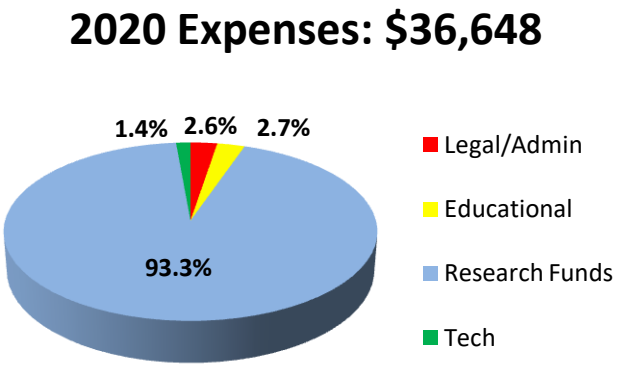
The MAP Gap

Bridging The Space Between The Science And You

Human Paratuberculosis Foundation Quarterly Newsletter April 2021

Human Para News

Human Para's **2020 tax filings** are complete, which again demonstrate our commitment to MAP research and low overhead costs. The below chart shows how your funds were utilized in 2020. Our dedicated volunteers make it possible to use the majority of our dollars on research and educational resources. We remain committed to this model and thank our entire community for their continued generosity.



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

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.

Is LRRK2 the missing link between inflammatory bowel disease and Parkinson's disease? (March 2021)

Links that implicate the gastrointestinal system in Parkinson's disease (PD) pathogenesis and progression have become increasingly common. PD shares several similarities with Crohn's disease (CD). Intestinal inflammation is common in both PD and CD and is hypothesized to contribute to PD neuropathology. Mutations in leucine-rich repeat kinase 2 (LRRK2) are one of the greatest genetic contributors to PD. Variants in LRRK2 have also been associated with increased incidence of CD. Since its discovery, LRRK2 has been studied intensely in neurons, despite multiple lines of evidence showing that LRRK2 is highly expressed in immune cells. Based on the fact that higher levels of LRRK2 are detectable in inflamed colonic tissue from CD patients and in peripheral immune cells from sporadic PD patients relative to matched controls, we posit that LRRK2 regulates inflammatory processes. Therefore, LRRK2 may

sit at a crossroads whereby gut inflammation and higher LRRK2 levels in CD may be a biomarker of increased risk for sporadic PD and/or may represent a tractable therapeutic target in inflammatory diseases that increase risk for PD. Here we will focus on reviewing how PD and CD share overlapping phenotypes, particularly in terms of LRRK2 in the context of the immune system, that could be targeted in future therapies.
Herrick MK, Tansey MG. NPJ Parkinsons Dis. 2021 Mar 9;7(1):26.❖

Identification and Characterization of *Mycobacterium smegmatis* and *Mycobacterium avium* subsp. *paratuberculosis* Zinc Transporters. (March 2021)

Zinc uptake in bacteria is essential to maintain cellular homeostasis and survival. ZnuABC is an important zinc importer of numerous bacterial genera, which is expressed to restore zinc homeostasis when the cytosolic concentration decreases beyond a critical threshold. Upon zinc limitation the fast-growing nonpathogenic organism *Mycobacterium smegmatis* (MSMEG) as well as the ruminant pathogen *M. avium* subsp. *paratuberculosis* (MAP) increases expression of genes encoding ZnuABC homologues, but also of genes encoding other transporters. This suggests an involvement of these transporters in zinc homeostasis. Here we characterized the putative zinc transporters of MSMEG (ZnuABC and ZnuABC2) and MAP (ZnuABC, MptABC, and MAP3774-76). Deletion of either ZnuABC or ZnuABC2 in MSMEG did not lead to growth defects, but to an increased expression of zinc marker genes in MSMEGΔznuABC, indicating cytosolic zinc limitation. However, chromatin immunoprecipitation proved direct binding of the global zinc regulator Zur to promoter regions of both *znu*ABC and *znu*ABC2. Simultaneous deletion of both transporters caused severe growth defects, which could be restored either by homologous complementation with single ZnuABC transporters or supplementation of growth media with zinc but not iron, manganese, cobalt, or magnesium. Heterologous complementation of the double mutant with MAP transporters also resulted in reconstitution of growth. Nonradioactive FluoZinTM-3AM zinc uptake assays directly revealed the competence of all transporters to import zinc. Finally, structural and phylogenetic analyses provided evidence of a novel class of ZnuABC transporters represented by the ZnuABC2 of MSMEG, which is present only in actinobacteria, mainly in the genera *Nocardia*, *Streptomyces* and fast growing *Mycobacteria*.
Goethe E et al. Journal of Bacteriology Mar 2021, JB.00049-21.❖

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;9(3):262..

Mycobacterium Avium Subspecies Paratuberculosis Infection and Biological Treatment of IBD: Cause or Consequence? (March 2021)

Mycobacterium avium subspecies *paratuberculosis* [MAP] is an obligate intracellular mycobacterium that causes Johne's disease [JD], a disease characterized by chronic granulomatous inflammation in the gut of ruminants and other mammalian species, including humans. The similarity between JD and Crohn's disease [CD], manifested through similar clinical symptoms such as diarrhoea and weight loss, similar transmural diffuse granulomatous inflammation in pathological reports [Figure 1] and overlapping epidemiological aspects [rising incidence, long incubation period and familial occurrence pattern], has raised the hypothesis of a possible aetiological connection between MAP and CD. This hypothesis is supported by the fact that various studies have reported a higher frequency of MAP in CD patients vs ulcerative colitis [UC] patients and healthy controls. However, small study sizes and inconsistent methodologies used in the detection and isolation of MAP have raised doubts regarding the causal relationship between this bacterium and CD.
E Proietti et al. J. of Crohn's and Colitis, 2021, jjab027.❖

Genetic Involvement of *Mycobacterium avium* Complex in the Regulation and Manipulation of Innate Immune Functions of Host Cells. (March 2021)

Mycobacterium avium complex (MAC), a collection of mycobacterial species representing nontuberculous mycobacteria, are characterized as ubiquitous and opportunistic pathogens. The incidence and prevalence of infectious diseases caused by MAC have been emerging

globally due to complications in the treatment of MAC-pulmonary disease (PD) in humans and the lack of understating individual differences in genetic traits and pathogenesis of MAC species or subspecies. Despite genetically close one to another, mycobacteria species belonging to the MAC cause diseases to different host range along with a distinct spectrum of disease. In addition, unlike *Mycobacterium tuberculosis*, the underlying mechanisms for the pathogenesis of MAC infection from environmental sources of infection to their survival strategies within host cells have not been fully elucidated. In this review, we highlight unique genetic and genotypic differences in MAC species and the virulence factors conferring the ability to MAC for the tactics evading innate immune attacks of host cells based on the recent advances in genetic analysis by exemplifying *M. avium* subsp. *hominissuis*, a major representative pathogen causing MAC-PD in humans. Further understanding of the genetic link between host and MAC may contribute to enhance host anti-MAC immunity, but also provide novel therapeutic approaches targeting the pangenesis-associated genes of MAC.
Shin MK et al. Int J Mol Sci. 2021;22(6):3011.❖

Novel CARMIL2 loss-of-function variants are associated with pediatric inflammatory bowel disease. (Match 2021)

CARMIL2 is required for CD28-mediated co-stimulation of NF-κB signaling in T cells and its deficiency has been associated with primary immunodeficiency and, recently, very early onset inflammatory bowel disease (IBD). Here we describe the identification of novel biallelic CARMIL2 variants in three patients presenting with pediatric-onset IBD and in one with autoimmune polyendocrine syndrome (APS). None manifested overt clinical signs of immunodeficiency before their diagnosis. The first patient presented with very early onset IBD. His brother was found homozygous for the same CARMIL2 null variant and diagnosed with APS. Two other IBD patients were found homozygous for a nonsense and a missense CARMIL2 variant, respectively, and they both experienced a complicated postoperative course marked by severe infections. Immunostaining of bowel biopsies showed reduced CARMIL2 expression in all the three patients with IBD. Western blot and immunofluorescence of transfected cells revealed an altered expression pattern of the missense variant. Our work expands the genotypic and phenotypic spectrum of CARMIL2 deficiency, which can present with either IBD or APS, aside from classic immunodeficiency manifestations. CARMIL2 should be included in the diagnostic work-up of patients with suspected monogenic IBD. Bosa L et al. Sci Rep. 2021 Mar 15;11(1):5945.❖

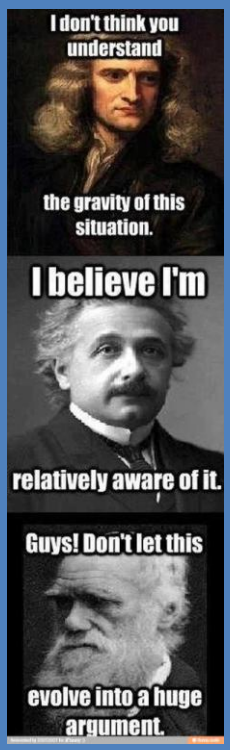
Other News

In February, the MAP community lost one of its most ardent champions. For decades, Patrick McLean dedicated his career to advancing research into the cause of IBD. His presentation at the 2015 MAP Conference reflected his enthusiasm for this cause. He will be missed by our entire community.

Qu Biologics Phase 2 RESTORE trial showed positive signed of clinical, histological and endoscopic response in Crohn's patients with moderate to severe Crohn's disease. The SSI therapy is designed to restore innate immune function. To hear more about this unique therapeutic approach to Crohn's disease, watch the presentation by CEO Hal Gunn, MD from our 2018 Berkeley conference.

Could MAP be involved in rheumatoid arthritis? This new article explores this question and highlights some of Dr. Saleh Naser's recent research showing a possible link.❖

Just for Fun



Phagomagnetic separation-quantitative PCR: A rapid, sensitive and specific surveillance tool for viable *Mycobacterium avium* ssp. *paratuberculosis* in bulk tank and individual cow milk. (March 2021)

Bulk tank milk samples from 392 Northern Ireland dairy farms and individual milk from animals (n = 293) on 4 of these farms were tested by a novel phagomagnetic separation (PhMS)-quantitative (q)PCR assay able to detect and quantify viable *Mycobacterium avium* ssp. *paratuberculosis* (MAP), to demonstrate its potential utility as a milk surveillance tool. Viable MAP were detected in 26.5% of the bulk tank milks, with MAP contamination levels ranging from 1 to 8,432 MAP/50 mL of milk; less than 2% of farms had MAP contamination levels >100 MAP/50 mL in their bulk tank milk. Follow-up PhMS-qPCR testing of milk from individual animals on 4 farms that had the highest numbers of MAP in their bulk tank milks indicated that 17 to 24% of animals in each herd were shedding viable MAP in their milk. Mean MAP numbers detected ranged between 6.7 and 42.1 MAP/50 mL of milk. No significant correlation was observed between the detection of viable MAP in bulk or individual milks by PhMS-qPCR and parallel milk ELISA results, or between PhMS-qPCR results and any other milk recording results (somatic cell count, total bacterial count, % butterfat, or % protein). Viable MAP was detected by IS900 qPCR in 52 (85.2%) Pozzato broth cultures of 61 PhMS-qPCR-positive individual milks after 12 wk of incubation, suggesting few PhMS-qPCR results were false positives. The mean sensitivities of the PhMS-qPCR assay and milk ELISA applied to individual milks were estimated by Bayesian latent class analysis to be 0.7096 and 0.2665, respectively, and mean specificities were similar (0.9626 and 0.9509). Our findings clearly demonstrate that the novel PhMS-qPCR assay could be a useful milk surveillance tool for dairy processors, or a milk monitoring tool for Johne's disease control or milk quality assurance programs.
Foddai ACG et al. J Dairy Sci. 2021 Mar 1:S0022-0302(21)00227-7.❖

Developing smarter vaccines for paratuberculosis: From early biomarkers to vaccine design. (Feb. 2021)

Vaccines for paratuberculosis have been used for over a hundred years but the disease continues to affect ruminant health and livestock industries globally. Mycobacterium avium subspecies paratuberculosis which causes the disease also known as Johne's disease is a subversive pathogen able to undermine both innate and adaptive host defense mechanisms. This review focuses on early protective immune pathways that lead to some animals becoming resilient to infection to provide a road map for designing better vaccines and emphasizes the need for harnessing the potential of mucosal immunity.
de Silva K. Immunol Rev. 2021 Feb 23.❖

Alfalfa Plants (*Medicago sativa* L.) Expressing the 85B (MAP1609c) Antigen of *Mycobacterium avium* subsp. *paratuberculosis* Elicit Long-Lasting Immunity in Mice. (March 2021)

Mycobacterium avium subsp. *paratuberculosis* (MAP) is the etiological agent of Paratuberculosis, a contagious, untreatable, and chronic granulomatous enteritis that results in diarrhea, emaciation, and death in farmed ruminants (i.e., cattle, sheep, and goats). In this study, the Ag85B antigen from MAP was expressed in transgenic alfalfa as an attractive vaccine candidate. Agrobacterium-mediated transformation allowed the rescue of 56 putative transformed plants and transgenesis was confirmed in 19 lines by detection of the *Ag85B* gene (*MAP1609c*) by PCR. Line number 20 showed the highest Ag85B expression [840 ng Ag85B per gram of dry weight leaf tissue, 0.062% Total Soluble Protein (TSP)]. Antigenicity of the plant-made Ag85B was evidenced by its reactivity with a panel of sera from naturally MAP-infected animals, whereas immunogenicity was assessed in mice immunized by either oral or subcutaneous routes. The plant-made Ag85B antigen elicited humoral responses by the oral route when co-administered with cholera toxin as adjuvant; significant levels of anti-85B antibodies were induced in serum (IgG) and feces (IgA). Long-lasting immunity was evidenced at day 180 days post-first oral immunization. The obtained alfalfa lines expressing Ag85B constitute the first model of a plant-based vaccine targeting MAP. The initial immunogenicity assessment conducted in this study opens the path for a detailed characterization of the properties of this vaccine candidate.
Monreal-Escalante E et al. Mol Biotechnol. 2021;1-13.❖

Bacteriophage-Based Methods for Detection of Viable *Mycobacterium avium* subsp. *paratuberculosis* and Their Potential for Diagnosis of Johne's Disease. (March 2021)

Bacteriophage-based methods for detecting *Mycobacterium avium* subsp. *paratuberculosis* (MAP) are a potential new approach for diagnosis of Johne's disease (JD). The basis of these tests is a mycobacteriophage (D29) with a lytic lifecycle that is able to infect a range of *Mycobacterium* spp., not just MAP. When added to a test sample, the phages will bind to and infect mycobacterial cells present. If the

host mycobacterial cells are viable, the phages will take over the metabolic machinery of the cells to replicate and produce multiple copies of themselves (phage amplification), before weakening the host cell walls by enzyme action and causing cell lysis. Cell lysis releases the host cell contents, which will include ATP, various enzymes, mycobacterial host DNA and progeny D29 phages; all of which can become the target of subsequent endpoint detection methods. For MAP detection the released host DNA and progeny phages have principally been targeted. As only viable mycobacterial cells will support phage amplification, if progeny phages or host DNA are detected in the test sample (by plaque assay/phage ELISA or qPCR, respectively) then viable mycobacteria were present. This mini-review will seek to: clearly explain the basis of the phage-based tests in order to aid understanding; catalog modifications made to the original plaque assay-based phage amplification assay (FASTPlaqueTB™) over the years; and summarize the available evidence pertaining to the performance of the various phage assays for testing veterinary specimens (bovine milk, blood and feces), relative to current JD diagnostic methods (culture, fecal PCR, and blood-ELISA).

Grant IR. Front Vet Sci. 2021;8:632498. Published 2021 Mar 11.❖

Isotype specific antibody responses to Mycobacterium Avium subspecies Paratuberculosis antigens are associated with the use of biological therapy in Inflammatory Bowel Disease. (Dec. 2020)

Background: The role of Mycobacterium avium subspecies paratuberculosis (MAP) in inflammatory bowel disease (IBD), especially Crohn's disease (CD) is controversial due conflicting results, lack of reproducibility and standardized tests. The current study focuses on the role of MAP in disease progression and genetic susceptibility as MAP is likely one of many factors involved in the complex pathogenesis of IBD, potentially affecting a subgroup depending on genetic susceptibility.

Methods: Serum from 812 patients was evaluated with seven immunoglobulin (Ig) isotype-specific serology tests assessing humoral response to three different MAP antigens. For each of these in total 21 tests, the intra-assay and inter-assay coefficients were used to evaluate test accuracy. Reliable assays were subsequently analyzed in relation to disease characteristics and need for biological therapy/surgery. Genome wide genotyping was available for all participants. Genetic determinants of humoral response to MAP antigens were evaluated using genome-wide association analysis and polygenic risk scores (PRS).

Results: High IgA or IgM response to MAP2609 was associated with increased use of biological therapy in CD and UC (odds ratio 2.69; 95% confidence interval 1.44-5.01; 2.60, 1.46-4.64, respectively). No associations were seen for risk of surgery (p-values>0.29). We could not identify genetic determinants nor polygenic risk scores for MAP response with genome-wide significance.

Conclusions: Extensive assays for serological response to MAP were evaluated using stringent criteria for reliability. Increased IgA and IgM response to MAP antigens was seen in patients exposed to biological therapy, while no genetic determinants underlying this humoral response were found.

van der Sloot KWJ, et al. J. Crohn's & Colitis, 2020 Dec..❖

Host-directed Therapy Alleviates Intracellular Mycobacterial Infection via Mediating Innate Immune Responses (2021)

Aims and objectives: Mycobacterium bovis (M. bovis) is the causative agent of bovine tuberculosis; however, it also causes infection in a wide range of hosts including human beings. Johne's disease (JD) is characterized by chronic granulomatous enteritis predominantly observed in ruminants, caused by Mycobacterium avium subsp. paratuberculosis (MAP). Autophagy is an innate immune defense mechanism for the control of intracellular bacteria, regulated by diverse signaling pathways. It plays a key role in the innate immune defense mechanism for controlling intracellular mycobacterium. Nilotinib, a tyrosine kinase inhibitor (TKI), has been studied extensively in various tumor models; however, no information exists about the pharmacological action of nilotinib in bacterial infections. We hypothesized to investigate the effect of nilotinib on the regulation of host innate immune responses against Mycobacterial infection. In addition, to devise best possible therapeutic remedy towards achieving the goal of mycobacterial diseases treatment based on molecular mechanism both in vitro and in vivo, which may provide useful strategy to treat animal's tuberculosis.

Methods: In the current study, we conducted both in vitro and in vivo experiments to investigate the role of nilotinib in the regulation of autophagy. Western blot, confocal microscopy, electron microscopy and immunohistochemistry were performed to investigate the induction of autophagy upon nilotinib treatment during M. bovis infection. Gross and histological analysis were conducted to observe the effect of nilotinib on the development of lung's lesions in mice infected with M. bovis. CFU assay was performed to determine the effect of nilotinib on the intracellular growth of M. bovis.

Results: We found that nilotinib improved the host immune responses by inhibiting intracellular survival and

growth of both M. bovis and MAP. Nilotinib also attenuated PI3k/Akt/mTOR axis for the regulation of autophagic degradation of intracellular mycobacterium mediated by inhibition of Abelson (c-ABL) tyrosine kinase. In addition, nilotinib promoted ubiquitination of intracellular bacteria through activation of E3 ubiquitin ligase parkin. Similarly, lung tissue sections of nilotinib treated mice also showed high intensity of parkin protein post M. bovis infection. From in-vivo experiment, we found that nilotinib effectively controlled M. bovis growth and the severity of disease in infected mice.

Conclusions: Altogether, our data shows that nilotinib regulates protective host innate immune responses against intracellular pathogen both in-vitro and in-vivo and can be exploited as a novel host-directed therapeutic agent in the control of M. bovis and MAP infections.

Tariq H, et al. Intl J Myco 2021, Vol. 10, Issue 5.❖

Detection of Mycobacterium avium Sub sp. paratuberculosis in Pasteurized Milk Samples in Northeast of Iran by Culture, Direct Nested PCR and PCR Methods. (Dec. 2020)

Mycobacterium avium Subsp. paratuberculosis (MAP) is a gram-positive, small, acid-fast bacillus with high environmental resistance. In animals, especially ruminants, it leads to Paratuberculosis (PTB) or Johne's disease, which is chronic granulomatous enteritis. This bacterium as the main causative agent of Crohn's disease can be a serious threat to human health. This study aimed to detect MAP in pasteurized milk samples produced in Khorasan Razavi province, Iran, using Direct Nested PCR, PCR, and culture methods. In this study, 544 milk samples from Pasteurized Milk Production Companies were selected randomly during the 3-month period. DNA was extracted from milk fat after centrifugation. In order to identify the bacteria, Direct Nested PCR and PCR tests were applied using IS900 and f57, respectively. Furthermore, to detect viable MAP, positive samples resulted from Direct Nested PCR assays were cultured on Herrold's egg medium. For identification of mycobacterial isolates, all colonies were processed by PCR based on f57. A total of 544 pasteurized milk samples were assayed, and Mycobacterium paratuberculosis was detected in 39% of them by IS900 Nested PCR, and only 4.9% of samples were positive in the culture method. All the colonies were positive for the f57using PCR. The results of this study indirectly indicated a high level of contamination of pasteurized milk to Mycobacterium paratuberculosis which is due to the large number of affected animals in livestock farms in Khorasan Razavi province. However, in comparison with the other researches, the low percentage of viable bacteria in pasteurized milk can be due to changes in temperature and time in pasteurizing systems of milk production companies in Khorasan Razavi province, Northeast of Iran.

Sadeghi, N. et al. Iranian Journal of Chemistry and Chemical Engineering (IJCCE), 39(6), 251-258. ❖

Effectiveness of Antibiotics as a Treatment Option For Patients With Crohn's Disease: A Meta Analysis (Jan. 2021)

Crohn's Disease (CD) is an Inflammatory Bowel Disease (IBD) whose etiology has been suspected to include bacterial antigens. A treatment option, therefore, would be the usage of antibiotics. To that end, the purpose of this study was to perform a meta-analysis on the ability of antibiotics inducing remission or a favorable clinical response in Crohn's disease (CD) patients. Thirty-two randomized, double-blind, placebo-controlled trials of antibiotics for treatment of CD in adults, totaling 3269 patients, were reviewed. Log-odds ratio and probability difference were performed to estimate risk difference. The Number Needed to Treat (NNT) was also calculated for each antibiotic reviewed (rifaximin, metronidazole, clarithromycin, and ciprofloxacin). The analysis revealed that antibiotics greatly improved patients' Crohn's disease activity, with a total response (defined as clinical response plus remission) odds ratio of 0.715 (95% CI: 0.6319–0.7971), a clinical response odds-ratio of 0.7478 (95% CI: 0.6214–0.8715), and a remission odds-ratio of 0.6877 (95% CI: 0.5768–0.7987). The Number Needed to Treat for each of the antibiotics used in the clinical trials were 5.018 (Ciprofloxacin), 6.480 (Clarithromycin), 7.556 (Metronidazole), and 6.824 (Rifaximin). This is an important outcome because it not only opens up a new treatment option for those suffering from Crohn's Disease but it also leads credibility to the theory that Crohn's is caused, at least in part, by bacteria, such as Mycobacterium avium subspecies paratuberculosis and adherent invasive E. coli.

Patterson J. Inflammatory Bowel Diseases, Volume 27, Issue Supplement 1, January 2021, Page S6.❖

Phage Amplification Assay for Detection of Mycobacterial Infection: A Review. (Jan. 2021)

An important prerequisite for the effective control, timely diagnosis, and successful treatment of mycobacterial infections in both humans and animals is a rapid, specific, and sensitive detection technique. Culture is still considered the gold standard in the detection of viable mycobacteria; however, mycobacteria are extremely fastidious and slow-growing microorganisms, and therefore cultivation requires a very long incubation period to obtain results. Polymerase Chain Reaction (PCR) methods are

also frequently used in the diagnosis of mycobacterial infections, providing faster and more accurate results, but are unable to distinguish between a viable and non-viable microorganism, which results in an inability to determine the success of tuberculosis patient treatment or to differentiate between an active and passive infection of animals. One suitable technique that overcomes these shortcomings mentioned is the phage amplification assay (PA). PA specifically detects viable mycobacteria present in a sample within 48 h using a lytic bacteriophage isolated from the environment. Nowadays, an alternative approach to PA, a commercial kit called Actiphage™, is also employed, providing the result within 6–8 h. In this approach, the bacteriophage is used to lyse mycobacterial cells present in the sample, and the released DNA is subsequently detected by PCR. The objective of this review is to summarize information based on the PA used for detection of mycobacteria significant in both human and veterinary medicine from various kinds of matrices
Beinhauerova M et al. Microorganisms. 2021; 9(2):237.❖

What is the evidence that mycobacteria are associated with the pathogenesis of Sjogren's syndrome? (Feb. 2021)

Sjogren's syndrome (SS) is a common, systemic autoimmune disorder primarily affecting the exocrine glands resulting in xerostomia and xerophthalmia. SS may also manifest with polyarthralgia, polyarthritis, polymyalgia, cutaneous/other organ vasculitis, interstitial lung disease, and/or various other disorders. The primary autoantibodies associated with SS and used as adjuncts to diagnosis are anti-Ro (SSA) and anti-La (SSB). The pathogenesis of SS is considered to involve genetic susceptibility and environmental triggers. An identified genetic susceptibility for SS lies in variants of the tumor necrosis factor alpha inducible protein 3 (TNFAIP3) gene, the product of which is known as A20. Deficiency or dysfunction of A20 is known to induce macrophage inflammatory response to mycobacteria, potentially increasing the repertoire of mycobacterial antigens available and predisposing to autoimmunity via the paradigm of molecular mimicry; i.e., providing a mechanistic link between genetic susceptibility to SS and exposure to environmental non-tuberculous mycobacteria (NTM). *Mycobacterium avium* ss. *paratuberculosis* (MAP) is an NTM that causes Johne's disease, an enteritis of ruminant animals. Humans are broadly exposed to MAP or its antigens in the environment and in food products from infected animals. MAP has also been implicated as an environmental trigger for a number of autoimmune diseases via cross reactivity of its heat shock protein 65 (hsp65) with host-specific proteins. In the context of SS, mycobacterial hsp65 shares epitope homology with the Ro and La proteins. A recent study showed a strong association between SS and antibodies to mycobacterial hsp65. If and when this association is validated, it would be important to determine whether bacillus Calmette-Guerin (BCG) vaccination (known to be protective against NTM likely through epigenetic alteration of innate and adaptive immunity) and anti-mycobacterial drugs (to decrease mycobacterial antigenic load) may have a preventive or therapeutic role against SS. Evidence to support this concept is that BCG has shown benefit in type 1 diabetes mellitus and multiple sclerosis, autoimmune diseases that have been linked to MAP via hsp65 and disease-specific autoantibodies. In conclusion, a number of factors lend credence to the notion of a pathogenic link between environmental mycobacteria and SS, including the presence of antibodies to mycobacterial hsp65 in SS, the homology of hsp65 with SS autoantigens, and the beneficial effects seen with BCG vaccination against certain autoimmune diseases. Furthermore, given that BCG may protect against NTM, has immune modifying effects, and has a strong safety record of billions of doses given, BCG and/or anti-mycobacterial therapeutics should be studied in SS
Dow CT et al. J Transl Autoimmun. 2021 Feb 5;4: 100085.❖

SARS-CoV-2 vaccination for patients with inflammatory bowel disease: a British Society of Gastroenterology Inflammatory Bowel Disease section and IBD Clinical Research Group position statement. (Jan. 2021)

SARS-CoV-2 has caused a global health crisis and mass vaccination programmes provide the best opportunity for controlling transmission and protecting populations. Despite the impressive clinical trial results of the BNT162b2 (Pfizer/BioNTech), ChAdOx1 nCoV-19 (Oxford/AstraZeneca), and mRNA-1273 (Moderna) vaccines, important unanswered questions remain, especially in patients with pre-existing conditions. In this position statement endorsed by the British Society of Gastroenterology Inflammatory Bowel Disease (IBD) section and IBD Clinical Research Group, we consider SARS-CoV-2 vaccination strategy in patients with IBD. The risks of SARS-CoV-2 vaccination are anticipated to be very low, and we strongly support SARS-CoV-2 vaccination in patients with IBD. Based on data from previous studies with other vaccines, there are conceptual concerns that protective immune responses to SARS-CoV-2 vaccination may be diminished in some patients with IBD, such as those taking anti-TNF drugs. However, the benefits of vaccination, even in patients treated with anti-TNF drugs, are likely to outweigh these theoretical

concerns. Key areas for further research are discussed, including vaccine hesitancy and its effect in the IBD community, the effect of immunosuppression on vaccine efficacy, and the search for predictive biomarkers of vaccine success.
Alexander JL et al. Lancet Gastroenterol Hepatol. 2021 Mar;6(3):218-224.❖

Bovine Neutrophils Release Extracellular Traps and Cooperate With Macrophages in *Mycobacterium avium* subsp. *paratuberculosis* clearance *In Vitro*. (March 2021)

Mycobacterium avium subsp. *paratuberculosis* (Map) is the underlying pathogen causing bovine paratuberculosis (PTB), an enteric granulomatous disease that mainly affects ruminants and for which an effective treatment is needed. Macrophages are the primary target cells for Map, which survives and replicates intracellularly by inhibiting phagosome maturation. Neutrophils are present at disease sites during the early stages of the infection, but seem to be absent in the late stage, in contrast to healthy tissue. Although neutrophil activity has been reported to be impaired following Map infection, their role in PTB pathogenesis has not been fully defined. Neutrophils are capable of releasing extracellular traps consisting of extruded DNA and proteins that immobilize and kill microorganisms, but this mechanism has not been evaluated against Map.

Our main objective was to study the interaction of neutrophils with macrophages during an in vitro mycobacterial infection. For this purpose, neutrophils and macrophages from the same animal were cultured alone or together in the presence of Map or *Mycobacterium bovis* Bacillus-Calmette-Guérin (BCG). Extracellular trap release, mycobacteria killing as well as IL-1β and IL-8 release were assessed. Neutrophils released extracellular traps against mycobacteria when cultured alone and in the presence of macrophages without direct cell contact, but resulted inhibited in direct contact. Macrophages were extremely efficient at killing BCG, but ineffective at killing Map. In contrast, neutrophils showed similar killing rates for both mycobacteria. Co-cultures infected with Map showed the expected killing effect of combining both cell types, whereas co-cultures infected with BCG showed a potentiated killing effect beyond the expected one, indicating a potential synergistic cooperation. In both cases, IL-1β and IL-8 levels were lower in co-cultures, suggestive of a reduced inflammatory reaction. These data indicate that cooperation of both cell types can be beneficial in terms of decreasing the inflammatory reaction while the effective elimination of Map can be compromised. These results suggest that neutrophils are effective at Map killing and can exert protective mechanisms against Map that seem to fail during PTB disease after the arrival of macrophages at the infection site.
Ladero-Auñon I et al. Front Immunol. 2021 Mar 17; 12:645304.❖

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