Mycobacterium avium subsp. paratuberculosis is an important pathogen that causes Johne's disease in animals and has been implicated in Crohn's disease in man yet few data exist on its physiological adaptation in either the host or the environment. In this study, the proteomic responses of the two distinct strains of M. a. paratuberculosis, cattle (C) and sheep (S), to hypoxia and starvation were studied in vitro. Nutrient starvation inhibited growth of both strains and was lethal for S strain after 12 weeks. Hypoxia induced a state of very low metabolic activity but rapid resuscitation occurred upon restoration of an aerobic atmosphere, consistent with the dormancy response of other mycobacteria. A total of 55 protein spots differentially expressed in response to starvation and/or hypoxic stress in one or both strains were identified from 2D gels and classified based on biological function. Antioxidant enzymes, oxidoreductase enzymes and proteins involved in amino acid metabolism, fatty acid metabolism, ATP and purine biosynthesis, proteolysis, cell wall synthesis, protein synthesis, signal recognition and hypothetical proteins with putative functions including dormancy response regulators and universal stress proteins were identified. These proteins are potential screening targets for future diagnosis, prevention and control of M. a. paratuberculosis infection and their identification will assist understanding the pathogenesis of diseases caused by this organism. (c) 2008 Elsevier B.V. All rights reserved

Paratuberculosis (Johne's disease), caused by Mycobacterium avium subsp. paratuberculosis, is an important disease for bovines, although its genetic basis is poorly understood. In this study, three candidate genes were typed to study the associations between single nucleotide polymorphisms (SNPs) and paratuberculosis susceptibility (measured in a 1 or 0 form) at the haplotype level. A significant risk haplotype, constructed by a variant allele (C) at the first SNP and a common allele (A) at the second SNP, within the CARD15 gene was detected to trigger genetic effects on paratuberculosis infection in an overdominance manner. Marginally significant haplotypes were identified for the other two genes. The results obtained will provide scientific guidance about the selection and prediction of resistance types in bovines

The objective of the present study was to determine whether offspring of dams infected with Mycobacterium avium subsp. paratuberculosis (Map) have all increased risk for Map infection. Antemortem and postmortem disease surveillance data were used to identify positive and test-negative ruminants born at the Zoological Society of San Diego (ZSSD) between 1991 and 2007 and to estimate cumulative lifetime incidence. A matched
case-control Study, nested within the Population, was conducted and conditional logistic regression analyses were used to quantify the association between infection status of offspring and their dams. Cases (infected ruminants, n = 47) were matched to controls (test-negative ruminants, n = 152) by species, birth date, birth enclosure, and follow-up time to control for confounding factors. The overall Cumulative lifetime incidence of infection was estimated at 2.2%, but it decreased over time and varied by species. There was a significant association between infection status of offspring and their dams (odds ratio [OR] = 6.8, P < 0.01), which is consistent with studies in domestic livestock species. The association was stronger for animals whose dam was diagnosed within 2 years of their birth (OR = 9.0, P < 0.01) than for animals whose dam was diagnosed more than 2 years after their birth (OR = 6.0, P < 0.01) compared to animals with test-negative dams. For positive animals born to a positive dam, 85.3% of the Map infections were attributable to having a positive dam. For the entire Population of ZSSD ruminants, 36.8% of the cases were attributable to having a positive dam. Findings will help guide future management of Map infection in zoo ruminant populations.


Cases Of tuberculosis due to Mycobacterium avium subsp. avium in 52 adult red deer (Cervus elaphus) from a farm were studied using different diagnostic techniques. Immunological probes consisted of the comparative cervical tuberculin (CCT) skin test, the interferon-gamma (IFN-gamma) assay, and 2 enzyme-linked immunosorbent assays (ELISAs) employing either avian purified protein derivatives or protoplasmatic antigen (PPA-3) as antigens. Three of the animals were euthanized due to severe weakness, loss of weight, and emaciation. Macroscopically, the 3 animals showed tuberculous lesions located mainly in lymph nodes of the digestive system and small intestine but also in other organs and lymph nodes. Polymerase chain reaction was carried out on samples from the 3 deer using primers to detect IS901, IS900, and IS6110, specific for Mycobacterium avium subsp. avium, Mycobacterium avium subsp. paratuberculosis, and Mycobacterium tuberculosis complex, including Mycobacterium bovis, respectively. The last 2 agents cause pathologies very similar to avian tuberculosis in deer. The 3 deer were strongly positive by both ELISAs, slightly positive by the IFN-gamma test, and 1 of 2 was positive by the CCT test. As with domestic ruminants, ELISA could detect deer in an advanced stage of infection, with large numbers of mycobacteria.


Abstract not available.

Gao, A.L., Odumeru, J., Raymond, M., Hendrick, S., Duffield, T., Mutharia, L. (2009) Comparison of milk culture, direct and nested polymerase chain reaction (PCR) with fecal culture based on samples from dairy herds infected with Mycobacterium avium subsp paratuberculosis Canadian Journal of Veterinary Research-Revue Canadienne de Recherche Veterinaire, 73, 58-64

Mycobacterium avium subsp. paratuberculosis (MAP) is the etiologic agent of Johne's disease in cattle and other farm ruminants, and is also a suspected pathogen of Crohn's disease in humans. Development of diagnostic methods for MAP infection has been a challenge over the last few decades. The objective of this study was to investigate the relationship between different methods for detection of MAP in milk and fecal samples. A total of 134 milk samples and 110 feces samples were collected from 146 individual cows in 14 MAP-infected herds in southwestern Ontario. Culture, IS900 polymerase chain reaction (PCR) and nested PCR methods were used for detecting MAP in milk; results were compared with those of fecal culture. A significant relationship was found between milk culture, direct PCR, and nested PCR (P < 0.05). The fecal culture results were not related to any of the 3 assay methods used for the milk samples (P > 0.10). Although fecal culture showed a higher sensitivity than the milk culture method, the difference was not significant (P = 0.2473). The number of MAP colony-forming units (CFU) isolated by culture from fecal samples was, on average, higher than that isolated from milk samples (P = 0.0083). There was no significant correlation between the number of CFU cultured from milk and from feces.
(Pearson correlation coefficient = 0.1957, N = 63, P = 0.1243). The animals with high numbers of CFU in milk culture may not be detected by fecal culture at all, and vice versa. A significant proportion (29% to 41%) of the positive animals would be missed if only 1 culture method, instead of both milk and feces, were to be used for diagnosis. This suggests that the shedding of MAP in feces and milk is not synchronized. Most of the infected cows were low-level shedders. The proportion of low-level shedders may even be underestimated because MAP is killed during decontamination, thus reducing the chance of detection. Therefore, to identify suspected Johne's-infected animals using the tests in this study, both milk and feces samples should be collected in duplicate to enhance the diagnostic rate. The high MAP kill rate identified in the culture methods during decontamination may be compensated for by using the nested PCR method, which had a higher sensitivity than the IS900 PCR method used.


Background: Infection of cattle with Mycobacterium avium subspecies paratuberculosis (M. ap) causes severe economic losses to the dairy industry in the USA and worldwide. In an effort to better examine diversity among M. ap strains, we used optical mapping to profile genomic variations between strains of M. ap K-10 (sequenced strain) and M. ap ATCC 19698 (type strain). Results: The assembled physical restriction map of M. ap ATCC 19698 showed a genome size of 4,839 kb compared to the sequenced K-10 genome of 4,830 kb. Interestingly, alignment of the optical map of the M. ap ATCC 19698 genome to the complete M. ap K-10 genome sequence revealed a 648-kb inversion around the origin of replication. However, Southern blotting, PCR amplification and sequencing analyses of the inverted region revealed that the genome of M. ap K-10 differs from the published sequence in the region starting from 4,197,080 bp to 11,150 bp, spanning the origin of replication. Additionally, two new copies of the coding sequences > 99.8% were identified, identical to the MAP0849c and MAP0850c genes located immediately downstream of the MAP3758c gene. Conclusion: The optical map of M. ap ATCC 19698 clearly indicated the miss-assembly of the sequenced genome of M. ap K-10. Moreover, it identified 2 new genes in M. ap K-10 genome. This analysis strongly advocates for the utility of physical mapping protocols to complement genome sequencing projects.


The presence of acid-fast bacilli compatible with Mycobacterium avium subsp. paratuberculosis in fecal and tissues smears was investigated using the Ziehl-Neelsen staining. A total of 26 fecal smears and 104 tissues smears collected from 26 sheep with confirmed paratuberculosis were analyzed. Sixteen (61.5%) fecal smears showed compatible with acid-fast bacilli on microscopic examination after staining. Twenty animals (76.9%) were diagnosed based on the positivity of tissues smears. The Ziehl-Neelsen sensitivities to faecal smears, tissues smears, and a combination of both were 61.5%, 76.9%, and 80.8%, respectively.

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Changes in the glycan structures of some glycoproteins have been observed in autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis. A deficiency of alpha-mannosidase II, which is associated with branching in N-glycans, has been found to induce SLE-like glomerular nephritis in a mouse model. These findings suggest that the alteration of the glycosylation has some link with the development of SLE. An analysis of glycan alteration in the disordered tissues in SLE may lead to the development of improved diagnostic methods and may help to clarify the carbohydrate-related pathogenic mechanism of inflammation in
SLE. In this study, a comprehensive and differential analysis of N-glycans in kidneys from SLE-model mice and control mice was performed by using the quantitative glycan profiling method that we have developed previously. In this method, a mixture of deuterium-labelled N-glycans from the kidneys of SLE-model mice and non-labelled N-glycans from kidneys of control mice was analysed by liquid chromatography/mass spectrometry. It was revealed that the low-molecular-mass glycans with simple structures, including agalactobiantennary and paucimannose-type oligosaccharides, markedly increased in the SLE-model mouse. On the other hand, fucosylated and galactosylated complex type glycans with high branching were decreased in the SLE-model mouse. These results suggest that the changes occurring in the N-glycan synthesis pathway may cause the aberrant glycosylations on not only specific glycoproteins but also on most of the glycoproteins in the SLE-model mouse. The changes in glycosylation might be involved in autoimmune pathogenesis in the model mouse kidney.


While glial cells are recognized for their roles in maintaining neuronal function, there is growing appreciation that resident central nervous system (CNS) cells initiate and/or augment inflammation following trauma or infection. We have recently demonstrated that microglia and astrocytes constitutively express nucleotide-binding oligomerization domain-2 (NOD2), a member of the novel nucleotide-binding domain leucine-rich repeat region containing a family of proteins (NLR) that functions as an intracellular receptor for a minimal motif present in all bacterial peptidoglycans. In this study, we have confirmed the functional nature of NOD2 expression in astrocytes and microglia and begun to determine the relative contribution that this NLR makes in inflammatory CNS responses to clinically relevant bacterial pathogens. We demonstrate the increased association of NOD2 with its downstream effector molecule, Rip2 kinase, in primary cultures of murine microglia and astrocytes following exposure to bacterial antigens. We show that this cytosolic receptor underlies the ability of muramyl dipeptide to augment the production of inflammatory cytokines by glia following exposure to specific ligands for disparate Toll-like receptor homologues. In addition, we demonstrate that NOD2 is an important component in the in vitro inflammatory responses of resident glia to N. meningitidis and B. burgdorferi antigens. Finally, we have established that NOD2 is required, at least in part, for the astrogliosis, demyelination, behavioral changes, and elevated inflammatory cytokine levels observed following in vivo infection with these pathogens. As such, we have identified NOD2 as an important component in the generation of damaging CNS inflammation following bacterial infection. (C) 2008 Wiley-Liss, Inc


Once dismissed as just the icing on the cake, sugar molecules are emerging as vital components in life's intricate machinery. Our understanding of their function within the context of the proteins and lipids to which they are attached has matured rapidly, and with it the far reaching clinical implications are becoming understood. Recent advances in high-throughput glycomic techniques, glyco biomarker profiling, glyco-bioinformatics and development of increasingly sophisticated glyco-arrays, combined with our increased understanding of the molecular details of glycosylation have facilitated the linkage between aberrant glycosylation and human diseases, and highlighted the possibility of using glyco-biomarkers as potential determinants of disease and its progression. The focus of this review is to give an insight into the biological significance of these glycomodifications, highlight some specific examples of glyco-biomarkers in relation to autoimmunity and in particular rheumatoid arthritis, and to explore the exciting possibility of exploiting these for diagnostic and prognostic strategies

NOD2, an intracellular sensor of bacteria-derived muramyl dipeptide (MDP) has been implicated as a key player in intestinal immune health and disease. Mast cells (MCs) have been reported to be increased in the gut of patients with inflammatory bowel disease. However, NOD2 expression and its role in human primary MCs are unknown. The number of NOD2(+) intestinal MCs was significantly increased in the Crohn's disease (CD) specimens compared to Ulcerative colitis (UC) specimens and controls. IFN-gamma upregulated NOD2 expression in MCs. CXCL10 and urokinase-type plasminogen activator (uPA) upregulation was specific to MCs activated by MDP compared to MCs activated by LIDS and IgE/anti-IgE. MDP-induced upregulation of ICAM-1, VCAM-1, and uPA was specific to MCs compared to mononuclear cells. The number of CXCL10(+)NOD2(+) intestinal MCs was significantly increased in the CD patients. Our results suggest that NOD2(+) MCs have specific pathogenic roles that involve the recruitment of inflammatory cells in CD. (C) 2008 Elsevier Inc. All rights reserved

Pattern recognition receptors (PRRs) are an integral part of the innate immune system and govern the early control of foreign microorganisms. Single nucleotide polymorphisms (SNPs) in the intracellular pattern recognition receptor nucleotide-binding oligomerization domain-containing protein (NOD2, nucleotide oligomerization domain 2) are associated with Crohn's disease (CD). We investigated the impact of NOD2 polymorphisms on cytokine secretion and proliferation of peripheral blood mononuclear cells (PBMCs) in response to Toll-like receptor (TLR) and NOD2 ligands. Based on NOD2 SNP analyses, 41 CD patients and 12 healthy controls were studied. PBMCs were stimulated with NOD2 and TLR ligands. After 18 h culture supernatants were measured using multiplex assays for the presence of human cytokines granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-1 beta and tumour necrosis factor (TNF)-alpha. In CD patients, TLR-induced GM-CSF secretion was impaired by both NOD2-dependent and -independent mechanisms. Moreover, TNF-alpha production was induced by a TLR-2 ligand, but a down-regulatory function by the NOD2 ligand, muramyl dipeptide, was impaired significantly in CD patients. Intracellular TLR ligands had minimal effect on GM-CSF, TNF-alpha and IL-1 beta secretion. CD patients with NOD2 mutations were able to secrete TNF-alpha, but not GM-CSF, upon stimulation with NOD2 and TLR-7 ligands. CD patients have impaired GM-CSF secretion via NOD2-dependent and -independent pathways and display an impaired NOD2-dependent down-regulation of TNF-alpha secretion. The defect in GM-CSF secretion suggests a hitherto unknown role of NOD2 in the pathogenesis of CD and is consistent with the hypothesis that impaired GM-CSF secretion in part constitutes a NOD2-dependent disease risk factor.