A special service of the OIE World Organization for Animal Health Reference Laboratory for Paratuberculosis, Brno, Czech Republic

APOLOGY: The database has not been updated since 23 April 2009. The pleasant reason was the international meeting and discussion on the bacterial teriggers in the etiology of Crohn's disease in May, the unpleasant one my illness and hospitalization. It may take some time to arrange your requests in time of vacationsnow. Thanks for kind understanding.


How to request full papers from PTB databases

New book
The Ecology of Mycobacteria: Impact on Animal’s and Human’s Health
The book, edited by J. Kazda, I. Pavlik, J. O. Falkingham and K. Hruska has been published by Springer
See the comprehensive contents

New publications in the PARATUBERCULOSIS database (537-594)

First Isolation of Mycobacterium Avium Subsp Paratuberculosis from Wild Guanacos (Lama Guanicoe) on Tierra del Fuego Island
Journal of Wildlife Diseases, 45, 295-301

The aim of this study was to search for Mycobacterium avium Subsp. paratuberculosis (Map) infection in a free-ranging wild animal species in a region where Johne’s disease has yet to be reported and to classify Map isolates using a genomic typing method. Fecal samples were obtained from 501 wild guanacos (Lama guanicoe) from Tierra del Fuego Island, Chile, in August 2006. Samples were cultured using Herrold’s egg yolk medium with mid without mycobactin J. After 9 mo of incubation, suspected Map colonies showing mycobactin dependence were confirmed by real-time polymerase chain reaction (PCR) based on IS900 and F57. Isolates were further tested using IS1311 PCR with restriction endonuclease analysis in order to type the guanaco Map strains. Twenty-one of 501 (4.2%) animals were fecal culture-positive for Map; identity was confirmed by real-time PCR and isolates were classified as cattle-type. Most culture-positive animals were located in four contiguous geographic areas, and the infection was most commonly found among adult animals. Prevalence was higher in females (5.9%) than males (3.1%) but the difference was not statistically significant. This represents the first isolation of Map from a free-ranging wildlife species in Chile. It expands the geographic range of paratuberculosis and the diversity of wildlife species that can become infected with Map

Comparative potential of modified indigenous, indigenous and commercial ELISA kits for diagnosis of Mycobacterium avium subspecies paratuberculosis in goat and sheep
Indian Journal of Experimental Biology, 47, 379-382
In the present study, modified indigenous ELISA kit (kit 1) was compared with indigenous ELISA kit (kit 2) and commercial ELISA kit (kit 3) for its sensitivity and specificity with respect to faecal culture for diagnosis of Johne's disease in goats and sheep under natural conditions. Of the 64 positive animals, serum of 42.1, 48.4 and 18.7% animals yielded positive infection in kit 1, 2 and 3, respectively. Specificity of kit 1 (95.1%) was maximum followed by kit 3 (93.7%) and kit 2 (83.4%). Kit 1 showed superior diagnostic potential than the other two kits. Kit 1 may be used as single screening test regimen for diagnosis of MAP infection in the population of goats and sheep in India


Johne's disease or paratuberculosis is a chronic granulomatous inflammation of the small intestine of ruminants caused by Mycobacterium avium subsp. paratuberculosis (MAP). Recent studies suggest an association between MAP and Crohn's disease in humans. MAP can become widely distributed within the tissues of infected animals, and meat may be a possible route of exposure of MAP to humans. In this study, 47 dairy and beef cattle were examined for the occurrence of viable MAP in diaphragm muscle. At the slaughterhouse, gut tissues, diaphragm muscle, blood, and feces of the 47 animals were collected for bacteriological culture, as well as gut samples for histopathological analysis. MAP was detected by bacteriological culture and conventional and real-time IS900 polymerase chain reaction in the diaphragm muscle of six infected cattle at slaughter (13%). The six animals showing evidence of MAP in diaphragm muscle had diffuse lesions and severe granulomatous inflammation in ileocecal lymph nodes, jejunal lymph nodes, ileocecal valve, and ileum. All six had heavy bacterial load in mesenteric lymph nodes, ileocecal valve, ileum, and jejunum, and four showed clinical signs of paratuberculosis. Two animals did not show clinical signs but had viable MAP in intestinal tissues and in diaphragm muscle as well. MAP was found in blood of only one of the six animals showing evidence of MAP in diaphragm muscle and in feces of three of them. In general, there was a positive association between enteric lesion severity, clinical signs of paratuberculosis, heavy bacterial load in intestinal tissues, fecal shedding of MAP, and the presence of disseminated MAP infection in diaphragm muscle. The results of this study demonstrated that MAP can be detected and cultured from muscle of MAP-infected cattle destined for human consumption and suggest a possible risk of exposure of humans to MAP via contaminated meat


Crohn's disease and ulcerative colitis are the two principal forms of inflammatory bowel disease (IBD). The root causes of these chronic and acute immunological disorders are unclear, but intestinal microorganisms are known to play a key role in the initiation and maintenance of disease. However, at present, there is no clear evidence for a single transmissible agent being involved in IBD aetiology. Although marked alterations occur in faecal and mucosal bacterial communities in IBD, it is unclear whether they are responsible for causing disease, or are due to changes in the gut environment that result from inflammatory reactions and extensive tissue destruction. Despite the involvement of microorganisms in inflammatory processes, antibiotic therapy has generally been unsuccessful in IBD. However, recent studies involving the use of probiotics, prebiotics and synbiotics suggest that there is potential for controlling these diseases through manipulation of the composition of the gut microbiota, and direct interactions with the gut immune system

PCR quantification is regarded as one of the most promising techniques for real-time identification of bio-aerosols. We have, therefore, validated a QPCR assay for quantification of a viral aerosol sample using the double-stranded DNA-binding dye SYBR green I, an economical alternative for quantification of target microorganisms. To achieve this objective we used mycobacteriophage D29 as model organism. Phage D29 aerosol was produced in an aerosol cabinet and then collected by use of an AGI liquid sampler. A standard curve was created by use of purified genomic DNA from the phage in liquid culture of known concentration measured by titration. To prevent false-positive results caused by formation of primer-dimers, an additional data-acquisition step was added to the three-step QPCR procedure; the new technique was called four-step QPCR. The standard curve was then used to quantify the total amount of phage D29 in liquid culture and aerosol samples. For liquid culture samples there was no significant difference (P > 0.05) between results from quantification of the virus using double-agar culture and QPCR. For aerosol samples, however, the result determined by the QPCR method was significantly (P < 0.05) higher than that from the double-agar culture method. The four-step SYBR green I QPCR method is a quick quantitative method for mycobacteriophage D29 aerosol. We believe that QPCR using SYBR green I dye will be an economical method for detection of airborne bio-aerosols.

Clinical trial on type of calving pen and the risk of disease in Holstein calves during the first 90 d of life
Preventive Veterinary Medicine, 89, 8-15

The objective of this study was to evaluate the efficacy of single cow calving pens that are cleaned between calvings vs. multiple cow calving pens for the prevention of calf diarrhea (scours), respiratory disease (pneumonia) and morbidity attributable to any cause. Every other pregnant cow or heifer was moved to either the single cow calving pen (treatment) or the multiple cow calving pen (control) within 48-72 h prior to actual calving. The calves born in the single cow calving pens were assigned to the treatment group while the calves born in the multiple calving pens were assigned to the control group. Fecal materials, placental remains, and any other conspicuous dirt were removed from the single cow calving pens between each calving prior to the introduction of the next pregnant cow. The calves were then separated from their dams within 2 h of birth. Multiple cow calving pens were managed as usual at the producers' discretion. Upon birth, the calf managers monitored each enrolled calf for signs of diarrhea, pneumonia plus other morbidity up to 90 d of age. The effects of single cow calving pens (vs. multiple cow calving pens) that are cleaned between calvings on the risk of neonatal calf diseases were evaluated using multivariable logistic regression models. Risk of diarrhea (OR = 0.93, P = 0.75), pneumonia (OR = 1.23, P = 0.64), and morbidity due to any cause (OR = 0.93, P = 0.74) were not significantly different between calves born in single cow vs. multiple cow calving pens. The current study found that, given the management situation evaluated, calves born in single cow calving pens were no different than calves born in multiple cow calving pens with respect to calf diseases risk. Long-term follow-up of the calves enrolled in the present study is ongoing to determine the efficacy of single cow calving pen use for the possible prevention of transmission of Mycobacterium avium subsp. paratuberculosis in Holstein calves. (C) 2009 Elsevier B.V. All rights reserved

Seroprevalence and epidemiological characteristics of Mycobacterium avium subsp paratuberculosis on 114 cattle farms in south west England
Preventive Veterinary Medicine, 89, 102-109

Between December 2002 and April 2006, 114 cattle farms in the southwest of England were visited at least once, with 100 farms visited three times. A total of 29,782 serum samples were collected from 15,736 individually identified cattle. The sera were tested for the presence of antibodies against Mycobacterium avium subsp. paratuberculosis (MAP) using an indirect enzyme-linked immunosorbent assay (ELISA). The mean seroprevalence in herds sampled three times was 7.1%; 10.1% of cattle had at least one positive result. There were 78%, 75% and 75% dairy herds with at least one positive bovine at the first, second and third routine visits, respectively. In comparison, 44%, 42% and 46% suckler herds had at least one positive
bovine for the first, second and third routine visits, respectively. In most herds (>90%), within herd seroprevalence of MAP remained stable over time. Markov chain Monte Carlo (MCMC) simulation methods were used to re-estimate the test sensitivity and specificity. The sensitivity results were 33.3% (95% CI, 28.8-37.8%), 34.5% (95% CI, 30.3-38.8%) and 34.8% (95% CI, 30.8-38.9%) for the first, second and third routine visits and the specificity results were 99.7% (95% CI, 99.3-99.9%), 99.8% (95% CI, 99.4-99.9%) and 99.7% (95% CI, 99.3-99.9%) for the first, second and third routine visits, respectively. The expected true prevalence was also estimated, 11 (21.1%) suckler herds and 1 (2.1%) dairy herd were predicted to be truly free from infection during the study period. The seroprevalence of antibodies against MAP increased with cattle age. There was a significantly higher seroprevalence of MAP in dairy breeds of cattle compared with suckler breeds of cattle. This was more pronounced in Channel Island breeds. Smaller dairy herds (<100 cattle) had a relatively lower seroprevalence of MAP than dairy herds with >= 100 cattle. In 8 (42%) of the 19 herds with >= 100 cattle born into the same herd, seropositive cattle were clustered by birth month whilst in the remaining herds clustering was not apparent. Daughters were significantly more likely to be MAP seropositive when born to a seropositive dam. (C) 2009 Elsevier B.V. All rights reserved

Association of farm soil characteristics with ovine Johne's disease in Australia
Preventive Veterinary Medicine, 89, 110-120

Speculation about the association of soil characteristics with the expression of ovine Johne's disease (OJD) prompted this cross-sectional study. We enrolled 92 sheep flocks in Australia during 2004-2005 and in each enrolled flock collected pooled faecal samples from an identified cohort (group of same age and sex) of sheep and soil samples from the paddocks grazed by this cohort of sheep. Faecal pools were cultured to create three outcome variables: positive or negative status of faecal pools (pool OJD status, binary): the log number of viable Mycobacterium avium subsp. paratuberculosis (MAP) organisms per gram of faeces (log pool MAP number, continuous); and the prevalence of faecal shedders (cohort OJD prevalence level, ordinal: low <2%, medium 2-10% and high >10%). Separate statistical models were then developed to investigate the association between soil characteristics and each outcome variable. Sheep raised on soils with a higher percentage of organic carbon and clay had a higher OJD prevalence whereas, sheep grazing on soils with a higher content of sand and nitrogen had a lower OJD prevalence. Iron content of the soil was positively associated with OJD infection but the association between soil pH and OJD was inconclusive. Parent soil type, the only farm level factor, was not significant in any of the final models. Study results indicate a higher risk of OJD in sheep raised on soils with greater organic matter and clay content. We hypothesise that this is due to adsorption of MAP to clay and the consequent retention of the bacteria in the topsoil, thus making them available in higher numbers to grazing sheep. (C) 2009 Elsevier B.V. All rights reserved

Efficacy of feeding plasma-derived commercial colostrum replacer for the prevention of transmission of Mycobacterium avium subsp paratuberculosis in Holstein calves
Javma-Journal of the American Veterinary Medical Association, 234, 1167-1176

Objective-To estimate the relative risk of paratuberculosis (Johne's disease [JD]) in calves fed a plasma-derived colostrum-replacement (CR) product versus raw bovine maternal colostrum (MC). Study Design-Randomized controlled clinical trial. Animals-497 heifer calves born in 12 JD-endemic commercial Holstein dairy farms located in Minnesota and Wisconsin. Procedures-Every calf was separated from its dam within 30 to 60 minutes after birth and systematically assigned to be fed raw bovine MC (control group, n = 261 calves) or CR (treatment group, 236 calves). The calves were monitored to adulthood and tested for Mycobacterium avium subsp paratuberculosis (MAP) infection by use of an ELISA to detect serum antibodies against MAP and bacterial culture for MAP in feces at approximately 30, 42, and 54 months of age. Weibull regression models were used to evaluate the effect of feeding CR (vs raw bovine MC) on the risk of developing JD infection. Results-Calves fed CR at birth were less likely (hazard ratio = 0.559) to become infected with MAP (as determined by use of an ELISA, bacterial culture, or both diagnostic tests), compared with the likelihood for calves
fed MC at birth. Conclusions and Clinical Relevance—This study revealed that feeding CB reduced the risk of developing MAP infection in Holstein calves born in JD-endemic herds, which implied that feeding raw bovine MC may be a source of MAP for calves. Plasma colostrum-replacement products may be an effective management tool for use in dairy herds attempting to reduce the prevalence of JD. (J Am Vet Med Assoc 2009; 234: 1167-1176)

Rapid Mycobacterial Liquid Culture-Screening Method for Mycobacterium avium Complex Based on Secreted Antigen-Capture Enzyme-Linked Immunosorbent Assay
Clinical and Vaccine Immunology, 16, 613-620

Sensors in automated liquid culture systems for mycobacteria, such as MGIT, Bact/Alert 3D, and Trek ESP II, flag growth of any type of bacteria; a positive signal does not mean that the target mycobacteria are present. All signal-positive cultures thus require additional and often laborious testing. An immunoassay was developed to screen liquid mycobacterial cultures for evidence of Mycobacterium avium complex (MAC). The method, called the MAC-enzyme-linked immunosorbent assay (ELISA), relies on detection of MAC-specific secreted antigens in liquid culture. Secreted MAC antigens were captured by the MAC-ELISA with polyclonal anti-Mycobacterium avium subsp. paratuberculosis chicken immunoglobulin Y (IgY), detected using rabbit anti-MAC IgG, and then revealed using horseradish peroxidase-conjugated goat anti-rabbit IgG. When the MAC-ELISA was evaluated using pure cultures of known mycobacterial (n = 75) and nonmycobacterial (n = 17) organisms, no false-positive or false-negative MAC-ELISA results were found. By receiver operator characteristic (ROC) analysis of 1,275 previously identified clinical isolates, at the assay optimal cutoff the diagnostic sensitivity and specificity of the MAC-ELISA were 92.6% (95% confidence interval [95% CI], 90.3 to 94.5) and 99.9% (95% CI, 99.2 to 100), respectively, with an area under the ROC curve of 0.992. Prospective evaluation of the MAC-ELISA with an additional 852 clinical samples inoculated into MGIT ParaTB medium and signaling positive per the manufacturer’s instructions found that the MAC-ELISA was effective in determining those cultures that actually contained MAC species and warranting the resources required to identify the organism by PCR. Of these 852 MGIT-positive cultures, the MAC-ELISA correctly identified 96.8% (of 219 MAC-ELISA-positive cultures) as truly containing MAC mycobacteria, based on PCR or high-performance liquid chromatography (HPLC) as reference tests. Only 6 of 433 MGIT signal-positive cultures (1.4%) were MAC-ELISA false negative, and only 7 of 219 MGIT signal-negative cultures (3.2%) were false positive. The MAC-ELISA is a low-cost, rapid, sensitive, and specific test for MAC in liquid cultures. It could be used in conjunction with or independent of automated culture reading instrumentation. For maximal accuracy and subspecies-specific identification, use of a confirmatory multiplex MAC PCR is recommended.

Gumber, S., Whittington, R.J. (2009)
Analysis of the growth pattern, survival and proteome of Mycobacterium avium subsp paratuberculosis following exposure to heat
Veterinary Microbiology, 136, 82-90

Mycobacterium avium subsp. paratuberculosis (MAP) causes Johne’s disease in ruminants and may be involved in Crohn’s disease in humans. The aim of this study was to evaluate the in vitro growth pattern and proteome of MAP after heat stress following prior observations that MAP may exist in a dormant state in the environment when protected from extreme temperature flux and may survive pasteurization. Data were obtained for two genomically distinct strains of MAP, sheep (S) and cattle (C), from 50 degrees C to 80 degrees C. When assessed by comparing accumulated time at a given high temperature, cycles of heating and cooling resulted in shorter survival than holding at the high temperature, for example MAP survived exposure to 60 degrees C for only 9 min during repeated cycles of 12-60 degrees C flux but survived to 28 min when continuously exposed at 60 degrees C. This helps to explain the observed die off of MAP in natural environments. A prolonged lag phase was observed following sub-lethal exposure to heat, specifically repeated temperature flux in the range 10-50 degrees C, and this was suggestive of dormancy. 2-D PAGE analysis and identification of differentially expressed spots detected 23 proteins in the C strain and 10 in the S strain associated with heat stress. These proteins represented a range of metabolic pathways, including 12 previously identified in M. tuberculosis during heat stress. These proteins may be
A triplex real-time (TRT-PCR) assay was developed to ensure a rapid and reliable detection of Mycobacterium avium subsp. paratuberculosis (Map) in faecal samples and to allow routine detection of Map in farmed livestock and wildlife species. The TRT-PCR assay was designed using IS900, ISMAP02 and f57 molecular targets. Specificity of TRT-PCR was first confirmed on a panel of control mycobacterial Map and non-Map strains and on faecal samples from Map-negative cows (n = 35) and from Map-positive cows (n = 20). The TRT-PCR assay was compared to direct examination after Ziehl-Neelsen (ZN) staining and to culture on 197 faecal samples collected serially from five calves experimentally exposed to Map over a 3-year period during the sub-clinical phase of the disease. The data showed a good agreement between culture and TRT-PCR (kappa score = 0.63), with the TRT-PCR limit of detection of 2.5 x 10^2 microorganisms/g of faeces spiked with Map. ZN agreement with TRT-PCR was not good (kappa = 0.02). Sequence analysis of IS900 amplicons from three single IS900 positive samples confirmed the true Map positivity of the samples. Highly specific IS900 amplification suggests therefore that each single IS900 positive sample from experimentally exposed animals was a true Map-positive specimen. In this controlled experimental setting, the TRT-PCT was rapid, specific and displayed a very high sensitivity for Map detection in faecal samples compared to conventional methods. (C) 2008 Elsevier B.V. All rights reserved

A goal of Johne’s disease control programs is to accurately detect Mycobacterium avium ssp. paratuberculosis (MAP) infected cattle as quickly as possible to reduce disease transmission. A newly introduced real-time PCR provides results rapidly, but its accuracy in the field has not been evaluated. Fecal and serum samples collected from dairy cows in northern Indiana were used to estimate the sensitivity and specificity of a newly licensed real-time PCR test for direct fecal detection of Mycobacterium avium ssp. paratuberculosis (MAP). Results of the real-time PCR were evaluated in parallel with solid and liquid media culture systems and a serum ELISA for detection of MAP antibodies to determine the accuracy of the real-time PCR and the tests’ potential usefulness in the field. A total of 143 samples were tested by all four methods. Using prior published estimates for sensitivity and specificity of each of the tests and Bayesian methodology, the sensitivity and specificity of the real-time PCR test was estimated to be 0.60 and 0.97, respectively. The accuracy of real-time PCR (0.90) was comparable to both solid (0.91) and liquid (0.93) culture. Because real-time PCR accuracy is comparable to standard culture methods, it is a useful new test. In addition, test results are obtained as rapidly as an ELISA, but are more accurate than the ELISA (0.82). This makes real-time PCR an attractive test and should shorten the quarantine period required for new purchases of unknown MAP-status animals into herds participating in an MAP control program. (C) 2008 Elsevier B.V. All rights reserved

Two thousand tissue samples of terminal ileum and mesenteric lymph nodes (MLN) cattle (Bos taurus) and buffalo (Bubalus bubalis) obtained randomly from abattoirs in Lahore district were used for detection of Mycobacterium paratuberculosis and Mycobacterium bovis using acid fast staining and PCR analysis. Acid fast staining revealed the presence of acid fast
bacilli in 17.4% intestinal and 16.4% MLN tissues in buffalo, while in cattle 19.2% intestinal and 17.8% MLN were found positive for bacilli. In PCR analysis, 12.8% and 12.4% intestinal and MLN tissues were positive for Mycobacterium paratuberculosis in buffalo. However, in cattle, PCR analysis showed respectively, 14.2% MLN and intestinal tissues positive for Mycobacterium avium subsp. paratuberculosis. Both types of tissues from cattle (5.8%) and buffalo (5%) were also positive for M. bovis by PCR. It is concluded that infections by various mycobacterium species can be differentiated by PCR amplification, which is not possible by acid fast staining technique.


Johne's disease is a chronic granulomatous enteritis in ruminants caused by Mycobacterium avium subsp. paratuberculosis (MAP). The disease is responsible for considerable economic losses in the livestock industry and in particular within the dairy sector. A more effective vaccine against Johne's disease would be of major benefit. In this study, we developed an efficient procedure for identifying mutants of MAP with reduced virulence that are potential live vaccine candidates against Johne's disease. A mariner transposon was used to create random insertional libraries in two different MAP strains (989 and k10), an effective cattle macrophage survival system was developed, and a total of 1890 insertion mutants were screened by using a 96-prong multi-blot replicator (frogger) system. Two of the transposon mutants with poor survival ability in macrophages were tested in mice. These strains were found to be attenuated in vivo, thereby validating the further use of this macrophage screening system to identify MAP mutants with potential as candidate vaccines against Johne's disease. (C) 2009 Elsevier B.V. All rights reserved


Mycobacterium avium subspecies Paratuberculosis infection is chronic disease that infect young animals and remains undetected for long period. The immune responses to the disease is characterised by the cell mediated immunity at the early stage while antibody response dominates the late stage of the infection. Early detection of the disease is vital to prevent its transmission to the susceptible animals. ELISA was seen one the sensitive tests in detection of the disease. The application of bovine test in detecting the disease was sought to unravel its versatility in camel. 95 serum samples [2-3 years-old (3 samples), 4-6 years-old (21 samples), 7-9 years old (24 samples) and 10-15 years-old (47 samples)] were collected from dromedary camels. The analysis of serum samples with commercial ELISA indicated only 8 positive (8.4%) and one inconclusive samples. The positive samples were restricted to older ages (7-9 years-old and 10-15 years-old). Despite the emaciation of few animals, the postmortem revealed no significant changes in intestine. The results proved that bovine ELISA is feasible in detecting anti-MAP antibodies in camel. However, the limitation of the ELISA sensitivity in detecting the infection in young animals will leave wide range of infected young animals undetected. The results encourage the application of ELISA concurrent with PCR and/or faecal culture in study of MAP infection prevalence in camel in Saudi Arabia


INFECTIONS with Mycobacterium avium subspecies paratuberculosis (MAP) can cause significant economic losses in dairy herds. Control of paratuberculosis in dairy cattle requires changes in management procedures to break transmission routes, and primarily involves immediate removal of calves from their dam at birth, frequent cleaning of calving areas and separation of calves from cow manure. These measures can be time consuming to implement. Since only a proportion of the cows in a herd may be shedding MAP, control measures need
only apply to infectious cows. However, continuous monitoring is required to detect newly infectious individuals in the herd. This article describes an approach to risk-based control of paratuberculosis in dairy herds.


Livestock herbivores are at risk of inter- and intra-specific exposure to parasites/pathogens via the faecal-oral route during grazing. Each contact between livestock and faeces in the environment is a potential parasite/pathogen transmission event. Cattle grazing contact with faeces varies in relation to the species depositing the faeces and the distribution of the faeces. We used a foraging model to simulate the grazing behaviour of beef cattle in two grazing systems to compare the relative inter-specific and intra-specific exposure risks to parasites/pathogens. Overall, there is a greater level of intra- vs. inter-specific risk via the faecal-oral route. However, under certain conditions, particularly for microparasite infections, e.g. paratuberculosis in rabbits and bovine tuberculosis in badgers, wildlife may pose a significant exposure risk to parasites/pathogens. These risks can be enhanced when cattle are first turned out onto pasture and in situations where intra-specific variations in wildlife behaviour result in more dispersed defecation patterns.


The importance of infectious pathogens in Crohn’s disease (CD) is still under debate. Therefore, we examined a panel of potential viral and bacterial pathogens in a large series of CD patients and controls. Archival tissue from 76 patients, 56 with CD and 20 control patients, with normal colon mucosa (n = 10) and nonsteroid anti-inflammatory drug (NSAID)-induced colitis (n = 10) were examined using PCR-based detection methods for human cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex virus 1, 2 (HSV1,2), adenovirus (AD), varicella-zoster virus (VZV), human herpes virus 6 (HHV6), human herpes virus 8 (HHV8), Mycobacterium tuberculosis complex (Mtbc), atypical mycobacteria (nM/MG1), including Mycobacterium avium (subspecies paratuberculosis, MAP), Stenotrophomonas maltophilia (Sm), and Yersinia enterocolitica (Ye). In CD patients, positive PCR results were achieved in 19 cases (34%). Sm was most frequent in 10 of 56 cases (17.9%) followed by EBV (6/56, 10.7%), nM/MG1 (4/56, 7.1%), including MAP, HHV6, and CMV (2/56, 3.6%), and finally Mtbc and AD (1/56, 1.8%). The control patients showed positive PCR results in 12 patients (12/20, 60%), nine of them with only weak signals, suggesting a persistent infection. In addition, we compared typical pathomorphological features of CD patients with the PCR results and found a significant correlation between EBV infection and mural abscesses (P = 0.014). Our data demonstrate that several potential pathogens can be detected in a sizeable fraction of specimens from patients with CD, but also in control patients, suggesting that the analyzed infectious pathogens may be associated with the disease, but do not represent an obligatory cause. (c) 2009 Elsevier GmbH. All rights reserved.


Accurate immunodiagnosis of bovine paratuberculosis is among others hampered by the lack of specific antigens. One of the most frequently used antigen preparations is purified protein derivative (PPD), also known as tuberculin. This crude extract has limitations when used in diagnostic assays due to the presence of cross-reactive antigens. The aim of the current study was to systematically analyze the qualitative protein composition of PPD of the major mycobacterial pathogens. One-dimensional gel electrophoresis followed by tandem mass spectrometry analysis of PPD from Mycobacterium avium subspecies paratuberculosis (MAP),
Mycobacterium avium subspecies avium (MAA) and Mycobacterium bovis (MB) identified 156, 95 and 132 proteins, respectively. Comparative sequence analysis led to the selection of a MAP-specific protein (MAP1718c), and finally heterologous expression in Escherichia coli of MAP1138c (LprG) enabled evaluation of their immunogenicity. Lymphocyte proliferation responses did not indicate substantial diagnostic potential of the antigens tested. In contrast serum antibody levels for MAP1138c in paratuberculosis infected cows (N=20) were significantly higher (p < 0.01) than in control animals (N=20), despite the conserved nature of this protein. In conclusion, this study showed that a combination of proteomics and genomics, starting from complex protein mixtures, present in tuberculins, can reveal novel proteins aiding the development of immunodiagnostics for mycobacterial diseases. (C) 2009 Elsevier B.V. All rights reserved

Concurrent occurrence of visceral linguatulosis and paratuberculosis in alpine cross goats (Capra hircus)
Veterinarski Arhiv, 79, 301-314

Concurrent visceral linguatulosis and paratuberculosis was diagnosed in five Alpine Cross goats (Capra hircus). Severe, gross and histopathological changes were observed, with the occurrence of multibacillary paratuberculosis and parasite-induced damage. The PCR-based technique was employed, using IS 900, to confirm paratuberculosis, and light, stereo- and scanning electron-microscopy were carried out to study the third-instar larvae of Linguatula serrata, Frohlich, 1789. The morphological changes were mainly ill the intestines and mesenteric lymph nodes and paratuberculosis-associated lesions were principal, of a diffuse multibacillary type, with a severe granulomatous reaction, consisting of macrophages laden with large numbers of acid-fast bacilli and variously sized cyst-like spaces in the lymph nodes, histologically associated with tired moth-eaten appearance of the parenchyma. Severely oedematous and haemorrhagic lymph nodes. having areas of calcification with profuse numbers of Mycobacterium avium subsp. paratuberculosis (Map), seemed to be characteristic of the Concurrent Occurrences of the diseases. The present investigation suggests that the parasite, being lymphovorous, might predispose to the multibacillary form of paratuberculosis

Study of Microbiological and Molecular Typing Aspects of Paratuberculosis in Sheep and Goats in Northern Greece
Transboundary and Emerging Diseases, 56, 285-290

P>Mycobacterium avium subsp. paratuberculosis, an acid-fast bacterium is the agent of Johne's disease, an intestinal disease that causes poor nutrient intake in ruminants. During the period 1987-2003, 322 of 777 (41.4%) goat herds and 97 of 458 (21.1%) sheep flocks were found to be infected with M.a.paratuberculosis in Northern Greece. From goats, mycobacteria were isolated from 238 of 652 (36.5%) of intestinal tissues, 14 of 119 (11.8%) of lymph nodes and five of 369 (1.4%) of faecal samples. From sheep, mycobacteria were isolated from 25 of 162 (15.4%) of intestinal tissues, three of 41 (7.3%) of lymph nodes and two of 322 (0.6%) of faecal samples. Isolates were typed by pulsed-field gel electrophoresis (PFGE) and restriction fragment length polymorphism followed by hybridization to IS900 [IS900- restriction fragment-length polymorphism (RFLP)]. IS900-RFLP BstEII profiles C1 and C5 and PFGE profiles [2-19] and [29-15] were identified. These PFGE profiles have not been found outside Greece to date

Economic evaluation of Johne's disease control programs implemented on six Michigan dairy farms
Preventive Veterinary Medicine, 90, 223-232

Johne's disease (JD) is an incurable, chronic infectious disease prevalent in dairy herds throughout the US and the world. The substantial economic losses caused by JD have been well documented. However. information on the costs of controlling the disease is limited, yet necessary, if producers are to make sound decisions regarding JD management. The purpose of this paper is to describe a method for evaluating the cost-effectiveness of management
changes to control JD on infected dairy farms. A 5-year longitudinal study of six dairy herds infected with JD was performed. Each herd implemented a JD control program upon study enrollment. Prevalence of JD within each herd was monitored with annual testing of all adult cows using fecal culture and/or serum ELISA. Individual cow production and culling information was collected to estimate the annual economic losses caused by JD. An economic questionnaire was developed and administered to each herd annually to estimate costs directly attributable to the JD control program. Based on the costs of the control program, and using the losses to estimate the potential benefits of the control program, the net present value (NPV) of the control program was calculated for each herd during the study and projected into the future for a total of 20 years. The NPV was calculated for four different scenarios: (1) assuming a linear decline in losses beyond the observed period of the study with JD eradication by year 20 of the control program; (2) assuming losses and JD prevalence remain constant at the rate equal to that of the last observed year while continuing the control program; (3) assuming linear increase in losses at rate equal to that in scenario 1 with no control program; and (4) assuming losses remain constant at same level as the beginning of the study with no control plan implemented. The NPV varied greatly across the herds. For scenario 1, only three herds had a positive NPV: and only two herds had a positive NPV under scenario two. In the absence of a control program, the NPV's were always negative. The costs of the JD control programs implemented on these herds averaged $30/cow/year with a median of $24/cow/year. The annual losses due to JD averaged $79/cow/year with a median of $66/cow/year. Investing in a JD control program can be cost-effective. (C) 2009 Published by Elsevier B.V

Sample handling substantially affects Johne's ELISA
Preventive Veterinary Medicine, 90, 278-283

Detection methods for Mycobacterium avium subsp. paratuberculosis (MAP) are imperfect, yet crucial for diagnosis of Johne's disease. Our purpose was to test for significant and biologically relevant changes in Johne's ELISA results associated with how field-collected blood samples were transported to the laboratory, prepared and stored prior to testing, while removing potential confounding by test kit and laboratory variables. Blood samples were collected from 21 cows that previously had MAP ELISA scores ranging from negative to highly positive. Samples for immediate laboratory processing were subjected to different transportation temperatures (on ice, 26 degrees C) and preparation methods (serum separated, hemolyzed and serum separated, clotted whole blood), but were tested using the same ELISA kit in the same laboratory. Samples for laboratory processing after one week of storage were subjected to different storage temperatures (4 degrees C, -20 degrees C) and preparation methods (serum separated, hemolyzed and serum separated, clotted whole blood), and again were tested using the same ELISA kit in the same laboratory. Finally, samples were evaluated by time to processing (one day, one week) and storage temperature (4 degrees C, -20 degrees C). Data were checked for normality and analyzed with repeated measures ANOVAs. Significantly (P = 0.027) higher MAP ELISA scores were recorded for whole blood and hemolzyed samples transported at 26 degrees C than serum separated samples. Sample storage for one week at -20 degrees C resulted in significantly (P < 0.001) lower MAP ELISA scores, regardless of handling method, compared to samples stored at 4 T for one week. Method of sample preparation, as well as transportation temperature and medium-term storage temperature, affects MAP ELISA results. Such discrepancies will inevitably result in improper classification of MAP-infected cattle, impeding both biosecurity measures on uninfected farms and MAP control programs. (C) 2009 Elsevier BY. All rights reserved

Prevalence of Mycobacterium avium subsp paratuberculosis in Ileocecal Lymph Nodes and on Hides and Carcasses from Cull Cows and Fed Cattle at Commercial Beef Processing Plants in the United States
Journal of Food Protection, 72, 1457-1462

Clinical associations between Crohn's disease in humans and Mycobacterium avium subsp.
paratuberculosis (MAP) have been suggested but not confirmed. Cattle could be sources for MAP, but little information on MAP prevalence with beef has been reported. Samples of ileocecal lymph nodes and swabs of hides and carcasses from 343 animals at cull cattle slaughtering facilities and 243 animals at fed cattle slaughtering facilities across the United States were analyzed for the presence of MAP. Amplification of genetic sequences detected MAP DNA predominantly on hides and in lymph nodes of samples taken at both types of processing facilities. More than 34% of the cattle at cull cow slaughtering facilities had ileocecal lymph nodes that tested positive for MAP DNA. From these same cattle, hide prevalence was more than twofold greater than the prevalence in ileocecal lymph nodes, suggesting that cross-contamination could be occurring during transport and lairage. The prevalence of MAP DNA decreased during processing, and less than 11% of the carcasses tested positive after interventions in the cull cow processing facilities. Using standard double-decontamination and Culture techniques, less than 1% of the postintervention carcasses tested positive for viable MAP at cull cow facilities. In samples from the facilities processing only fed cattle, MAP prevalence of 1% or less was detected for ileocecal lymph node, hide, and carcass samples, and viable MAP was not detected. Based on this study, fed cattle carcasses are unlikely sources of MAP, and carcasses at cull cow plants have only a slight risk for transmitting viable MAP, due to current interventions.


To evaluate the bactec(TM) Mgit(TM) 960/Mgit para tb (Mgit) System for drug susceptibility testing of mycobacterium avium subsp. Paratuberculosis (Map), A pathogen implicated in some forms of crohn's disease. Mics of 11 drugs for 10 map strains were determined using the mgit system, the bactec(TM)460Tb system (Bactec) And conventional agar dilution methods. Mics determined by mgit methods showed 80%-100% agreement (+/- 1 Log(2) Dilution) With those determined by the bactec and agar dilution methods for ciprofloxacin, levofloxacin, azithromycin and clofazimine. The mgit and bactec methods showed 70%, 80% and 90% agreement (+/- 1 Log(2) Dilution) For mics of ethambutol, rifabutin and rifampicin; Agreement for all drugs increased to 100% at 2 log(2) Dilution differences. For clarithromycin, the mgit method had greater agreement with the agar dilution method (70% At the same dilution) Than the bactec method (60% At +/- 1 log(2) Dilution); Agreement increased to 100% at +/- 2 log(2) Dilutions in both cases. The mgit and agar dilution methods agreed 60% and 100% for amikacin mics at +/- 1 log(2) Dilution and +/- 2 log(2) Dilutions, respectively. By all methods mics were higher than achievable serum concentrations for isoniazid and dapsone. There was 100% agreement between all three methods for azithromycin, clarithromycin and ciprofloxacin, and 80% agreement for rifampicin using published mic thresholds available for m. Avium complex strains. This study shows that the mgit system can be used for rapid and reliable drug susceptibility testing of map.


In this experiment Toll-like receptor expression pattern in monocytes and monocyte-derived macrophages by lipopolysaccharide (LPS) stimulation was examined. Jugular venous blood was collected from four Japanese calves, and the peripheral blood mononuclear cells (PBMCs) were isolated. The cells were directly used for collecting monocytes by magnetic cell sorting or cultured for 7 days to collect monocyte-derived macrophages in Repcell. Then we analyzed the mRNA expression pattern of TLRs and cytokines in monocytes and monocyte-derived macrophages after LPS stimulation for 24 h. LPS stimulation of both monocytes and monocyte-derived macrophages resulted in an increase in the levels of mRNA transcripts for TNF-alpha, IL-6 and IL-8. Moreover, TNF-alpha and IL-6 mRNA expressions were significantly augmented by LPS stimulation in monocyte-derived macrophages. TLRs mRNA expressions were unchanged after LPS stimulation of monocytes, while TLRs mRNA expressions in monocyte-derived macrophages were complicated. TLR1, 3, 5, 8 and 10 were significantly
decreased after LPS stimulation and there were no differences in the mRNA expressions of TLR2, 4, 6 and 7 between the groups of control and LPS stimulation. Besides, no expression of TLR9 was found. As antigen presenting cells, monocytes and monocyte-derived macrophages respond differently to LPS, so they may have different functions in the innate immune system. Cellular & Molecular Immunology. 2009;6(3):223-229

564 Bhide, M.R., Mucha, R., Mikula, I., Kisova, L., Skrabana, R., Novak, M., Mikula, I. (2009) Novel mutations in TLR genes cause hyporesponsiveness to Mycobacterium avium subsp paratuberculosis infection Bmc Genetics, 10, Background: Toll like receptors (TLR) play the central role in the recognition of pathogen associated molecular patterns (PAMPs). Mutations in the TLR1, TLR2 and TLR4 genes may change the ability to recognize PAMPs and cause altered responsiveness to the bacterial pathogens. Results: The study presents association between TLR gene mutations and increased susceptibility to Mycobacterium avium subsp. paratuberculosis (MAP) infection. Novel mutations in TLR genes (TLR1: Ser150Gly and Val220Met; TLR2: Phe670Leu) were statistically correlated with the hindrance in recognition of MAP legends. This correlation was confirmed subsequently by measuring the expression levels of cytokines (IL-4, IL-8, IL-10, IL-12 and IFN-gamma) in the mutant and wild type moDCs (mocyte derived dendritic cells) after challenge with MAP cell lysate or LPS. Further in silico analysis of the TLR1 and TLR4 ectodomains (ECD) revealed the polymorphic nature of the central ECD and irregularities in the central LRR (leucine rich repeat) motifs. Conclusion: The most critical positions that may alter the pathogen recognition ability of TLR were: the 9(th) amino acid position in LRR motif (TLR1-LRR10) and 4(th) residue downstream to LRR domain (extra-LRR region of TLR4). The study describes novel mutations in the TLRs and presents their association with the MAP infection.


The objective of this research was to assess the association between 4 cow reproductive and weight traits, and 2 preweaning calf traits and ELISA scores for paratuberculosis (0 = negative, 1 = suspect, 2 = weak-positive, and 3 = positive) in a multibreed herd of cows ranging from 100% Angus (A) to 100% Brahman (B). Cow data were 624 gestation lengths (GL), 358 records of time open (TO), 605 calving intervals (CI), and 1240 weight changes from November to weaning in September (WC) from 502 purebred and crossbred cows. Calf data consisted of 956 birth weights (BWT), and 923 weaning weights adjusted to 205 d of age (WW205) from 956 purebred and crossbred calves. Traits were analyzed individually using multibreed mixed models that assumed homogeneity of variances across breed groups. Covariances among random effects were assumed to be zero. Fixed effects were year, age of cow, sex of calf, year x age of cow interaction (except WC), age of cow x sex of calf interaction (only for WC), and covariables for B fraction of sire and cow, heterosis of cow and calf, and ELISA score. Random effects were sire (except for TO and CI), dam, and residual. Regression estimates of cow and calf traits on ELISA scores indicated that lower cow fertility (longer TO), lower ability of cows to maintain weight (negative WC), lower calf BWT, and lower calf WW205 were associated with higher cow ELISA scores. Further research on the effects of subclinical paratuberculosis in beef cattle at regional and national levels seems advisable considering the large potential economic cost of this disease.


The aim of the present study is to evaluate the efficiency of three methods to determine the molecular diversity of 34 Mycobacterium avium subsp. paratuberculosis (MAP) strains isolated from 17 cattle herds. The applied methods included the analysis of sequence polymorphism of the mononucleotide (G1 and G2) and trinucleotide sequences (GGT) of the Short Sequence
Repeats (SSR) and the determination of size polymorphism of 9 different Mycobacterial Interspersed Repetitive Units (MIRU) and 6 Variable Number Tandem Repeats (VNTR). Sequence analysis of SSR of 34 isolates showed 4, 6, and 2 alleles of G1, G2, and GGT repeats, respectively. The amplification of the investigated 9 MIRU units revealed only two discriminatory genotyping systems (MIRU2 and MIRU3). Out of 6 VNTR PCR differentiation methods, only one method could be recommended for genotyping purposes. The profile 7g-12g-4ggt-ll-b-2 of the combination systems G1-G2-GGT-MIRU2-MIRU3-VNTR1658 dominates among the examined isolates and was detected in 14.7% of the isolates. The use of certain repetitive loci of SSR, MIRU, and VNTR techniques in this study showed greater potential than others for the characterization of MAP isolates. The recommended loci can be used for the epidemiological tracing of MAP field strains and to determine the relationships between isolates in different herds.


Clinical isolates of Mycobacterium avium (n=81) from patients with pulmonary infections who were HIV-negative and isolates (n=33) from HIV-positive patients were subjected to genetic analysis by PCR detection of three M. avium-specific insertion sequences (IS901, IS 1245 and IS 1311), and nucleotide sequencing of the heat-shock protein 65 (hsp65) gene. All clinical isolates were identified as W. avium subspecies hominisuis' by sequence analysis of hsp65. Compared with clinical isolates of M. avium reported elsewhere, IS 1245 was found less frequently in Japanese isolates (96/114 isolates, 84%) and IS901 was detected more frequently (76/114 isolates, 67%). One isolate was found to lack IS 1311, which has not been reported previously for W. avium subsp. hominissuis'. Nucleotide sequence analysis of the PCR products for IS901 revealed that all clinical isolates had the same new insertion sequence, designated ISMav6, which had 60 point mutations compared with the nucleotide sequence of the original IS901. These results suggest that V avium subsp. hominissuis with ISMav6 is prevalent in Japan. ISMav6 may have implications for the virulence of M. avium and contribute to an increase of M. avium infections in this country.


Mycobacterium avium complex (MAC) infections are increasing annually in various countries, including Japan, but the route of transmission and pathophysiology of the infection remain unclear. Currently, a variable-number tandem-repeat (VNTR) typing method using the Mycobacterium avium tandem repeat (MATR) loci (MATR-VNTR) is employed in Japan for epidemiological studies using clinical isolates of M. avium. In this study, the usefulness of this MATR-VNTR typing method was compared with that of the IS1245-restriction fragment length polymorphism (IS1245-RFLP) typing method and a mycobacterial interspersed repetitive-unit (MIRU)-VNTR typing method reported previously (V. C. Thibault, M. Grayon, M. L. Boschiroli, C. Hubbans, P. Overduin, K. Stevenson, M. C. Gutierrez, P. Supply, and F. Biet, J. Clin. Microbiol. 45: 2404-2410, 2007). Seventy clinical isolates identified as M. avium from human immunodeficiency virus-negative patients with MAC infections were used. MATR-VNTR typing using 15 loci distinguished 56 patterns of different allele profiles, yielding a Hunter-Gaston discriminatory index (HGDI) of 0.990. However, IS1245-RFLP and MIRU-VNTR typing yielded HGDI of 0.960 and 0.949, respectively, indicating that MATR-VNTR has an excellent discriminatory power compared with MIRU-VNTR and IS1245-RFLP typing. Moreover, concomitant use of the MATR-VNTR method and IS1245-RFLP typing increased the HGDI to 0.999. MATR-VNTR typing is inexpensive and easy to perform and could thus be useful in establishing a digital multifacility database that will greatly contribute to the clarification of the transmission route and pathophysiology of M. avium infections.
Single Nucleotide Polymorphisms in the IS900 Sequence of Mycobacterium avium subsp paratuberculosis Are Strain Type Specific
Journal of Clinical Microbiology, 47, 2260-2264

Insertion sequence IS900 is used as a target for the identification of Mycobacterium avium subsp. paratuberculosis. Previous reports have revealed single nucleotide polymorphisms within IS900. This study, which analyzed the IS900 sequences of a panel of isolates representing M. avium subsp. paratuberculosis strain types I, II, and III, revealed conserved type-specific polymorphisms that could be utilized as a tool for diagnostic and epidemiological purposes.

570 Shanahan, F., Bernstein, C.N. (2009)
The evolving epidemiology of inflammatory bowel disease
Current Opinion in Gastroenterology, 25, 301-305

Purpose of review Epidemiologic studies in inflammatory bowel disease (IBD) include assessments of disease burden and evolving patterns of disease presentation. Although it is hoped that sound epidemiologic studies provide etiological clues, traditional risk factor-based epidemiology has provided limited insights into either Crohn's disease or ulcerative colitis etiopathogenesis. In this update, we will summarize how the changing epidemiology of IBD associated with modernization can be reconciled with current concepts of disease mechanisms and will discuss studies of clinically significant comorbidity in IBD. Recent findings The increased frequency of IBD, which has been consistently observed as society becomes developed or modernized, may be linked with changes in the gastrointestinal microbiota which, in turn, may affect the development of the immune system and influence the risk of inflammatory diseases. Although extra-intestinal disease associations have long been recognized to be linked to IBD, there is a disturbing increase in comorbidity with Clostridium difficile-associated disease, arterial and venous thromboembolism and abnormalities of cervical cytology. These have important implications in an era of increased use of immunomodulatory drugs. Summary Advances in understanding the basic biology of IBD with rapidly emerging therapeutic strategies have prompted a shift in traditional epidemiologic approaches away from risk factor anthologies toward rapprochement with disease mechanisms and pursuit of changing patterns of comorbidity of clinical relevance.

ATP release by infected bovine monocytes increases the intracellular survival of Mycobacterium avium subsp paratuberculosis
Comparative Immunology Microbiology and Infectious Diseases, 32, 365-377

Mycobacterium avium subsp. paratuberculosis is the etiologic agent of Johne's disease, a chronic intestinal infection in ruminants. Adenosine 5'-Triphosphate (ATP) has been reported to induce killing of several Mycobacterium species in human and murine macrophages. We investigated whether ATP secreted from M. avium subsp. paratuberculosis-infected bovine monocytes affects intracellular survival of the bacilli. Bovine monocytes constitutively secreted ATP during an 8-day incubation period in vitro; however, M. avium subsp. paratuberculosis infection did not enhance ATP release. Removal of extracellular ATP by the addition of apyrase increased the viability of infected monocytes, but surprisingly decreased the number of viable intracellular bacilli. In contrast to previous reports, addition of extracellular ATP (1 mM) increased intracellular survival of M. avium subsp. paratuberculosis in bovine monocytes. Neither apyrase nor ATP altered production of reactive oxygen intermediates (ROI) or reactive nitrogen intermediates (RNI) by bovine monocytes. These results suggest that ATP release from infected bovine monocytes improves, rather than decreases, the intracellular survival of M. avium subsp. paratuberculosis. (C) 2008 Elsevier Ltd. All rights reserved

Neutralization of Interleukin-10 from CD14(+) Monocytes Enhances Gamma Interferon Production in Peripheral Blood Mononuclear Cells from Mycobacterium avium subsp paratuberculosis-Infected Goats
The gamma interferon assay is used to identify Mycobacterium avium subsp. paratuberculosis-infected animals. It has been suggested that regulatory mechanisms could influence the sensitivity of the test when it is performed with cells from cattle and that the neutralization of interleukin-10 (IL-10) in vitro would increase the gamma interferon responses. To investigate the regulatory mechanisms affecting the gamma interferon assay with cells from goats, blood was collected from M. avium subsp. paratuberculosis-infected, M. avium subsp. paratuberculosis-exposed, and noninfected goats. Neutralization of IL-10 by a monoclonal antibody resulted in increased levels of gamma interferon production in M. avium subsp. paratuberculosis purified protein derivative (PPDj)-stimulated samples from both infected and exposed goats. However, the levels of gamma interferon release were also increased in unstimulated cells and in PPDj-stimulated cells from some noninfected animals following neutralization. Depletion of putative regulatory CD25(high) T cells had no clear effect on the number of gamma-interferon-producing cells. The IL-10-producing cells were identified to be mainly CD14(+) major histocompatibility complex class II-positive monocytes in both PPDj-stimulated and control cultures and not regulatory T cells. However, possible regulatory CD4(+) CD25(+) T cells produced IL-10 in response to concanavalin A stimulation. The numbers of CD4(+), CD8(+), and CD8(+) gamma delta T-cell receptor-positive cells producing gamma interferon increased following IL-10 neutralization. These results provide insight into the source and the role of IL-10 in gamma interferon assays with cells from goats and suggest that IL-10 from monocytes can regulate both innate and adaptive gamma interferon production from several cell types. Although IL-10 neutralization increased the sensitivity of the gamma interferon assay, the specificity of the test could be compromised.

Paratuberculosis is a chronic infectious disorder and a major problem in farmed ruminants. This disease is caused by Mycobacterium avium subsp. paratuberculosis. M. avium subsp. paratuberculosis is an important pathogen that causes Johne’s disease in animals and also has been implicated as a possible cause of Crohn’s disease in humans, but little is known about the protective immune responses to this microorganism. Fibronectin attachment protein (FAP) is a member of a family of fibronectin-binding proteins produced by several species of mycobacteria which is important in the pathogenesis of M. avium. Addition of recombinant FAP to human respiratory tract organ cultures inhibits M. avium binding to areas where there is epithelial damage. We characterized the role of FAP in promoting adaptive and innate immune responses. FAP functionally activated dendritic cells by augmenting the expression of CD80, CD86, major histocompatibility complex class I, and major histocompatibility complex class II. Moreover, FAP induced the allogeneic immunostimulatory capacity of dendritic cells by stimulating dendritic cell production of Th1-promoting interleukin-12. FAP also increased the production of gamma interferon by T cells in mixed-lymphocyte reactions, which would be expected to contribute to the Th1 polarization of the immune response. The expression of surface markers and cytokine production in dendritic cells was mediated by both mitogen-activated protein kinases and NF-kappa B pathways. These results show that FAP modulates the adaptive immune responses to M. avium subsp. paratuberculosis by inducing maturation and activation of dendritic cells, which drives Th1 polarization.

The aim of this study was to assess the immunogenicity of recombinant stress-associated proteins of Mycobacterium avium subsp. paratuberculosis in sheep infected with the organism compared to control sheep. Five proteins - MAP2411, ClpP, Ppa, MAP0593c and GreA -
which were identified previously in in vitro stress or dormancy responses of M. paratuberculosis to hypoxia, nutrient starvation and heat, were cloned, expressed and purified as His-tag recombinant proteins from the pET-15b vector in a BL21(DE3)pLysS strain of E. coli. The immunogenicity of MAP2411 did not differ between infected and control sheep. However, the serological reactivity of the other recombinant antigens, and combinations of them, varied according to the histological stage of paratuberculosis. Interestingly, the sera from some animals with paucibacillary lesions, which were not immunoreactive in a commercial paratuberculosis ELISA that was based on non-defined native antigens, recognised the recombinant antigens. We infer from their differential immunogenicity in infected and control sheep that four of the stress-associated proteins were expressed by M. paratuberculosis in vivo. These data provide fundamental information on host-mycobacterial interactions and have conceptual implications for the development of future diagnostic tests for early immune responses in animals infected with mycobacteria. (C) 2009 Elsevier B.V. All rights reserved

Feeding heat-treated colostrum to neonatal dairy heifers: Effects on growth characteristics and blood parameters
Journal of Dairy Science, 92, 3265-3273

Newborn Holstein heifer calves were studied to compare absorption of immunoglobulin G (IgG(1) and IgG(2)), total serum protein concentration, lymphocyte counts, health scores, growth, and starter intake after receiving unheated or heat-treated colostrum. First-milking colostrum was collected from Holstein cows and frozen at -20 degrees C to accumulate a large batch. After thawing and mixing, half of the colostrum was transferred into 1.89-L plastic containers and frozen at -20 degrees C until needed for feeding. The remaining half was heated at 60 degrees C for 30 min, transferred into 1.89-L plastic containers, and then frozen at -20 degrees C until needed for feeding. Forty heifer calves weighing >= 32 kg at birth were enrolled into 1 of 2 treatment groups before suckling occurred. For the first feeding, 3.8 L of colostrum was bottle fed by 1.5 to 2 h of age. For the second and third feedings, pasteurized whole milk at 5% of birth body weight (BW) was fed. Subsequently, calves received milk replacer containing 20% crude protein and 20% fat at 10% of birth BW/d until wk 5. Milk replacer was reduced to 1 feeding of 5% birth BW until weaning at 6 wk of age. Blood samples and growth data were collected through wk 8. Batch heat-treatment of colostrum at 60 degrees C for 30 min lowered colostrum bacteria concentration while maintaining colostral IgG concentration and viscosity. Calves fed heat-treated colostrum had significantly greater IgG concentrations at 24 h and greater apparent efficiency of IgG absorption (IgG = 23.4 g/L; apparent efficiency of absorption = 33.2%) compared with calves fed unheated colostrum (IgG = 19.6 g/L; apparent efficiency of absorption = 27.7%). There was no difference between treatment groups in growth measurements, calf starter intake, lymphocyte counts, or health scores

Genetics of tuberculosis in Irish Holstein-Friesian dairy herds
Journal of Dairy Science, 92, 3447-3456

Information is lacking on genetic parameters for tuberculosis (TB) susceptibility in dairy cattle. Mycobacterium bovis is the principal agent of tuberculosis in cattle. The objective of this study was to quantify the genetic variation present among Irish Holstein-Friesian dairy herds in their susceptibility to M. bovis infection. A total of 15,182 cow and 8,104 heifer single intradermal comparative tuberculin test (SICTT, a test for M. bovis exposure and presumed infection) records from November 1, 2002, to October 31, 2005, were available for inclusion in the analysis. Data on observed carcass TB lesions from abattoirs were also available for inclusion in the analysis. The only animals retained were those present in a herd during episodes in which at least 2 animals showed evidence of infection; this ensured a high likelihood of exposure to M. bovis. Linear animal models, and sire and animal threshold models were used to estimate the variance components for susceptibility to M. bovis-purified protein derivative (PPD) responsiveness and confirmed M. bovis infection. The heritability estimates from the threshold sire models were biased upward because the relatedness between dam-daughter
pairs was ignored. The threshold animal model produced heritability estimates of 0.14 in cows and 0.12 in heifers for susceptibility to M. bovis-PPD responsiveness, and 0.18 in cows for confirmed M. bovis infection susceptibility. Therefore, exploitable genetic variation exists among Irish dairy cows for susceptibility to M. bovis infection. Sire rankings from the linear and threshold animal models were similar, indicating that either model could be used for the analysis of susceptibility to M. bovis-PPD responsiveness. A favorable genetic correlation close to unity was observed between susceptibility to confirmed M. bovis infection and M. bovis-PPD responsiveness, indicating that direct selection for resistance to M. bovis-PPD responsiveness will indirectly reduce susceptibility to confirmed M. bovis infection. Data from the national TB eradication program could be used routinely to estimate breeding values for susceptibility to M. bovis infection.


Microarrays represent a modern powerful technology, which have potential applications in many areas of biological research and provide new insights into the genomics and transcriptomics of living systems. The aim of this review is to describe the application of microarray technology for Mycobacterium avium subsp. paratuberculosis (MAP) research. The main focus points include a summary of results obtained for MAP using microarrays, examination of the fields of MAP research which are currently being investigated and possible areas of future research. This article is divided into two parts according to the type of nucleic acid used for array hybridisation. Articles related to MAP research using microarray technology are then divided according to the field of study, such as comparative genome analysis, diagnostics, expression or environmental studies.


Effective control of paratuberculosis and investigations of potential link to Crohn's disease have been hampered by the lack of effective assays for easy and accurate diagnosis of Mycobacterium avium subspecies paratuberculosis (Map). Map is extremely fastidious and depends on iron chelator (Mycobactin). Map strains from humans and sheep are very difficult to isolate and may require years to emerge. Therefore, small. numbers of Map isolates have been maintained in available collections. This situation has limited the study of biodiversity of Map. Though, much is known about environmental. and host factors that contribute to paratuberculosis disease, but Little is known about bacterial. genetic mechanism of infection. Diagnostic and strain typing markers still demand improvements. Complete genome sequence of Map K10 strain is available in public domain for comparative genomics with other mycobacteria and clinical isolates of Map. It is anticipated that the genome sequence will. help in carrying molecular diagnosis and strain typing with respect to Map forward at rapid pace. This paper reviews the current diagnostic and strain typing markers, which may be useful in typing of clinical isolates in near future. (C) 2007 Elsevier GmbH. All rights reserved


An epizootic of nontuberculous mycobacteriosis occurred in a captive herd of aoudad (Ammotragus lervia) over a period of 18 mo. Each of the affected animals was subject to a thorough postmortem examination that included histopathology, tissue concentration and acid-fast staining, aerobic and anaerobic bacterial culture, mycobacterial Culture, and real-time polymerase chain reaction specific for Mycobacterium tuberculosis DNA. Histopathologic lesions consistent with pulmonary mycobacteriosis, including the presence of acid-fast
bacteria, were identified in two captive adult male aoudad. M. avium was isolated in culture from the pulmonary parenchyma, and M. parafortuitum was isolated from a mesenteric lymph node of a third animal, an adult female, euthanized subsequent to an illness characterized by progressive dyspnea and tachypnea. M. intracellularum was isolated within the bronchial lymph node of a fourth aoudad, an adult female that was euthanized due to chronic weight loss.

Diagnostic testing of the 34 individuals in the herd included collection of blood for an interferon-gamma assay, intradermal tuberculin testing, and radiometric fecal culture for M. avium subsp. paratuberculosis. On the basis of this investigation, mycobacteriosis associated with M. bovis, M. tuberculosis, and/or M. avium subsp. paratuberculosis was ruled out and nontuberculous mycobacteriosis was confirmed in this herd.

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**Uptake and transport of foreign particles in Peyer’s patches of both distal ileum and jejunum of calves**

Cell and Tissue Research, 337, 125-135

To investigate the uptake and transport patterns of variously sized particles in Peyer's patches (PPs) of calves, intestinal loops were created in four newborn and two 2-month-old calves, and the loops were inoculated with various particles, including carbon black, fluorescein isothiocyanate (FITC)-labeled latex, FITC-labeled dextran, bovine serum, and recombinant mouse prion protein (rMPrP). The intestinal loops were recovered at 3, 6, 9, and 24 h in newborn calves and at 24 h in 2-month-old calves after inoculation, and the transport of the particles was examined by histological and immunohistochemical means. The uptake of the particles was quantified by estimation of signal intensities. A greater intensity was found in newborn calves compared with the 2-month-old calves. The peak uptake of carbon black, FITC-labeled latex, and rMPrP in the PPs of the distal ileum occurred at 6 h after inoculation in newborn calves and then progressively decreased with time. Uptake was also dependent on the site within the small intestine and the size of the particle studied. The transport of carbon black, FITC-labeled latex, and FITC-labeled dextran occurred via the bloodstream, the mesenteric lymph nodes, and the liver of newborn calves. rMPrP was found primarily in the interfollicular regions of the submucosa of the distal ileum of newborn calves. Thus, distal ileal PPs are probably more effective at particle absorption than the jejunal PPs, and the peak uptake of the PPs within the newborn calf occurs at 6 h after inoculation.

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**Optimization of a Phage Amplification Assay To Permit Accurate Enumeration of Viable Mycobacterium avium subsp paratuberculosis Cells**

Applied and Environmental Microbiology, 75, 3896-3902

A commercially available phage amplification assay, FASTPlaqueTB (Biotec Laboratories, Ipswich, United Kingdom), when used according to the manufacturer's instructions, does not permit accurate enumeration of Mycobacterium avium subsp. paratuberculosis. The aim of this study was to optimize the phage amplification assay conditions to permit accurate quantification of viable M. avium subsp. paratuberculosis cells. The burst time for M. avium subsp. paratuberculosis was initially determined to inform decisions about optimal incubation time before plating, and then other test parameters were altered to evaluate how the correlation between plaque and colony counts was affected. The D29 mycobacteriophage replicates more slowly in M. avium subsp. paratuberculosis than in Mycobacterium smegmatis (used to optimize the commercial test originally), and the mean burst time for four M. avium subsp. paratuberculosis strains was 210 +/- 36.8 min at 37 degrees C compared to 63 +/- 17.5 min for M. smegmatis mc(2) 155. To achieve 100% correlation between plaque and colony counts, the optimized phage assay includes the following: (i) resuspension of the samples to be tested in Middlebrook 7H9 broth containing 10% oleic acid-albumin-dextrose-catalase and 2 mM calcium chloride, followed by overnight incubation at 37 degrees C before performance of the phage assay; (ii) a 2-h incubation of the sample with D29 mycobacteriophage before viricide treatment; and (iii) a further 90-min incubation after viricide treatment and neutralization up to the burst time (total incubation time, 210 min) before plating with M. smegmatis mc2 155 in 7H9 agar. The optimized phage amplification assay was able to detect 1 to 10 CFU/ml of M. avium subsp. paratuberculosis in spiked milk or broth within 48 h, as demonstrated by the results of several blind trials.
A cross-sectional, stratified random survey of Michigan dairy herds was conducted to estimate the prevalence of herds infected with Mycobacterium avium paratuberculosis (MAP), the causative agent of Johne's disease, in Michigan using targeted environmental sampling. One pooled sample each from the primary manure storage area and a high-traffic common cow area from each herd was collected and cultured for MAP using the ESP (R) culture system II. A herd was classified as positive if at least one sample was culture positive for MAP. State, agricultural district, and herd size stratum prevalence were calculated. Information on past MAP testing and cattle purchase history was collected, and logistic regression was performed to determine their importance to the MAP status of the herd. One hundred twenty-seven herds were contacted, and 94 agreed to participate in the study. The environment of 38 (40.4%) herds cultured positive for MAP. MAP was found in all herds (n = 15) with greater than 200 lactating cows. Herds that had tested for MAP or purchased cattle in the previous 5 years were 4.6 and 3.1 times, respectively, more likely to be infected than herds that had not. MAP continues to be prevalent on Michigan dairy farms, especially those with greater than 200 lactating cows. The environmental sampling protocol used in this study is an economically attractive alternative for monitoring herd level prevalence and the progress of Johne's disease control programs at the state or national level. Implementation of such a program would aid states in monitoring Johne's control program progress, and guide changes over time. (C) 2009 Elsevier B.V. All rights reserved

The possibility that Mycobacterium avium subsp. paratuberculosis (MAP) plays some role in the development of Crohn's disease in humans is attracting attention to milk and milk products originating from infected animals. In this study, we focused on the detection of MAP in 220 bulk tank milk (BTM) samples from all dairy cattle herds in Cyprus. In total, 63 (28.6%) BTM milk samples were found to be positive for MAP using quantitative real-time PCR assays for IS900 and F57. The presence of MAP in BTM was low, and was assessed to be several tens of MAP cells per one ml of BTM. Milk samples examined by cultivation were found to be negative for MAP in all 220 BTM. In two BTM samples cultivation and subsequent sequencing of 16S rRNA revealed two isolates of M. fortuitum. (C) 2009 Elsevier B.V. All rights reserved

Knowledge about nitrogen metabolism and control in the genus Mycobacterium is sparse, especially compared to the state of knowledge in related actinomycetes like Streptomyces coelicolor or the close relative Corynebacterium glutamicum. Therefore, we screened the published genome sequences of Mycobacterium smegmatis, Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium avium ssp. paratuberculosis and Mycobacterium leprae for genes encoding proteins for uptake of nitrogen sources, nitrogen assimilation and nitrogen control systems, resulting in a detailed comparative genomic analysis of nitrogen metabolism-related genes for all completely sequenced members of the genus. Transporters for ammonium, nitrate, and urea could be identified, as well as enzymes crucial for assimilation of these nitrogen sources, i.e. glutamine synthetase, glutamate dehydrogenase, glutamate synthase, nitrate reductase, nitrite reductase, and urease proteins. A reduction of genes encoding proteins for nitrogen transport and metabolism was observed for the pathogenic mycobacteria, especially for M. leprae. Signal transduction components identified for the different species include adenyllyl- and uridylyltransferase and a P-II-type signal transduction protein. Exclusively for M. smegmatis, two homologs of putative nitrogen regulatory proteins were found, namely GlnR and AmtR, while in other mycobacteria, AmtR was absent and GlnR
seems to be the nitrogen transcription regulator protein. Copyright (C) 2008 S. Karger AG, Basel

A longitudinal study on the impact of Johne's disease status on milk production in individual cows
Journal of Dairy Science, 92, 2653-2661

Longitudinal data from 3 commercial dairy herds in the northeast United States were collected from 2004 to 2007. Johne's disease status, as indicated by Mycobacterium avium ssp. paratuberculosis infection levels, was determined through quarterly ELISA serum testing, biannual fecal culture, and culture of tissues at slaughter. Milk production data were collected from the Dairy Herd Improvement Association. The effect of Johne's disease status on milk production was analyzed using a mixed linear model with an autocorrelation random effect structure. Infected animals produced more milk than uninfected cows before they began shedding M. avium ssp. paratuberculosis. Cows infected with M. avium ssp. paratuberculosis had monthly decreases of 0.05 to 1 kg in daily milk production relative to uninfected animals, with greater decreases in progressive disease categories. Animals with fecal culture results of >30 cfu/g produced approximately 4 kg less milk per day compared with uninfected cows. These results will be valuable in calculating the economic effect of Johne's disease.

Demographics of cattle positive for Mycobacterium avium subspecies paratuberculosis by faecal culture, from submissions to the Cork Regional Veterinary Laboratory
Irish Veterinary Journal, 62, 398-405

The demography of bovine infections caused by Mycobacterium avium subspecies paratuberculosis (MAP) in Ireland is poorly defined. The objective of this study was to describe the demographics of cattle positive to MAP on faecal culture, based on submissions to the Cork Regional Veterinary Laboratory (Cork RVL) from 1994 to 2006. The study focused on all available faecal samples from adult cattle with non-responsive chronic diarrhoea that were submitted by private veterinary practitioners to Cork RVL for MAP culture. For each MAP-positive by faecal culture animal, data were collated from Cork RVL and Cattle Movement Monitoring Scheme (CMMS) records. Johne's disease (JD) was confirmed in 110 animals from 86 herds by the Cork RVL between 1994 and 2006, with a rate of positive cases between 15% and 18% over last four years of the study. Two breeds (Holstein/Friesian or Limousin) made up 78% of submissions. Movements were assessed for the 57 study animals with available movement information, 90% died within one year of the test and 26% tested positive in the herd they were born into. The study provides preliminary information about movement trends and demographics of animals with MAP positive submissions. Although the study area is restricted, it includes the most intensive (and economically-important) dairy region in Ireland. The demographics of JD infection from the study area are in agreement with international reports. Further work is required to determine demographic trends, incidence and prevalence of JD throughout Ireland. It is hoped this work may contribute to the development of a surveillance strategy for MAP by regional veterinary laboratories.

Pathogenesis of Mycobacterium avium subsp paratuberculosis in neonatal calves after oral or intraperitoneal experimental infection
Veterinary Microbiology, 136, 306-313

Understanding the host response to Mycobacterium avium subsp. paratuberculosis is critical to the development of effective vaccines and therapeutics for the control of this disease in the field. The current study compared the effectiveness of oral and intraperitoneal (IP) methods of experimental inoculation and two strains of M. avium subsp. paratuberculosis (strain K-10 and clinical isolate 509) on the level of infection and lesion development. Calves were inoculated with 4 x 10(11) to 8 X 10(12) cfu live bacteria, depending upon treatment group. Fecal shedding of M. avium subsp. paratuberculosis was minimal and infrequent over the course of the study for calves that received strain K-10 (oral and IP), however, calves orally inoculated
with the clinical isolate shed high numbers of bacteria in their feces up to 4 months post-inoculation. Colonization was present in a number of intestinal tissues and lymph nodes with the lowest number of affected tissues in the IP calves and the highest for calves receiving the clinical isolate via oral inoculation. Microscopic lesions were predominantly found in the ileal and jejunal sections of small intestine and their associated lymph nodes, as well as the ileocecal valve and node. These data suggest that a variety of experimental infection regimes can be effective but oral inoculation with a clinical isolate may result in greater colonization of tissues and fecal shedding of M. avium subsp. paratuberculosis. (C) Published by Elsevier B.V

The role of enteric microflora in inflammatory bowel disease
Przeglad Gastroenterologiczny, 4, 1-6

Enteric microflora play an important role in the development and course of ulcerative colitis (UC) and Crohn's disease (CD), the two major forms of inflammatory bowel disease (IBD). No infectious agent has been proved as a causative factor of IBD, although in numerous studies Mycobacterium avium subspecies paratuberculosis has been proposed to be an inciting agent of CD. Indigenous intestinal microorganisms have a significant impact on normal host physiology. In normal conditions commensal bacteria remain in a state of homeostasis with the gastrointestinal immunological system. Disturbances of protective functions or regulatory mechanisms can lead to inappropriate activation of the mucosal immune system and inflammation resulting in tissue injury. The composition and function of enteric microflora in patients with IBD reveal substantial changes as compared to healthy persons. Therapeutic manipulation of intestinal microorganisms offers great potential for the treatment of active disease and maintenance of remission in IBD

589 Pierce, E.S. (2009)
Where Are All the Mycobacterium avium Subspecies paratuberculosis in Patients with Crohn's Disease?
Plos Pathogens, 5, Mycobacterium avium subspecies paratuberculosis (MAP) causes a chronic granulomatous inflammation of the intestines, Johne's disease, in dairy cows and every other species of mammal in which it has been identified. MAP has been identified in the mucosal layer and deeper bowel wall in patients with Crohn's disease by methods other than light microscopy, and by direct visualization in small numbers by light microscopy. MAP has not been accepted as the cause of Crohn's disease in part because it has not been seen under the microscope in large numbers in the intestines of patients with Crohn's disease. An analysis of the literature on the pathology of Crohn's disease and on possible MAP infection in Crohn's patients suggests that MAP might directly infect endothelial cells and adipocytes and cause them to proliferate, causing focal obstruction within already existing vessels (including granuloma formation), the development of new vessels (neoangiogenesis and lymphangiogenesis), and the "creeping fat" of the mesentery that is unique in human pathology to Crohn's disease but also occurs in bovine Johne's disease. Large numbers of MAP might therefore be found in the mesentery attached to segments of intestine affected by Crohn's disease rather than in the bowel wall, the blood and lymphatic vessels running through the mesentery, or the mesenteric fat itself. The walls of fistulas might result from the neoangiogenesis or lymphangiogenesis that occurs in the bowel wall in Crohn's disease and therefore are also possible sites of large numbers of MAP. The direct visualization of large numbers of MAP organisms in the tissues of patients with Crohn's disease will help establish that MAP causes Crohn's disease

A questionnaire-based cross-sectional study of clinical Johne's disease on dairy farms in New Zealand
New Zealand Veterinary Journal, 57, 34-43

AIM: To investigate associations between both farm management factors and breed of dairy cow, and the incidence of farmer-observed clinical Johne's disease (JD) on dairy farms in four major dairying regions in the North Island of New Zealand. METHODS: A questionnaire-based cross-sectional study was conducted to identify associations between both farm management practices and breed of dairy cow, and the incidence of clinical cases of JD suspected by
farmers, on dairy farms in the Waikato, Taranaki, Wellington-Manawatu-Wanganui, and Wairarapa regions of New Zealand. Using multinomial logistic regression, the frequency of management practices was compared between farms on which no clinical cases of JD were observed, farms on which the observed incidence was low, and farms on which the observed incidence was high. RESULTS: Of the 427 responding farmers, 201 (47%) had suspected clinical cases of JD in their herd in the preceding 5 years. Only 56/427 (13%) farmers observed an average annual incidence of >0.5 cases/100 cows during this period. Ninety percent (203/225) of farmers that had not observed clinical cases and 21% (42/201) of farmers that had observed clinical cases did not consider the disease a serious problem. Farmers and veterinarians had a moderate level of agreement regarding the JD status of a farm. Their perceptions were in agreement for 86% (38/44) of the high-incidence herds for which both a farmer's and a veterinarian's perception were available. The presence of Jersey cows in the herd and the purchase of bulls were most strongly associated with the incidence of clinical JD. Grazing calves in the hospital paddock, purchase of a large percentage of heifers, larger-than-average herds within our sample, and the use of induction were also positively associated with JD. Farmers who ensured heifers were at least 2 years old, rather than younger, when mixed with adult stock were likely to observe fewer cases of clinical JD.

CONCLUSIONS AND CLINICAL RELEVANCE: The annual incidence of farmer-observed clinical cases of JD was low, and the disease was generally regarded as of little importance by farmers. Farmers that had a high proportion of Jersey cows or that purchased bulls from either one or more than four sources were most likely to report clinical cases of JD. Management practices that could aid in the control of JD are the purchase of bulls free of JD, ensuring that calves do not graze in the hospital paddock, and ensuring that young stock are at least 2 years old prior to contact with adult stock.

Biochemistry, 48, 4344-4353

The PLL(PTE-like lactonase)-group of enzymes within the amidohydrolase superfamily hydrolyze N-acyl-homoserine lactones (AHLs) that are involved in bacterial quorum-sensing pathways. These enzymes possess the (beta/alpha)(8) -barrel fold and serve as attractive templates for in vitro evolution and engineering of quorum-quenching biological molecules that can serve as antivirulence therapeutic agents. Using a quorum-quenching lactonase from Mycobacterium avium subsp. paratuberculosis K-10 (GI: 41409766) as the initial template for in vitro evolution experiments, we enhanced the catalytic efficiency and increased the substrate range of the wild-type enzyme through a single point mutation on the loop at the C-terminal end of the eighth beta-strand. This N266Y mutant had an increased value of k(cat)/K-m of 30- and 32-fold toward 3-oxo-N-octanoylL-homoserine lactone and N-hexanoyl-L-homoserine lactone, respectively; the evolved mutant also exhibited lactonase activity toward 3-oxo-N-hexanoyl-L-homoserine lactone and N-butyryl-L-homoserine lactone, AHLs that were previously not hydrolyzed by the wild-type enzyme. This article reinforces the evolutionary potential of the (beta/alpha)(8)-barrel fold and highlights the possibility of using quorum-quenching lactonases in the amidohydrolase superfamily as templates for engineering biomolecules of therapeutic use.

Research in Veterinary Science, 86, 371-372

In this experiment 63 animals from a paratuberculosis (PTB) and tuberculosis-free herd were tested by Intradermal Tuberculin Tests (ITT) and blood samples were collected before PPD inoculation and on days 3, 15, 30, 60 and 90 post-inoculation (p.i.). Sera were tested for PTB-specific antibodies by ELISA-PPA and confirmed by a commercial ELISA. Three (4.76%) animals were positive by ELISA-PPA and five (7.93%) in the commercial ELISA, between days 30 and 90 p.i. These results suggest that ITT can interfere in the reliability of ELISAs and that serological testing for PTB should be avoided for 90 d after PPD inoculation. (C) 2008 Elsevier Ltd. All rights reserved
Different methods for the detection of Mycobacterium avium ssp. avium (MAA) in naturally infected hens were compared. They included the conventional culture method (solid Herrold's and Stonebrink media and liquid Sula medium) and newly developed liquid culture systems, the manual mycobacteria growth indicator tube (M-MGIT) and the fully automated BACTEC MGIT 960 system (A-MGIT). 152 tissues originating from 15 naturally infected hens have been processed. The overall detection rates (percentage of positive cultures from the number of positive cultures determined by all the methods together) were 60, 70 and 76 % for the conventional media, M-MGIT and A-MGIT systems, respectively, the mean time of mycobacteria detection being 32.6, 17.6 and 14.6 d, respectively. The lowest contamination rate (2.0 %) was found in A-MGIT compared with M-MGIT (4.6 %) and conventional media (10.4 %).

Purpose of review The authors present evidence published during the past 2 years of the roles of commensal and pathogenic bacteria in the pathogenesis of the inflammatory bowel diseases. Recent findings Rodent models conclusively implicate commensal enteric bacteria in chronic, immune-mediated, experimental colitis, and genetically determined defects in bacterial killing by innate immune cells are found in a subset of patients with Crohn's disease. There is no evidence that a single pathogen, including Mycobacterium avium subspecies paratuberculosis, causes Crohn's disease or ulcerative colitis. However, adherent/invasive Escherichia coli are associated with ileal Crohn's disease, with the mechanisms and genetics of adherent/invasive E. coli virulence being elucidated. Molecular characterization of the microbiota in patients with inflammatory bowel diseases reveals decreased biodiversity of commensal bacteria, most notably the phyla Bacteroidetes and Firmicutes, including the clinically relevant Faecalibacterium prausnitzii, and increased E. coli concentrations. VSL#3 is one probiotic preparation shown to be efficacious in certain clinical situations in small clinical trials. Summary Further characterization of altered microbiota in patients with inflammatory bowel diseases and linking dysbiosis with host genetic alterations in immunoregulation, innate microbial killing and barrier function are critical, so that individualized treatments to increase beneficial commensals and their metabolic products (probiotic and prebiotic administration) and diminish deleterious species such as adherent/invasive E. coli can be tailored for defined patient subsets.

Anti-TNF immunotherapy has revolutionized the treatment of some inflammatory diseases, such as RA. However, a major concern is that patients receiving this therapy have an increased risk of fungal and bacterial infection, particularly of reactivating latent tuberculosis (TB). In this issue of the JCI, in an effort to understand how anti-TNF immunotherapy affects host mechanisms required to control TB, Bruns and colleagues examined the effects of the anti-TNF therapeutic infliximab on Mycobacterium tuberculosis-specific human lymphocytes (see the related article beginning on page 1167). The authors report that a granulysin-expressing CD45RA(+) subset of effector memory CD8(+) T cells that contributes to the killing...
of intracellular M. tuberculosis is depleted in vivo by infliximab in patients with RA, and that these cells are susceptible to complement-mediated lysis in the presence of infliximab in vitro. The study provides insight into host defense mechanisms that act to control TB infection and how they are affected during anti-TNF immunotherapy for autoimmune disease.

Anti-TNF immunotherapy reduces CD8(+) T cell-mediated antimicrobial activity against Mycobacterium tuberculosis in humans
Journal of Clinical Investigation, 119, 1167-1177

The incidence of tuberculosis is increased during treatment of autoimmune diseases with anti-TNF antibodies. This is a significant clinical complication, but also provides a unique model to study immune mechanisms in human tuberculosis. Given the key role for cell-mediated immunity in host defense against Mycobacterium tuberculosis, we hypothesized that anti-TNF treatment impairs T cell-directed antimicrobial activity. Anti-TNF therapy reduced the expression in lymphocytes of perforin and granulysin, 2 components of the T cell-mediated antimicrobial response to intracellular pathogens. Specifically, M. tuberculosis-reactive CD8(+)CCR7(-)CD45RA(+) effector memory T cells (T-EMRA cells) expressed the highest levels of granulysin, lysed M. tuberculosis, and infected macrophages and mediated an antimicrobial activity against intracellular M. tuberculosis. Furthermore, T-EMRA cells expressed cell surface TNF and bound the anti-TNF therapeutic infliximab in vitro, making them susceptible to complement-mediated lysis. Immune therapy with anti-TNF was associated with reduced numbers of CD8(+) T-EMRA cells and decreased antimicrobial activity against M. tuberculosis, which could be rescued by the addition of CD8(+) T-EMRA cells. These results suggest that anti-TNF therapy triggers a reduction of CD8(+) T-EMRA cells with antimicrobial activity against M. tuberculosis, providing insight into the mechanism whereby key effector T cell subsets contribute to host defense against tuberculosis.

285 Philpott, D.J., Girardin, S.E. (2009)
Crohn's disease-associated Nod2 mutants reduce IL10 transcription
Nature Immunology, 10, 455-457

The 3020insC mutation in Nod2 is associated with Crohn's disease, but how it influences disease pathogenesis is unknown. A new study shows that the 3020insC mutant protein fails to activate a key transcription factor that drives interleukin 10 expression, resulting in reduced production of this anti-inflammatory cytokine.

Autophagy genes in immunity
Nature Immunology, 10, 461-470

In its classical form, autophagy is a pathway by which cytoplasmic constituents, including intracellular pathogens, are sequestered in a double-membrane-bound autophagosome and delivered to the lysosome for degradation. This pathway has been linked to diverse aspects of innate and adaptive immunity, including pathogen resistance, production of type I interferon, antigen presentation, tolerance and lymphocyte development, as well as the negative regulation of cytokine signaling and inflammation. Most of these links have emerged from studies in which genes encoding molecules involved in autophagy are inactivated in immune effector cells. However, it is not yet known whether all of the critical functions of such genes in immunity represent 'classical autophagy' or possible as-yet-undefined autophagolysosome-independent functions of these genes. This review summarizes phenotypes that result from the inactivation of autophagy genes in the immune system and discusses the pleiotropic functions of autophagy genes in immunity.

Immunohistochemical detection of NOD1 and NOD2 in the healthy murine and canine eye
Veterinary Ophthalmology, 12, 269-275
The innate immune system provides the immediate defense against pathogens. NOD1 and NOD2 proteins are intracytoplasmic signaling receptors of the innate immune system and recognize conserved microbial molecular structures. The aim of this study was to analyze the expression of NOD1 and NOD2 proteins in healthy mouse and dog eyes using immunohistochemistry on formalin-fixed, paraffin-embedded globes. In both the mouse and dog globes, a strong immunosignal for NOD1 and NOD2 was present within corneal epithelium, corneal endothelium and conjunctival epithelium. Scattered cells in the conjunctival substantia propria displayed moderate immunopositivity for NOD1 and NOD2. Additionally, in dog eyes, nonpigmented iridal epithelium was immunopositive for NOD1 and NOD2. No other examined ocular tissues were immunopositive for NOD1 or NOD2. To the best of the authors' knowledge, this is the first description of an immunohistochemical study on NOD1 and NOD2 expression in healthy mouse and dog eyes. Since signaling molecules of the innate immune system mediate pro-inflammatory responses in numerous organs, they likely also contribute to the pathogenesis of ocular inflammation.

The Kinase Activity of Rip2 Determines Its Stability and Consequently Nod1-and Nod2-mediated Immune Responses
Journal of Biological Chemistry, 284, 19183-19188

Rip2 (RICK, CARD3) has been identified as a key effector molecule downstream of the pattern recognition receptors, Nod1 and Nod2; however, its mechanism of action remains to be elucidated. In particular, it is unclear whether its kinase activity is required for signaling or for maintaining protein stability. We have investigated the expression level of different retrovirally expressed kinase-dead Rip2 mutants and the role of Rip2 kinase activity in the signaling events that follow Nod1 and Nod2 stimulation. We show that in primary cells expressing kinase-inactive Rip2, protein levels were severely compromised, and stability could not be reconstituted by the addition of a phospho-mimetic mutation in its autophosphorylation site. Consequently, inflammatory cytokine production in response to Nod1 and Nod2 ligands was abrogated both in vitro and in vivo in the absence of Rip2 kinase activity. Our results highlight the central role that Rip2 kinase activity plays in conferring stability to the protein and thus in the preservation of Nod1- and Nod2-mediated innate immune responses.

Autoimmune disease in the era of the metagenome
Autoimmunity Reviews, 8, 677-681

Studies of autoimmune disease have focused on the characteristics of the identifiable antibodies. But as our knowledge of the genes associated with the disease states expands, we understand that humans must be viewed as superorganisms in which a plethora of bacterial genomes - a metagenome - work in tandem with our own. The NIH has estimated that 90% of the cells in Homo sapiens are microbial and not human in origin. Some of these microbes create metabolites that interfere with the expression of genes associated with autoimmune disease. Thus, we must re-examine how human gene transcription is affected by the plethora of microbial metabolites. We can no longer assume that antibodies generated in autoimmune disease are created solely as autoantibodies to human DNA. Evidence is now emerging that the human microbiota accumulates during a lifetime, and a variety of persistence mechanisms are coming to light. In one model, obstruction of VDR nuclear-receptor-transcription prevents the innate immune system from making key antimicrobials, allowing the microbes to persist. Genes from these microbes must necessarily impact disease progression. Recent efforts to decrease this VDR-perverting microbiota in patients with autoimmune disease have resulted in reversal of autoimmune processes. As the NIH Human Microbiome Project continues to better characterize the human metagenome, new insights into autoimmune pathogenesis are beginning to emerge. (C) 2009 Elsevier B.V. All rights reserved.

Infection and type 1 diabetes mellitus - A two edged sword?
Autoimmunity Reviews, 8, 682-686
Infection by various viral and bacterial pathogens has long been proposed as one of the etiologies of autoimmune diabetes. Many theories, ranging from direct cytolysis of pancreatic islet cells to immunological processes such as antigen mimicry and polyclonal lymphocyte activation, tried to explain the epidemiological correlation between infections and diabetes, supported by information from human and animal studies. However, a direct correlation and exact mechanism continue to elude investigators due to scarce and conflicting data. Interestingly, there is also data to support an opposite role for infection in the development of type 1 diabetes, as several pathogens demonstrated a protective effect from this disease. This article reviews the current data available from clinical studies and animal models, while trying to explain the different mechanisms underlying these findings. (C) 2009 Elsevier B.V. All rights reserved


Innate immunity is the earliest response to invading microbes and acts to contain infection in the first minutes to hours of challenge. Unlike adaptive immunity that relies upon clonal expansion of cells that emerge days after antigenic challenge, the innate immune response is immediate. Soluble mediators, including complement components and the mannose binding lectin (MBL) make an important contribution to innate immune protection and work along with epithelial barriers, cellular defenses such as phagocytosis, and pattern-recognition receptors that trigger pro-inflammatory signaling cascades. These four aspects of the innate immune system act in concert to protect from pathogen invasion. Our work has focused on understanding the protection provided by this complex defense system and, as discussed in this review, the particular contribution of soluble mediators such as MBL and phagocytic cells. Over the past two decades both human epidemiological data and mouse models have indicated that MBL plays a critical role in innate immune protection against a number of pathogens. As demonstrated by our recent in vitro work, we show that MBL and the innate immune signaling triggered by the canonical pattern-recognition receptors (PRRs), the Toll-like receptors (TLRs), are linked by their spatial localization to the phagosome. These observations demonstrated a novel role for MBL as a TLR co-receptor and establishes a new paradigm for the role of opsonins, which we propose to function not only to increase microbial uptake but also to spatially coordinate, amplify, and synchronize innate immune defenses mechanism. In this review we discuss both the attributes of MBL that make it a unique soluble pattern recognition molecule and also highlight its broader role in coordinating innate immune activation


Autophagy is a cell biological process, enabling cells to autodigest their own cytosol when starved, remove cytoplasmic protein aggregates too large for proteasomal degradation, eliminate aberrant or over-proliferated organelles, and sanitize the cytoplasm by killing intracellular microbes. The role of autophagy has been expanded in recent years to include diverse immunological effector and regulatory functions. In this review, we summarize the multiple immunological roles of autophagy uncovered to date and focus primarily on details of induction of autophagy by pattern recognition receptors, as a newly established Toll-like receptor output. Taken together with other links between autophagy and innate and adaptive immunity processes, this cell-autonomous antimicrobial defense may be evolutionarily positioned at the root of immunity with the multiple innate and adaptive immunity connections uncovered to date reflecting a co-evolution of this ancient cell-defense mechanism and more advanced immunological systems in metazoans. Cell Death and Differentiation (2009) 16, 976-983; doi: 10.1038/cdd.2009.40; published online 15 May 2009

Cellular inhibitor of apoptosis proteins (cIAPs) block apoptosis, but their physiological functions are still under investigation. Here, we report that cIAP1 and cIAP2 are E3 ubiquitin ligases that are required for receptor-interacting protein 2 (RIP2) ubiquitination and for nucleotide-binding and oligomerization (NOD) signaling. Macrophages derived from Birc2(-/-) or Birc3(-/-) mice, or colonocytes depleted of cIAP1 or cIAP2 by RNAi, were defective in NOD signaling and displayed sharp attenuation of cytokine and chemokine production. This blunted response was observed in vivo when Birc2(-/-) and Birc3(-/-) mice were challenged with NOD agonists. Defects in NOD2 signaling are associated with Crohn's disease, and muramyl dipeptide (MDP) activation of NOD2 signaling protects mice from experimental colitis. Here, we show that administration of MDP protected wild-type but not Ripk2(-/-) or Birc3(-/-) mice from colitis, confirming the role of the cIAPs in NOD2 signaling in vivo. This discovery provides therapeutic opportunities in the treatment of NOD-dependent immunologic and inflammatory diseases.

Deretic, V., Levine, B. (2009)
*Autophagy, Immunity, and Microbial Adaptations*
Cell Host & Microbe, 5, 527-549

Autophagy adjusts cellular biomass and function in response to diverse stimuli, including infection. Autophagy plays specific roles in shaping immune system development, fueling host innate and adaptive immune responses, and directly controlling intracellular microbes as a cell-autonomous innate defense. As an evolutionary counterpoint, intracellular pathogens have evolved to block autophagic microbicidal defense and subvert host autophagic responses for their survival or growth. The ability of eukaryotic pathogens to deploy their own autophagic machinery may also contribute to microbial pathogenesis. Thus, a complex interplay between autophagy and microbial adaptations against autophagy governs the net outcome of host-microbe encounters.

*Anti glycans antibodies establish an unexpected link between C. albicans and Crohn disease*
M S-Medecine Sciences, 25, 473-481

Almost 80% of the dry weight of the yeast cell wall is composed of glycans including mannans, glucans and chitin. Within this variable and complex edifice, glycans play a major role in their relation with the environment. Experimental antibodies allowed to define the localization, the variability of expression and the biological role of numerous natural oligosaccharidic sequences. These glycans and their synthetic analogues were used to study the human humoral response during invasive candidiasis (IC) determined by Candida albicans and Crohn's disease (CD) where antibodies against the dietary yeast Saccharomyces cerevisiae have been reported. On these bases, it was established experimentally and clinically that a large panel of CD biomarkers consisting in anti glycans antibodies were also generated during IC establishing a link never suspected between C. albicans and CD. We describe here the principle of this serological analysis and its perspectives related to the use of multianalyte profiling technology for a better understanding of IC and CD pathophysiology. This may contribute to improve disease management in terms of diagnosis and therapy.

Gaisford, W., Cooke, A. (2009)
*Can infections protect against autoimmunity?*
Current Opinion in Rheumatology, 21, 391-396

Purpose of review It has often been suggested that autoimmune diseases are initiated by certain infectious agents that mimic self-antigens or polyclonally activated autoreactive lymphocytes. An alternative, and not necessarily mutually exclusive, hypothesis that some infections might inhibit the onset of some autoimmune conditions has more recently been explored. In this review, the evidence suggesting that the current rise in the incidence of some autoimmune diseases is attributable to a decrease in the incidence of exposure to certain infections will be discussed. Recent findings Studies using animal models have shown that
some infectious agents or products derived from them have the potential to inhibit the onset of autoimmunity. These studies have led to the suggestion that human autoimmune or allergic diseases might be alleviated by the use of microbial products. There are some data that would support such an observation. Summary: The incidence of some autoimmune diseases has increased dramatically in recent years in the developed world. Many autoimmune diseases are governed by both genetic and environmental factors. Our immune system has coevolved with infectious agents. There have been marked changes in the exposure to certain infectious agents over the last 70 years. It has been proposed that certain infections of historical importance might inhibit the development of autoimmune disorder. This review highlights studies addressing the ways in which infectious agents might inhibit onset of autoimmunity, and how this might lead to the development of novel therapeutic approaches.


OBJECTIVES: We sought to evaluate whether two novel immunoglobulin A (IgA) cell wall polysaccharide antibodies, anti-laminarin (anti-L) and anti-chitin (anti-C), aid in the diagnosis and phenotype differentiation of Crohn’s disease (CD) and ulcerative colitis (UC). METHODS: A cohort of 818 individuals with inflammatory bowel disease (IBD; 517 CD and 301 UC) from two IBD tertiary referral centers, with median ages of 33 and 39 years, respectively, and disease duration of 8.9 years, were phenotyped using the Montreal classification, and analyzed for seven anti-glycan antibodies (gASCA (anti-Saccharomyces cerevisiae) IgG, gASCA IgA, antichitobioside (GlcNAC(bet1,4) GlcNAC(bet1)), anti-laminaribioside (Glc(bet1,3) Glb(bet1)), anti-mannobioside (Man(alp1,3) Man(alp1)), anti-L, and anti-C) and perinuclear atypical neutrophil cytoplasmic antibodies (pANCA). RESULTS: In the CD patient population, 73% were positive for >1 anti-glycan antibody. All glycan markers were specific for CD (85.4-97.7%) and more prevalent in CD vs. UC (P<0.0015). gASCA IgG and IgA best differentiated CD from UC followed by anti-L (area under the curve 0.818, 0.815, and 0.702, respectively). The addition of anti-L and anti-C to gASCA IgG and pANCA improved discrimination between CD and UC (P<0.001). Adding anti-L to gASCA and pANCA differentiated colonic CD and UC (P=0.02). An increasing number of positive antibodies was associated with early CD onset, penetrating phenotype, perianal disease, and the need for surgery (P<0.001). Anti-L was associated with ileocolonic CD (odds ratio (OR) 2.28, 95% confidence interval (CI) 1.40-3.69; P=0.001), and anti-C with penetrating (OR 2.75, 95% CI 1.50-5.04; P=0.001) and perianal disease (OR 1.95, 95% CI 1.06-3.59; P=0.03). CONCLUSIONS: Anti-L and anti-C improve differentiation between CD and UC. Anti-L may also differentiate between isolated colonic CD and UC. Both anti-L and anti-C are independently associated with a more aggressive CD phenotype.


Background & Aims: During the pathogenesis of Crohn’s disease (CD), interleukin (IL)-12, a cytokine produced by mucosal CD14(+) monocyte-like cells, promotes tissue-damaging Th helper cell (Th1) 1-mediated inflammation through mechanisms that are not fully understood. IL-25 promotes Th2 cell responses by activating major histocompatibility complex class II-positive non-T and non-B cells. Because Th1 and Th2 cells, and the cytokines they release, are often mutually antagonistic, we examined whether IL-25 affects IL-12 production or Th1 cell-mediated inflammation in the gut. Methods: Studies were performed using colonic samples from patients and mice with peptidoglycan (PGN)-, 2,4,6-trinitrobenzenesulphonic acid (TNBS)-, or oxazolone-induced colitis. IL-25 receptor (IL-25R) levels were evaluated in intestinal lamina propria mononuclear cells by flow cytometry, and IL-25 levels were measured by real-time polymerase chain reaction, immunoblotting, and immunohistochemistry. Mucosal CD14(+) cells from patients with CD were incubated with IL-25 and/or lipopolysaccharide or
PGN. Mice were injected with IL-25, and some mice first received injections of an IL-13 blocking antibody. Cytokines were quantified by real-time polymerase chain reaction and enzyme-linked immunosorbent assay. Results: CD14(+) cells from the mucosa of CD patients expressed IL-25R and responded to IL-25 by decreasing the synthesis of IL-12 and IL-23. IL-25 prevented PGN-induced colitis in mice. IL-25 induced IL-13 production in the colon, but IL-13 was not required for suppression of PGN colitis. IL-25 ameliorated TNBS- and oxazolone-colitis. Patients with CD or ulcerative colitis produced significantly less IL-25 compared with controls. Conclusions: IL-25 inhibits CD14(+) cell-derived cytokines and experimental colitis. IL-25 could be a useful treatment of CD and ulcerative colitis.

O'Reilly, M.K., Paulson, J.C. (2009)
**Siglecs as targets for therapy in immune-cell-mediated disease**
Trends in Pharmacological Sciences, 30, 240-248

The sialic-acid-binding immunoglobulin-like lectins (siglecs) comprise a family of receptors that are differentially expressed on leukocytes and other immune cells. The restricted expression of several siglecs to one or a few cell types makes them attractive targets for cell-directed therapies. The anti-CD33 (also known as Siglec-3) antibody gemtuzumab (Mylotarg(TM)) is approved for the treatment of acute myeloid leukemia, and antibodies targeting CD22 (Siglec-2) are currently in clinical trials for treatment of B cell non-Hodgkins lymphomas and autoimmune diseases. Because siglecs are endocytic receptors, they are well suited for a 'Trojan horse' strategy, whereby therapeutic agents conjugated to an antibody, or multimeric glycan ligand, bind to the siglec and are efficiently carried into the cell. Although the rapid internalization of unmodified siglec antibodies reduces their utility for induction of antibody-dependent cellular cytotoxicity or complement-mediated cytotoxicity, antibody binding of Siglec-8, Siglec-9 and CD22 has been demonstrated to induce apoptosis of eosinophils, neutrophils and depletion of B cells, respectively. Here, we review the properties of siglecs that make them attractive for cell-targeted therapies.

Pierce, E.S. (2009)
**Where Are All the Mycobacterium avium Subspecies paratuberculosis in Patients with Crohn’s Disease?**
PloS Pathogens, 5, Mycobacterium avium subspecies paratuberculosis (MAP) causes a chronic granulomatous inflammation of the intestines, Johne's disease, in dairy cows and every other species of mammal in which it has been identified. MAP has been identified in the mucosal layer and deeper bowel wall in patients with Crohn's disease by methods other than light microscopy, and by direct visualization in small numbers by light microscopy. MAP has not been accepted as the cause of Crohn's disease in part because it has not been seen under the microscope in large numbers in the intestines of patients with Crohn's disease. An analysis of the literature on the pathology of Crohn's disease and on possible MAP infection in Crohn's patients suggests that MAP might directly infect endothelial cells and adipocytes and cause them to proliferate, causing focal obstruction within already existing vessels (including granuloma formation), the development of new vessels (neoangiogenesis and lymphangiogenesis), and the "creeping fat" of the mesentery that is unique in human pathology to Crohn's disease but also occurs in bovine Johne's disease. Large numbers of MAP might therefore be found in the mesentery attached to segments of intestine affected by Crohn's disease rather than in the bowel wall, the blood and lymphatic vessels running through the mesentery, or the mesenteric fat itself. The walls of fistulas might result from the neoangiogenesis or lymphangiogenesis that occurs in the bowel wall in Crohn's disease and therefore are also possible sites of large numbers of MAP. The direct visualization of large numbers of MAP organisms in the tissues of patients with Crohn's disease will help establish that MAP causes Crohn's disease.

**Combined Polymorphisms in Genes Encoding the Inflammasome Components NALP3 and CARD8 Confer Susceptibility to Crohn’s Disease in Swedish Men**
American Journal of Gastroenterology, 104, 1180-1188

OBJECTIVES: Crohn’s disease (CD) is characterized by overproduction of proinflammatory
cytokines like interleukin (IL)-1 beta. Production of mature IL-1 beta is dependent on a caspase-1-activating protein complex called the NALP3 inflammasome, composed of NALP3, ASC, and CARD8. NALP3 shares structural similarities with Nod2, and both of these proteins are required for bacteria-induced IL-1 beta secretion. The combination of the polymorphisms CARD8 (C10X) and NALP3 (Q705K) was recently shown to be associated with rheumatoid arthritis. Our aim was to investigate whether these combined polymorphisms play a role in the susceptibility to CD. METHODS: The study included 498 CD patients in two cohorts from different regions and 742 control individuals from a Swedish population. DNA was isolated from whole blood. Polymorphisms of (Q705K) NALP3 and (C10X) CARD8, as well as the Nod2 variants, R702W and G908R, were genotyped using the Taqman single nucleotide polymorphism assay. The Nod2 frameshift mutation, L1007fs, was detected by Megabace SNuPe genotyping. RESULTS: Our results show that men who have both the C10X and Q705K alleles in CARD8 and NALP3, and who express wild-type alleles of Nod2 are at an increased risk of developing CD (odds ratio, OR: 3.40 (range: 1.32-8.76); P = 0.011). No association with these polymorphisms was found in women (OR: 0.89 (range: 0.44-1.77); P = 0.74). CONCLUSIONS: We suggest a role for combined polymorphisms in CARD8 and NALP3 in the development of CD in men, with obvious sex differences in the genetic susceptibility pattern. These findings give further support to the importance of innate immune responses in CD

Commensal bacteria, traditional and opportunistic pathogens, dysbiosis and bacterial killing in inflammatory bowel diseases
Current Opinion in Infectious Diseases, 22, 292-301

Purpose of review The authors present evidence published during the past 2 years of the roles of commensal and pathogenic bacteria in the pathogenesis of the inflammatory bowel diseases. Recent findings Rodent models conclusively implicate commensal enteric bacteria in chronic, immune-mediated, experimental colitis, and genetically determined defects in bacterial killing by innate immune cells are found in a subset of patients with Crohn’s disease. There is no evidence that a single pathogen, including Mycobacterium avium subspecies paratuberculosis, causes Crohn’s disease or ulcerative colitis. However, adherent/invasive Escherichia coli are associated with ileal Crohn’s disease, with the mechanisms and genetics of adherent/invasive E coli virulence being elucidated. Molecular characterization of the microbiota in patients with inflammatory bowel diseases reveals decreased biodiversity of commensal bacteria, most notably the phyla Bacteroidetes and Firmicutes, including the clinically relevant Faecalibacterium prausnitzii, and increased E coli concentrations. VSL#3 is one probiotic preparation shown to be efficacious in certain clinical situations in small clinical trials. Summary Further characterization of altered microbiota in patients with inflammatory bowel diseases and linking dysbiosis with host genetic alterations in immunoregulation, innate microbial killing and barrier function are critical, so that individualized treatments to increase beneficial commensals and their metabolic products (probiotic and prebiotic administration) and diminish deleterious species such as adherent/invasive E coli can be tailored for defined patient subsets

303 Deretic, V. (2009)
Multiple regulatory and effector roles of autophagy in immunity
Current Opinion in Immunology, 21, 53-62

Autophagy is a cytoplasmic homeostasis pathway, enabling cells to digest their own cytosol, remove toxic protein aggregates, and eliminate defective or surplus organelles. A plenitude of studies has now expanded roles of autophagy to both effector and regulatory functions in innate and adaptive immunity. In its role of an immunological effector, autophagy plays many parts: (i) In its most primeval manifestation, it captures and digests intracellular microbes, (ii) it is an antimicrobial output of Toll-like receptor (TLR) response to pathogen associated molecular patterns (PAMP), and (iii) it is an effector of Th1-Th2 polarization in resistance or susceptibility to intracellular pathogens. As a regulator of immunity, autophagy plays a multitude of functions: (i) It acts as a topological inversion device servicing both innate and adaptive immunity by delivering cytosolic antigens to the lumen of MHC II compartments and cytosolic PAM Ps to endosomal TLRs, (ii) it is crucial in T cell repertoire selection in the
thymus and control of central tolerance, (iii) it plays a role in T and B cell homeostasis, and (iv) it is of significance for inflammatory pathology. A properly functioning autophagy helps prevent autoimmunity and assists in clearing pathogens. When aberrant, it contributes to human inflammatory disorders such as Crohn's disease.

Modulation of muramyl dipeptide stimulation of cytokine production by blood components 
Clinical and Experimental Immunology, 156, 428-433

Muramyl dipeptide (MDP) is the minimal active fragment of peptidoglycan of the cell wall of Gram-positive bacteria, with potential beneficial effects as a vaccine adjuvant. Peptidoglycans and MDP are recognized by the intracellular receptor NOD2 (nucleotide-binding oligomerization domain 2), leading to production of proinflammatory cytokines. In the present study, it is shown that, despite stimulatory effects on isolated human mononuclear cells, MDP does not stimulate production of tumour necrosis factor-alpha, interleukin-1 beta or interleukin-6 in a whole-blood assay. However, MDP retains synergistic effects on lipopolysaccharide-induced cytokines in whole blood. Screening tests of NOD2 function based on whole-blood stimulation should therefore employ strategies based on the synergistic effects of MDP on Toll-like receptor-induced cytokine production. Plasma was not responsible for the inhibition of MDP in whole blood. The inhibition of MDP stimulation was dependent upon cellular components, with erythrocyte-derived haemoglobin and neutrophils collaborating in the inhibition of MDP effects in whole blood.