New publications in the PARATUBERCULOSIS database (1064-1068)

1064 Badr, H.M. (2011)
Inactivation of Mycobacterium paratuberculosis and Mycobacterium tuberculosis in fresh soft cheese by gamma radiation
Radiation Physics and Chemistry, 80, 1250-1257

The effectiveness of gamma irradiation on the inactivation of Mycobacterium paratuberculosis, Mycobacterium bovis and Mycobacterium tuberculosis in fresh soft cheese that prepared from artificially inoculated milk samples was studied. Irradiation at dose of 2 kGy was sufficient for the complete inactivation of these mycobacteria as they were not detected in the treated samples during storage at 4 +/- 1 degrees C for 15 days. Moreover, irradiation of cheese samples, that were prepared from un-inoculated milk, at this effective dose had no significant effects on their gross composition and contents from riboflavin, niacin and pantothenic acid, while significant decreases in vitamin A and thiamin were observed. In addition, irradiation of cheese samples had no significant effects on their pH and nitrogen fractions contents, except for the contents of ammonia, which showed a slight, but significant, increases due to irradiation. The analysis of cheese fats indicated that irradiation treatment induced significant increase in their oxidation parameters and contents from free fatty acids: however, the observed increases were relatively low. On the other hand, irradiation of cheese samples induced no significant alterations on their sensory properties. Thus, irradiation dose of 2 kGy can be effectively applied to ensure the safety of soft cheese with regards to these harmful mycobacteria. (C) 2011 Elsevier Ltd. All rights reserved

Evaluation of the in vitro activity of gallium nitrate against Mycobacterium avium subsp paratuberculosis
American Journal of Veterinary Research, 72, 1243-1246

Objective-To evaluate the in vitro susceptibility of various field isolates of Mycobacterium avium subsp paratuberculosis (MAP) to gallium nitrate. Sample-10 isolates of MAP, including 4 isolated from cattle, 2 isolated from bison, 1 isolated from an alpaca, and 3 isolated from humans. Procedures-The in vitro susceptibility to gallium nitrate was tested by use of broth culture with detection of MAP growth by means of a nonradiometric automated detection method. For each MAP isolate, a series of 7 dilutions of gallium nitrate (concentrations ranging from 200 to 1,000 mu M) were tested. Gallium nitrate was considered to have caused 90% and 99% inhibition of the MAP growth when the time to detection for culture of the MAP stock solution and a specific concentration of gallium nitrate was delayed and was similar to that obtained for culture of the MAP stock solution (without the addition of gallium nitrate) diluted 1:10 and 1:100, respectively. Results-Gallium nitrate inhibited MAP growth in all 10 isolates. The susceptibility to gallium nitrate was variable among isolates, and all isolates of MAP were inhibited in a dose-dependent manner. Overall, the concentration that resulted in 90% inhibition ranged from <200 mu M for the most susceptible isolates to 743 mu M for the least susceptible isolates. Conclusions and Clinical Relevance-Gallium nitrate had activity against all 10 isolates of MAP tested in vitro and could potentially be used as a prophylactic agent to aid in the control of MAP infections during the neonatal period. (Am J Vet Res 2011;72:1243-1246)

Diverse Cytokine Profile from Mesenteric Lymph Node Cells of Cull Cows Severely...
Affected with Johne’s Disease
Clinical and Vaccine Immunology, 18, 1467-1476

Mycobacterium avium subsp. paratuberculosis, the causative agent of Johne’s disease, is able to dampen or distort immune responses at the mucosal sites and coexist with a massive infiltration of immune cells in the gastrointestinal tract. Knowledge of the mechanism by which M. avium subsp. paratuberculosis subverts the immune response at the mucosal level in cattle is important for the development of improved disease control strategies, including new vaccines and diagnostic tests. In this study, 38 cull cows from herds infected with M. avium subsp. paratuberculosis were divided into four groups, based on M. avium subsp. paratuberculosis culture from gut tissues and histopathological lesion scores. Cytokine gene expression and secretion from M. avium subsp. paratuberculosis sonicate-stimulated peripheral blood mononuclear cell (PBMC) and mesenteric lymph node (MLN) cultures of the animals were compared. Antigen stimulation of MLN cells from the severely lesioned group resulted in significant upregulation of the mRNA expression of five cytokines, gamma interferon (IFN-gamma), interleukin-10 (IL-10), IL-13, IL-17A, and tumor necrosis factor alpha (TNF-alpha), which have a diverse range of functions, while there was no significant upregulation of these cytokines by the other groups. There were major differences between the responses of the PBMC and MLN cultures, with higher levels of secreted IFN-gamma released from the MLN cultures and, conversely, higher levels of IL-10 released from the PBMC cultures. The upregulation of all five cytokines from cells at the site of infection in the severely lesioned animals suggested a dysregulated immune response, contributing to a failure to clear infection in this group of animals.

Dipstick immunoassay for rapid diagnosis of paratuberculosis in small ruminants
Small Ruminant Research, 99, 214-221

Highly sensitive and specific gold nanoparticle based dipstick immunoassay using protoplasmic antigen of Mycobacterium avium subspecies paratuberculosis (MAP) for diagnosis of paratuberculosis was introduced. In this method the colloidal gold nanoparticles (GNPs) were coated with MAP protoplasmic antigen using alkanethiols derivatives and anti-MAP rabbit antibodies. These antigen coated GNPs acts as a detector reagent in this assay. The antibody (test sera) immobilized onto nitrocellulose (NC) membrane binds with antigen coated GNPs and this was detected visually by development of red color (due to gold nanoparticles) on the nitrocellulose membrane (NC). This immunoassay was specific to MAP when compared with other common mycobacterial species. In addition, the dipstick immunoassay was able to detect an antibody dilution of 1:50 of polyclonal anti-MAP antibody raised in rabbit. Further the efficacy of this dipstick immunoassay was evaluated by comparing this test to other serological tests like agar gel immunodiffusion (AGID) and absorbed ELISA in detecting MAP antibodies in sheep and goats. Out of 536 sera samples (271 sheep and 265 goats) collected from different parts of India, positive results recorded were; AGID 79 (14.74%), dipstick immunoassay 83 (15.49%), and absorbed ELISA 88(16.42%). Though dipstick immunoassay was less sensitive compared to absorbed ELISA, but it was simple to perform in field conditions and requires less time. This dipstick immunoassay was also compared in live animals using intradermal Johnin test and nested PCR (detecting mycobacterial DNA in feces) in 65 animals (35 sheep and 30 goats) of our institutional animal houses. Of which, positive results recorded in animals were; Johnin test 21 (32%), dipstick immunoassay 26 (40%) and fecal PCR detected mycobacterial DNA in 28 (43%) animals. Though fecal nested PCR for mycobacterial DNA gave best results, dipstick immunoassay might be considered for large scale use in field condition for on-site screening of paratuberculosis. (C) 2011 Elsevier B.V. All rights reserved

Possible association between Mycobacterium avium subsp paratuberculosis infection and Crohn’s disease
Revista Medica de Chile, 139, 794-801
Paratuberculosis is a chronic intestinal disease of animals caused by Mycobacterium avium subsp. paratuberculosis (MAP), which has some pathological features similar to Crohn's disease (CD) in humans. The presence of MAP in food for human consumption and in affected tissues of patients with CD has been detected. Therefore, a causal association between this microorganism and the disease in humans, has been postulated. However, several related studies have failed to confirm this hypothesis and the scientific acceptance of MAP as a zoonotic agent remains controversial. This review presents the main findings related to this issue, contrasting evidences for and against an association between MAP and CD. The need to promote national studies focusing on this area is suggested. (Rev Med Chile 2011; 139: 794-801)

New publications in the CROHN'S DISEASE AND PARATUBERCULOSIS database (589-592)


Objective: The mammalian commensal gut microbiota is highly diverse and displays an individual-specific composition determined by host genotype and environmental factors. The temporal development of host-microbial homeostasis in the digestive tract is recognised as a major function of the immune system. However, the underlying cellular and molecular mechanisms are just beginning to come to light. Nucleotide-binding, oligomerisation domain 2 (NOD2) recognises bacterial muramyl dipeptide and is regarded as a pivotal sensor molecule of the intestinal barrier. The aim of this study was to investigate its influence on the development and composition of the intestinal microbiota using a Nod2-deficient mouse model. Methods: The dynamics of faecal and ileal microbial composition were investigated in Nod2(+/+) and Nod2(-/-) mice on a C57BL/6J background. We assessed microbial diversity and composition using 16S ribosomal RNA gene-based clone library sequencing and high throughput pyrosequencing and quantified the observed changes by real-time PCR. Changes in the major bacterial phyla were investigated in human samples by quantitative real-time PCR. Results: We found that adult Nod2-deficient mice display a substantially altered microbial community structure and a significantly elevated bacterial load in their faeces and terminal ileum compared to their wild-type counterparts. Interestingly, we demonstrate that these findings are also present in weaning mice, indicating a profound influence of Nod2 on the early development and composition of the intestinal microbiota. We demonstrate that NOD2 genotypes also influence the microbial composition in humans. Conclusions: Our results point to an essential role of Nod2 for the temporal development and composition of the host microbiota, both in mice and in humans, which may contribute to the complex role of NOD2 for the aetiopathogenesis of Crohn's disease.


Dendritic cells (DCs) as sentinels of the immune system are important for eliciting both primary and secondary immune responses to a plethora of microbial pathogens. Cooperative stimulation of a complex set of pattern-recognition receptors, including TLR2 and nucleotide-binding oligomerization domain (NOD)-like receptors on DCs, acts as a rate-limiting factor in determining the initiation and mounting of the robust immune response. It underscores the need for "decoding" these multiple receptor interactions. In this study, we demonstrate that TLR2 and NOD receptors cooperatively regulate functional maturation of human DCs.
Intriguingly, synergistic stimulation of TLR2 and NOD receptors renders enhanced refractoriness to TGF-beta- or CTLA-4-mediated impairment of human DC maturation. Signaling perturbation data suggest that NOTCH1-PI3K signaling dynamics assume critical importance in TLR2- and NOD receptor-mediated surmounting of CTLA-4- and TGF-beta-suppressed maturation of human DCs. Interestingly, the NOTCH1-PI3K signaling axis holds the capacity to regulate DC functions by virtue of PKC delta-MAPK-dependent activation of NF-kappa B. This study provides mechanistic and functional insights into TLR2-and NOD receptor-mediated regulation of DC functions and unravels NOTCH1-PI3K as a signaling cohort for TLR2 and NOD receptors. These findings serve in building a conceptual foundation for the design of improved strategies for adjuvants and immunotherapies against infectious diseases.

591 Werts, C., Rubino, S., Ling, A., Girardin, S.E., Philpott, D.J. (2011) 
Nod-like receptors in intestinal homeostasis, inflammation, and cancer 
Journal of Leukocyte Biology, 90, 471-482

NLRs have been shown in a number of models to protect against microbial infection through their ability to participate in "pattern recognition" and their triggering of inflammatory pathways to control infection. Over the past few years, however, the role of NLRs, especially Nod1, Nod2, and NLRP3, in intestinal homeostasis has been highlighted. Indeed, these specific NLRs have been implicated in IBD, in particular, the association of Nod2 with CD, yet a clear understanding of how dysfunctional NLR activation leads to aberrant inflammation is still the focus of much investigation. In this review, we will examine how NLRs participate in the maintenance of gut homeostasis and how upset of this regulation can tip the balance toward chronic inflammation and intestinal cancer. J. Leukoc. Biol. 90: 471-482; 2011

ATG16L1 polymorphisms are associated with NOD2-induced hyperinflammation 
Autophagy, 7, 1074-1075

In recent years considerable advances in understanding the pathogenesis of Crohn disease have been achieved, with the identification of susceptibility variants of genes that are part of the autophagy machinery, i.e., ATG16L1 and IRGM. Subsequent functional studies have been conducted to unravel the underlying mechanism of this genetic association. For the ATG16L1 Thr300Ala polymorphism (c.898A > G, rs2241880), it was demonstrated that the risk variant is associated with a reduced capacity of innate immune cells to induce autophagy upon triggering with specific microbial structures such as peptidoglycans, that are specifically recognized by the intracellular pattern-recognition receptor nucleotide oligomerization domain-2 (NOD2). Due to the impaired autophagy activation, autophagosome formation and the subsequent antigen presentation through the major histocompatibility complex are diminished, leading to decreased immune activation. However, these findings arguing for defective host defense mechanisms in individuals bearing the ATG16L1 300Ala variant, and subsequent bacterial persistence in the gut mucosa, provide no conclusive explanation for the excessive inflammation observed in Crohn disease.