The MAP Gap

Bridging The Space Between The Science And You

Human Paratuberculosis Foundation Quarterly Newsletter October 2019

Human Para News

The **Human Para Forum** needs you! If you haven't checked it out lately, there are new threads visible only to registered members. Registration is free and easy. Come on over and join us!

Human Para has been narrowing down potential partners in anticipation of a **pediatric MAP testing study**. Study design and methodology is critical, and our team participated in a conference call in September to discuss what type of study may be most needed. As soon as we have a final protocol in place, we will release more information to the community.

Testing is now complete for the first phase of our inaugural **Joint MAP Testing Study** and all sites have delivered results. The study was recently unblinded, and data analysis is ongoing. Whole genome sequencing has begun on selected mycobacterial cultures. **Look for a post with more up to date information coming soon!** We appreciate your patience while we work diligently to provide accurate data as soon as possible.

Black Friday. Cyber Monday.

#GIVINGTUESDAY

December 3, 2019

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

Synthetic cathelicidin LL-37 reduces Mycobacterium avium subsp. paratuberculosis internalization and pro-inflammatory cytokines in macrophages. (Sept. 2019)

The main Mycobacterium avium subsp. paratuberculosis (MAP) causes chronic diarrheic intestinal infections in domestic and wild ruminants (paratuberculosis or Johne's disease) for which there is no effective treatment. Critical in the pathogenesis of MAP infection is the invasion and survival into macrophages, immune cells with ability to carry on phagocytosis of microbes. In a search for effective therapeutics, our objective was to determine whether human cathelicidin LL-37, a small peptide secreted by leuckocytes and epithelial cells, enhances the macrophage ability to clear MAP infection. In murine (J774A.1) macrophages, MAP was quickly internalized, as determined by confocal microscopy using green fluorescence protein expressing MAPs. Macrophages infected with MAP had increased transcriptional gene expression of pro-

inflammatory TNF-α, IFN-γ, and IL-1β cytokines and the leukocyte chemoattractant IL-8. Pretreatment of macrophages with synthetic LL-37 reduced MAP load and diminished the transcriptional expression of TNF-α and IFN-γ whereas increased IL-8. Synthetic LL-37 also reduced the gene expression of Toll-like receptor (TLR)-2, key for mycobacterial invasion into macrophages. We concluded that cathelicidin LL-37 enhances MAP clearance into macrophages and suppressed production of tissue-damaging inflammatory cytokines. This cathelicidin peptide could represent a foundational molecule to develop therapeutics for controlling MAP infection.
Cirone AM et al. Cell Tissue Res. 2019 Sep 2.❖

Potential Vaccines for Treating Crohn's Disease. (Aug. 2019)

Crohn's disease (CD) is an inflammatory disease of the gastrointestinal tract (GIT) tract and can affect several parts of the digestive system. There is a relationship between impaired mucosal barrier in the GIT of inflammatory bowel disease patients and the role of bacteria such as Mycobacterium avium paratuberculosis in CD. Apart from different therapeutic approaches for treating CD, development of a vaccine is a novel modality. In the present article, most available therapeutic opportunities in the last decade, especially the possibility of vaccines against CD, are reviewed. According to search, availability of a new generation of vaccines against CD is expected specially tolerogenic ex vivo-derived dendritic cell-based vaccines. Regarding different locations of the challenge and the variety of clinical manifests of CD and also the type of resident antigen-presenting cells and their traffic in different parts of gastrointestinal tract, the results of immunotherapy with dendritic cell-based vaccines may vary case by case.
Rostami-Nejad M et al. Iran Biomed J. 2019 Aug 26.❖

Alteration of Gut Microbiota in Inflammatory Bowel Disease (IBD): Cause or Consequence? IBD Treatment Targeting the Gut Microbiome. (August 2019)

Inflammatory bowel disease (IBD) is a chronic complex inflammatory gut pathological condition, examples of which include Crohn's disease (CD) and ulcerative colitis (UC), which is associated with significant morbidity. Although the etiology of IBD is unknown, gut microbiota alteration (dysbiosis) is considered a novel factor involved in the pathogenesis of IBD. The gut microbiota acts as a metabolic organ and contributes to human health by performing various physiological functions; deviation in the gut flora composition is involved in various disease pathologies, including IBD. This review aims to summarize the current knowledge of gut microbiota alteration in IBD and how this contributes to intestinal inflammation, as well as explore the potential role of gut microbiota-based treatment approaches for the prevention and treatment of IBD. The current literature has clearly demonstrated a perturbation of the gut microbiota in IBD patients and mice colitis models, but a clear causal link of cause and effect has not yet been presented. In addition, gut microbiota-based therapeutic approaches have also shown good evidence of their effects in the amelioration of colitis in animal models (mice) and IBD patients, which indicates that gut flora might be a new promising therapeutic target for the treatment of IBD. However, insufficient data and confusing results from previous studies have led to a failure to define a core microbiome associated with IBD and the hidden mechanism of pathogenesis, which suggests that well-designed randomized control trials and mouse models are required for further research. In addition, a better understanding of this ecosystem will also determine the role of prebiotics and probiotics as therapeutic agents in the management of IBD.
Khan I et al. Pathogens. 2019 Aug 13;8(3).❖

Seroprevalence of anti-microbial antibodies in the normal healthy population with implications in chronic diseases. (July 2019)

We have previously discovered a panel of anti-microbial antibodies from patients with Crohn's disease (CD) and Sjogren's syndrome (Sjo). We have also demonstrated the increase of these anti-microbial antibodies in other autoimmune disease such as rheumatoid arthritis, systemic lupus erythematosus and multiple sclerosis in a small number of cases. The seroprevalence of these antibodies in the normal healthy population is unknown. We set to survey the normal population for these anti-microbial antibodies. We collected 288 blood samples from the donor units of the leukocyte-reduced red blood cells from the American Red Cross, and examined the presence of the anti-microbial antibodies in these blood samples using direct ELISA assays established in our laboratory using the recombinant microbial protein antigens. Our results showed that the prevalence of RPOB antibody in the normal blood donor population is 2.4% (7 positive of 288 samples). The prevalence of EF-G antibody is 4.2% (12 positive of 288 samples), ATP5a 5.2% (15 positive), Hsp65 2.8% (8 positive), EF-Tu 5.6% (16 positive), and NMPC 4.2% (12 positive). Meanwhile, in 109 patients with Crohn's disease and 28 patients with Sjogren's syndrome,

these anti-bacterial antibodies are significantly increased ($p<0.001$). These results indicate that the specific anti-microbial antibodies within the normal general population are uncommon, but frequent in chronic disease states. The presence of increased anti-microbial antibodies in the blood of patients but not in normal controls can serve as biomarkers for chronic diseases such as Crohn's disease and Sjogren's, and their presence indicates abnormal B-cell/plasma cell function in response to the commensal/pathogenic microbes. Since the antigens were derived from the common microbes present on the surface of the normal population, the antimicrobial antibodies in patients with diseases but not in the normal population suggest a deficient clearance of the microbes from the circulation by the innate immunity system in chronic diseases. These results also raise questions of bacterial vaccination using whole bacterial extracts as these anti-bacterial antibodies appear pathogenic rather than protective, offering fresh thinking in designing bacterial vaccines as preventive or therapeutic measures in chronic diseases such as Crohn's disease and Sjogren's Disease.
Zhang P et al. bioRxid doi: https://doi.org/10.1101/693655 ❖

Unraveling the Structure of the Mycobacterial Envelope. (July 2019)

The mycobacterial cell envelope consists of a typical plasma membrane of lipid and protein surrounded by a complex cell wall composed of carbohydrate and lipid. In pathogenic species, such as Mycobacterium tuberculosis, an outermost "capsule" layer surrounds the cell wall. This wall embraces a fundamental, covalently linked "cell-wall skeleton" composed of peptidoglycan, solidly attached to arabinogalactan, whose penta-saccharide termini are esterified by very-long-chain fatty acids (mycolic acids). These fatty acids form the inner leaflet of an outer membrane, called the mycomembrane, whose outer leaflet consists of a great variety of non-covalently linked lipids and glycolipids. The thickness of the mycomembrane, which is similar to that of the plasma membrane, is surprising in view of the length of mycoloyl residues, suggesting dedicated conformations of these fatty acids. Finally, a periplasmic space also exists in mycobacteria, between the plasma membrane and the peptidoglycan. This article provides a comprehensive overview of this biologically important and structurally unique mycobacterial cell compartment.
Daffé M, Microbiol Spectr. 2019 Jul;7(4). ❖

Other News

The British Society of Gastroenterology published **updated Guidelines for IBD** in June. A discussion of antibiotic therapy in Crohn's disease can be found in Section 4.2.4. The Guideline outlines information on paratuberculosis, pediatric research and the RedHill and Selby trials, but does not draw firm conclusions.

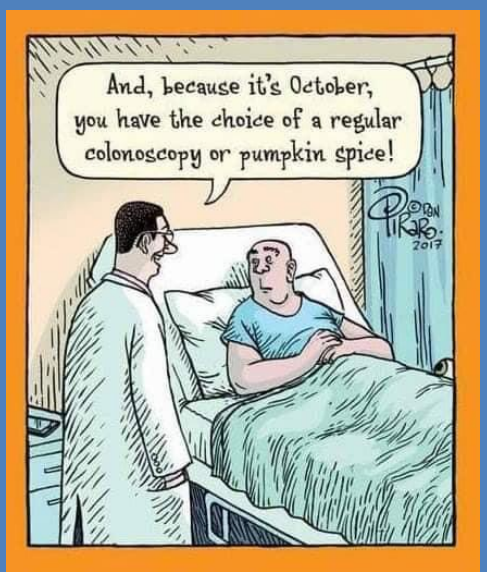
See the recently updated page on **Antimicrobial Therapy on Johnes.org**. While this site is primarily focused on MAP in a veterinary context, it is an excellent resource on MAP in general, with updated news items weekly.

SEE WHO'S POSTING IN OUR FORUM

RedHill Biopharma will be presenting positive data on their Stage 3 trial of RHB-104 in Crohn's disease at the **American College of Gastroenterology Annual Scientific Meeting** on October 25-30th.

Abstract submission for the 15th International Colloquium on Paratuberculosis to be held in Dublin, Ireland on June 14th to 18th has opened and will remain open until December 1st. See the **Conference website** for more details about how to submit an abstract.❖

Just for Fun



Insights into the Physiology and Metabolism of a Mycobacterial Cell in an Energy-Compromised State. (Sept. 2019)

Mycobacterium tuberculosis, a bacterium that causes tuberculosis, poses a serious threat, especially due to the emergence of drug-resistant strains. *M. tuberculosis* and other mycobacterial species, such as *M. smegmatis*, are known to generate an inadequate amount of energy by substrate-level phosphorylation and mandatorily require oxidative phosphorylation (OXPHOS) for their growth and metabolism. Hence, antibacterial drugs, such as bedaquiline, targeting the multisubunit ATP synthase complex, which is required for OXPHOS, have been developed with the aim of eliminating pathogenic mycobacteria. Here, we explored the influence of suboptimal OXPHOS on the physiology and metabolism of *M. smegmatis*. *M. smegmatis* harbors two identical copies of *atpD*, which codes for the β subunit of ATP synthase. We show that upon deletion of one copy of *atpD* (*M. smegmatis* Δ*atpD*), *M. smegmatis* synthesizes smaller amounts of ATP and enters into an energy-compromised state. The mutant displays remarkable phenotypic and physiological differences from the wild type, such as respiratory slowdown, reduced biofilm formation, lesser amounts of cell envelope polar lipids, and increased antibiotic sensitivity compared to the wild type. Additionally, *M. smegmatis* Δ*atpD* overexpresses genes belonging to the dormancy operon, the β-oxidation pathway, and the glyoxylate shunt, suggesting that the mutant adapts to a low energy state by switching to alternative pathways to produce energy. Interestingly, *M. smegmatis* Δ*atpD* shows significant phenotypic, metabolic, and physiological similarities with bedaquiline-treated wild-type *M. smegmatis*. We believe that the identification and characterization of key metabolic pathways functioning during an energy-compromised state will enhance our understanding of bacterial adaptation and survival and will open newer avenues in the form of drug targets that may be used in the treatment of mycobacterial infections.
Patil V et al, J Bacteriol. 2019 Sep 6;201(19). pii: e00210-19.❖

Novel Microbial-Based Immunotherapy Approach for Crohn's Disease. (July 2019)

Current Crohn's disease (CD) therapies focus on suppressing immune function and come with consequent risk, such as infection and cancer. Notwithstanding, most CD patients still experience disease progression. There is a need for new CD treatment strategies that offer better health outcomes for patients.

Aims: To assess safety, efficacy, and tolerability of a novel microbial-derived immunotherapy, QBECO, that aims to restore rather than suppress immune function in CD.

Methods: A randomized, double-blind, placebo-controlled trial was conducted in 68 patients with moderate-to-severe CD. Primary endpoints: safety and Week 8 clinical improvement. Secondary endpoints: Week 8 clinical response and remission. Week 8 responders continued blinded treatment through Week 16; non-responders received open-label QBECO from Weeks 9-16. Exploratory analyses included immune biomarker and genotype assessments.

Results: QBECO was well-tolerated. Mean reduction in Crohn's Disease Activity Index (CDAI) score was -68 for QBECO vs. -31 for placebo at Week 8. Improvement with QBECO continued through Week 16 (-130 CDAI reduction). Week 8 QBECO clinical response, improvement and remission rates were 41.2%, 32.4%, 29.4% vs. 26.5%, 23.5%, 23.5% for placebo. TNFα inhibitor-naïve subjects achieved higher response rates at Week 8 with QBECO (64%) vs. placebo (26%). Specific immune biomarkers were identified that linked to QBECO response.

Conclusion: This proof-of-concept study supports further investigation for the use of QBECO as a novel immunotherapy approach for CD. Biomarker analyses suggests it may be feasible to personalize CD treatment with QBECO. Larger trials are now needed to confirm clinical improvement and the unique biological findings.
Sutcliffe S et al. Front Med (Lausanne) 2019 Jul 19;6: 170.

Phage therapy as a renewed therapeutic approach to mycobacterial infections: a comprehensive review. (Sept. 2019)

Mycobacterial infections are considered to a serious challenge of medicine, and the emergence of MDR and XDR tuberculosis is a serious public health problem. Tuberculosis can cause high morbidity and mortality around the world, particularly in developing countries. The emergence of drug-resistant Mycobacterium infection following limited therapeutic technologies coupled with the serious worldwide tuberculosis epidemic has adversely affected control programs, thus necessitating the study of the role bacteriophages in the treatment of mycobacterial infection. Bacteriophages are viruses that are isolated from several ecological specimens and do not exert adverse effects on patients. Phage therapy can be considered as a significant alternative to antibiotics for treating MDR and

XDR mycobacterial infections. The useful ability of bacteriophages to kill *Mycobacterium* spp has been explored by numerous research studies that have attempted to investigate the phage therapy as a novel therapeutic/ diagnosis approach to mycobacterial infections. However, there are restricted data about phage therapy for treating mycobacterial infections. This review presents comprehensive data about phage therapy in the treatment of mycobacterial infection, specifically tuberculosis disease. Azimi T et al, Infect Drug Resist. 2019; 12: 2943–2959. ❖

Possible role of L-form switching in recurrent urinary tract infection. (Sept. 2019)

Recurrent urinary tract infection (rUTI) is a major medical problem, especially in the elderly and infirm, but the nature of the reservoir of organisms responsible for survival and recolonisation after antibiotic treatment in humans is unclear. Here, we demonstrate the presence of cell-wall deficient (L-form) bacteria in fresh urine from 29 out of 30 older patients with rUTI. In urine, E. coli strains from patient samples readily transition from the walled state to L-form during challenge with a cell wall targeting antibiotic. Following antibiotic withdrawal, they then efficiently transition back to the walled state. E. coli switches between walled and L-form states in a zebrafish larva infection model. The results suggest that L-form switching is a physiologically relevant phenomenon that may contribute to the recurrence of infection in older patients with rUTI, and potentially other infections.
Mickiewicz KM et al, Nat Commun. 2019 Sep 26;10(1): 4379. ❖

Vaccine approaches for the 'therapeutic management' of *Mycobacterium avium* subspecies *paratuberculosis* infection in domestic livestock. (Sept. 2019)

High endemicity of Johne's disease (JD) in herds adversely affects heavy milk yielding breeds by reducing the per animal productivity and 'productive life-span'.This review evaluates different vaccines used for its control and summarizes the benefits of 'global vaccine' in the four major domestic livestock species, namely goat, sheep, buffalo and cattle. Vaccines developed by using 'native strains' revealed both 'therapeutic' and preventive effects in domestic livestock. The 'therapeutic' role of vaccine in animals suffering from clinical JD turned out to be valuable in some cases by reversing the disease process and animals returning back to health and production. Good herd management, improved hygiene, 'test and cull' methodology, disposal of animal excreta and monitoring of MAP bio-load were also regarded as crucial in the 'therapeutic' management of JD. Vaccine approaches have been widely adopted in JD control programs and may be considered as a valuable adjunct in order to utilize huge populations of otherwise un-productive livestock. It has been shown that vaccination was the preeminent strategy to control JD, because it yielded approximately 3-4 times better benefit-to-cost ratios than other strategies. Internationally, 146 vaccine trials/studies have been conducted in different countries for the control of JD and have shown remarkable reduction in its national prevalence. It is concluded that for Johne's disease, there cannot be global vaccines or diagnostic kits as solutions have to come from locally prevalent strains of MAP. Despite some limitations, vaccines might still be an effective strategy to reduce or eradicate JD.
Gupta S et al, Vet Q. 2019 Sep 16:1-18. ❖

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