compared with healthy samples resulted in a single antigen sensitivity/specificity of 91/100
This work has identified promising antigens for further large-scale testing against both MAP in cattle and Crohn's disease. Additionally, the first test of a flow through device shows promise in developing a rapid point of care device for the detection of MAP.
Mason, Paul (2021) Masters thesis, Durham University..❖

Hyperbaric oxygen therapy in inflammatory bowel disease: a systematic review and meta-analysis. (April 2021)

Background: Translational data suggest a potential role of hyperbaric oxygen therapy (HBOT) in a subset of patients

with inflammatory bowel disease (IBD). We performed a systematic review and meta-analysis for the efficacy and safety of HBOT in IBD.
Methods: We searched Pubmed, Embase and CENTRAL to identify studies reporting the efficacy of HBOT in ulcerative colitis or Crohn's disease. We pooled the response rates for HBOT in ulcerative colitis and Crohn's disease separately.
Results: A total 18 studies were included in the systematic review and 16 in the analysis. The overall response rate of HBOT in ulcerative colitis was 83.24% (95% confidence interval: 61.90-93.82), while the response in Crohn's disease was 81.89 (76.72-86.11). The results of randomized trials for HBOT as adjuvant therapy in ulcerative colitis were conflicting. The complete healing of fistula in fistulizing Crohn's disease was noted 47.64% (22.05-74.54), while partial healing was noted in 34.29% (17.33-56.50%). Most of the adverse events were minor.
Conclusion: Observational studies suggest benefit of use of HBOT in ulcerative colitis flares and Crohn's disease. However, adequately powered randomized trials are needed to draw a definite conclusion.
Singh AK et al. Eur J Gastroenterol Hepatol. 2021 Apr 19. Epub ahead of print. PMID: 33905214.❖

Early onset leprosy reveals a joint effect of *LRRK2* and *NOD2* variants. (April 2021)

Leprosy, caused by *Mycobacterium leprae*, has a long incubation period and cases with age-of-onset <5 years are rare. Here, we studied a three-generational multiplex leprosy family which included monozygotic twins age <24 months suffering from paucibacillary leprosy. Whole genome sequencing identified a homozygous double mutation in the *LRRK2* gene (N551K, R1398H) and a heterozygous mutation in *NOD2* (R702W) as candidate variants underlying the early onset phenotype in the twins. The same amino acid substitutions had previously been identified as shared risk-modulating factors for Crohn's disease and Parkinson's disease. To evaluate the functional impact of the *LRRK2* mutations, we employed genome editing in RAW264.7 cells. Cells expressing the *LRRK2* variants displayed reduced respiratory burst and apoptosis following mycobacterial challenge. Moreover, the BCG-induced respiratory burst was significantly lower in *LRRK2* wild-type-expressing cells transfected with *NOD2* R702W compared with *NOD2* wild-type constructs. Employing co-immunoprecipitation, we showed that *LRRK2* and *NOD2* wild-type proteins interact in RAW cells. This interaction was independent of the LRRK2 variants but strongly reduced for *NOD2* R702W. However, N-glycolyl MDP-triggered RIP2 phosphorylation and NF-kB activation were additively reduced by both *LRRK2* and *NOD2* mutations. Finally, we observed a joint effect of *LRRK2* and *NOD2* variants on cytokine/chemokine secretion with the most significant reduction of secretion observed for the mutant genotypes carried by the twins. These data demonstrated the pleiotropic effects of *LRRK2* and *NOD2* in response to mycobacterial infection consistent with a role of the identified mutations in the development of early onset leprosy.
Dallmann-Sauer M et al. medRxiv 2021.03.25.21253623❖

Phase II Investigation of the Efficacy of Antimycobacterial Therapy in Chronic Pulmonary Sarcoidosis. (May 2021)

Background: A Phase I, single-center investigation found that 8 weeks of antimycobacterial therapy improved sarcoidosis FVC. Safety and efficacy assessments have not been performed in a multicenter cohort.
Research question: The objective of this study was to determine the safety and efficacy of antimycobacterial therapy on the physiological and immunologic end points of sarcoidosis.
Study design and methods: In a double-blind, placebo-controlled, multicenter investigation, patients with pulmonary sarcoidosis were randomly assigned to receive 16 weeks of concomitant levofloxacin, ethambutol, azithromycin, and rifabutin (CLEAR) or matching placebo to investigate the effect on FVC. The primary outcome was a comparison of change in percentage of predicted FVC among patients randomized to receive CLEAR or placebo in addition to their baseline immunosuppressive regimen. Secondary outcomes included 6-min walk distance (6MWD), St. George's Respiratory Questionnaire (SGRQ) score, adverse events, and decrease in mycobacterial early secreted antigenic target of 6 kDa (ESAT-6) immune responses.
Results: The intention-to-treat analysis revealed no significant differences in change in FVC among the 49 patients randomized to receive CLEAR (1.1% decrease) compared with the 48 randomized to receive placebo (0.02% increase) (P = .64). Physiological parameters such as the change in 6MWD were likewise similar (P = .91); change in SGRQ favored placebo (-8.0 for placebo vs -1.5 for CLEAR; P = .028). The per-protocol analysis revealed no significant change in FVC at 16 weeks between CLEAR and placebo. There was no significant change in 6MWD (36.4 m vs 6.3 m; P = .24) or SGRQ (-2.3 vs -7.0; P = .14). A decline in ESAT-6 immune responses at 16 weeks was noted among CLEAR-treated patients (P = .0003) but not patients receiving placebo (P = .24).
Interpretation: Despite a significant decline in ESAT-6 immune responses, a 16-week CLEAR regimen provided

no physiological benefit in FVC or 6MWD among patients with sarcoidosis.
Drake WP et al. Chest. 2021 May;159(5):1902-1912.❖

Hyperbaric oxygen therapy for the treatment of rectovaginal fistulas in patients with Crohn's disease: results of the HOT-REVA pilot study. (May 2021)
Background: Positive effects of hyperbaric oxygen (HBO) on perianal fistulas in Crohn's disease (CD) have been described, but the effect on rectovaginal fistulas (RVFs) has not yet been studied. The aim was to investigate the efficacy, safety and feasibility of HBO in patients with RVF in CD.
Methods: In this prospective study, consecutive CD patients between November 2018 and February 2020 presenting with RVF at the outpatient fistula clinic of the Amsterdam University Medical Centre were included and selected to receive treatment with 30 daily HBO sessions, if fistulas were actively draining and any concomitant treatment regimen was stable at least 6 weeks prior to start of HBO. Patients with a stoma were excluded. The primary endpoint was clinical closure at 3-month follow-up, defined as cessation of complaints and/or closure of the external orifice if visible at baseline. Secondary outcomes were improvement of concomitant perianal fistulas as measured by the perianal disease activity index (PDAI) and fistula drainage assessment (FDA), as well as improvement in patient-reported outcomes (visual analogue scale (VAS), inflammatory bowel disease questionnaire (IBDQ), faecal incontinence quality of life scale (FIQL) and female sexual functioning index (FSFI)) at 3-month follow-up.
Results: Out of 14 eligible patients, nine patients (median age 50 years) were treated, all of whom had previously had one or more unsuccessful medical and/or surgical treatments for their RVF. Clinical closure occurred in none of the patients at 3-month follow-up. There was no improvement in PDAI and patient-reported outcomes (VAS, IBDQ, FIQL and FSFI). Two patients had concomitant perianal fistulas; using FDA, one patient had a clinical response and one patient was in clinical remission 3 months after HBO. There were two treatment-related adverse events during HBO concerning claustrophobia and fatigue. Furthermore, two patients had a surgical intervention due to RVF and two patients were treated with antibiotics for a urinary tract infection during follow-up. One patient had a dose reduction of ustekinumab because of decreased luminal complaints.
Conclusion: Treatment with HBO was feasible, but in this therapy-refractory cohort without deviating ostomy no clinical closure of RVF or improvement in quality of life was seen 3 months after HBO. Treatment with HBO alone in this specific group of patients therefore appears to be ineffective.
Lansdorp CA et al. BJS Open. 2021 May 7;5(3):zrab042.❖

Other News

Kimberley Coleman, a Crohn's-MAP warrior and speaker at our 2018 Berkley Conference, was recently **featured in the New Zealand news**. Her new program, Let's Go Wahine, transforms the lives of out of work women. Hear more about Kimberley's amazing journey in this **recent interview**.

A **study presented** at the 2021 virtual Digestive Disease Week in May found that IBD patients were more than twice as likely as the general patient population to develop Alzheimer's disease. More research into the potential role that MAP may play in these two conditions is warranted.

Results from Qu Biologsics' Crohn's disease RESTORE trial were **presented at ECCO 2021** in July. Data from this trial suggests that Crohn's disease can be treated in an entirely different way by restoring innate immune function.

The technology used to detect Covid-19 in wastewater is now being used to help dairy **farmers manage Johne's disease** in their herds. This new test provides farmers with a cost-effective way to screen their herd for Johne's disease.

In case you missed it... Mycobacteria found in **100% of tissue samples** from Crohn's disease patients, but 0% in controls.❖

In Memoriam

This month, the world lost a true MAP pioneer. Prof. John Hermon-Taylor devoted decades of his career and retirement to MAP research. His articles formed the bedrock of human MAP science and were life-changing to many in our community. We mourn his passing, and honor his life and work with **a tribute** by one of his fellow researchers. ❖

Nicotine Increases Macrophage Survival through α7nAChR/NF-κB Pathway in *Mycobacterium* avium *paratuberculosis* Infection. (May 2021)

Recently, we reported that nicotine plays a role in the failure of the macrophage in the clearance of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) during infection in Crohn's disease smokers. We also demonstrated that nicotine enhances macrophages cellular survival during MAP infection. Blocking α7 nicotinic acetylcholine receptor (α7nAChR) with the pharmacological antagonist-mecamylamine-subverted the anti-inflammatory effect of nicotine in macrophages. Yet, it is still unknown how α7nAChR is involved in the modulation of the macrophage response during MAP infection. Here, we studied the mechanistic role of nicotine-α7nAChR interaction in modulating NF-κB survival pathway, autophagy, and effect on cathelicidin production in MAP-infected macrophages using THP-1 cell lines. Our results showed that nicotine upregulated α7nAChR expression by 5-folds during MAP infection compared to controls. Bcl-2 expression was also significantly increased after nicotine exposure. Moreover, Nicotine inhibited autophagosome formation whereas infection with MAP in absence of nicotine has significantly increased LC-3b in macrophages. Nicotine also further upregulated NF-κB subunits expression including Rel-B and p100, and increased nuclear translocation of p52 protein. We also discovered that cathelicidin production was significantly suppressed in MAP-infected macrophages, treatment with nicotine showed no effect. Overall, the study provides new insight toward understanding the cellular role of nicotine through α7nAChR/NF-κB p100/p52 signaling pathway in inducing anti-apoptosis and macrophage survival during MAP infection in Crohn's disease smokers.
AlQasrawi D et al. Microorganisms. 2021 May 18;9(5): 1086.❖

Safety and Immunogenicity of Adenovirus and Poxvirus Vectored Vaccines against a Mycobacterium Avium Complex Subspecies. (March 2021)

Heterologous prime-boost strategies are known to substantially increase immune responses in viral vectored vaccines. Here we report on safety and immunogenicity of the poxvirus Modified Vaccinia Ankara (MVA) vectored vaccine expressing four Mycobacterium avium subspecies paratuberculosis antigens as a single dose or as a booster vaccine following a simian adenovirus (ChAdOx2) prime. We demonstrate that a heterologous prime-boost schedule is well tolerated and induced T-cell immune responses.
Folegatti PM et al. Vaccines (Basel). 2021 Mar 16;9(3):262.❖

Long-term dietary patterns are associated with pro-inflammatory and anti-inflammatory features of the gut microbiome. (May 2021)

Objective: The microbiome directly affects the balance of pro-inflammatory and anti-inflammatory responses in the gut. As microbes thrive on dietary substrates, the question arises whether we can nourish an anti-inflammatory gut ecosystem. We aim to unravel interactions between diet, gut microbiota and their functional ability to induce intestinal inflammation.
Design: We investigated the relation between 173 dietary factors and the microbiome of 1425 individuals spanning four cohorts: Crohn's disease, ulcerative colitis, irritable bowel syndrome and the general population. Shotgun metagenomic sequencing was performed to profile gut microbial composition and function. Dietary intake was assessed through food frequency questionnaires. We performed unsupervised clustering to identify dietary patterns and microbial clusters. Associations between diet and microbial features were explored per cohort, followed by a meta-analysis and heterogeneity estimation.
Results: We identified 38 associations between dietary patterns and microbial clusters. Moreover, 61 individual foods and nutrients were associated with 61 species and 249 metabolic pathways in the meta-analysis across healthy individuals and patients with IBS, Crohn's disease and UC (false discovery rate<0.05). Processed foods and animal-derived foods were consistently associated with higher abundances of Firmicutes, *Ruminococcus* species of the *Blautia* genus and endotoxin synthesis pathways. The opposite was found for plant foods and fish, which were positively associated with short-chain fatty acid-producing commensals and pathways of nutrient metabolism.
Conclusion: We identified dietary patterns that consistently correlate with groups of bacteria with shared functional roles in both, health and disease. Moreover, specific foods and nutrients were associated with species known to infer mucosal protection and anti-inflammatory effects. We propose microbial mechanisms through which the diet affects inflammatory responses in the gut as a rationale for future intervention studies.
Bolte LA et al. Gut 2021;70:1287-1298.❖

Early life exposures and the risk of inflammatory bowel disease: Systematic review and meta-analyses. (May 2021)

Background: Early life exposures impact immune system development and therefore the risk of immune-mediated diseases, including inflammatory bowel disease (IBD). We systematically reviewed the impact of pre-, peri-, and

postnatal exposures up to the age of five years on subsequent IBD diagnosis.
Methods: We identified case-control and cohort studies reporting on the association between early life environmental factors and Crohn's disease (CD), ulcerative colitis (UC), or IBD overall. Databases were search from their inception until May 24th, 2019 until July 14th, 2020. We conducted meta-analyses for quantitative review of relevant risk factors that were comparable across studies and qualitative synthesis of the literature for a wide range of early life exposures, including maternal health and exposures during pregnancy, perinatal factors, birth month and related-factors, breastfeeding, hygiene-related factors and social factors, immigration, antibiotics, offspring health, including infections, and passive smoking. PROSPERO registration: CRD42019134980.
Findings: Prenatal exposure to antibiotics (OR 1.8; 95% CI 1.2-2.5) and tobacco smoke (OR 1.5; 95% CI 1.2-1.9), and early life otitis media (OR 2.1; 95% CI 1.2-3.6) were associated with IBD. There was a trend towards an association between exposure to antibiotics in infancy and IBD (OR: 1.7, 95% CI 0.97, 2.9), supported by positive data on population-based data. Breastfeeding was protective against IBD. Other early life risk factors had no association with IBD, but data were limited and heterogenous.
Interpretation: Early life is an important period of susceptibility for IBD development later in life. Tobacco smoke, infections and antibiotics were associated positively, and breastfeeding was associated negatively with IBD. Our findings offer an opportunity to develop primary prevention strategies.
Agrawal M et al. EClinicalMedicine. 2021 May 15;36: 100884.❖

Adjunctive Hyperbaric Oxygen Therapy in Refractory Crohn's Disease: An Observational Study (April 2021)

Background and aims: Patients may experience complications of Crohn's disease (CD) even when treated with optimal medical therapy strategies. Previous data have shown the efficacy of hyperbaric oxygen therapy (HBOT) in the management of complicated CD. However, there is no consensus regarding the optimal number of sessions or duration of treatment regimens. The aim of the present study was to investigate the efficacy of HBOT in CD patients who were refractory to conventional medical management.
Methods: This study included patients who underwent HBOT for the treatment of the following complications: perianal fistulizing Crohn's disease (pCD), enterocutaneous fistulas (ECF), or pyoderma gangrenosum (PG). Complete healing was defined as the closure of external orifice and the absence of active draining (in pCD), complete wound healing (in PG), and granulation or complete wound epithelialization with no enteric draining (in ECF). The persistence of draining and the absence of wound granulation were defined as incomplete healing.
Results: Forty patients were included. The mean CD duration was 10.6 ± 5.8 years. pCD comprised most of the included patients (25/62.5%), followed by ECF (n = 13/32.5%) and PG (n = 6/15%). In two patients (5%), a combination of ECF and PG was diagnosed, and in one patient (2.5%), all three complications were observed. A total of 32 patients (82.5%) had complete healing. Patients with PG had the highest healing rates (100%), followed by those with ECF (84.6%) and pCD (80%).
Conclusions: Adjunctive HBO was associated with significant healing rates for CD-associated complications such as pCD, ECF, and PG.
Feitosa MR et al. Gastroenterol Res Pract. 2021 Apr 26; 2021:6628142..❖

Rationally designed bacterial consortia to treat chronic immune-mediated colitis and restore intestinal homeostasis. (May 2021)

Environmental factors, mucosal permeability and defective immunoregulation drive overactive immunity to a subset of resident intestinal bacteria that mediate multiple inflammatory conditions. GUT-103 and GUT-108, live biotherapeutic products rationally designed to complement missing or underrepresented functions in the dysbiotic microbiome of IBD patients, address upstream targets, rather than targeting a single cytokine to block downstream inflammation responses. GUT-103, composed of 17 strains that synergistically provide protective and sustained engraftment in the IBD inflammatory environment, prevented and treated chronic immune-mediated colitis. Therapeutic application of GUT-108 reversed established colitis in a humanized chronic T cell-mediated mouse model. It decreased pathobionts while expanding resident protective bacteria; produced metabolites promoting mucosal healing and immunoregulatory responses; decreased inflammatory cytokines and Th-1 and Th-17 cells; and induced interleukin-10-producing colonic regulatory cells, and IL-10-independent homeostatic pathways. We propose GUT-108 for treating and preventing relapse for IBD and other inflammatory conditions characterized by unbalanced microbiota and mucosal permeability.
van der Lelie, D. et al. Nat Commun 12, 3105 (2021).❖

Comparison of a mycobacterial phage assay to detect viable *Mycobacterium avium* subspecies *paratuberculosis* with standard diagnostic modalities in cattle with naturally infected Johne disease (May 2021)

Background: *Mycobacterium avium* subspecies *paratuberculosis* (MAP), the cause of Johne disease, is a slow growing mycobacterium. Viable MAP detection is difficult, inconstant and time-consuming. The purpose of this study was to compare a rapid phage/qPCR assay performed on peripheral blood mononuclear cells (PBMCs) with three standard methods of MAP detection: fecal MAP PCR; plasma antigen-specific IFN-γ & serum MAP ELISA hypothesizing that, if sensitive and specifc, Johne animals would be positive and Control animals negative. We studied a well characterized herd of Holstein cattle that were naturally infected with MAP and their Controls.
Results: With phage/qPCR 72% (23/32) of Johne and 35% (6/17) of Controls were MAP positive. With fecal PCR 75% (24/32) of Johne and 0% (0/17) of Controls were MAP positive. With plasma antigen-specific IFN-γ 69% (22/32) of Johne and 12% (2/17) of Controls were MAP positive. With serum MAP ELISA, 31% (10/32) of Johne and 0% (0/17) of Controls were MAP positive. When phage / qPCR and fecal PCR results were combined, 100% (32/32) Johne and 35% (6/17) of Control animals were MAP positive. Younger Control animals (1–3 years) had significantly fewer plaques (25±17 SEM) than older Controls (4–12 years) (309±134 p=0.04). The same trend was not observed in the Johne animals (p=0.19).
Conclusions: In contrast to our hypothesis, using the phage/qPCR assay we find that viable circulating MAP can rapidly be detected from the blood of animals infected with, as well as those in the Control group evidently colonized by MAP. These data indicate that the presence of viable MAP in blood does not necessarily signify that an animal must of necessity be demonstrably ill or be MAP positive by standard diagnostic methods.
Greenstein, Robert et al. (2021). Gut Pathogens. 13. 10.1186/s13099-021-00425-5. ❖

Effect of the deletion of *lprG* and *p55* genes in the *K10* strain of *Mycobacterium avium* subspecies *paratuberculosis*. (Sept. 2021)

The *lprG-p55* operon of *Mycobacterium tuberculosis*, *M. bovis* and *M. avium* strain D4ER has been identified as a virulence factor involved in the transport of toxic compounds. LprG is a lipoprotein that modulates the host immune response against mycobacteria, whereas P55 is an efflux pump that provides resistance to several drugs. In the present study we search for, and characterize, *lprg* and *p55*, putative virulence genes in *Mycobacterium avium* subsp. *paratuberculosis* (MAP) to generate a live-attenuated strain of MAP that may be useful in the future as live-attenuated vaccine. For this purpose, we generated and evaluated two mutants of MAP strain *K10*: one mutant lacking the *lprG* gene (⊿*lprG*) and the other lacking both genes *lprG* and *p55* (⊿*lprG-p55*). None of the mutant strains showed altered susceptibility to first-line and second-line antituberculosis drugs or ethidium bromide, only the double mutant had two-fold increase in clarithromycin susceptibility compared with the wild-type strain. The deletion of *lprG* and of *lprG-p55* reduced the replication of MAP in bovine macrophages; however, only the mutant in *lprG-p55* grew faster in liquid media and showed reduced viability in macrophages and in a mouse model. Considering that the deletion of both genes *lprG-p55*, but not that of *lprG* alone, showed a reduced replication in vivo, we can speculate that *p55* contributes to the survival of MAP in this animal model.
Viale MN et al. Res Vet Sci. 2021 Sep;138:1-10.❖

Highly Specific and Quick Detection of *Mycobacterium avium* subsp. *paratuberculosis* in Feces and Gut Tissue of Cattle and Humans by Multiple Real-Time PCR Assays. (April 2021)

Mycobacterium avium subsp. *paratuberculosis* is the causative agent of Johne's disease (JD) in cattle and may be associated with Crohn's disease (CD) in humans. It is the slowest growing of the cultivable mycobacteria, and culture from clinical, veterinary, food, or environmental specimens can take 4 months or even longer. Currently, the insertion element IS*900* is used to detect *M. avium* subsp. *paratuberculosis* DNA. However, closely related IS*900* elements are also present in other mycobacteria, thus limiting its specificity as a target. Here we describe the use of novel primer sets derived from the sequences of two highly specific single copy genes, MAP2765c and MAP0865, for the quantitative detection of *M. avium* subsp. *paratuberculosis* within 6 h by using real-time PCR. Specificity of the target was established using 40 *M. avium* subsp. *paratuberculosis* isolates, 67 different bacterial species, and two intestinal parasites. Using the probes and methods described, we detected 27 (2.09%) *M. avium* subsp. *paratuberculosis*-positive stool specimens from 1,293 individual stool samples by the use of either IS*900* or probes deriving from the MAP2765c and MAP0865 genes described here. In general, bacterial load due to *M. avium* subsp. *paratuberculosis* was uniformly low in these samples and we estimated 500 to 5,000 *M. avium* subsp. *paratuberculosis* bacteria per gram of stool in assay-positive samples. Thus, the methods described here are useful for

rapid and specific detection of *M. avium* subsp. *paratuberculosis* in clinical samples.
Imirzalioglu C et al. J Clin Microbiol. 2011;49(5):1843-1852. doi:10.1128/JCM.01492-10.❖

Revealing immune responses in the Mycobacterium avium subsp. paratuberculosis-infected THP-1 cells using single cell RNA-sequencing (July 2021)

Mycobacterium avium subsp. paratuberculosis (MAP) is a causative agent of Johne's disease, which is a chronic and debilitating disease in ruminants. MAP is also considered to be a possible cause of Crohn's disease in humans. However, few studies have focused on the interactions between MAP and human macrophages to elucidate the pathogenesis of Crohn's disease. We sought to determine the initial responses of human THP-1 cells against MAP infection using single-cell RNA-seq analysis. Clustering analysis showed that THP-1 cells were divided into seven different clusters in response to phorbol-12-myristate-13-acetate (PMA) treatment. The characteristics of each cluster were investigated by identifying cluster-specific marker genes. From the results, we found that classically differentiated cells express CD14, CD36, and TLR2, and that this cell type showed the most active responses against MAP infection. The responses included the expression of proinflammatory cytokines and chemokines such as CCL4, CCL3, IL1B, IL8, and CCL20. In addition, the Mreg cell type, a novel cell type differentiated from THP-1 cells, was discovered. Thus, it is suggested that different cell types arise even when the same cell line is treated under the same conditions. Overall, analyzing gene expression patterns via scRNA-seq classification allows a more detailed observation of the response to infection by each cell type.
Park HT et al. PLoS One. 2021 Jul 2;16(7):e0254194.❖

Anti-MAP Triple Therapy Supports Immunomodulatory Therapeutic Response in Crohn's Disease Through Downregulation of NF-kB Activation in the Absence of MAP Detection (2021)

The triple antibiotic formulation, known as anti-MAP therapy, exhibits unique synergistic antimicrobial activity and should be effective for treatment of Crohn's disease (CD) associated with Mycobacterium avium subspecies paratuberculosis (MAP). The absence of MAP detection in some CD cases may be linked to poor diagnostics or lack of association with the disease. To understand the therapeutic response of some CD patients to anti-MAP therapy in absence of MAP detection, the immunomodulatory potency of anti-MAP therapy and its major ingredients, clarithromycin (CLA) and rifabutin (RIF), in THP-1, Caco-2, and Jurkat T-cells were investigated. Anti-MAP formulation at 2.0 μg/mL decreased MAP viability in macrophages by 18-fold over 72 h. Additionally, M1/M2 macrophage polarization ratio was reduced by 6.7-fold, and expression and protein levels of TNF-a and IL-6 were reduced by 2.9-fold, whereas IL-10 increased by 5.0-fold in these cells. Mechanistically, the effect of anti-MAP formulation on NF-kB activation was dose-dependent and decreased to 13.4% at 2.0 μg/mL. Anti-MAP therapy also reversed the pro-inflammatory response in lipopolysaccharide (LPS)-induced macrophages, which shows that the anti-inflammatory effect of the treatment is not just due to a decrease in MAP viability. Furthermore, this study shows that anti-MAP therapy exhibits anti-cytotoxic effects in Caco-2 monolayers infected with MAP or treated with dextran sodium sulfate (DSS). Anti-MAP therapy decreased T-cell proliferation by up to 4.8-fold following treatment with phytohemagglutinin (PHA) or MAP purified protein derivative (PPD). Overall, the data demonstrate that anti-MAP therapy plays a significant role in modulating and eliciting a protective immune response in macrophages, endothelial cells, and T lymphocytes, even in absence of infection. This may explain the therapeutic response of some CD patients to treatment, even in absence of MAP detection, infection, or total eradication. The study supports anti-MAP therapy as an alternate treatment option for CD, especially in absence of reliable MAP diagnostics.
Elkamel, Erij, (2021). Electronic Theses and Dissertations, 2020-. 499.❖

Targeting emerging *Mycobacterium avium* infections: perspectives into pathways and antimicrobials for future interventions. (July 2021)

Mycobacterium avium is an emerging opportunistic pathogen, globally. Infections caused by *M. avium* are laborious to treat and could result in drug resistance. This review discusses the importance of many factors including the cell wall in *M. avium* pathogenesis, since this unique structure modulates the pathogen's ability to thrive in various hosts and environmental niches including conferring resistance to killing by antimicrobials. More research efforts in future are solicited to develop novel therapeutics targeting *M. avium*. The complete eradication of *M. avium* infection in immunocompromised individuals would need a deeper understanding of the source of infection, unique underlying mechanisms and its uncharacterized pathways. This could, perhaps in future, hold the key to target and treat *M. avium* more effectively.
Mattoo R. Future Microbiol. 2021 Jul;16:753-764.❖

Efficacy of using antibiotic cocktail in medically refractory colitis/inflammatory bowel disease. (May 2021)

Background: Antibiotic cocktail targeting intestinal bacteria may offer a promising approach in certain patients with medically refractory IBD. It may find a place in serving as a bridge to more effective long-term medical therapies and in some cases help in achieving clinical remission in patients with refractory disease as an adjunctive therapy. Its main attractions include efficacy in about 50% of medically refractory patients and a non-immunosuppressive adjunct in treatment of children who may otherwise suffer from complications of untreated disease or heavy immunosuppression. The main drawbacks are lack of understanding of true mechanism of action, potential development of drug resistant bacterial colonies, and gaps in knowledge about optimal duration of therapy and the patient phenotypes most likely to benefit. Pediatric data is sparse regarding efficacy, indications and treatment protocol.
Objectives/Goal: By conducting this retrospective analysis, we hope to contribute towards knowledge that can verify efficacy of antibiotic cocktail therapy and further identify various patient and treatment related factors that are associated with its response.
Methods/Design: Patients with IBD between the ages of 1 year and 22 years who were treated with an antibiotic cocktail consisting of at least 3 drugs (most commonly: metronidazole, amoxicillin, doxycycline, and vancomycin) for a minimum of 1 week for medically refractory disease were included for retrospective chart review. Children who had undergone simultaneous changes in IBD therapy within 4 weeks of starting antibiotics were excluded.
Results: 19 children were included: mean age 10.8 ± 4.6 years, 8 females (42%), 18 (95%) with moderate to severe disease activity, 8 (42%) with Crohn's colitis, 5 (29%) with ulcerative colitis, 4 (21%) with indeterminate colitis. 7 (37%) patients were corticosteroid-dependent or resistant and 18 (95%) had shown poor response to anti-TNF therapy. The antibiotic cocktail was definitely effective in 12 of 19 patients who entered clinical remission following therapy (PUCAI < 10). By diagnosis, 2 (40%) with ulcerative colitis, 6 (75%) with Crohn's colitis, and 4 (80%) with indeterminate colitis responded. 4 (21%) patients developed Clostridium difficile infection after undergoing therapy. 4 patients (21%) ultimately required colectomy for medically refractory disease.
Conclusions: The use of oral wide-spectrum antibiotic cocktail in pediatric IBD seems promising and safe in children refractory to other salvage therapy. Further study, including a randomized controlled study, is warranted to further assess the efficacy of this intervention.
Simon, David Aaron. (2021) Research Days 15.❖

Is *Mycobacterium avium* subspecies *paratuberculosis* (MAP) associated with Crohn's Disease? (Sept. 2021)

Objective This retroactive meta-analysis assesses the relationship between *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and Crohn's Disease (CD).
Methods A meta-analysis of PCR– and culture–based studies was conducted to determine if there is a relationship between MAP and Crohn's Disease. The chi-squared test of independence was also conducted to determine if MAP infection and Crohn's disease onset are independent events.
Results The studies analyzed were able to provide evidence that *Mycobacterium avium* subspecies *paratuberculosis* is highly associated with Crohn's disease. It is also shown that, regardless of detection method, MAP can effectively be detected in Crohn's patients.
Conclusion MAP is strongly connected with Crohn's Disease.
Patterson JM et al, medRxiv 2021.09.27.21263960.❖

Do you enjoy this newsletter? Sign up for our mailing list to get the latest content. We value your privacy as much as you do, your information with never be sold or shared.

Have a question or comment? We'd love to hear from you! Visit our Contact page.