The MAP Gap

Bridging The Space Between The Science And You

Human Paratuberculosis Foundation Quarterly Newsletter October 2020

Human Para News

We hope this edition of our quarterly newsletter finds you safe and healthy as we head into the end of 2020.

We are excited that MAP research has resumed after a pause this summer. The initial portion of our **MAP/Crohn's Testing Study** is complete, and our research team resubmitted the paper for publication in September. The remaining WGS portion on the 40 selected MAP isolates has been put on hold due to COVID. However, individual participant results will be mailed as soon as the initial study is accepted for publication.

The **pediatric MAP testing pilot study** is nearly complete. All samples have been received by Otakaro Pathways and results are being finalized. The research team will meet in October to discuss the results.

A **third MAP study** is in the planning phase. Human Para staff have been meeting regularly with potential partners. Details will be shared as soon as the protocol is in place.

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 content this quarter.

Systematic Assessment of *Mycobacterium avium* Subspecies *Paratuberculosis* Infections from 1911–2019: A Growth Analysis of Association with Human Autoimmune Disease (Aug. 2020)

Mycobacterium avium subsp. *paratuberculosis* (MAP) is an understudied pathogen worldwide with continuous implications in human autoimmune diseases (ADs). The awareness of MAP appears to be low in many places and its research is at infant stage in many countries. The lack of worldwide coverage of the MAP research landscape calls for urgent research attention and prioritization. This present study aimed to assess MAP global research productivity with an emphasis on its implications in ADs via bibliometric and growth analytic frameworks from authors, countries, institutions, international, disciplines and collaboration network perspectives. MAP primary articles were retrieved from the Scopus database and the Web of Science from 1911 to 2019 via title-specific algorithm. Analytic results of dataset yielded a total of 3889 articles from 581 journals and 20.65 average citations per

documents. The annual growth rate of MAP research for the period was 6.31%. Based on a country's productivity (articles (%), freq. of publication (%)), the USA (887 (22.81%), 26.72%), and Australia (236 (6.07%), 6.07%) ranked the top 2 countries but Egypt and Germany had the highest average growth rate (AGR, 170%) in the last 3 years. MAP studies are generally limited to Europe, Australia, Asia, South America and few nations in Africa. It had positive growth rate (30%–100%) in relation to type 1 diabetes mellitus and rheumatoid arthritis ADs; food science and technology, immunology, agriculture, pathology, and research and experimental medicine, wildlife, environments, virulence, disease resistance, meat and meat products, osteopontin, waste milk and slurry/sludge digestion subjects; but negative growth (−130% to −30%) in ulcerative colitis and Parkinson's disease and no growth in multiple sclerosis, sarcoidosis, thyroid disorders, psoriasis, and lupus. The mapping revealed a gross lack of collaboration networking in terms of authorship, (intra- and inter-) nationally and institutionally with a generalized collaboration index of 1.82. In conclusion, inadequate resources-, knowledge- and scientific-networking hampered growth and awareness of MAP research globally. The study recommends further research to strengthen evidence of MAP's epidemiologic prevalence in ADs and proffer practical solution(s) for drug development and point-of-care diagnostics amongst other extended themes.
Ekundayo, T.C. et al. Microorganisms 2020, 8, 1212.❖

Is Multiple Sclerosis an Extra-Intestinal Manifestation of Inflammatory Bowel Disease? Food for Thought. (July 2020)

For many years there has been a suggested association between multiple sclerosis (MS) and inflammatory bowel disease (IBD). Aside from their common epidemiological and immunological similarities, there appears to be an association between the incidence of both diseases coexisting. We report a case of a 41-year-old man with chronic diarrhea and weakness, who was found to have concomitant MS and Crohn's Disease. Our report underscores the importance clinicians of maintaining a high degree of suspicion about the potential association of these conditions among these patient populations.
Dziadkowiec, K et al, Cureus. 2020 Jul; 12(7): e9485.❖

Antibiotics for Crohn's Disease: What Are We Treating? (Sept. 2020)

What causes Crohn's disease (CD)? This is a question that almost all patients ask for which no answer exists, despite great strides in the understanding genetic and environmental risk factors for CD. One of the most commonly accepted theories of CD pathogenesis is a triggering environmental exposure in a genetically susceptible host. An additional factor complicating understanding of the causative factors for CD is that an exposure may trigger the onset of intestinal inflammation, but may not be necessary for perpetuation of inflammation if the innate immune system is dysregulated. The implication of this concept is that even if a causative agent is identified and eliminated, eradication of that agent may not stop the inflammatory cascade that has already been set in motion. [Continue reading this editorial by Dr. Hou, where he discusses two microbial infections that are potentially pathogenic for Crohn's disease.]
Hou, J.K. Dig Dis Sci (2020). ❖

Mycobacterium avium Subspecies paratuberculosis Infects and Replicates within Human Monocyte-Derived Dendritic Cells. (July 2020)

Background: *Mycobacterium avium* subspecies *paratuberculosis* (MAP), a member of the mycobacteriaceae family, causes Johne's disease in ruminants, which resembles Crohn's disease (CD) in humans. MAP was proposed to be one of the causes of human CD, but the evidence remains elusive. Macrophages were reported to be the only cell where MAP proliferates in ruminants and humans and is likely the major producer of TNFα-associated inflammation. However, whether human dendritic cells (DCs), another major antigen-presenting cell (APC), have the ability to harbor MAP and disseminate infection, remains unknown.

Methods: Human monocyte-derived dendritic cells (moDCs) were infected with MAP and phagocytosis and intracellular survival were quantified by immunofluorescence (IF) and colony counts, respectively. MoDC cytokine expression was measured via ELISA and their activation state was measured via flow cytometry.

Results: We showed that MAP can infect and replicate in human moDCs as means to evade the immune system for successful infection, through inhibition of the phago-lysosome fusion via the secretion of protein tyrosine phosphatase PtpA. This mechanism initially led to a state of tolerance in moDCs and then subsequently caused a pro-inflammatory response as infection persisted, characterized by the upregulation of IL-6 and TNFα, and downregulation of IL-10. Moreover, we showed that moDCs have the ability to phagocytose up to 18% of MAP, when exposed at a multiplicity of infection of 1:1.

Conclusion: Infection and subsequent proliferation of MAP within moDCs could provide a unique means for the

dissemination of MAP to lymphoid tissue, while altering immune responses to facilitate the persistence of infection of host tissues in CD.
Rees, W.D. et al. Microorganisms 2020, 8, 994.❖

Discerning novel drug targets for treating *Mycobacterium avium ss. paratuberculosis*-associated autoimmune disorders: an *in silico* approach (Sept. 2020)

Mycobacterium avium subspecies *paratuberculosis* (MAP) exhibits 'molecular mimicry' with the human host resulting in several autoimmune diseases such as multiple sclerosis, type 1 diabetes mellitus (T1DM), Hashimoto's thyroiditis, Crohn's disease (CD), etc. The conventional therapy for autoimmune diseases includes immunosuppressants or immunomodulators that treat the symptoms rather than the etiology and/or causative mechanism(s). Eliminating MAP–the etiopathological agent might be a better strategy to treat MAP-associated autoimmune diseases. In this case study, we conducted a systematic in silico analysis to identify the metabolic chokepoints of MAP's mimicry proteins and their interacting partners. The probable inhibitors of chokepoint proteins were identified using DrugBank. DrugBank molecules were stringently screened and molecular interactions were analyzed by molecular docking and 'off-target' binding. Thus, we identified 18 metabolic chokepoints of MAP mimicry proteins and 13 DrugBank molecules that could inhibit three chokepoint proteins viz. katG, rpoB and narH. On the basis of molecular interaction between drug and target proteins finally eight DrugBank molecules, viz. DB00609, DB00951, DB00615, DB01220, DB08638, DB08226, DB08266 and DB07349 were selected and are proposed for treatment of three MAP-associated autoimmune diseases namely, T1DM, CD and multiple sclerosis. Because these molecules are either approved by the Food and Drug Administration or these are experimental drugs that can be easily incorporated in clinical studies or tested in vitro. The proposed strategy may be used to repurpose drugs to treat autoimmune diseases induced by other pathogens.
Garg A et al, Briefings in Bioinformatics, bbaa195.❖

Heterogeneity and clonal relationships of adaptive immune cells in ulcerative colitis revealed by single-cell analyses (Aug. 2020)

Inflammatory bowel disease (IBD) encompasses a spectrum of gastrointestinal disorders driven by dysregulated immune responses against gut microbiota. We integrated single-cell RNA and antigen receptor sequencing

Other News

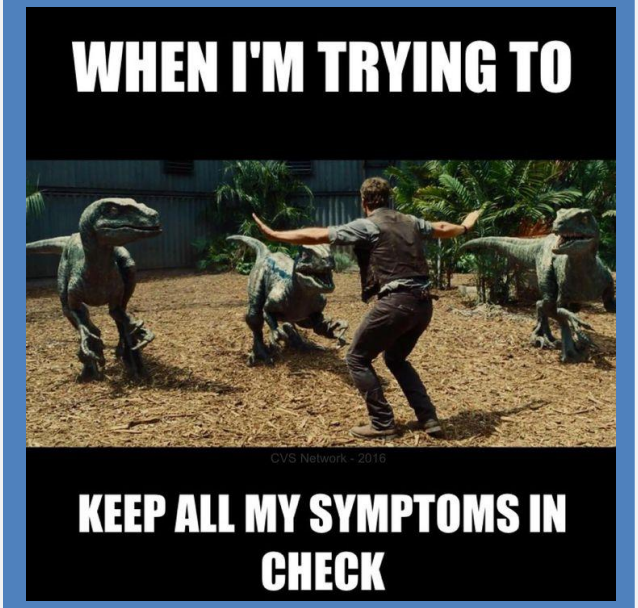
Plant Based News recently published an article exploring the link between MAP and diabetes and Crohn's disease. The authors conclude that eliminating dairy products may be the best way to minimize risk.

In a **similar publication**, Dr. Gilles R.G. Monif recently outlined his recommendations on dietary manipulation in Crohn's disease. This thoughtful article sets out a well-cited hypothesis showing how diet may augment healing for patients. Read the full abstract below.

A local newspaper in California recently reported on the **contamination risk of MAP-infected cattle** and wild elk to the public who visit Point Reyes National Seashore. The land is shared between 3 million visitors and 6,000 cattle, posing a risk to both.

In case you missed it... Can phages be used as an additive to eliminate MAP in food products? **See how one company is developing this novel product**.(Plus, read more about novel phage research on Pages 7 and 8 below!❖

Just for Fun



to elucidate key components, cellular states, and clonal relationships of the peripheral and gastrointestinal mucosal immune systems in health and ulcerative colitis (UC). UC was associated with an increase in IgG1+ plasma cells in colonic tissue, increased colonic regulatory T cells characterized by elevated expression of the transcription factor ZEB2, and an enrichment of a γδ T cell subset in the peripheral blood. Moreover, we observed heterogeneity in CD8+ tissue-resident memory T (TRM) cells in colonic tissue, with four transcriptionally distinct states of differentiation observed across health and disease. In the setting of UC, there was a marked shift of clonally related CD8+ TRM cells toward an inflammatory state, mediated, in part, by increased expression of the T-box transcription factor Eomesodermin. Together, these results provide a detailed atlas of transcriptional changes occurring in adaptive immune cells in the context of UC and suggest a role for CD8+ TRM cells in IBD.
Boland B et al, Sci Immunol. 2020 Aug 21;5(50):eabb4432.

General Overview of Nontuberculous Mycobacteria Opportunistic Pathogens: *Mycobacterium avium* and *Mycobacterium abscessus*. (Aug. 2020)

Nontuberculous mycobacteria (NTM) are emerging human pathogens, causing a wide range of clinical diseases affecting individuals who are immunocompromised and who have underlying health conditions. NTM are ubiquitous in the environment, with certain species causing opportunistic infection in humans, including *Mycobacterium avium* and *Mycobacterium abscessus*. The incidence and prevalence of NTM infections are rising globally, especially in developed countries with declining incidence rates of *M. tuberculosis* infection. *Mycobacterium avium*, a slow-growing mycobacterium, is associated with *Mycobacterium avium* complex (MAC) infections that can cause chronic pulmonary disease, disseminated disease, as well as lymphadenitis. *M. abscessus* infections are considered one of the most antibiotic-resistant mycobacteria and are associated with pulmonary disease, especially cystic fibrosis, as well as contaminated traumatic skin wounds, postsurgical soft tissue infections, and healthcare-associated infections (HAI). Clinical manifestations of diseases depend on the interaction of the host's immune response and the specific mycobacterial species. This review will give a general overview of the general characteristics, vulnerable populations most at risk, pathogenesis, treatment, and prevention for infections caused by *Mycobacterium avium*, in the context of MAC, and *M. abscessus*.
To K et al, J Clin Med. 2020 Aug; 9(8): 2541. ❖

Molecular and Serological Footprints of *Mycobacterium avium* Subspecies Infections in Zoo Animals. (Aug. 2020)

Background: Mycobacteria of the *Mycobacterium avium* complex (MAC) pose a significant risk to zoological collections. *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is a member of MAC and the causative agent of Johne's disease. Despite many reports in animals kept in zoological gardens, systemic surveillance has rarely been reported.
Methods: In this study, archived serum samples collected from animal species at the Wilhelma Zoological and Botanical Gardens in Stuttgart, Germany, were screened for the presence of antibodies against MAC and MAP. In addition, molecular investigations were performed on necropsy, fecal, and environmental samples.
Results: In total, 30/381 serum samples of various mammalian species were positive for MAC antibodies in ELISA, while one sample of a reticulated giraffe (Giraffa camelopardalis reticulata) was positive in MAP-specific ELISA. Samples from many species were positive in pan-*Mycobacterium* real-time PCR (40/43 fecal samples, 27/43 environmental samples, and 31/90 necropsy samples). Surprisingly, no sample was positive in the MAP-specific molecular assays. However, two environmental samples from primate enclosures were positive in *Mycobacterium avium* subspecies *hominissus* (MAH)-specific real-time PCR.
Conclusions: The results reveal serological indications of MAC infections in the zoological collection. However, the presence of a MAP-contaminated environment by a high-shedding individual animal or MAP-infected population is unlikely.
Roller M et al, Vet Sci. 2020 Aug 23;7(3):E117. ❖

Gallium Porphyrin and Gallium Nitrate Synergistically Inhibit *Mycobacterial* Species by Targeting Different Aspects of Iron/Heme Metabolism (Aug. 2020)

There is an urgent need for new effective and safe antibiotics active against pathogenic mycobacterial species. Gallium (Ga) nitrate (Ga(NO3)3) and Ga porphyrin (GaPP) have each been shown to inhibit the growth of a variety of mycobacterial species. Ga(III) ion derived from Ga(NO3)3 has the potential to disrupt mycobacterial Fe(III) uptake mechanisms and utilization, including replacing iron (Fe) in the active site of enzymes, resulting in disruption of function. Similarly, non-iron metalloporphyrins like heme mimetics, which can be transported across the bacterial membrane via heme-uptake pathways, would potentially block the acquisition of iron-containing heme and bind to heme-utilizing proteins,

making them nonfunctional. Given that they likely act on different aspects of mycobacterial Fe metabolism, the efficacy of combining Ga(NO3)3 and GaPP was studied in vitro against *Mycobacterium avium*, *Mycobacterium abscessus*, and *Mycobacterium tuberculosis* (M. tb). The combination was then assessed in vivo in a murine pulmonary infection model of *M. abscessus*. We observed that Ga(NO3)3 in combination with GaPP exhibited synergistic inhibitory activity against the growth of *M. avium*, *M. tb*, and *M. abscessus*, being most active against *M. abscessus*. Activity assays indicated that Ga(NO3)3 and GaPP inhibited both catalase and aconitase at high concentrations. However, the combination showed a synergistic effect on aconitase activity of *M. abscessus*. The Ga(NO3)3/GaPP combination via intranasal administration showed significant antimicrobial activity in mice infected with *M. abscessus*. *M. abscessus* CFU from the lungs of the Ga(NO3)3/GaPP treated mice were significantly less compared to non-treated or single Ga(III)-treated mice. These findings suggest that combinations of different Ga(III) compounds can synergistically target multiple iron/heme-utilizing mycobacterial enzymes. The results support the potential of combination Ga therapy for development against mycobacterial pathogens.
Choi S et al, (2020). ACS Infectious Diseases. XXXX. 10.1021/acsinfecdis.0c00113.❖

Dietary Manipulation in Crohn's Disease? (Aug. 2020)

In isolated cases of Crohn's disease, dietary manipulations have produced what biologics and steroids have yet to document: permanent remissions/cures. The questions of how and why are addressed. The postulates advanced are 1) that clinical amelioration remissions achieved through dietary manipulation are the consequence of limiting MAP antigen/cytokine interactions at the sites of Mycobacterium avium subspecies paratuberculosis mucosal attachment and ultimately, 2) by dietary restoration and enhancement of host cellular immunity, resulting in the destruction of the MAP-template which drives the dysfunctional pro-inflammatory immune response. The syngergistic coupling of diet and biologics is discussed.
Monif GRM, J Gast Hepa Rep, 2020. Volume 1(1): 3-3.❖

Preclinical Models of Nontuberculous Mycobacteria Infection for Early Drug Discovery and Vaccine Research (Aug. 2020)

Nontuberculous mycobacteria (NTM) represent an increasingly prevalent etiology of soft tissue infections in animals and humans. NTM are widely distributed in the environment and while, for the most part, they behave as saprophytic organisms, in certain situations, they can be pathogenic, so much so that the incidence of NTM infections has surpassed that of *Mycobacterium tuberculosis* in developed countries. As a result, a growing body of the literature has focused attention on the critical role that drug susceptibility tests and infection models play in the design of appropriate therapeutic strategies against NTM diseases. This paper is an overview of the in vitro and in vivo models of NTM infection employed in the preclinical phase for early drug discovery and vaccine development. It summarizes alternative methods, not fully explored, for the characterization of anti-mycobacterial compounds.
Rampacci E et al, Pathogens. 2020 Aug 6;9(8):641.❖

Extrapulmonary *Mycobacterium avium* complex infections in immunocompetent patients. (July 2020)

The Mycobacterium avium complex (MAC) includes ubiquitous bacteria that typically cause infection in immunocompromised patients. This paper reviews the presentation, diagnosis, pathogenesis, and treatment of extrapulmonary MAC infections in immunocompetent patients by compiling information from case reports identified by a PubMed search. Mycobacterium avium complex infections in immunocompetent patients can present primarily with extrapulmonary symptoms, and this makes the diagnosis of MAC infection in these patients more difficult. The American Thoracic Society has not established criteria for the diagnosis of extrapulmonary MAC infections; testing for MAC should be based on clinical suspicion or after the exclusion of all other causative agents. Methods of testing should include molecular and biochemical tests, since these tests provide more definitive identification than routine cultures. Extrapulmonary presentations usually respond well to macrolide based multi-drug regimens started as soon as a MAC infection is identified.
Yim V et al (2020). The Southwest Respiratory and Critical Care Chronicles, 8(35), 1-6.❖

An eco-friendly decontaminant to kill *Mycobacterium avium* subsp. *paratuberculosis.* (Sept. 2020)

Mycobacteria are difficult to kill due to the complexity of their cell wall. Further, *Mycobacterium avium* subsp. *paratuberculosis* (MAP) has one of the more elaborate cell wall compositions of all the mycobacteria. As a working pathogen within a research laboratory setting or as an environmental contaminant shed in the manure from infected animals, MAP is highly resistant to typical disinfectants. In the past, the most successful disinfectants

to kill mycobacteria were based upon phenolics, harsh compounds that can break down the lipids within the cell wall. New disinfectants have been developed that are less toxic to the environment, however, it is unknown how well they perform compared to more traditional disinfectants. In the present study, we present comparative data on the utility of a commercial eco-friendly disinfectant, Benefect®, compared to Amphyl®, a phenolic-based disinfectant, and Lysol®, a quaternary ammonium-based disinfectant, to kill MAP in pure culture, tissues, and manure. Results demonstrated that Benefect was highly effective with up to 100% kill of MAP within 30 min in all experiments, paralleling results obtained with Amphyl. Lysol performed the most poorly, requiring longer contact times to kill MAP. These results suggest that natural, nontoxic ingredients can be used to disinfect even hearty pathogens such as MAP effectively, both within the laboratory and on-farm

Stabel JR et al. J Microbiol Methods. 2020 Sep;176: 106001.❖

Anti-*Mycobacterium paratuberculosis* (MAP) therapy for Crohn's disease: an overview and update. (July 2020)

The role of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in the pathogenesis of Crohn's disease (CD) has been strongly debated for many years. MAP is the known aetiological agent of Johne's disease, a chronic enteritis affecting livestock. At present, due to the paucity of high-quality data, anti-MAP therapy (AMT) is not featured in international guidelines as a treatment for CD. Although the much-quoted randomised trial of AMT did not show sustained benefits over placebo, questions have been raised regarding trial design, antibiotic dosing and the formulation used. There are several lines of evidence supporting the CD and MAP association with uncontrolled and controlled trials demonstrating effectiveness, including a retrospective review of cases treated at our own institution. Here, we provide an overview of the evidence supporting and refuting AMT in CD before focusing on updates of the current research in the field, including the ongoing trials with the novel RHB-104 formulation and the MAP vaccine trial. While controversial, gastroenterologists are often asked about long-term combination antibiotic therapy for CD. There has been broadcast and social media coverage surrounding this, particularly with regard to current trials. Although patients should not be deterred from treatments of proven effectiveness, this review aims to help with commonly asked questions and highlights our own approach for the use of anti-MAP in specific circumstances.

Honap S et al. Frontline Gastroenterology, 28 July 2020..❖

Seroprevalence of Immunoglobulin G Antibodies Against *Mycobacterium avium* subsp. *paratuberculosis* in Dogs Bred in Japan. (July 2020)

In this study, the seroprevalence of immunoglobulin G (IgG) antibodies against *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in dogs bred in Japan was evaluated. Ninety-two non-clinical samples were obtained from three institutes and fifty-seven clinical samples were obtained from a veterinary hospital in Japan. Serum titers of total IgG, IgG1 and IgG2 isotype antibodies against MAP were measured using an indirect enzyme-linked immunosorbent assay (ELISA). The IgG antibodies against MAP in non-clinical serum obtained from three institutes was observed to be 2.4%, 20% and 9.0%. Similarly, the IgG1 antibodies titers against MAP were observed to be 7%, 20% and 0%. Lastly, the IgG2 antibodies against MAP were observed to be 7%, 20% and 4.4%. No significance differences in these titers were observed among the three institutes. The IgG, IgG1 and IgG2 antibodies in serum obtained from a veterinary hospital were observed to be 55.3%, 42% and 42%, respectively. Significant differences were found between the non-clinical and clinical samples. The titers in the clinical samples showed a high degree of variance, whereas low variance was found in the non-clinical samples. The IgG antibody levels were thought to be induced following exposure to MAP-contaminated feed. The difference in titers between the clinical and non-clinical samples is likely to be related to the amount of MAP antigen contamination in dog foods.

Kuribayashi T et al. Vet Sci. 2020 Jul 17;7(3):E93.❖

Anti-Mycobacterial Antibiotic Therapy Induces Remission in Active Paediatric Crohn's Disease. (July 2020)

Crohn's disease is increasing in incidence and prevalence in younger people and is of a particularly aggressive nature. One emerging treatment targets Mycobacterium avium paratuberculosis (MAP), an organism implicated in the causation of Crohn's disease. This study reviewed a cohort of paediatric patients with active Crohn's disease treated with Anti-Mycobacterial Antibiotic Therapy (AMAT). Sixteen paediatric patients, the majority of whom had failed conventional immunosuppressive therapy, were treated with AMAT. Endoscopic remission was scored using the Simple Endoscopic Score for Crohn's Disease and clinical remission was assessed using the Weighted Paediatric Crohn's Disease Activity Index (wPCDAI). Inflammatory blood markers were also routinely recorded. Patients were followed up clinically and endoscopically during treatment after an average of two months (range 1–6) and 17 months

(range 2–49), respectively. A significant reduction in both scores assessing clinical improvement ($p < 0.001$) and mucosal healing ($p < 0.0078$) was observed at these timepoints; 47% of patients had achieved clinical remission and 63% endoscopic remission. Haemoglobin and serum inflammatory markers normalised for more than 50% of the cohort by six months of treatment. No adverse effects were reported throughout treatment. This is the first report of Anti-Mycobacterial Antibiotic Therapy offering a safe and efficacious therapy for paediatric patients with Crohn's disease. Further larger randomised studies are required in order to validate these findings.
Agrawal G et al. Microorganisms. 2020 Aug; 8(8): 1112..❖

Role of Infections in the Pathogenesis of Rheumatoid Arthritis: Focus on Mycobacteria (Sept. 2020)

Rheumatoid arthritis (RA) is a systemic inflammatory autoimmune disease characterized by chronic erosive polyarthritis. A complex interaction between a favorable genetic background, and the presence of a specific immune response against a broad-spectrum of environmental factors seems to play a role in determining susceptibility to RA. Among different pathogens, mycobacteria (including *Mycobacterium avium* subspecies *paratuberculosis*, MAP), and *Epstein–Barr virus* (EBV), have extensively been proposed to promote specific cellular and humoral response in susceptible individuals, by activating pathways linked to RA development. In this review, we discuss the available experimental and clinical evidence on the interplay between mycobacterial and EBV infections, and the development of the immune dysregulation in RA.
Bo, M et al. Microorganisms 2020, 8, 1459.❖

Notch-1 Signaling Modulates Macrophage Polarization and Immune Defense against *Mycobacterium avium paratuberculosis* Infection in Inflammatory Diseases (July 2020)

Despite the extensive research on Notch signaling involvement in inflammation, its specific role in macrophage response in autoimmune disease and defense mechanisms against bacterial infection, such as *Mycobacterium avium paratuberculosis* (MAP), remains unknown. In this study, we investigated the molecular role of Notch-1 signaling in the macrophage response during MAP infection. In particular, we measured the in vitro effect of MAP on Notch-1 signaling and downstream influence on interleukin (IL)-6 and myeloid cell leukemia sequence-1 (MCL-1) and consequent cellular apoptosis, MAP viability, and macrophage polarization. Overall, the data show significant upregulation in Notch-1, IL-6, and MCL-1 in MAP-infected macrophages, parallel with a decrease in apoptosis and elevated pro-inflammatory response in these infected cells. On the contrary, blocking Notch signaling with γ-secretase inhibitor (DAPT) decreased MAP survival and burden, increased apoptosis, and diminished the pro-inflammatory response. In particular, the treatment of infected macrophages with DAPT shifted macrophage polarization toward M2 anti-inflammatory phenotypic response. The outcome of this study clearly demonstrates the critical role of Notch signaling in macrophage response during infection. We conclude that MAP infection in macrophages activates Notch-1 signaling and downstream influence on IL-6 which hijack MCL-1 dependent inhibition of apoptosis leading to its chronic persistence, and further inflammation. This study supports Notch-1 signaling as a therapeutic target to combat infection in autoimmune diseases such as Crohn's disease and Rheumatoid Arthritis.
Keewan, E et al. Microorganisms 2020, 8, 1006.❖

Randomized Trial of Ciprofloxacin Doxycycline and Hydroxychloroquine Versus Budesonide in Active Crohn's Disease. (July 2020)

Fifty-nine patients were recruited across 8 sites. Including crossover, 39 patients received antibiotics/ hydroxy-chloroquine and 39 received budesonide. At 10 weeks, 24 weeks, and 52 weeks on initial therapy, only 2/27, 2/27, and 1/27 were in remission on antibiotics/ hydroxychloroquine compared with 8/32, 1/32, and 1/32 on budesonide ($P = 0.092$ at 10 weeks). Withdrawals by 10 weeks due to adverse events were seen in 15 receiving antibiotics/hydroxychloroquine and 6 budesonide. Results including crossover were more promising with 9/24 patients receiving antibiotics/hydroxychloroquine per protocol in remission by 24 weeks. No correlation was seen between response to antibiotics/hydroxychloroquine and ASCA/OmpC antibody status or disease location. Overall results with this antibiotic/hydroxychloroquine combination were unimpressive, but long-term remission is seen in some patients and justifies further study.
Rhodes, J.M. et al. Dig Dis Sci (2020).❖

Bacteriophages and the One Health Approach to Combat Multidrug Resistance: Is This the Way? (July 2020)

Antimicrobial resistance necessitates action to reduce and eliminate infectious disease, ensure animal and human health, and combat emerging diseases. Species such as *Acinetobacter baumanniii*, vancomycin resistant *Enterococcus*, methicillin resistance *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, as well as other WHO priority pathogens, are

becoming extremely difficult to treat. In 2017, the EU adopted the "One Health" approach to combat antibiotic resistance in animal and human medicine and to prevent the transmission of zoonotic disease. As the current therapeutic agents become increasingly inadequate, there is a dire need to establish novel methods of treatment under this One Health Framework. Bacteriophages (phages), viruses infecting bacterial species, demonstrate clear antimicrobial activity against an array of resistant species, with high levels of specificity and potency. Bacteriophages play key roles in bacterial evolution and are essential components of all ecosystems, including the human microbiome. Factors such are their specificity, potency, biocompatibility, and bactericidal activity make them desirable options as therapeutics. Issues remain, however, relating to their large-scale production, formulation, stability, and bacterial resistance, limiting their implementation globally. Phages used in therapy must be virulent, purified, and well characterized before administration. Clinical studies are warranted to assess the in vivo pharmacokinetics and pharmacodynamic characteristics of phages to fully establish their therapeutic potential.
Garvey, M. Antibiotics 2020, 9, 414.❖

A novel one-day phage-based test for rapid detection and enumeration of viable *Mycobacterium avium* subsp. *paratuberculosis* in cows' milk. (Sept. 2020)

Bacteriophage-based methods for the rapid detection of viable *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in veterinary specimens are a recent addition to the Johne's disease diagnostic toolbox. Here, we report the use of D29 mycobacteriophage-coated tosylactivated paramagnetic beads to capture and concentrate MAP cells from samples (termed phagomagnetic separation, PhMS) and then naturally lyse viable MAP cells (from the inside out) to provide DNA for IS900 qPCR purposes. Transmission electron microscopy confirmed that D29 phages had bound to beads in the correct orientation and that the phage-coated beads captured MAP cells from a suspension. During test optimization, conventional IS900 PCR results were used to subjectively assess the effect of different phage:bead coating ratios, differing amounts of coated beads during PhMS, optimal incubation time post-PhMS to obtain maximal MAP DNA, and the potential benefit of a brief heat shock (55 °C/1 min) prior to IS900 TaqMan qPCR. The limit of detection 50% (LOD50%) of the optimised PhMS-qPCR assay was 10.00 MAP cells/50 ml milk (95% CI 1.20–82.83). Finally, in order to demonstrate the new assay's ability to detect viable MAP in naturally contaminated milk, bulk tank milk samples from 100 dairy farms were tested. Forty-nine (49%) of these tested PhMS-qPCR-positive, with viable MAP numbers detected ranging from 3–126 MAP/50 ml. The novel PhMS-qPCR assay is a sensitive, specific and easy-to-apply phage-based assay for viable MAP, with potential application for milk surveillance or diagnosis of Johne's disease.
Foddai, A.C.G., Grant, I.R. Appl Microbiol Biotechnol (2020).❖

Directed Evolution of a Mycobacteriophage. (April 2019)

Bacteriophages represent an alternative strategy to combat pathogenic bacteria. Currently, *Mycobacterium tuberculosis* infections constitute a major public health problem due to extensive antibiotic resistance in some strains. Using a non-pathogenic species of the same genus as an experimental model, *Mycobacterium smegmatis*, here we have set up a basic methodology for mycobacteriophage growth and we have explored directed evolution as a tool for increasing phage infectivity and lytic activity. We demonstrate mycobacteriophage adaptation to its host under different conditions. Directed evolution could be used for the development of future phage therapy applications against mycobacteria.
Cebriá-Mendoza M, et al. Antibiotics (Basel). 2019;8(2): 46.❖
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