Sale of Raw Milk in Northern Italy: Food Safety Implications and Comparison of Different Analytical Methodologies for Detection of Foodborne Pathogens
Foodborne Pathogens and Disease, 9, 293-297

The safety of raw milk sold in Northern Italy was investigated in relation to hygiene quality parameters and presence of Salmonella spp., Listeria monocytogenes, thermotolerant Campylobacter, and Verocytotoxin producing Escherichia coli O157:H7. The performance of different analytical methods used-official culture method (ISO), modified Bacteriological Analytical Manual cultural method (mBAM), and polymerase chain reaction (PCR)-was evaluated. The presence of Mycobacterium avium subsp. paratuberculosis (Map) was investigated only by PCR. All samples met regulations for alkaline phosphatase and inhibitory substance, while 18% and 44.8% of samples collected from vending machines had, respectively, somatic cell count (SCC) >300,000/mL and total bacterial count (TBC) >50,000 CFU/mL. The correlation between hygienic quality parameters in samples collected from bulk tank and vending machines showed a significant increase of TBC in vending machines meaning that raw milk was mishandled during distribution and sale. All pathogens investigated were detected in raw milk sold at vending machines; a total of five samples (5%) had at least one pathogen, of which two were detected by PCR and three by mBAM. None of the samples was positive by cultural ISO methods. Even if the comparison of analytical methods showed that none performs significantly better than the others, testing a higher volume of milk (25 versus 210 mL) affects significantly the detection rate of pathogens. Three samples (3%) were positive for Map, suggesting that raw milk is a significant source of Map exposure for consumers. The observed TBC increase and the detection of several pathogenic bacteria pose questions on the safety of raw milk; the use of ISO seems inefficient in detecting a low contamination level of pathogens in milk and consequently not appropriate as official method for testing. In order to ensure consumer's safety, a new approach for the raw milk chain is required.

Identification of a novel tetrapeptide structure of the Mycobacterium avium glycopeptidolipid that functions as a specific target for the host antibody response
Biochemical and Biophysical Research Communications, 419, 687-691

Mycobacterium avium complex (MAC) is a group of non-tuberculous mycobacteria that cause tuberculosis-like diseases in humans. Unlike Mycobacterium tuberculosis, MAC expresses high levels of glycopeptidolipids (GPLs) containing a well-defined tetrapeptide-amino alcohol core, composed of D-phenylalanine, D-allo-threonine, D-alanine, and L-alaninol, that is modified with a fatty acid and sugar residues. Surprisingly, however, a careful scrutiny of the mass spectrum of MAC GPLs revealed the presence of ions that could not readily accountable for the known GPL structure. The magnitude of the ions was increased prominently when GPLs were isolated from the valine-supplemented culture, and the ions representing the authentic GPL species were diminished, suggesting the possibility that the basic structure of the peptide backbone might be altered in response to the exogenously added valine. Indeed, further mass spectrometry (MS)/MS and gas chromatography-MS analysis indicated a substitution of D-valine for the N-terminal D-phenylalanine of the tetrapeptide core, and the presence of D-valine and the absence of D-phenylalanine was confirmed by high-performance liquid chromatography, using the derivatized amino acid residues that were released from the
tetrapeptide. Finally, specific antibodies to the purified valine-containing GPL species were detected in the serum of a MAC-infected guinea pig. Therefore, these results identify a new molecular species of MAC GPLs with immunogenic potential. (C) 2012 Elsevier Inc. All rights reserved


Paratuberculosis, or Johne's disease (JD), is caused by Mycobacterium avium subspecies paratuberculosis (MAP), is found in ruminants worldwide and can cause considerable economic losses in cattle. Control efforts and programs for JD in cattle are very diverse among European states, in Austria clinical JD is rated as a notifiable disease since 2006. The voluntary control programs established in many European countries, show different aims, measurements and acceptance. Most control programs for JD are based on a test and cull strategy, combined with hygienic precautions. Unfortunately, the willingness to participate in such programs by farmers and veterinarians is limited due to high costs, intensive workload, long duration and limited success. To overcome this drawback and to harmonize the control of MAP in Europe, a basic program with defined minimum standards is suggested. This "minimal program" for the control of JD in cattle consists of 3 steps. Step 1 includes diagnostic evaluation of every case of diarrhea in adult cattle and culling of animals with clinical JD. Step 2 is the implementation of basic management measures, adapted to the potentials of the individual farm. Step 3 consists of regularly evaluation of the MAP-herd status with the focus on MAP-shedding animals. This basic control program can be performed with reasonable costs and work load in most cattle herds and might serve as an international minimum standard for MAP-control in cattle. Such a program can also pose an incentive to decrease MAP-infections for those not willing to participate in more sophisticated control programs


Paratuberculosis (Ptb), caused by Mycobacterium avium subsp. paratuberculosis (Map), is a chronic and progressive granulomatous enteritis that affects many livestock and wild animals worldwide. The clinical disease is called Johne's disease (JD). In Japan, all dairy cattle (half million head) are examined for Ptb every five years. About 1000 the officially examined cattle are diagnosed annually as positive for Ptb, but most of these exhibit only minor or no clinical signs and typical lesions in recent years. In contrast to the situation in Japan, the disease prevalence in western countries is very high. We have used ELISA and a culture examination of Map, and recently real-time PCR to diagnose this disease. In this review, the author outlines the history of the epidemic and national practical strategies to control paratuberculosis in Japan


The United States of America (U.S.) has made several attempts over the years to develop a producer accepted voluntary program. The focus of the U.S. Voluntary Bovine Johne's Disease Control Program (VBJDCP) is to provide producers with the tools to control Johne's disease on their farms and identify herds with a low risk for the presence of Mycobacterium avium subspecies paratuberculosis (MAP) infection. The VBJDCP includes an evaluation of producers' operations to identify practices that could allow the transmission of MAP among animals or between premises. Once risky practices have been identified, a herd management plan is developed to assist the producer in correcting risky practices. In addition to management changes, vaccination is a control tool allowed in the U.S. because it reduces the clinical signs of Johne's disease and the shedding of MAP. Testing is used in addition to management changes. While the classification component of the VBJDCP dictates the
amount and type of testing herd owners are required to conduct, the education and management components of the VBJDCP does not specify testing protocols. The testing for control is intended to fit the needs and resources of producers and can be quite flexible. Management changes on the farm remain the key to control and programs cannot replace well thought out plans by producers that are specific to their resources, facilities, and operation

New publications in the CROHN’S DISEASE AND PARATUBERCULOSIS database (670-676)


Since its discovery, the unique properties of the naturally occurring amino acid, L-ergothioneine (EGT; 2-mercaptohistidine trimethylbetaine), have intrigued researchers for more than a century. This widely distributed thione is only known to be synthesized by non-yeast fungi, mycobacteria and cyanobacteria but accumulates in higher organisms at up to millimolar levels via an organic cation transporter (OCTN1). The physiological role of EGT has yet to be established. Numerous in vitro assays have demonstrated the antioxidant and cytoprotective capabilities of EGT against a wide range of cellular stressors, but an antioxidant role has yet to be fully verified in vivo. Nevertheless the accumulation, tissue distribution and scavenging properties, all highlight the potential for EGT to function as a physiological antioxidant. This article reviews our current state of knowledge. This article is part of a Special Issue entitled: Antioxidants and Antioxidant Treatment in Disease. (C) 2011 Elsevier B.V. All rights reserved

671 Van Eden, W., Spiering, R., Broere, F., van der Zee, R. (2012) A case of mistaken identity: HSPs are no DAMPs but DAMPErs Cell Stress & Chaperones, 17, 281-292

Until recently, the immune system was seen solely as a defense system with its primary task being the elimination of unwanted microbial invaders. Currently, however, the functional significance of the immune system has obtained a much wider perspective, to include among others the maintenance and restoration of homeostasis following tissue damage. In this latter aspect, there is a growing interest in the identification of molecules involved, such as the so-called danger or damage-associated molecular patterns (DAMPs), also called alarmins. Since heat shock proteins are archetypical molecules produced under stressful conditions, such as tissue damage or inflammation, they are frequently mentioned as prime examples of DAMPs (Bianchi, J Leukoc Biol 81:1-5, 2007; Kono and Rock, Nat Rev Immunol 8:279-289, 2008; Martin-Murphy et al., Toxilcol Lett 192:387-394, 2010). See for instance also a recent review (Chen and Nunez, Science 298:1395-1401, 2010). Contrary to this description, we recently presented some of the arguments against a role of heat shock protein as DAMPs (Broere et al., Nat Rev Immunol 11:565-c1, 2011). With this perspective and reflection article, we hope to elaborate on this debate and provide additional thoughts to further ignite this discussion on this critical and evolving issue


Clinically available anti-tumour necrosis factor (TNF) biologics, which inhibit both soluble (sTNF) and transmembrane forms (tmTNF) of TNF, eliminating all TNF signalling, have successfully treated autoimmune diseases including uveitis. These have potentially serious side effects such as reactivation of latent Mycobacterium tuberculosis and, therefore, more specific inhibition of TNF signalling pathways may maintain clinical efficacy while reducing
adverse effects. To determine the effects of specific pharmacological inhibition of sTNF on macrophage activation and migration, we used a mouse model of uveitis (experimental autoimmune uveoretinitis; EAU). We show that selective inhibition of sTNF is sufficient to suppress EAU by limiting inflammatory CD11b+ macrophages and CD4+ T cell migration into the eye. However, inhibition of both sTNF and tmTNF is required to inhibit interferon-γ-induced chemokine receptor 2, CD40, major histocompatibility complex class II and nitric oxide (NO) up-regulation, and signalling via tmTNF is sufficient to mediate tissue damage. In confirmation, intravitreal inhibition of sTNF alone did not suppress disease, and inflammatory cells that migrated into the eye were activated, generating NO, thus causing structural damage to the retina. In contrast, intravitreal inhibition of both sTNF and tmTNF suppressed macrophage activation and therefore disease. We conclude that sTNF is required for inflammatory cell infiltration into target tissue, but at the tissue site inhibition of both sTNF and tmTNF is required to inhibit macrophage activation and to protect from tissue damage.


Interleukin-23 (IL-23), a member of the IL-12 family, is a heterodimeric cytokine composed of p19 and p40 subunits. IL-23 plays crucial roles in the activation, proliferation and survival of IL-17-producing helper T cells which induce various autoimmune diseases. Human p19 and p40 subunits were cloned and coexpressed in N-acetylglucosaminyltransferase I-negative 293S cells, which produce high-mannose-type glycosylated proteins in order to diminish the heterogeneity of modified N-linked glycans. The glycosylated human IL-23 was purified and crystallized by the hanging-drop vapour-diffusion method. X-ray diffraction data were then collected to 2.6 angstrom resolution. The crystal belonged to space group P6(1) or P6(5), with unit-cell parameters a = b = 108.94, c = 83.79 angstrom, gamma = 120 degrees. Assuming that the crystal contains one molecule per asymmetric unit, the calculated Matthews coefficient was 2.69 angstrom(3) Da(-1), with a solvent content of 54.2%. The structure was determined by the molecular-replacement method, with an initial R factor of 52.6%. After subsequent rigid-body and positional refinement, the R-work and R-free values decreased to 31.4% and 38.7%, respectively.


The objective of the study was to investigate the association of caspase activating and recruitment domain 8 (CARD8) and nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain containing 3 (NLRP3) polymorphisms with rheumatoid arthritis (RA) in Tunisian and French populations. CARD8 (c.30T>A, rs2043211) and NLRP3 (c.2113C>A, rs35829419) single nucleotide polymorphisms (SNPs) were genotyped in 100 French RA trio families and 141 Tunisian patients with RA and 191 unrelated healthy controls, using TaqMan (R) allelic discrimination assay. The genetic analyses for the association and linkage in French families were performed using the comparison of allelic frequencies (AFBAC), the genotype relative risk (GRR) and the transmission disequilibrium test (TDT). Data for case and control samples were analysed by chi-square-test, GRR and odds ratio (OR). No significant differences between alleles and genotypes frequencies were detected in French trio and Tunisian patients with RA and controls, either with CARD8 or with NLRP3 SNPs both in French and in Tunisian populations. Moreover, stratifying patients according to the presence of rheumatoid factor (RF), anti-cyclic peptides antibodies (ACPA), erosion, nodules, other autoimmune disease or HLA-DRB1*04-positive subgroups did not show any significant association with CARD8 or NLRP3 (P = 0.05). This study suggests that variations in the innate
immunity genes CARD8 (p.C10X) and NLRP3 (p.Q705K) have no effect on RA susceptibility either in the Tunisian or in the French population

Redundant and Antagonistic Functions of Galectin-1,-3, and-8 in the Elicitation of T Cell Responses
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Galectins, a family of mammalian lectins, have emerged as key regulators of the immune response. We previously demonstrated that galectin (Gal)-8, from the tandem-repeat subgroup, exerts two well-defined effects on mouse naive peripheral CD4 T cells: Ag-specific costimulation and Ag-independent proliferation. These stimulatory signals on naive T cells have not been described for any other Gal. Therefore, we investigated whether Gal-1 and Gal-3, two prominent members of the Gal family, share the stimulatory effects exerted by Gal-8 on naive T cells. We found that Gal-1 costimulated Ag-specific T cell responses similarly to Gal-8, as evaluated in the DO11.10 TCROVA-transgenic mouse model, by acting simultaneously on APCs and target CD4 T cells. In contrast, Gal-3 failed to costimulate Ag-specific T cell responses; moreover, it antagonized both Gal-1 and Gal-8 signals. We observed that both Gal-1 and Gal-3 were unable to induce Ag-independent proliferation; however, when two Gal-1 molecules were covalently fused, the resulting chimeric protein efficiently promoted proliferation. This finding indicates that Gal-1 might eventually induce proliferation and, moreover, stresses the requirement of a tandem-repeat structure. Remarkably, a single dose of recombinant Gal-1 or Gal-8 administered together with a suboptimal Ag dose to DO11.10 mice strengthened weak responses in vivo. Taken together, these findings argue for the participation of Gals in the initiation of the immune response and allow the postulation of these lectins as enhancers of borderline Ag responses, thus representing potential adjuvants for vaccine formulations. The Journal of Immunology, 2012, 188: 2991-2999

Regulatory Circuits Mediated by Lectin-Glycan Interactions in Autoimmunity and Cancer
Immunity, 36, 322-335

Numerous regulatory programs have been identified that contribute to the restoration of homeostasis at the conclusion of immune responses and to safeguarding against the detrimental effects of chronic inflammation and autoimmune pathology. Malignant cells may usurp these pathways to create immunosuppressive networks that thwart antitumor responses. Herein we review the role of endogenous lectins (C-type lectins, siglecs, and galectins) and specific N- and O-glycans generated by the coordinated action of glycosyltransferases and glycosidases that together promote regulatory signals that control immune cell homeostasis. We also discuss the mechanisms by which glycan-dependent regulatory programs integrate into canonical circuits that amplify or silence immune responses related to autoimmunity and neoplastic disease