2013-03-27-015 Paratuberculosis databases updated (2013-03-22)
To: (04) Mycobacterial diseases; (12) Scientific Information, research and education;

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New publications in the PARATUBERCULOSIS database (1387-1391)

1387 Chandra, S. , Faisal, S.M., Chen, J.W., Chen, T.T., McDonough, S.P., Liu, S., Moreira, M.A.S., Akey, B.L., Chang, C.F., Chang, Y.F.

Immune response and protective efficacy of live attenuated Salmonella vaccine expressing antigens of Mycobacterium avium subsp paratuberculosis against challenge in mice
Vaccine, (2012) 31, 242-251

Mycobacterium avium subsp. paratuberculosis (MAP) causes chronic granulomatous enteritis in ruminants that leads to diarrhea and eventually death. Existing vaccines have proven useful in limiting disease progression but have not been effective in preventing infection. To address this problem we constructed an attenuated Salmonella (Delta yejE; Delta ssaV) strain harboring a plasmid that expressed a fusion protein comprised of the Salmonella Type III secretion system (T3SS) effector SopE and MAP antigens (85A, 85B, SOD, 74F) and evaluated its potential as vaccine candidate against MAP infection in mice. Of various SopE-MAP fusion proteins analyzed, only SopE104-Ag85A C-terminal(202-347)-SOD N-terminal(1-72)-Ag85B C-terminal(173-330) and SopE104-74F(1-148+669-786) were successfully expressed and secreted into culture media as revealed by western blot analysis. Mice immunized with attenuated Salmonella (Delta yejE; Delta ssaV) harboring the SopE104-Ag85A C-terminal(202-347)-SOD N-terminal(1-72)-Ag85B C-terminal(173-330) and SopE104-74F(1-148+669-786) plasmid generated a potent and long lasting Th1 response characterized by production of IFIN-gamma). The cytokine profile varied at various time points after immunization and challenge, which showed down regulation of Th2 cytokines (IL-4, IL-10) and up-regulation of proinflammatory cytokines (IL-12 and IL-17). Further, the immune response correlated with protection as revealed by reduced bacterial load and improved histopathology of spleen and liver, which showed fewer granulomas and lower numbers of acid-fast bacilli as compared to PBS controls. Interestingly, vaccination with antigens mixed with Ribi adjuvant (Agmix + Ribi) imparted better protection than the attenuated salmonella vectored vaccine. Thus, priming with a live recombinant Salmonella strain that secretes MAP antigens represents a promising approach that could lead to development of an efficacious and cost effective vaccine for johne's disease. (c) 2012 Elsevier Ltd. All rights reserved.

1388 Barry, A.O. , Boucherit, N., Mottola, G., Vadovic, P., Trouplin, V., Soubeyran, P., Capo, C., Bonatti, S., Nebreda, A., Toman, R., Lemichez, E., Mege, J.L., Ghigo, E.

Impaired Stimulation of p38 alpha-MAPK/Vps41-HOPS by LPS from Pathogenic Coxiella burnetii Prevents Trafficking to Microbicidal Phagolysosomes
Cell Host & Microbe, (2012) 12, 751-763

Variations in lipopolysaccharide (LPS), a bacterial outer membrane component, determine virulence of the obligate intracellular bacterium Coxiella burnetii, but the underlying mechanisms are unknown. We find that while avirulent C. burnetii LPS (avLPS) stimulates host p38 alpha-MAPK signaling required for proper trafficking of bacteria containing compartments to lysosomes for destruction, pathogenic C. bumetii LPS (vLPS) does not. The defect in vLPS and pathogenic C. bumetii targeting to degradative compartments involves an antagonistic engagement of TLR4 by vLPS, lack of p38 alpha-MAPK-driven phosphorylation, and block in recruitment of the homotypic fusion and protein-sorting complex component Vps41 to vLPS-containing vesicles. An upstream activator of p38 alpha-MAPK or phosphomimetic mutant Vps41-S796E expression overrides the inhibition, allowing vLPS and pathogenic C. bumetii targeting to phagolysosomes. Thus, p38 alpha-MAPK and its crosstalk

with Vps41 play a central role in trafficking bacteria to phagolysosomes. Pathogenic C. burnetii has evolved LPS variations to evade this host response and thrive intracellularly.

1389 Pribylova, R., Kubickova, L., Babak, V., Pavlik, I., Kralik, P.

Effect of short- and long-term antibiotic exposure on the viability of Mycobacterium avium subsp paratuberculosis as measured by propidium monoazide F57 real time quantitative PCR and culture
Veterinary Journal, (2012) 194, 354-360

Mycobacterium avium subsp. paratuberculosis (MAP), the causative agent of paratuberculosis in ruminants, has a lipid-rich cell wall which facilitates its survival and persistence in the environment. This property of the organism is exploited when it is cultured as decontaminating agents and antibiotics are used to suppress the growth of contaminating microflora, but such treatments can also negatively affect the isolation of MAP itself. The objective of this study was to assess the effect of the 'VAN' antibiotics (vancomycin, amphotericin B and nalidixic acid) on the viability of MAP using a propidium monoazide real time quantitative PCR (PMA qPCR) and culture. Long-term (5 week) treatment with VAN antibiotics resulted in a larger decrease in bacterial numbers compared to short-term (3 day) exposure. The PMA qPCR assay indicated that 50 mu g/mL of vancomycin, 50 mu g/mL of nalidixic acid, and 200 mu g/mL of amphotericin B were 'threshold' concentrations, respectively, above which the decline in the viability of MAP was statistically significant. Using culture, these threshold concentrations were 100 mu g/mL of vancomycin, 50-100 mu g/mL of nalidixic acid, and 100 mu g/mL of amphotericin B, respectively. Given that the two methods were found to be comparable, the PMA qPCR is a potentially more convenient and effective alternative to culture in detecting MAP. (C) 2012 Elsevier Ltd. All rights reserved.

1390 Brugere-Picoux, J.

Update on Bovine Paratuberculosis
Bulletin de l Academie Veterinaire de France, (2012) 165, 9-20

Ruminant paratuberculosis (Johne's disease) is caused by Mycobacterium avium subsp. paratuberculosis (Map). It has been known for a long time, but it is still a problem for livestock farmers. This digestive disease associated with cachexia affects mainly domestic and wild adult ruminants. It has spread progressively to areas previously considered as disease-free, due to livestock trading. One of the main causes of infection is a contaminated environment due to fecal shedding by adult cows, particularly those with diarrhea or asymptomatic heavy shedders. Control measures must target these animals specifically, and continuous monitoring is required to detect newly infected individuals in the herd. This article looks at the survival of Map in the environment and the transmission factors, as well as the improvement of methods for the diagnosis and control of paratuberculosis. The hypothesis of a zoonotic risk linked to Map is also considered due to its presence in milk and meat.

1391 Ghazarian, L., Diana, J., Simoni, Y., Beaudoin, L., Lehuen, A.

Prevention or acceleration of type 1 diabetes by viruses
Cellular and Molecular Life Sciences, (2013) 70, 239-255

Type 1 diabetes is an autoimmune disease characterized by the destruction of insulin-producing pancreatic beta-cells. Even though extensive scientific research has yielded important insights into the immune mechanisms involved in pancreatic beta-cell destruction, little is known about the events that trigger the autoimmune process. Recent epidemiological and experimental data suggest that environmental factors are involved in this process. In this review, we discuss the role of viruses as an environmental factor on the development of type 1 diabetes, and the immune mechanisms by which they can trigger or protect against this pathology.

New publications in the CROHN'S DISEASE AND PARATUBERCULOSIS database (767-789)

767 Mundo, S.L., Gilardoni, L.R., Hoffman, F.J., Lopez, O.J.

Rapid and Sensitive Method To Identify Mycobacterium avium subsp paratuberculosis in Cow's Milk by DNA Methylase Genotyping
Applied and Environmental Microbiology, (2013) 79, 1612-1618

Paratuberculosis is an infectious, chronic, and incurable disease that affects ruminants, caused by Mycobacterium avium subsp. paratuberculosis. This bacterium is shed primarily through feces of infected cows but can be also excreted in colostrum and milk and might survive pasteurization. Since an association of genomic sequences of M. avium subsp. paratuberculosis in patients with Crohn's disease has been described; it is of interest to rapidly detect M. avium subsp. paratuberculosis in milk for human consumption. IS900 insertion is used as a target for PCR amplification to identify the presence of M. avium subsp. paratuberculosis in biological samples. Two target sequences were selected: IS1 (155 bp) and IS2 (94 bp). These fragments have a 100% identity among all M. avium subsp. paratuberculosis strains sequenced. M. avium subsp. paratuberculosis was specifically concentrated from milk samples by immunomagnetic separation prior to performing PCR. The amplicons were characterized using DNA methylase Genotyping, i.e., the amplicons were methylated with 6-methyl-adenine and digested with restriction enzymes to confirm their identity. The methylated amplicons from 100 CFU of M. avium subsp. paratuberculosis can be visualized in a Western blot format using an anti-6-methyl-adenine monoclonal antibody. The use of DNA methyltransferase genotyping coupled to a scintillation proximity assay allows for the detection of up to 10 CFU of M. avium subsp. paratuberculosis per ml of milk. This test is rapid and sensitive and allows for automation and thus multiple samples can be tested at the same time.

768 Hruska, K., Kaevska, M.

Mycobacteria in water, soil, plants and air: a review
Veterinarni Medicina, (2012) 57, 623-679

Amazingly, despite the 24 143 papers on mycobacteria, indexed in the Web of Science database during the last six years, published by 67 008 authors from 13 128 organizations located in 166 countries or territories, internationally accepted legal directives on how to control the public health risk associated with environmental mycobacteria have yet to be developed. Mycobacteria are human and animal pathogens, causing not only tuberculosis and leprosy, but mycobacterioses of skin, soft tissues and lung. Due to their cell wall composition and their adaptability mycobacteria can survive in different habitats for years. Their immunomodulatory ability has been recognised for more than 50 years and hundreds of papers published during the last two decades have demonstrated that small chemical products derived from mycobacterial cells participate in inflammatory pathways involved the pathogenesis of important human diseases like Crohn's disease, asthma, type 1 diabetes mellitus, psoriasis, arthrosis, Blau syndrom, sarcoidosis, autism etc. Mycobacteria can influence inflammatory pathways not only as live organisms, but also by means of components derived from dead cells. Pasteurisation or cooking does not affect this ability. Hence, how many mycobacterial cells are ingested, what factors play a role concurrently, and how long the harmful effect persists become important questions. This paper presents only a short review based on selected papers about mycobacteria in water, soil, plants and air with the aim of attracting attention to this significant global problem and of making the first steps towards protection of people. Selected bibliographic references of published data from 2007 to 2012 are presented in easy-to-navigate tables.

769 Rosenstiel, P.

Stories of love and hate: innate immunity and host-microbe crosstalk in the intestine
Current Opinion in Gastroenterology, (2013) 29, 125-132

Purpose of review Recent advances in molecular techniques have enabled a deep view into the structure and function of the host's immune system and the stably associated commensal intestinal flora. This review outlines selected aspects of the interplay of innate immune recognition and effectors that shape the ecological niches for the intestinal microbiota. Recent findings Several studies have demonstrated a pivotal role of innate immune receptor pathways (NOD-like receptors and Toll-like receptors) for the maintenance of microbial communities in the gut. Genetic deficiencies in these pathways have been associated with increased susceptibility to inflammation that in animal models can be transmitted via direct contact or by stool transplantation in the absence of abundant pathogens. Summary The genetic architecture of the human host shapes the diversity and function of its stably associated intestinal microflora. Innate immune receptors such as NOD2 or the inflammasome component NOD-like receptor, pyrin-domain containing 6 play a major role in licensing the microbiota under physiological conditions. Understanding the symbiotic interplay in the intestinal tract should help develop procedures and therapeutic interventions aiming at the identification and restoration of disturbed microbiota states. Indeed, these states may be the missing trigger factor for the manifestation of a multitude of civilization disorders including inflammatory bowel disease and gastrointestinal cancer.

770 Hanifian, S. , Khani, S., Barzegari, A., Shayegh, J.

Quantitative real-time PCR and culture examination of Mycobacterium avium subsp paratuberculosis at farm level
Veterinary Microbiology, (2013) 162, 160-165

Mycobacterium avium subsp. paratuberculosis (MAP) causes Johne's disease in ruminants and may contribute to Crohn's disease in humans. The aim of this study was to determine the occurrence and quantity of MAP in cattle feces and milk in the Iranian context. In addition, we evaluated the effect of cattle age as well as farming system as risk factors contributing to MAP load. For this, a total sample of 373 consisting of 150 cattle feces (CF), 150 individual cow's milk (ICM), as well as 73 bulk-tank milk (BTM) was collected randomly and regardless of the cattle health status. The samples were assayed using F57 quantitative real-time PCR (qPCR) and culture method. According to the results of qPCR which was found similar to 10 times more sensitive than culture assay, MAP was detected in 68.66% (103/150) of the CF, 12% (18/150) of the ICM and 52.05% (38/73) of the BTM samples. In contrast to the previous reports, the quantity of MAP in the BTM (2.03-5.97 log cfu/50 ml) was statistically ($p < 0.01$) higher than the ICM (0.90-1.97 log cfu/50 ml). Data suggested a direct relation ($p < 0.01$) between the cattle age and the quantity of MAP in the CF samples, while the relation was not statistically significant ($p > 0.05$) for the ICM. In addition, MAP load in the BTM samples obtained from traditional farms was significantly ($p < 0.01$) higher than that of the industrial ones, while the differences in CF and ICM was not significant ($p > 0.05$).

771 Abendano, N. , Juste, R.A., onso-Hearn, M.

Anti-Inflammatory and Antiapoptotic Responses to Infection: A Common Denominator of Human and Bovine Macrophages Infected with Mycobacterium avium Subsp paratuberculosis
Biomed Research International, (2013Article Number: 908348 DOI: 10.1155/2013/908348-..

Mycobacterium avium subsp. paratuberculosis (Map) is the causative agent of a chronic intestinal inflammation in ruminants named Johne's disease or paratuberculosis and a possible etiopathological agent of human Crohn's disease (CD). Analysis of macrophage transcriptomes in response to Map infection is expected to provide key missing information in the understanding of the role of this pathogen in establishing an inappropriate and persistent infection in a susceptible host and of the molecular mechanisms that might underlie the early phases of CD. In this paper we summarize transcriptomic studies of human and bovine

peripheral blood mononuclear cells (PBMC), monocyte-derived macrophages (MDMs), and macrophages-like cell lines in vitro infected with Map. Most studies included in this paper consistently reported common gene expression signatures of bovine and human macrophages in response to Map such as enhanced expression of the anti-inflammatory cytokines IL-10 and IL-6, which promote bacterial survival. Overexpression of IL-10 could be responsible for the Map-associated reduction in the expression of the proapoptotic TNF-alpha gene observed in bovine and human macrophages.

772 Karunasena, E., Kurkure, P.C., Lackey, R.D. , McMahon, K.W., Kiernan, E.P., Graham, S., Alabady, M.S., Campos, D.L., Tatum, O.L., Brashears, M.M.

Effects of the probiotic Lactobacillus animalis in murine Mycobacterium avium subspecies paratuberculosis infection
Bmc Microbiology, (2013) 13, Article Number: 8 DOI: 10.1186/1471-2180-13-8 Published: JAN 16 2013-..

Background: MAP is a suspected zoonotic pathogen and the causative agent of Johne's Disease in cattle and other ruminant animals. With over $ 1 billion dollars in loss to the dairy industry due to Johne's Disease, efforts to eliminate or reduce MAP from cattle are of importance. The purpose of this study was to determine if daily intake of probiotics could eliminate or reduce Johne's Disease associated symptoms and pathogenesis by MAP. Post infection, animals are often asymptomatic carriers with limited shedding of the pathogen, proving early detection to be difficult. Disease and symptoms often appear 3-4 years after infection with antibiotic treatment proving ineffective. Symptoms include chronic gastrointestinal inflammation leading to severe weight-loss from poor feed and water intake cause a wasting disease. These symptoms are similar to those found in individuals with Crohn's Disease (CD); MAP has been implicated by not proven to be the causative agent of CD. Probiotics administered to livestock animals, including dairy and beef cattle have demonstrated improvements in cattle performance and health. Our objectives included determining the benefits of Lactobacillus animalis (strain name: NP-51) in MAP infected BALB/c mice by evaluating systemic and gastrointestinal response by the host and gut microbiota. Male and female animals were fed 1x10(6) CFU/g probiotics in sterile, powdered mouse chow daily and infected with 1 x 10(7) CFU/ml MAP and compared to controls. Animals were evaluated for 180 days to assess acute and chronic stages of disease, with sample collection from animals every 45 days. MAP concentrations from liver and intestinal tissues were examined using real time-PCR methods and the expression of key inflammatory markers were measured during MAP infection (interferon-gamma [IFN-gamma], Interleukin-1 alpha, IL-12, IL-10, IL-6, and Tumor necrosis factor alpha [TNF-alpha]). Results: Our results demonstrate administration of probiotics reduces production of IFN-gamma and IL-6 while increasing TNF-alpha and IL-17 in chronic disease; healthful immune responses that reduce chronic inflammation associated to MAP infection. Conclusions: We observed that the immune system's response in the presence of probiotics to MAP contributes towards host health by influencing the activity of the immune system and gut microbial populations.

773 Vinh, D.C., Behr, M.A.

Crohn's as an immune deficiency: from apparent paradox to evolving paradigm
Expert Review of Clinical Immunology, (2013) 9, 17-30

Expert Rev. Clin. Immunol. 9(1), 17-30 (2013) Crohn's disease is often considered an autoimmune condition, based on the observations of a histopathological inflammatory process in the absence of identifiable causal microorganism(s) and that immune-modulating therapeutics result in diminished host-directed inflammatory pathology. However, the evidence for a self-targeted immune response is unproven; thus, the instigating and perpetuating forces that drive this chronic inflammation remain unknown. In recent years, a convergence of findings from different fields of investigation has led to a new paradigm, where Crohn's disease appears to be the consequence of an intrinsic innate immune deficiency. While genomic/postgenomic studies and functional immunologic investigations offer a common perspective, critical details of the processes involved require further elaboration. In this review,

we place this new model in the context of the emerging literature on non-HIV immune deficiencies, to compare and contrast what is known about proven intrinsic (primary) immune deficiencies to the nascent understanding of Crohn's disease. We then re-evaluate postgenomic research, looking at the functional importance of Crohn's disease-associated mutations and polymorphisms, to delineate points of consensus and issues requiring further study. We ask whether the immunologic profile can guide predictions as to which microbial triggers could exploit these defects and thereby initiate and/or perpetuate chronic enteritis. Finally, we outline potential clinical implications of this model, from immunologic assessment of patients to the selection of therapeutic interventions.

774 Miheller, P. , Kiss, L.S., Juhasz, M., Mandel, M., Lakatos, P.L.

Recommendations for identifying Crohn's disease patients with poor prognosis
Expert Review of Clinical Immunology, (2013) 9, 65-76

Expert Rev. Clin. Immunol. 9(1), 65-76 (2013) Clinical presentation at diagnosis and the disease course of Crohn's disease is heterogeneous and variable over time. The majority of patients with Crohn's disease will develop at least one stricturing or perforating complication requiring surgery during follow-up. New data support a change in the natural history of the disease associated with the advent of biologicals and tailored treatment strategy. Therefore, it is important to identify patients at risk for disease progression as soon as possible. In recent years, much emphasis has been placed on determining important predictive factors. Complex evaluation of factors such as clinical and endoscopic presentation, fecal, serological and routine laboratory tests, and genetic factors is needed. This review summarizes the available evidence and will hopefully assist clinicians when choosing a treatment strategy in everyday practice.

775 Lauc, G., Huffman, J.E., Pucic, M., Zgaga, L., Adamczyk, B., Muzinic, A., Novokmet, M., Polasek, O., Gornik, O., Kristic, J., Keser, T., Vitart, V., Scheijen, B., Uh, H.W., Molokhia, M., Patrick, A.L., McKeigue, P., Kolcic, I., Lukic, I.K., Swann, O., van Leeuwen, F.N., Ruhaak, L.R., Houwing-Duistermaat, J.J., Slagboom, P.E., Beekman, M., de Craen, A.J.M., Deelder, A.M., Zeng, Q., Wang, W., Hastie, N.D., Gyllensten, U., Wilson, J.F., Wuhrer, M., Wright, A.F., Rudd, P.M., Hayward, C., Aulchenko, Y., Campbell, H., Rudan, I.

Loci Associated with N-Glycosylation of Human Immunoglobulin G Show Pleiotropy with Autoimmune Diseases and Haematological Cancers
Plos Genetics, (2013) 9, Article Number: e1003225 DOI: 10.1371/journal.pgen.1003225 Published: JAN 2013-..

Glycosylation of immunoglobulin G (IgG) influences IgG effector function by modulating binding to Fc receptors. To identify genetic loci associated with IgG glycosylation, we quantitated N-linked IgG glycans using two approaches. After isolating IgG from human plasma, we performed 77 quantitative measurements of N-glycosylation using ultra-performance liquid chromatography (UPLC) in 2,247 individuals from four European discovery populations. In parallel, we measured IgG N-glycans using MALDI-TOF mass spectrometry (MS) in a replication cohort of 1,848 Europeans. Meta-analysis of genome-wide association study (GWAS) results identified 9 genome-wide significant loci ($P<2.27x10(-9)$) in the discovery analysis and two of the same loci (B4GALT1 and MGAT3) in the replication cohort. Four loci contained genes encoding glycosyltransferases (ST6GAL1, B4GALT1, FUT8, and MGAT3), while the remaining 5 contained genes that have not been previously implicated in protein glycosylation (IKZF1, IL6ST-ANKRD55, ABCF2-SMARCD3, SUV420H1, and SMARCB1-DERL3). However, most of them have been strongly associated with autoimmune and inflammatory conditions (e. g., systemic lupus erythematosus, rheumatoid arthritis, ulcerative colitis, Crohn's disease, diabetes type 1, multiple sclerosis, Graves' disease, celiac disease, nodular sclerosis) and/or haematological cancers (acute lymphoblastic leukaemia, Hodgkin lymphoma, and multiple myeloma). Follow-up functional experiments in haplodeficient Ikzf1 knock-out mice showed the same general pattern of changes in IgG glycosylation as identified in the meta-analysis. As IKZF1 was associated with multiple IgG N-glycan traits, we explored biomarker potential of affected N-glycans in 101 cases with SLE

and 183 matched controls and demonstrated substantial discriminative power in a ROC-curve analysis (area under the curve=0.842). Our study shows that it is possible to identify new loci that control glycosylation of a single plasma protein using GWAS. The results may also provide an explanation for the reported pleiotropy and antagonistic effects of loci involved in autoimmune diseases and haematological cancer.

776 Garcia-Vallejo, J.J., Ambrosini, M., Overbeek, A., van Riel, W.E., Bloem, K., Unger, W.W.J., Chiodo, F., Bolscher, J.G., Nazmi, K., Kalay, H., van Kooyk, Y.

Multivalent glycopeptide dendrimers for the targeted delivery of antigens to dendritic cells
Molecular Immunology, (2013) 53, 387- 397

Dendritic cells are the most powerful type of antigen presenting cells. Current immunotherapies targeting dendritic cells have shown a relative degree of success but still require further improvement. One of the most important issues to solve is the efficiency of antigen delivery to dendritic cells in order to achieve an appropriate uptake, processing, and presentation to Ag-specific T cells. C-type lectins have shown to be ideal receptors for the targeting of antigens to dendritic cells and allow the use of their natural ligands - glycans - instead of antibodies. Amongst them, dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN) is an interesting candidate due to its biological properties and the availability of its natural carbohydrate ligands. Using Le(b)-conjugated poly(amido amine) (PAMAM) dendrimers we aimed to characterize the optimal level of multivalency necessary to achieve the desired internalization, lysosomal delivery, Ag-specific T cell proliferation, and cytokine response. Increasing DC-SIGN ligand multivalency directly translated in an enhanced binding, which might also be interesting for blocking purposes. Internalization, routing to lysosomal compartments, antigen presentation and cytokine response could be optimally achieved with glycopeptide dendrimers carrying 16-32 glycan units. This report provides the basis for the design of efficient targeting of peptide antigens for the immunotherapy of cancer, autoimmunity and infectious diseases. (c) 2012 Elsevier Ltd. All rights reserved.

777 Warner, N., Burberry, A., Franchi, L., Kim, Y.G., McDonald, C., Sartor, M.A., Nunez, G.

A Genome-Wide siRNA Screen Reveals Positive and Negative Regulators of the NOD2 and NF-kappa B Signaling Pathways
Science Signaling, (2013) 6, Article Number: rs3 DOI: 10.1126/scisignal.2003305 Published: JAN 15 2013-..

The cytoplasmic receptor NOD2 (nucleotide-binding oligomerization domain 2) senses peptidoglycan fragments and triggers host defense pathways, including activation of nuclear factor kappa B (NF-kappa B) signaling, which lead to inflammatory immune responses. Dysregulation of NOD2 signaling is associated with inflammatory diseases, such as Crohn's disease and Blau syndrome. We used a genome-wide small interfering RNA (siRNA) screen to identify regulators of the NOD2 signaling pathway. Several genes associated with Crohn's disease risk were identified in the screen. A comparison of candidates from this screen with other "omics" data sets revealed interconnected networks of genes implicated in NF-kappa B signaling, thus supporting a role for NOD2 and NF-kappa B pathways in the pathogenesis of Crohn's disease. Many of these regulators were validated in secondary assays, such as measurement of interleukin-8 secretion, which is partially dependent on NF-kappa B. Knockdown of putative regulators in human embryonic kidney 293 (HEK 293) cells followed by stimulation with tumor necrosis factor-alpha revealed that most of the genes identified were general regulators of NF-kappa B signaling. Overall, the genes identified here provide a resource to facilitate the elucidation of the molecular mechanisms that regulate NOD2- and NF-kappa B-mediated inflammation.

778 Wuhrer, M.

Glycomics using mass spectrometry
Glycoconjugate Journal, (2013) 30, 11-22

Mass spectrometry plays an increasingly important role in structural glycomics. This review provides an overview on currently used mass spectrometric approaches such as the characterization of glycans, the analysis of glycopeptides obtained by proteolytic cleavage of proteins and the analysis of glycosphingolipids. The given examples are demonstrating the application of mass spectrometry to study glycosylation changes associated with congenital disorders of glycosylation, lysosomal storage diseases, autoimmune diseases and cancer.

779 Zoldos, V., Novokmet, M., Beceheli, I., Lauc, G.

Genomics and epigenomics of the human glycome
Glycoconjugate Journal, (2013) 30, 41-50

The majority of all proteins are glycosylated and glycans have numerous important structural, functional and regulatory roles in various physiological processes. While structure of the polypeptide part of a glycoprotein is defined by the sequence of nucleotides in the corresponding gene, structure of a glycan part results from dynamic interactions between hundreds of genes, their protein products and environmental factors. The composition of the glycome attached to an individual protein, or to a complex mixture of proteins, like human plasma, is stable within an individual, but very variable between individuals. This variability stems from numerous common genetic polymorphisms reflecting in changes in the complex biosynthetic pathway of glycans, but also from the interaction with the environment. Environment can affect glycan biosynthesis at the level of substrate availability, regulation of enzyme activity and/or hormonal signals, but also through gene-environment interactions. Epigenetics provides a molecular basis how the environment can modify phenotype of an individual. The epigenetic information (DNA methylation pattern and histone code) is especially vulnerable to environmental effects in the early intrauterine and neo-natal development and many common late-onset diseases take root already at that time. The evidences showing the link between epigenetics and glycosylation are accumulating. Recent progress in high-throughput glycomics, genomics and epigenomics enabled first epidemiological and genome-wide association studies of the glycome, which are presented in this mini-review.

780 Lipinski, S. , Grabe, N., Jacobs, G., Billmann-Born, S., Till, A., Hasler, R., Aden, K., Paulsen, M., Arlt, A., Kraemer, L. , Hagemann, N., Erdmann, K.S., Schreiber, S., Rosenstiel, P.

RNAi screening identifies mediators of NOD2 signaling: Implications for spatial specificity of MDP recognition
Proceedings of the National Academy of Sciences of the United States of America, (2012) 109, 21426-21431

The intracellular nucleotide-binding oligomerization domain-2 (NOD2) receptor detects bacteria-derived muramyl dipeptide (MDP) and activates the transcription factor NF-kappa B. Here we describe the regulatome of NOD2 signaling using a systematic RNAi screen. Using three consecutive screens, we identified a set of 20 positive NF-kappa B regulators including the known pathway members RIPK2, RELA, and BIRC4 (XIAP) as well as FRMPD2 (FERM and PDZ domain-containing 2). FRMPD2 interacts with NOD2 via leucine-rich repeats and forms a complex with the membrane-associated protein ERBB2IP. We demonstrate that FRMPD2 spatially assembles the NOD2-signaling complex, hereby restricting NOD2-mediated immune responses to the basolateral compartment of polarized intestinal epithelial cells. We show that genetic truncation of the NOD2 leucine-rich repeat domain, which is associated with Crohn disease, impairs the interaction with FRMPD2, and that intestinal inflammation leads to down-regulation of FRMPD2. These results suggest a structural mechanism for how polarity of epithelial cells acts on intestinal NOD-like receptor signaling to mediate spatial specificity of bacterial recognition and control of immune responses.

781 Jostins, L., Ripke, S., Weersma, R.K., Duerr, R.H., McGovern, D.P., Hui, K.Y., Lee, J.C., Schumm, L.P., Sharma, Y., Anderson, C.A., Essers, J., Mitrovic, M., Ning, K., Cleynen, I., Theatre, E., Spain, S.L., Raychaudhuri, S., Goyette, P., Wei, Z., Abraham, C., Achkar, J.P., Ahmad, T.,

Amininejad, L., Ananthakrishnan, A.N., Andersen, V., Andrews, J.M., Baidoo, L., Balschun, T., Bampton, P.A. , Bitton, A., Boucher, G., Brand, S., Buning, C., Cohain, A., Cichon, S., D'Amato, M., de Jong, D., Devaney, K.L., Dubinsky, M., Edwards, C., Ellinghaus, D., Ferguson, L.R., Franchimont, D., Fransen, K., Gearry, R., Georges, M., Gieger, C., Glas, J., Haritunians, T., Hart, A., Hawkey, C., Hedl, M., Hu, X.L., Karlsen, T.H., Kupcinskas, L., Kugathasan, S., Latiano, A., Laukens, D., Lawrance, I.C., Lees, C.W., Louis, E., Mahy, G., Mansfield, J., Morgan, A.R., Mowat, C., Newman, W., Palmieri, O., Ponsioen, C.Y., Potocnik, U., Prescott, N.J., Regueiro, M., Rotter, J.I., Russell, R.K., Sanderson, J.D., Sans, M., Satsangi, J., Schreiber, S., Simms, L.A., Sventoraityte, J., Targan, S.R., Taylor, K.D., Tremelling, M., Verspaget, H.W., De Vos, M., Wijmenga, C., Wilson, D.C., Winkelmann, J., Xavier, R.J., Zeissig, S., Zhang, B., Zhang, C.K., Zhao, H.Y. , Silverberg, M.S., Annese, V., Hakonarson, H., Brant, S.R., Radford-Smith, G., Mathew, C.G., Rioux, J.D., Schadt, E.E., Daly, M.J., Franke, A., Parkes, M., Vermeire, S., Barrett, J.C., Cho, J.H.

Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease
Nature, (2012) 491, 119-124

Crohn's disease and ulcerative colitis, the two common forms of inflammatory bowel disease (IBD), affect over 2.5 million people of European ancestry, with rising prevalence in other populations(1). Genome-wide association studies and subsequent meta-analyses of these two diseases(2,3) as separate phenotypes have implicated previously unsuspected mechanisms, such as autophagy(4), in their pathogenesis and showed that some IBD loci are shared with other inflammatory diseases(5). Here we expand on the knowledge of relevant pathways by undertaking a meta-analysis of Crohn's disease and ulcerative colitis genome-wide association scans, followed by extensive validation of significant findings, with a combined total of more than 75,000 cases and controls. We identify 71 new associations, for a total of 163 IBD loci, that meet genome-wide significance thresholds. Most loci contribute to both phenotypes, and both directional (consistently favouring one allele over the course of human history) and balancing (favouring the retention of both alleles within populations) selection effects are evident. Many IBD loci are also implicated in other immune-mediated disorders, most notably with ankylosing spondylitis and psoriasis. We also observe considerable overlap between susceptibility loci for IBD and mycobacterial infection. Gene co-expression network analysis emphasizes this relationship, with pathways shared between host responses to mycobacteria and those predisposing to IBD.

782 Scharl, M., Rogler, G.

Inflammatory Bowel Disease: Dysfunction of Autophagy?
Digestive Diseases, (2012) 30, 12-19

Recent genome-wide association studies identified single nucleotide polymorphisms within gene loci, encoding autophagy genes, e.g. the autophagy-related 16-like 1 (ATG16L1) and the immunity-related GTPase family M (IRGM), as an important risk factor for the onset of chronic inflammatory diseases such as Crohn's disease (CD) or rheumatoid arthritis. CD is characterized by a breakdown of the intestinal epithelial barrier function leading to an overwhelming and uncontrolled immune response to bacterial antigens. Autophagy, and therefore ATG16L1 and IRGM, are critically involved in the innate immune response to invading pathogens. Dysfunction of these molecules results in the increased survival of intracellular bacteria, defective antigen presentation and proinflammatory cytokine secretion. Interestingly, autophagy can also be regulated by other CD susceptibility genes, such as nucleotide oligomerization domain 2 or protein tyrosine phosphatase nonreceptor type 2, and the presence of the CD-associated variations within these genes results in comparable effects. ATG16L1 also plays a crucial role in maintaining Paneth cell function and morphology, while IRGM seems to be associated with mitochondrial function and apoptosis. Dysfunction of these molecules, i.e. of autophagy in vivo, is clearly associated with the increased bacterial infection and the onset of colitis. Interestingly, the phenotype of aberrant Paneth cells and dextran sodium sulphate-induced colitis in ATG16L1 hypomorphic mice closely resembles human CD. Taken together, the available data strongly suggest an important role for autophagy in maintaining intestinal homeostasis, and dysfunction of autophagy seems to be a

major risk factor for the onset of chronic intestinal inflammation. Copyright (C) 2012 S. Karger AG, Basel.

783 van Onkelen, R.S., Mitalas, L.E., Gosselink, M.P., van Belkum, A., Laman, J.D. , Schouten, W.R.

Assessment of microbiota and peptidoglycan in perianal fistulas
Diagnostic Microbiology and Infectious Disease, (2013) 75, 50-54

Transanal advancement flap repair has been advocated as the treatment of choice for high transsphincteric perianal fistulas, but fails in 1 of every 3 patients. Persistence of the fistula after flap repair might be the result of ongoing disease in the remaining fistula tract. In 10 specimens of the distal part of the fistula, microbiota was assessed by means of conventional microbiological culture and 165 rRNA gene sequencing. Proinflammatory bacterial peptidoglycan and recognition proteins were assessed by immunohistochemistry. Bacterial species were bowel derived, skin derived, or a combination of both. No mycobacterium species were identified. 165 rRNA gene sequencing failed to identify bacteria in all but 1 specimen, most likely as a result of low numbers of organisms. Peptidoglycan was detected in 90% of the patients, and a host response to peptidoglycan in 60%. Therefore, we suggest that peptidoglycan might play a role in the ongoing inflammation in perianal fistulas. (C) 2013 Elsevier Inc. All rights reserved.

784 Smith, S.L., West, D.M., Wilson, P.R., de Lisle, G.W., Collett, M.G., Heuer, C., Chambers, J.P.

The prevalence of disseminated Mycobacterium avium subsp paratuberculosis infection in tissues of healthy ewes from a New Zealand farm with Johne's disease present
New Zealand Veterinary Journal, (2013) 61, 41-44

AIM: To determine the prevalence of disseminated Mycobacterium avium subsp. paratuberculosis (Map) infection in healthy ewes in a flock with a history of clinical Johne's disease. METHODS: Twenty-four healthy ewes, from a large sheep and cattle farm with a history of clinical Johne's disease in the ewe flock, were randomly selected, euthanased, blood sampled, and examined at necropsy. BACTEC (TM) radiometric culture for Map was performed on samples of faeces, ileum, mesenteric lymph node, biceps femoris muscle and mononuclear cells in peripheral blood. Serum antibody ELISA tests were performed. Histological sections and Ziehl Neelsen (ZN) stains of impression smears of ileum and mesenteric lymph node were examined for pathological lesions characteristic of Johne's disease and acid fast organisms (AFO). Indirect quantification of Map was performed, using BACTEC radiometric growth indices measuring the time taken for the production of 14CO2. RESULTS: No histological evidence of Johne's disease or AFO was found in the ileum and mesenteric lymph nodes. Twelve of the 24 ewes (50%) had Map cultured from the ileum (n=6) and/or mesenteric lymph nodes (n=8) while none had Map cultured from the faeces, biceps femoris muscle or blood mononuclear cells. One of the 12 Map culture positive ewes was serum ELISA positive. The culture growth rates in liquid medium suggest low numbers of Map were present in the tissues of the culture positive ewes. CONCLUSION: Fifty per cent of clinically healthy ewes exposed to Map within a Johne's infected flock were Map culture positive in the ileum and/or mesenteric lymph node(s), while the ELISA was positive in 8% of those animals (n=1). There was no faecal shedding of Map and no Map was cultured from skeletal muscle or from blood mononuclear cells suggesting that systemic Map infection, defined as positive culture of Map from skeletal muscle and/or blood, may be uncommon in healthy mixed age ewes without clinical Johne's disease. CLINICAL RELEVANCE: ELISA serology detected 1 of 12 ewes infected with Map whilst none were detected from faecal BACTEC radiometric culture, suggesting biosecurity measures used to control the spread of Map may be of limited use. Map was not cultured from blood mononuclear cells or skeletal muscle, indicating that meat from healthy ewes, from farms where Johne's disease is present, is an unlikely source of Map exposure for humans. Further research is warranted to establish the prevalence and dissemination of Map in tissues outside the alimentary tract of healthy ewes from farms throughout New Zealand where Map is present.

785 Chargui, A., Cesaro, A., Mimouna, S., Fareh, M., Brest, P., Naquet, P., rfeuille-Michaud, A., Hebuterne, X., Mograbi, B., Vouret-Craviari, V., Hofman, P.

Subversion of Autophagy in Adherent Invasive Escherichia coli-Infected Neutrophils Induces Inflammation and Cell Death
Plos One, (2012) 7, Article Number: e51727 DOI: 10.1371/journal.pone.0051727
Published: DEC 14 2012-..

Invading bacteria are recognized, captured and killed by a specialized form of autophagy, called xenophagy. Recently, defects in xenophagy in Crohn's disease (CD) have been implicated in the pathogenesis of human chronic inflammatory diseases of uncertain etiology of the gastrointestinal tract. We show here that pathogenic adherent-invasive Escherichia coli (AIEC) isolated from CD patients are able to adhere and invade neutrophils, which represent the first line of defense against bacteria. Of particular interest, AIEC infection of neutrophil-like PLB-985 cells blocked autophagy at the autolysosomal step, which allowed intracellular survival of bacteria and exacerbated interleukin-8 (IL-8) production. Interestingly, this block in autophagy correlated with the induction of autophagic cell death. Likewise, stimulation of autophagy by nutrient starvation or rapamycin treatment reduced intracellular AIEC survival and IL-8 production. Finally, treatment with an inhibitor of autophagy decreased cell death of AIEC-infected neutrophil-like PLB-985 cells. In conclusion, excessive autophagy in AIEC infection triggered cell death of neutrophils.

786 Correa, R.G. , Milutinovic, S., Reed, J.C.

Roles of NOD1 (NLRC1) and NOD2 (NLRC2) in innate immunity and inflammatory diseases
Bioscience Reports, (2012) 32, 597-608

NOD1 {nucleotide-binding oligomerization domain 1; NLRC [NOD-LRR (leucine-rich repeat) family with CARD (caspase recruitment domain) 1]} and NOD2 (NLRC2) are among the most prominent members of the NLR (NOD-LRR) family -proteins that contain nucleotide-binding NACHT domains and receptor-like LRR domains. With over 20 members identified in humans, NLRs represent important components of the mammalian innate immune system, serving as intracellular receptors for pathogens and for endogenous molecules elaborated by tissue injury. NOD1 and NOD2 proteins operate as microbial sensors through the recognition of specific PG (peptidoglycan) constituents of bacteria. Upon activation, these NLR family members initiate signal transduction mechanisms that include stimulation of NF-kappa B (nuclear factor-kappa B), stress kinases, IRFs (interferon regulatory factors) and autophagy. Hereditary polymorphisms in the genes encoding NOD1 and NOD2 have been associated with an increasing number of chronic inflammatory diseases. In fact, potential roles for NOD1 and NOD2 in inflammatory disorders have been revealed by investigations using a series of animal models. In the present review, we describe recent experimental findings associating NOD1 and NOD2 with various autoimmune and chronic inflammatory disorders, and we discuss prospects for development of novel therapeutics targeting these NLR family proteins.

787 Sigal, L.H.

Basic Science for the Clinician 59 Polymorphonuclear Cells: Mechanisms in Human Defense and in the Pathogenesis of Autoimmune Disease
Jcr-Journal of Clinical Rheumatology, (2012) 18, 443- 449

When I learned about polymorphonuclear neutrophils (PMNs) in medical school, they were presented as pretty much 1-trick ponies: PMNs were phagocytes with no intrinsic specificity; their only specificity was supplied by the Fc gamma receptors on their surfaces and that would then be the specificity of the bound immunoglobulin G, nothing intrinsic to the PMN. My, how simple life was in those days! And how wrong! Turns out, these circulating cells are involved in bridging the innate immune system and the acquired immune response in some very interesting ways and may play a crucial role in the immunopathogenesis of some of "our" diseases. Polymorphonuclear neutrophils are often underappreciated as drivers of

inflammatory diseases, which is why I think it is time for us to turn our attention to this underappreciated component of the immune response.

788 Click, R.E.

Alteration of GI symptoms in a cow with Johne disease by the dietary organosulfur, 2-mercaptoethanol
Virulence, (2012) 3, 543-545

Sub-phenotypes of inflammatory bowel disease (IBD)-Crohn disease, ulcerative colitis and some cases of irritable bowel syndrome-are generally considered a consequence of gastrointestinal inflammation of unknown etiology. Conventional therapy and more recently biologic agents, all with varying degrees of drawbacks, have resulted in improved control of these diseases. However, as the incidence and prevalence continue to rise, needs for prevention, permanent remission and cures remain unmet, plus there still remain needs for improved control of symptoms, such as pain and diarrhea. The case report herein describes a serendipitous, novel means for curtailing these symptoms associated with a bovine gastrointestinal disease that may have applicability for patients with diseases characterized by abdominal-visceral pain and diarrhea.

789 Pierce, E.S.

Free-ranging Rocky Mountain bighorn sheep and an outbreak of inflammatory bowel disease along the Clark Fork River in Plains, Montana
Virulence, (2012) 3, 546-550

Nine individuals with ulcerative colitis or Crohn disease grew up or lived in Plains, Montana, a 1,200-person community adjacent to the Clark Fork River near herds of free ranging Rocky Mountain bighorn sheep. This inflammatory bowel disease outbreak is similar to others that have occurred along rivers contaminated by animal feces.

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