

Crohn's disease and related inflammatory diseases: from many single hypotheses to one "superhypothesis"¹

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ABSTRACT: The aetiology of Crohn's disease and paratuberculosis are the subjects of intensive study and also frequently, of dispute. However, a number of other nosological entities have a similar history, namely type 1 diabetes, multiple sclerosis, sarcoidosis, asthma, psoriasis, spondylarthritis, Blau syndrom etc. The zoonotic risk of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) has been discussed for more than one hundred years. „*The problem remains open, further research is needed*“, is the sentence which seems to be obligatory in the conclusions of many scientific articles. A number of hypotheses have been suggested, all with a grain of truth in them. The infection hypothesis has many supporters and opponents, but it does not fit to all Crohn's disease cases. The contribution of the genetic factor has been admitted a long time ago and has been experimentally confirmed by recent excellent studies. An environmental factor is expected and has been often mentioned, but has yet to be discovered. Muramyl dipeptide, derived from peptidoglycans of the bacterial cell wall is one of the triggers, mentioned in connection with chronic inflammatory diseases. The immunomodulatory ability of this compound has been recognised for decades and is exploited in Freund's adjuvant. A critical amount of muramyl dipeptide can affect immunity during some bacterial infections but the long latent period between infection and onset of the clinical form of the disease could explain why a causative relationship between the primary infection and chronic inflammation is not considered. Different species of mycobacteria can be found in the environment, in water, dust, soil and aerosol. Although severe infections with mycobacteria have been described, these species are not thought to be typical zoonotic pathogens. Muramyl dipeptide derived from mycobacteria obviously plays a starring role as a bacterial trigger in the aetiology of many autoimmune and autoinflammatory diseases. Paratuberculosis in cattle and other ruminants is a source of enormous contamination of the environment but also of milk and meat by MAP. Muramyl dipeptide from mycobacteria, namely MAP, and Crohn's disease as a representative of diseases often called civilization threats, are important pieces of the gigantic puzzle. Mycobacteria in the environment and foodstuffs have to be acknowledged as a public health risk, which can never be completely eliminated. There is no reason to push the panic button, but we must learn how to live together with this microorganism, how the pool of immunomodulator sources can be diminished, and how the pathogenic relationship between triggers and target tissues can be disrupted. The dissemination of knowledge, the availability of rapid and inexpensive tools for identification of mycobacteria in different matrices, and the establishment of a maximal allowed limit for mycobacteria in milk and meat should contribute to food safety and consumer protection.

Keywords: aerosolisation; asthma; autoimmune diseases; autoinflammatory diseases; bacterial triggers; biofilms; Blau syndrome; breast feeding; cattle; cervical lymphadenopathy; communal water; cow; dairy products; diabetes; dung; dust; environmental mycobacteria; fish-tank; food-borne pathogens; granulomatous lung disease; heat shock proteins; hot tub lung; immunomodulator; infant formula; inflammatory diseases; Johne's disease; lifeguard lung; machine operator's lung; manure; meat; milk; multiple sclerosis; muramyl dipeptide; mycobacterioses;

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Mycobacterium avium subsp. *paratuberculosis*; non-tuberculous mycobacteria; paratuberculosis; peptidoglycans; pneumonitis; potentially pathogenic mycobacteria; proinflammatory cytokines; psoriasis; rheumatoid arthritis; sarcoidosis; sheep; slurry; swimming pool; tumour necrosis factor; water; water chlorination

Abbreviations:

CD = Crohn's disease, **JD** = Johne's disease, **MAP** = *Mycobacterium avium* subsp. *paratuberculosis*, **NOD2** = nucleotide-binding oligomerisation domain-containing protein 2, **PTB** = paratuberculosis (animal disease), **T1D** = type 1 diabetes

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Chiodini et al. (2012):

"One is again reminded that transposable elements (Barbara McClintock; Nobel Laureate, 1983) and the bacterial etiology of peptic ulcers (Barry Marshall; Nobel Laureate, 2005) were once controversial and polarizing issues. It is time to resolve this controversial and polarizing issue and resolve the role, if any, of M. paratuberculosis in Crohn's disease. Patients cannot afford to wait another 25 years."

1. The introduction and conclusions together: The new paradigms

- Crohn's disease develops not only after simple infection with one zoonotic food pathogen but is the result of many complex pathways mainly of immunological nature with participating genetic factors.
- On one side of this maze of partially known pathways lies a set of triggers or pathogens; on the other side lies a set of inflammatory and autoimmune diseases.
- Muramyl dipeptide is not the only but rather the most important bacterial trigger or immunomodulator.
- *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the most important but not the only source of bacterial triggers or bacterial mimics.
- It is not possible to eliminate triggers and mimics completely from the food chain, drinking water and environment, but it is necessary to do what is possible to protect consumers.
- To realise the above requirement, it is necessary firstly to accept that not only *M. tuberculosis* complex members are dangerous pathogens, but also other mycobacteria, alive or dead, according to their number, species, growth conditions etc., can harm humans in different ways, according to the individual's age, genetic disposition, health or welfare conditions, length of exposure etc.
- Research should be directed at controlling paratuberculosis in ruminants, diminishing the number of MAP in manure and at manipulating the pathways leading to clinical changes using effective new therapeutic strategies in humans suffering

from inflammatory and autoimmune diseases. These approaches should complement efforts aimed at decreasing the number of mycobacteria in foods, water and the environment.

- The health risk associated with peptidoglycans and muramyl dipeptide can be diminished step by step (see the subtitle 10). More so than funds, a responsible approach of health and food safety and consumer protection authorities to the existing data is needed.
- Associations of medical, veterinary and environmental professionals, patient associations and journalists have to play their role in knowledge dissemination and in calling upon the relevant authorities to issue the necessary regulations and directives.

2. Civilization, new technologies and public health

New biotechnologies are so strictly controlled that any risk from their application is nearly impossible. However, negative effects can result from the interaction of different technologies between which mutual relations were not expected. Consequences are often insidious and their discovery can be difficult. They can be affected by factors, originating in another technology and under some circumstances may pose a risk. Problems are sometimes beyond the expertise and imagination of any single expert. To prevent the mutual influence of different technologies it is often necessary to disregard long-acknowledged principles and to accept a new principle, which may run counter to what we had imagined. This chapter can be considered as an imaginary story about friends, having quite different professions – a molecular pathologist, who might dream about the Nobel Prize, a dairy farmer, who does not like to spend a single day away from his farm, a gastroenterologist, who is fully engaged with his patients, a supplier of municipal water, whose main problem is to have enough water for treatment and distribution to the houses of residents and a meat inspector, doing a good job but enjoying every minute outside a slaughterhouse. These fellows are sailing on the same deck. Although their jobs greatly differ, now their common interest is that a good wind blows through their sails. They have known each other for a long time, but they are not yet aware of the common aim which will characterise their different professional careers as soon as their holidays will be over. Similarly, to reach the

harbour their working efforts must come together in order to eliminate, at least partially, the public health risk posed by mycobacteria in food, water and air. We hope they will read and understand this story.

New technologies are often strongly promoted. However, any negative effect means a great and urgent problem, mainly if a technology has a negative impact on the environment or public health. Huge investments can be lost, and new investments are needed without a chance of financial profit. Risk, even hypothetical, must be addressed. Sometimes it is difficult to discover the links between technologies and to understand their significance and impact on human health. The participants often have little willingness to accept even quite evident associations due to fears it will result in the loss of trust in a new technology and loss of prestige of the inventor of the technology.

More and more people seek their livelihood in cities and have to accept the advantages and disadvantages of their new environment and to change their lifestyle accordingly. These people are under the influence of new factors, which may be a threat to their health. Foodstuffs, which they could formerly produce themselves, are now substituted by low price products, transported sometimes from the other side of the globe. Pesticides, used to ensure high production and low loss during transportation enter the food chain. Humans, animals and plants are under the influence of industrial fumes. City residents quickly adapt to flats with central heating and a permanent supply of hot water and have no idea how these advantages of civilization can influence their health. Infants are breast-fed for a shorter time as their mothers have to return to their jobs. It is now so easy to substitute breast milk with a formula, available in the nearest drugstore.

This is a routine scenario which has played out innumerable times over the last century. Any performance has a list of scenes and settings where a story is told and includes a description of props used. Those, important in our story, are summarised under the subtitle 7 “A theatre of war” and in Table 1 (Players on the scene posing a risk). We do not know how many readers imagine the existence of any association between the items in Table 1 and Table 4 (What should be drawn in the map as the different battle fields with mycobacteria) but if this number were small it would not be surprising. We expect the readers will be amazed by the principle which unifies the listed subjects and how this principle can impact the health of spectators, who are direct participants in this performance.

The underlying causes of disease have always been a focus of interest. Advancements in technology and science brought forth such tools as the microscope and the first insight into bacteria as pathogens. Later, infectious diseases were also associated with viruses, and non-infectious diseases with metabolic disorders, defects in kidneys, liver or endocrine glands, allergens in the air or foodstuffs, genetic disorders, physical factors like ionizing or sun radiation, toxic elements and compounds, and an insufficient supply of certain elements, amino acids, vitamins, etc. Supernatural forces are not thought at present to be an underlying cause of disease, but some people still believe in their ability to treat incurable diseases.

Some diseases with still unknown causation have similar traits. They can be associated with previous infectious disease, which could be characterised by a massive replication of bacteria or viruses in the host's body. Such illnesses can be associated with inflammation at different locations and of different extents. Symptoms of such disease are sometimes linked to a known genetic disorder and often a protective effect of breastfeeding is reported. Inflammation during these illnesses is associated with mutation of the *NOD2* gene, linked with the identification of muramyl dipeptide, derived from peptidoglycan, a constituent of the bacterial cell wall. Some patients are treated with the same biological preparations, although the signs and characters of their illnesses are rather different. Nevertheless, they exhibit inflammation linked to the activation of similar pathways; hence, biological treatment which modulates immunity can be successful.

Examples of these illnesses are presented in Table 2. We would like to ask readers, if they suppose any association between the Tables 1 and 2. Most readers would probably be surprised that such an association is frequently reported, although the subjects listed in the tables are so different. They are linked together by the star of this story, which is formed by only 66 atoms. A tiny but potent immunomodulator is called muramyl dipeptide and has a molecular mass of only 492.5. Its structure and its potent ability of interfering with immunity are well known and sufficiently described (Figure 1). This molecule forms a part of the cell wall of bacteria. Mycobacteria, namely *Mycobacterium avium* subsp. *paratuberculosis* (MAP), play a role in connecting the subjects listed in Table 1 and some chronic inflammatory diseases (Table 2).

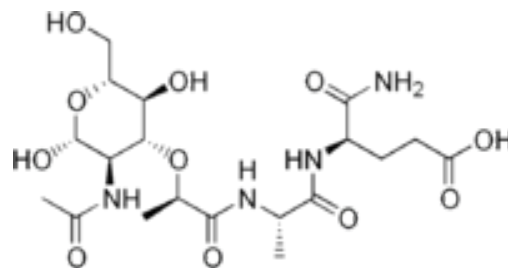


Figure 1. Muramyl dipeptide. Muramyl dipeptide is a peptidoglycan constituent of both Gram positive and Gram negative bacteria. It is composed of *N*-acetylmuramic acid linked by its lactic acid moiety to the *N*-terminus of an L-alanine D-isoglutamine dipeptide (http://en.wikipedia.org/wiki/Muramyl_dipeptide); Inohara et al. (2003), Martinon et al. (2004)

3. Muramyl dipeptide and inflammatory diseases

Muramyl dipeptide (MDP) is a potent immunomodulator. Its ability to increase the production of antibodies in immunised animals is well known and was first described in 1974. Complete Freund adjuvant, containing mycobacteria killed by boiling, is widely used. Its stimulatory effect on antibody formation is associated with muramyl dipeptide, the smallest entity in peptidoglycans, derived from mycobacteria. MDP increases the synthesis of tumour necrosis factor and triggers the proinflammatory cytokine cascade. The protein *NOD2* is able to recognise and bind MDP. Cells with a defective *NOD2* gene cannot bind MDP to the necessary extent. This increases the disposition to develop Crohn's disease at least in a proportion of patients. Peptidoglycans in the cell wall of different bacteria are associated with several inflammatory diseases (Table 2). *N*-glycolyl MDP, derived from peptidoglycan originating from mycobacteria is a more effective immunomodulator than *N*-acetyl MDP, derived from other bacteria (Coulombe et al. 2009).

MDP has a direct effect on inflammation, but it also affects innate immunity in the perinatal period. The sensitisation of T cells, epigenetic changes and modulation of apoptosis are often mentioned as effects of MDP. These pathways participate in the pathogenesis of Crohn's disease and some autoimmune diseases. When a human meets MDP for the first time, the number of MDP molecules which participate in this meeting, the origin of the MDP and how long the meeting takes place seem to be crucial factors. The subsequent meetings of the host with MDP also play an important role, again

with respect to its origin, amount, and duration of action and if the target person has been already sensitised by mycobacteria or MDP from other sources. The most important sources of MAP and other mycobacteria are foodstuffs and drinking and surface waters. Mycobacteria can promote the formation of lesions after a long time when a host is stressed or as a result of a booster effect of MDP of the same or different origin.

MAP in milk and mycobacteria from water are unique sources of MDP, which can come into contact with healthy new-borns already in the first days or weeks of life. Measuring mycobacteria concentrations in milk and water is not yet required by any directive and the maximum acceptable concentration is also not yet legally established. However, there is no doubt about the possible impact of mycobacteria on developing immunity with possible consequences later in life. Nevertheless, “feeding” new-borns and children with mycobacteria is completely tolerated.

4. Mycobacteria around us

Infectious diseases are usually characterised by distinctive symptoms like fever, diarrhoea, pain or acute inflammation. The pathogen usually replicates in the patient. Antibodies are produced as a defence of the host and after a few days or weeks can be detected in blood. Food-borne pathogens like salmonella or listeria are in an open war with the host: they either gain superiority over defence systems or are successfully eradicated by an effective immune system supported by pharmaceuticals. If the pathogen is not resistant to antibiotics or chemotherapeutics their application can inhibit the replication of the pathogen and the winner is known within a few days. A strong and healthy person can overcome an attack of a small number of salmonella or listeria cells and other pathogens even without treatment. Nobody seems to be surprised that the ratio of winners to losers in the battle against typhus was different in the Middle Ages, or if typhus outbreaks in Europe are very rare but represent daily problems for example in Sudan.

Of course, every disease is under the influence of many factors such as pathogen virulence and dose, host age, body condition, health history, gender, post-vaccination immunity etc. Hence, the intensity of symptoms, duration and outcome of illness may be different in each patient, although the disease has typical and pathognomonic symptoms.

Why do so many experts expect a uniform course of Crohn’s disease in every exposed person? Are the objections addressed to those who consider the public health risk of MAP as possible and call for measures to diminish it or to those, who are afraid of their own fecklessness?

Non-tuberculous mycobacteria (potentially pathogenic and/or environmental mycobacteria) can be the cause of different forms of “mycobacterioses” and can affect immunity and have a function of allergen-like compounds. They are found in milk and meat, in the air and in different waters, often in high concentrations. When they are digested or inhaled no apparent reaction appears immediately. Mycobacteria can enter the gut in sufficient numbers together with food or water and can reach the lungs together with air. Such exposure is often chronic. The struggle is secret and inconspicuous at the beginning, without any violent symptoms and with a patience not usual in other pathogens. The time interval between the crossing of the frontiers by enemy troops and the symptoms of a battle can be very long, e. g. many months or even years. Due to this long period between the exposure and clinical outcome of mycobacterioses it is often difficult to recognise an association between mycobacteria and the disease.

Also not fully recognised is the pathogenesis of mycobacterial disease. An involvement of mycobacteria or some compound from their cell wall is expected and partially described. The effect of MDP is well known; it acts in a similar way as chemical contaminants, pesticides or allergens. However, inflammation can be triggered also by other compounds from the mycobacterial cells or by compounds which are synthesised by other cells under the influence of mycobacteria, e.g. tumour necrosis factor alpha, heat shock proteins etc. (Clancy et al. 2007; Dow 2008; Dow 2012). Therefore, mycobacteria need not be alive, undamaged or capable of replication. The participation of dead cells in the struggle is a fascinating privilege of mycobacteria. The total number of mycobacteria entering the host organism by different routes is important for triggering the respective inflammatory process.

The “Army of Mycobacteria” currently consists of more than 160 species or subspecies. Some characteristics are common to all of them, while some are specific and surprising. Some mycobacteria can sometimes change according to the environment (Bull et al. 2013; Rosu et al. 2013), others can alter their cell wall and/or can form cell wall-deficient

forms or spheroplasts by losing the cell wall, or can form resistant cells similar to spores (Hines and Styer 2003; Beran et al. 2006).

Mycobacteria in water and soil can be ingested by amoebae and in the form of amoebal cysts they have the perfect defence against adverse conditions. In a similar way mycobacteria can utilise for their benefit the environment in human macrophages (Stabel and Stabel 1995; Alzuherri et al. 1996). Some mycobacterial species are more resistant to higher temperature than other bacteria so they have a higher possibility of surviving pasteurisation. Even if apparently dead, they can sometimes resuscitate and begin growth again after months or even years of recovering. Mycobacteria are more resistant to chlorine than other microorganisms, so their survival in chlorinated water is evident and was repeatedly confirmed in water supply systems. Water chlorination is paradoxically an advantage for mycobacteria, because they need not compete for nutrients with other organisms not surviving chlorination, especially in biofilms. Mycobacteria can be aerosolised from contaminated surface water and inshore water, from water in swimming pools and from communal water and can enter host organisms not only by ingestion but also by inhalation of aerosols or across scratched skin.

Some capabilities of mycobacteria are supported by the genetic disposition of the host. A well-known example is mutation of the *NOD2* gene, which increases the probability of developing Crohn's disease. In a comprehensive study a total of 163 inflammatory bowel disease loci that meet genome-wide significance thresholds have been identified. Many of these are implicated in immune-mediated

disorders, like psoriasis, ankylosing spondylitis and mycobacterial infections. From this study it is obvious that pathways are shared between host responses to mycobacteria and predisposition to Crohn's disease (Jostins et al. 2012). However, it is not easy to understand Crohn's disease: more than 44 thousand scientific papers are indexed in the Web of Science® database, 3000 papers annually during the recent years (Figure 2).

Causal agents of human and bovine tuberculosis (*M. tuberculosis*, *M. africanum*, *M. canettii*, *M. bovis*, and *M. caprae*) and *M. leprae* and *M. ulcerans* are recognised as dangerous pathogens. The risk of other mycobacteria for human health is often underestimated due to insufficient knowledge or because of difficulties in determining causal links. Traditional techniques, e. g. culture, can be used only in special labs and results are not fully reliable.

M. leprae is the causal agent of leprosy and is a representative of another extraordinary attribute of mycobacteria. This species is unculturable *in vitro* in the laboratory, but can successfully grow in the footpads of mice or in the liver of the nine-banded armadillo. Leprosy is a good example of how long some diseases must wait for a small step towards the discovery of their mystery. Leprosy has affected humanity for over 4000 years. The agent was discovered and named in 1873 as the first species of the genus *Mycobacterium*. The misery from leprosy was apparent and decision makers were scared out of their wits looking at the poor sufferers. Although the pathogenesis was not understood and suitable treatment of leprosy was not available, the application of a precautionary principle, e.g. opening the leprosaria was a good decision.

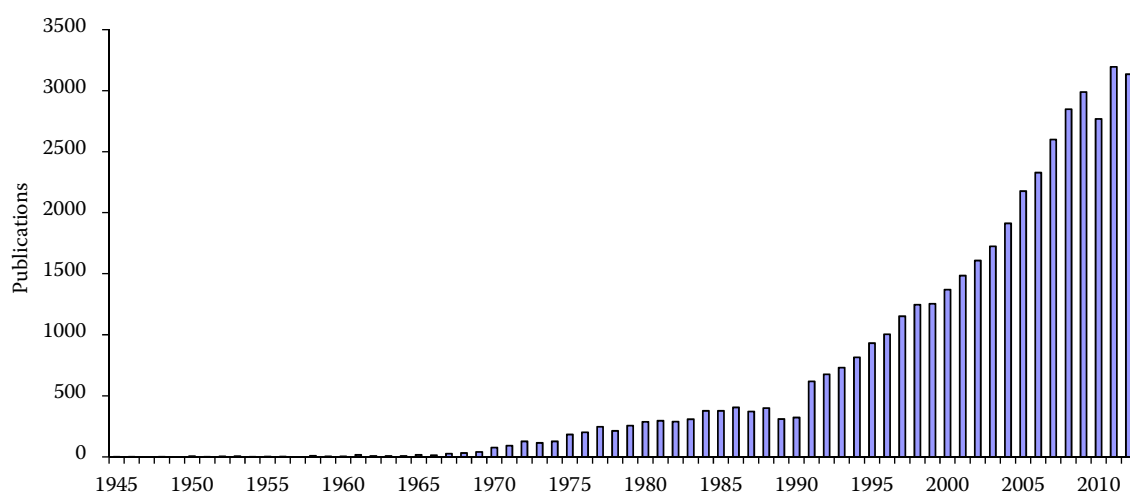


Figure 2. Publications on Crohn's disease retrieved from the database Web of Science (1945–2012)

Crohn's patients suffer discretely at home, close to the toilet, and they do not frighten those who almost do not believe that so cruel a disease exists. Hopefully, it will not be necessary to wait much longer before the association of paratuberculosis with Crohn's disease is acknowledged and legal measures for health risk elimination applied.

MAP is shed by diseased ruminants, mainly cows and sheep, in enormous amounts in milk and with faeces to the environment and surface waters. This mycobacterium enters the food chain and it is certain that MAP is an important, but not the only source of MDP. MAP and other mycobacteria have also been found in water and in fish, contaminated from water or water plants. Pork can also be contaminated by mycobacteria if pigs are kept on sawdust or peat bedding, fed by peat or compost, or drink contaminated water. The risk of mycobacterioses in pigs increases with the use of water, medicated with vitamins or mineral elements. The organic agriculture represents a high risk for the exposure of pigs to potentially pathogenic mycobacteria. The disease is economically important because often heads and livers have to be confiscated. The carcasses are usually conditionally approved for consumption as thermally processed products but with massive contamination of boiled mycobacteria. Meat products can be contaminated also from water or casings from ruminants suffering from paratuberculosis.

Mycobacteria are present in soil and surface waters, in protozoa, earthworms, and insects or on their surface. Mycobacteria can enter from soil into vegetables or can contaminate the surface. Hence, strawberries and other fruits, lettuce or parsley can be contaminated. Mycobacteria were found in high concentrations in sediments of drinking water for animals, in water reservoirs and in water plumbing. Higher concentrations are usually found in plumbing for hot water or in pipes installed superficially on walls exposed to direct sunlight. Aquaria represent an enormous risk of water contamination with mycobacteria. Skin infections in aquarists and other people handling infected fish, fish for human and animal consumption, as well as infections of injured skin of gardeners are often misdiagnosed and skin mycobacteriosis is not properly treated. Gloves should be used for cleaning aquaria and the detritus should not be used for fertilising vegetables. It is very likely that mycobacteria can be found in hydroponic solutions used for the industrial production of vegetables. Mycobacteria can also be

found in fertilisers based on dried manure. Pelleted faeces for fertilising are available in supermarkets.

4.1. Basic knowledge, important for assessment of mycobacteria as a public health risk:

- Mycobacteria are in milk, dairy products, and in meat and meat products, vegetables and water) in concentrations which warrant attention
- MDP derived from mycobacterial peptidoglycans has enormous immunomodulatory effects
- MDP affects immunological pathways not only after parenteral application but also after ingestion or inhalation
- MDP derived from bacteria and synthetic MDP have the same immunomodulatory effect
- MDP derived from live, dead or partially devitalised mycobacteria by pasteurisation, boiling, canning or cooking can trigger the inflammatory pathways similarly
- Food and water contamination by mycobacteria are not yet under legal control
- Similarly, paratuberculosis in cattle and ruminants is not yet a generally notifiable disease and is disseminated without any legal control within herds in the same country and across national borders
- MDP can affect the maturation of immunity in the first days and weeks of life with consequences which only appear later in life
- Infants can be affected by MDP owing to the great number of MAP in baby formula milk and water used for preparing formula milk
- Incidence of Crohn's disease and dissemination of this disease in different parts of the world is increasing in parallel with dissemination of paratuberculosis in animals; similar trends were observed in some other diseases
- MDP participation in other diseases such as multiple sclerosis, asthma, psoriasis, rheumatoid arthritis, sarcoidosis, Blau syndrome, and others should be considered based on many indirect but persuasive findings

The above-mentioned information has been regularly published, but as yet it is not generally considered as sufficient to justify the adoption of directives to limit the impact of mycobacteria on human health. The war continues. We should understand that if the attack has already begun, the defender cannot wait until its intelligence ser-

vice will have in its hands a detailed description of weapon systems, until spies infiltrate the general staff of the enemy, or until civilians agree in a referendum to send an army into the battlefield. We have to apply immediately all the knowledge we already have and we cannot constantly wait for data to be confirmed over and over again. The responsible bodies cannot be under the illusion that all data regarding the unquestionable risk will be tomorrow also unquestionably declared as invalid. The problem will not disappear; it will just become more difficult to treat it. We have given paratuberculosis a head start of 100 years (Salem et al. 2013a). Our strategy must be changed; we have to close the gap. At the very least.

5. Water, the main but not exclusive habitat of mycobacteria

Mycobacteria are present in most natural waters and piped water supplies. The main features of mycobacteria as a public health risk have been characterised by Collins et al. already in 1984 as evident from the sub-headings of their published review (Collins et al. 1984):

- Resistance of mycobacteria to chlorination
- Access, persistence and colonization in piped supplies
- Is water the natural habitat of free-living mycobacteria?
- Water as a vector for mycobacterial infections
- Immune response to environmental mycobacteria
- Mycobacteria as indicators of pollution

Most of the bacteria in drinking water distribution systems are associated with biofilms and can survive in water for a long time (Kazda et al. 2009). Water, regardless of origin and quality, can be contaminated by mycobacteria and, under specific conditions, can jeopardise the users (Falkinham 2009a; Falkinham 2009b; Whiley et al. 2012). Water is a habitat and vector for the transmission of different species of mycobacteria, associated with human mycobacterial infections and immune modulation. Examples can be found in spas and hot tubs, indoor swimming pools, footbaths etc. Mycobacteria were found in saltwater as well as in fresh underground water, in distillate and deionised water, in bottled drinking and mineral water, in dental unit waterlines and hospital water distribution systems. The classification of most mycobacteria as non-pathogenic and problems with their detection and

identification make public health authorities tolerant to ubiquitous bacteria ready to enter a host and able to wait indefinitely for suitable conditions both in the environment and inside a body.

Published data showed that MAP remained culturable in lake water microcosms for 632 days and persisted for up to 841 days. Mycobacteria legally share the environment with new-born babies, children, genetically disposed or immunocompromised people, patients after organ transplantation or with HIV/AIDS-positive persons. Nobody is responsible, as no limits of contamination are declared and no control is legally required. The persons under the highest risk of mycobacterioses and immunomodulatory disorders are not protected against high concentrations of MAP and other mycobacteria in food and water by public health inspections. Moreover, they are not sufficiently informed how to protect themselves. They do not know why they should be careful while cleaning their aquaria or while potting their flowers. Parents are not aware of the risk of infant swimming, and do not know if the showerhead in their bathroom is populated by mycobacteria or if drinking water used for cooking is not heavily contaminated. Although the association of MAP with Crohn's disease is our primary interest, we shall also describe a few case reports, well documented and published, to help explain the harmfulness of other mycobacteria, their power to cause harm to different target tissues and the diversity of symptoms of mycobacterial diseases. These will illustrate the hazards of underestimating this genus.

• Pickup et al. (2005) reported that *M. avium* subsp. *paratuberculosis* can be present in high concentrations in the river water in the catchment area of pastures. Data presented in this paper bring evidence of a higher incidence of Crohn's disease in districts bordering rivers. In South Wales, United Kingdom, a populated coastal region lies beneath hill pastures grazed by livestock in which MAP is endemic. The Taff is a spate river running off the hills and through the principal city of Cardiff. Previous epidemiological research in Cardiff demonstrated a highly significant increase in Crohn's disease in eleven districts. These bordered the river except for a gap on the windward side. A topographical relief map shows that this gap is directly opposite a valley open to the prevailing south-westerly winds. This would influence the distribution of aerosols carrying *M. avium* subsp. *paratuberculosis* from the river. How can river water be associated with Crohn's disease if the distance between pastures and

the city is 40 to 100 km and swimming in the river is not popular in the city? The answer to this question was given by determination of MAP DNA in 32% of samples of river water taken twice weekly for one year. Twelve samples were positive for MAP culture. How MAP, due to its high hydrophobicity, can be enriched in ejected droplets and transferred from water to air was described already in 1970 (Blanchard and Syzdek 1970). The spread of mycobacteria in this manner is one of their remarkable – and troublesome – properties, and something that can be observed in a simple experiment. Simply invert a Petri dish containing agar medium 10 cm above the surface of water contaminated with *M. avium*, and leave it there for 60 min. Droplets containing more than 10 000 times the concentration of bacteria in the water will contact the Petri dish and grow there. The mechanism by which *M. avium* becomes so concentrated in these ejected droplets is actually quite simple: Tiny air bubbles rise to the surface and collect the hydrophobic mycobacterial cells like an elevator collecting passengers. Hydrophobic bacteria prefer the air-water interface, so they tend to be in contact with bubbles rising up. Microscopic bubbles containing these hydrophobic cells are ejected by means of surface tension when they reach the surface. Mycobacteria can even be suspended in air since they survive desiccation and lend themselves to being absorbed on microparticles of dust.

- Exposure to waters whose catchments include heavily grazed pastures was associated with conspicuous clusters of Crohn's disease as is evident from other reports. The first of these involved a rural community of about 2000 people in England, in which 12 people developed Crohn's disease between 1960 and 1983. The village, which had its own water supply from local springs, lay in a hollow surrounded by upland pastures grazed by cattle in which clinical paratuberculosis was evident (Allan et al. 1986).

- A further suspicious cluster of seven cases of Crohn's disease amongst 285 graduates of the Mankato West High School class of 1980 was reported by Van Kruiningen and Freda (2001). All seven students had been swimming in local ponds and lakes.

- Not only river water but obviously also communal potable water poses a risk. Two case control epidemiological studies carried out independently in the United Kingdom each unexpectedly identified the availability of fixed hot water supplies in the early childhood home as a significant risk factor for the subsequent development of Crohn's disease (Gent et al. 1994; Duggan et al. 1998).

- An urban cluster of Crohn's disease possibly linked to fully treated drinking water has been described by Pierce. Her report describes three unrelated individuals who lived on the same block along a street in a Midwestern American city and developed Crohn's disease within four years (Pierce 2009).

- A familial cluster of cutaneous *M. avium* infection was reported by Sugita et al. (2000). A 45-year-old father, his 14-year-old son and 11-year-old daughter, among five persons in a family, presented with a 2-month history of inflammatory subcutaneous nodules and ulcerations. They all used a circulating, constantly heated bath water system. The bath water was continuously heated to about 40 °C, and *M. avium* was isolated from the filter of the bath tub heating unit. It is considered that this unusual familial cluster of cutaneous *M. avium* infection in healthy persons may have resulted from the use of contaminated bath water.

Data on potentially pathogenic mycobacteria of different species found in different habitats like surface, industrial and drinking water, metalworking fluids, soil, dust and air have been published in hundreds of papers. They have been presented together with descriptions of the clinical significance and manifestations of infection in humans and possibilities of prevention in the book "The Ecology of Mycobacteria: Impact on Animal's and Human's Health" (Kazda et al. 2009). The most frequently studied species are *M. abscessus*, *M. avium*, *M. fortuitum*, *M. chelonae*, *M. kansasii*, *M. intracellulare*, *M. malmoense*, *M. marinum*, and *M. xenopi*. Mycobacterioses are typical granulomatous inflammations, and manifest as chronic hypersensitivity pneumonitis, granulomatous lung disease, hot tub lung, lifeguard lung, machine operator's lung, cervical lymphadenopathy or lymphadenitis, skin infections (originally called swimming pool granuloma, later changed due to the most common source of infection to fish-tank granuloma), finger arthritis, stomatitis etc.

Some of these diseases are associated with professional or hobby activities, some with genetic factors, or with other diseases in the same patient or other conditions affecting health, like chlorine or bacterial endotoxins in air or free-living amoebae in drinking water. Some mycobacteria, such as *M. kansasii*, can colonise cold water distribution systems whilst *M. xenopi* and *M. avium* are more commonly associated with hot water systems and *M. marinum* with water in swimming pools or aquaria.

Mycobacteria are well equipped to trigger inflammation alone, but obviously they can cause health

problems together with other external factors or circumstances. Severe cervical lymphadenopathy in children is associated with tonsillectomy, teeth exchange or extraction. Catheters and bronchoscopes have been implicated as sources of mycobacterial infections. Mycobacteria from catheter-related infections are often resistant to antibiotics and their source is usually unknown. The disinfection solution used to sterilise the skin surface before the catheter emplacement can be sometimes responsible for infection. The removal of biofilms from instruments is difficult and the concentration of some disinfectants must be increased 100-fold over concentrations recommended for devitalisation of normal *Staphylococcus aureus* because mycobacteria are at least 100 times more resistant to disinfectants like chlorine, chloramine, chlorine dioxide and ozone (Taylor et al. 2000).

It can be concluded that mycobacteria, owing to their ubiquitous presence, are always ready to use their chance.

6. The cow – an efficient bioreactor for production of both milk and mycobacteria

Cattle breeding has changed in industrial countries during the recent decades. Traditionally, cows were mainly kept on pastures with a relatively low milk production of 1500 to 4000 litres from one cow between two successive calvings, usually a period of 12 to 14 months. A contemporary yield can be 15 000 to 25 000 litres in the same period. Pasture and the free movement of dairy cows in the fresh air is now an outdated practice. Buildings resemble more closely modern sophisticated factories than traditional farms. The transmission of mycobacteria from a heavily contaminated room shared by hundreds of animals is easier than transmission on a pasture or between eight to thirty cows, which was a usual number of cattle heads in a traditional farm only 20 or 30 years ago.

Animals are now expensive bioreactors, installed in industrial units with several hundred to twenty thousand cattle heads. Computerised feeding and milking systems ensure a high working productivity and efficient care for animals. Complicated technology and sophisticated logistics are needed because one thousand cows need about 80 000 kg of forage and 40 000 liters to 100 000 liters of drinking water and produce 20 000 to 25 000 liters of

milk and 60 000 kg of manure in one day. Two to ten calves are born and one to two cows are culled every day. No interruption of the process is possible, feeding and milking cannot be stopped. In a car factory the production lines can be switched off during whole factory vacations. In a nuclear power plant the reactor can be stopped and its production interrupted for several weeks. This is not possible in a factory for milk production equipped with one thousand live machines as bioreactors, producing 700 to 1000 g of pure fat, 700 to 900 g of pure protein and 1000 to 1300 g of sugar a day (Table 3). Unfortunately, some of these bioreactors also shed 250 million MAP cells in milk and 350 billion MAP cells in faeces daily. Hence, the dissemination of MAP infection is easy in this high density population inside such a closed space.

Cattle, sheep, goats and other ruminants suffer from paratuberculosis, or Johne's disease, a chronic bowel inflammation caused by MAP and known in England already by the end of the 19th century. The modern technology of dairy cattle breeding, local and international trade with animals and the enormous load of metabolic requirements is favourable for the dissemination of paratuberculosis within and between herds. The disease was considered to be mild, similar to diarrhoea, and something akin to dietary failure. In fact, until now it is not notifiable, so exact data of prevalence are not available. Nevertheless, it is estimated that 50% to 90% of herds have one or several cows shedding MAP. The disease is not aggressive, but one ill cow reliably transmits paratuberculosis to several other animals over the course of one or two years. Most calves born and left in such an environment catch the disease. The latent period without any signs of disease can last for many months or even years, often until a cow is stressed or challenged in some way and clinical signs are expressed. In the meantime billions of shed MAP cells have found their hosts and in luxurious "all-inclusive" conditions wait for the suitable time for the eruption of their replication.

MAP has been doing the job in secret for years. No specific antibodies can be detected with a sufficient sensitivity in the blood of host animals, no lameness, no cough, no sternutation and no specific skin reaction such as the tuberculation skin testing for bovine tuberculosis. MAP is not like most enteric pathogens, which can be cultured within hours or days and basically typed directly on selective media. Samples for MAP culture must be decontaminated prior to culture examination to avoid the growth of

other bacteria and moulds and culture media must be incubated for three to four months before colonies of mycobacteria are visible by the naked eye and can be identified and typed by different methods. The procedure of decontamination sometimes kills also a major part of the mycobacteria in the sample, so false negative results are common.

The detection of specific DNA sequences represents a new possibility for MAP detection. Results can be obtained in a few days or hours and the method is very sensitive. Unfortunately, MAP shedding is not regular during the course of the disease prior to clinical signs. As a result, MAP is permanently in the advantage and the battle is very uneven. There is no tiebreak and MAP can wait. Those who are at a disadvantage are farmers, producers and consumers.

7. A theatre of war

The battle between mycobacteria and hosts does not proceed according to a clear war strategy, offensive on the side of mycobacteria and defensive on the side of the attacked hosts. The war is global and permanent, just with different intensities or sometimes under an apparent ceasefire. Crohn's disease is one of the battlefields, which should not be extended. Beating it should have high priority. However other diseases, also associated with inflammatory pathways affected by MDP, are also the focus of interest. There are countless settings, utensils, vehicles, situations, traditional behaviours, new technologies, trade customs, food habits, social events and positions and enemy agents operating behind enemy lines, participating in these encounters mostly without an awareness of the subjects, participating in this war. The most important settings are listed in Table 4, some of them with details, which should not be neglected. Perhaps it will contribute to the development of new ideas on how to gain control over the battle field and to decrease the number of victims, not only those of Crohn's disease. Some wars in history lasted dozens of years and even holding one small hill or town can be a great success. The war with mycobacteria will be endless. Keeping them under control to stop their expansion would be a great success, similar to building an impassable line between troops in war. The enemy, which cannot be destroyed, must be discovered, its positions investigated and drawn on the map. As evident, you can meet MDP derived from MAP or other species of

mycobacteria from different sources in many quite unexpected places. Are you surprised that seven million mycobacterial cells can be found in one gram of house dust (Torvinen et al. 2010) or 10 million per one gram of pork lymph node (Tirkkonen et al. 2013)? Do you envy the blower operator his job in the fresh air or do you enjoy using an air blower to clean your garden or backyard? Are you happy if the pavement under your windows is cleaned by an air blower? Is it cleaning at all? Did you expect contamination of bottled mineral water with mycobacteria? Thousands of scientific papers and recently one book of 520 pages have addressed questions on the ecology of mycobacteria and their impact on animal and human health (Kazda et al. 2009). However, the incomparable properties of mycobacteria require more attention from microbiologists, ecologists, biomedical scientists, clinicians, gastroenterologists, immunologists, surgeons, dentists, veterinarians, public health and food inspectors, farmers, technicians in water industry and many others. If scientific papers are not read and the conclusions are not taken note of, it will be necessary to sound the alarm and both the authorities responsible for public health and consumer protection and consumers and the patients themselves have to be mobilised.

8. Two similar stories

8.1. First, Iceland, 80 years ago

Iceland was a relatively isolated territory in the first half of the 20th century. Prior to 1930 MAP infection and ruminant paratuberculosis in Iceland were virtually unknown (Pedley et al. 2004; Hruska 2012). Then in 1933, twenty Karakul sheep were imported from Germany and, after quarantine, were distributed to 14 farms (Fridriksdottir et al. 2000). Although apparently healthy, some of the Karakul sheep were subclinically infected with MAP. They transmitted MAP to the Icelandic sheep population though they never developed disease themselves. By 1938 clinical paratuberculosis appeared in Icelandic sheep on five of the original farms. By about 1945, clinical paratuberculosis was in the cattle on the same farms, although infection in the cattle was difficult to diagnose as the organisms would not grow in culture; a characteristic of sheep MAP strains. The microorganism from these cattle was later confirmed as the sheep strain of MAP by molecular methods (Whittington et al. 2001).

Slowly the MAP infection spread so that by the late 1950s the disease was epidemic with about 30% of sheep farms affected and with huge annual losses. The mean incidence of Crohn's disease (number of cases/ 10^5 per year) in the human population was 0.4 from 1950 to 1959, 0.45 from 1960 to 1969, 0.9 from 1970 to 1979, 3.1 from 1980 to 1989 and 5.6 from 1990 to 1994. Young people were particularly affected (Bjornsson 1989; Bjornsson et al. 1998; Bjornsson and Johannsson 2000). Can anybody believe that links between MAP and Crohn's disease do not exist?

8.2. Second, Czech Republic, 60 years later

Prior to 1990 MAP infection and animal paratuberculosis in Czechoslovakia were virtually unknown. Czechoslovakia, a small country in central Europe, had been isolated for political and economic reasons until 1990 as a satellite of the Soviet Union. The country was free from paratuberculosis like Iceland was before WW2. Foodstuffs were imported from Western Europe in very limited cases. Valuable breeding bulls and rams were imported rarely. Paratuberculosis in cattle was known only in a few cattle and sheep herds. The Iron Curtain was destroyed in 1989 after the "Velvet Revolution" and frontiers were opened for the import of foodstuffs and live animals. At least thirty thousand heifers and other ruminants including farmed red deer were imported with government and EC subsidies especially from Germany, France, Denmark and the Netherlands.

Paratuberculosis was not a reason for banning import, because it was not on the list of important and notifiable animal diseases in the countries of origin. Diagnostic methods were not sufficiently reliable in preclinical stages of the disease and classical quarantine at that time for 42 days was called off very often without any consequences. The now improved intravital methods are not absolutely reliable and cannot protect against a MAP infection with an incubation time of months or even years. After two decades paratuberculosis has appeared progressively not only in imported animals, but also in other autochthonous animals in the herd. Paratuberculosis in cattle is not yet a notifiable disease in many countries and it is not reported in slaughtered animals. Nevertheless, without any doubt, the incidence of paratuberculosis in the Czech Republic has increased enormously since that time and during the last 20 years MAP infection was diagnosed in most districts.

Baby food produced in the former Czechoslovakia (split into the Czech Republic and Slovakia in 1993) was, until the early nineties, produced only from milk originating from local breeding cattle herds from the districts free of MAP infection. The domestic brand has been substituted with many products, imported from Western Europe. In 2005, 51 products of formula milk produced by 10 producers in seven countries were tested. We found that 49% of samples were contaminated with MAP DNA (Hruska et al. 2005; Hruska et al. 2011).

The environmental factor is expected to be a trigger of Crohn's disease and some autoimmune diseases (Rosenstiel 2013). Nationwide statistics of human diseases are very reliable in the Czech Republic. The increase in Crohn's disease between 1995 and 2012 is more than 13-fold in all age categories and more than 12-fold in people of 65 and above (Figure 3A). Similarly the increase of type 1 diabetes (T1D) between 1995 and 2012 is 2.6-fold in children under 15 (Figure 3B). MAP is the single most evident novel food and environmental factor to have emerged in the Czech Republic in the mid-1990s. Can anybody be in doubt that links between MAP and Crohn's disease and between MAP and T1D do exist?

9. Speculation about Crohn's disease aetiology: From many single hypotheses to one "superhypothesis"

Mycobacteria were first suspected to be associated with Crohn's disease more than 100 years ago, when T. K. Dalziel, a surgeon from the Western Infirmary, Glasgow, published his paper on chronic interstitial enteritis in *The British Medical Journal* in 1913 (Dalziel 1913). He made a possible diagnosis of tuberculosis, but also noted the uniform character of the infection. The lymph nodes were enlarged but not caseous, and bowel consistence and smoothness resembled "an eel in a state of rigor mortis". Further, Dalziel cited an earlier description of a chronic bacterial enteritis in cattle, observed already in 1895 (Henny and Frothingham, cited by Dalziel 1913). Six cases of Johne's disease (paratuberculosis) described by McFadyen in cattle in England in 1911 and by Dalziel in humans in 1913 were so similar with regard to their histological profiles as to justify the proposition that the diseases may be one and the same. McFadyen described "an acid-fast bacillus, similar to but demonstrably not the tubercle bacillus, differing in size and

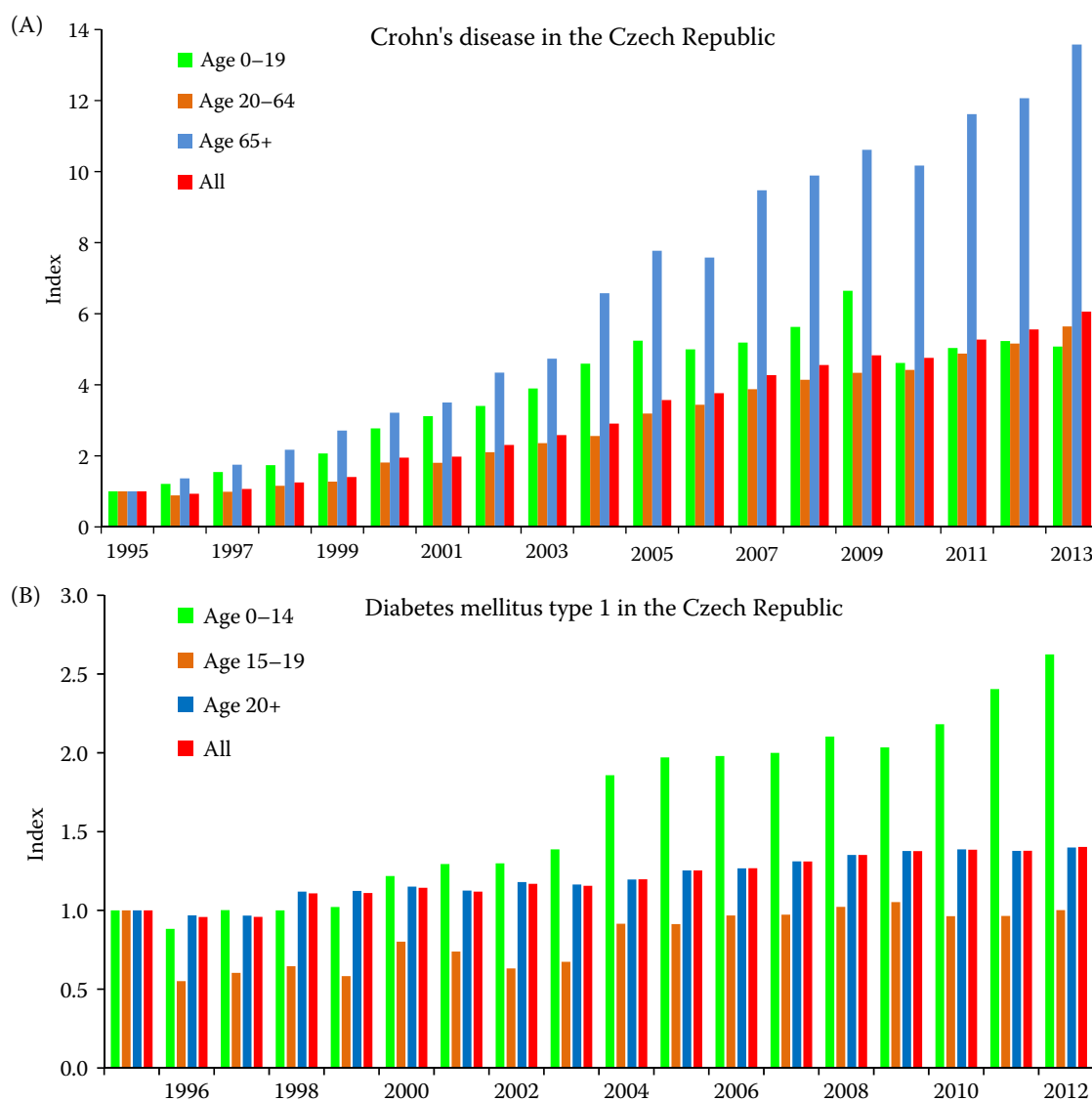


Figure 3A. Crohn's disease in the Czech Republic (Institute of the Health Information and Statistics of the Czech Republic, Prague; World Organization for Animal Health, Reference Laboratory for Paratuberculosis, Veterinary Research Institute, Brno). Index calculated from the number of patients under medical care

Total number of patients in 2012: 18 749

Figure 3B. Type 1 diabetes in the Czech Republic (Institute of the Health Information and Statistics of the Czech Republic, Prague; World Organization for Animal Health, Reference Laboratory for Paratuberculosis, Veterinary Research Institute, Brno). Index calculated from the number of patients under medical care

Total number of patients in 2012: 56 514

also not giving rise to tuberculosis in guinea-pigs" (cited by Dalziel 1913). This first hypothesis has run since that time like a thread through the history of Crohn's disease for more than a hundred years.

Countless hypotheses regarding the aetiology of Crohn's disease have been suggested, defended, approved, respected, declared as uncertain and disproved and still believed and cited (Table 5). Infection by different bacteria is mentioned

most frequently. *Mycobacteria* and specifically *Mycobacterium avium* subsp. *paratuberculosis* (MAP), suggested as an aetiological factor already by McFadyen, are regarded as prominent candidates for participation in the aetiology or pathogenesis of Crohn's disease. Infection by listeria or by viruses in anamnesis, namely measles, adherent invasive *Escherichia coli*, or imbalances in the intestinal microbiome are also suspected causes.

There are several good reasons to consider mycobacteria as the most important but not only aetiological factors in the development of Crohn's disease. Dead mycobacteria as a component of Freund's adjuvant are widely used as immunomodulators. The mechanism is known and experimentally confirmed. The muramyl dipeptide (MDP) derived from peptidoglycans, a component of the bacterial cell wall, is responsible for stimulation of antibody production in immunised animals. The interaction of MDP with the NOD2 protein in inflammatory pathways, resulting in gut inflammation, has been described in the magazine *Science* in 2005 (Kobayashi et al. 2005; Maeda et al. 2005). MDP can be derived from different bacterial species and it is very likely that the host will be affected if the dose of cells is sufficient and the host is vulnerable due to genetic disposition, age, stress or parallel infection. Early childhood is a period of life when mammals are sensitive to modulators of innate immunity and epigenetic changes (Zoldos et al. 2013). A new-born baby is surrounded with microflora from the first seconds of life and the innate immunological mechanisms adapt to this situation.

Mycobacteria, so strange and different from other bacteria, have high chances to be recognised and kept in the immunological memory. Changes induced by "peaceful" microbes or by pathogens during new-born infections or after vaccination make the individual ready to use defence mechanisms when necessary many years later and sometimes over the whole course of an individual's life. Synthetic MDP showed a priming effect in mice not only when administered parentally but also via the oral route (Takada et al. 2002). Hence, the participation of baby formula containing MAP from milk or other mycobacteria from water is likely a key step in the increased risk of later contraction of Crohn's disease or other diseases triggered by similar pathways.

Breast feeding is often mentioned as an important protection factor in prevention against Crohn's disease, type 1 diabetes, asthma and other diseases of a similar pathogenesis. Formula feeding is a popular alternative to breast feeding, beneficial and lifesaving if breast feeding is not possible, but available also for the comfort of less responsible mothers. It should be noted that no legal measures are applied to control the MAP contamination of milk used for baby food products. As many as 10 000 MAP cells per gram of powdered infant milk represent eight

million cells in an 800 g package. Over the course of eight to ten days eight million MAP cells are consumed by a baby, followed by an additional eight million if the next package is of the same batch, and even more, if tap water also contains mycobacteria.

Such a hypothesis is supported by observations of the lower incidence of Crohn's disease in Canadian Indians, Maoris in New Zealand, Arab residents in Israel, the Gypsy population in Hungary or residents with a lower social status in many countries. There is one common attribute for these ethnic or social groups: breastfeeding is practised and newborns are less frequently fed by formula. A global market made baby food easily accessible even in many developing countries, where Crohn's disease was nearly unknown twenty years ago and paratuberculosis in cattle did not exist.

The environmental factor in many hypotheses had been elusive and nobody was aware of the easy dissemination of mycobacteria as an environmental factor in milk as a result of the global boom in formula feeding. It is easy to understand that families of babies fed by formula have refrigerators, parents consider infant swimming as popular and their babies enjoy bathing or showering in hot tap water from a communal pipeline. Burger consumption by children is a common practice in western urban populations. Similarly, observations of higher incidences of Crohn's disease have been published in social groups with higher living standards. The smoking hypothesis, the hygiene and cold chain hypotheses, the differences between immigrants and long-term residents or between immigrants and indigenous citizens are shown to be plausible in the light of MAP and other mycobacteria as sources of the most important triggers for Crohn's disease and very likely also for some other human chronic inflammatory diseases. The environmental factor, missing but expected in many hypotheses, can be introduced as MDP. We dare to call this a "superhypothesis" and it fits to most published hypotheses. Nobody should be disappointed, previously suggested and passionately defended hypotheses are rational from different points of view.

9.1. The superhypothesis is based on the following facts

- Muramyl dipeptide (MDP) is the minimal activating structure produced by degradation of bacterial peptidoglycans

- MDP is a molecule associated with the pathogenesis of inflammatory diseases, the aetiology of which need not be unique
- MDP triggers pro-inflammatory cytokines
- MDP is able to modify immunity, namely early after birth
- Nucleotide-binding oligomerisation domain-containing protein 2 (NOD2), encoded by the *NOD2* gene, plays an important role in the immune system. It recognises bacterial molecules (peptidoglycans, their degradation products and MDP) and stimulates an immune reaction. *NOD2* is the most important gene among many others, involved in MDP detection
- Mycobacteria are important sources of MDP
- *M. avium* subsp. *paratuberculosis* (MAP) is the most important, but not exclusive source of MDP
- MAP is also a source of heat shock protein 65, a bacterial mimic probably participating in the aetiology of autoimmune disease type 1 diabetes mellitus (Naser et al. 2013)
- MAP is produced by cattle suffering from paratuberculosis, also called Johne's disease (PTB-JD)
- Enormous numbers of MAP are shed in faeces and contaminate the environment, milk, dairy products and meat
- PTB-JD is also a serious global financial problem in dairy cattle herds; Crohn's disease (CD) is a chronic inflammatory disease associated with MDP, at least as one of the triggers
- CD is difficult to cure and its treatment is very expensive; for most sufferers (600 000 in the USA and about two millions in European Union) the disease is only partially curable. CD incidence is increasing with similar dynamics like paratuberculosis in ruminants
- MDP may be associated with other non-specific inflammatory diseases: type 1 diabetes, multiple sclerosis, asthma, lupus erythematosus, degenerative arthritis, Blau syndrome, sarcoidosis etc.

Taking mycobacteria and namely MAP as the most important factor in Crohn's disease pathogenesis, the public health risk cannot be totally eliminated, but should be greatly decreased. We believe it can be achieved with relatively low expenses in contrast with the current losses in dairy farming and the huge costs for treatment of victims of this battle between mycobacteria and civilization. This uneven combat (for MAP and the alliance of mycobacteria do not follow the rules of war) must be finished. The strategy will be described in the following lines.

10. Step by step towards a partial reduction of Crohn's disease and paratuberculosis incidence

Both science and practice are at least fifty years late in controlling paratuberculosis in cattle. The public health risk associated with mycobacteria could be diminished if MAP would be reduced in milk and dairy products, and all mycobacterial species should be controlled in water. Maximum acceptable concentrations of MAP must be established for milk processed in dairies and MAP DNA in infant formula must be lower than the limit of detection. Governments have to be able to answer two simple questions: how many dairy herds are affected by paratuberculosis according to the reports from slaughterhouses, and how many cows displayed MAP-associated intestinal lesions at slaughter. These legal measures are an easy and cheap way for governments to begin protecting infants and all consumers against MDP originating from MAP, and to motivate dairy farmers and the dairy industry to control paratuberculosis in cattle and to follow the incidence of paratuberculosis in cattle in the future. Such measures should start in the USA, Canada and the European Union, because the decisions of these three entities influence a great part of global milk production and the dairy product market. A few simple directives would be a first step towards the protection of 972 000 000 consumers. Up to three million patients suffering from Crohn's disease will appreciate every step towards a reduction of their annoyances. Hopefully, the participation of other countries, namely Australia, Brazil, Israel, Japan, New Zealand and the Republic of Korea should follow.

Obviously the importance and consequences of the "superhypothesis" on MDP as a public health risk will not be palatable to many experts and managers. Hence, knowledge dissemination and gradual improvements in understanding this superhypothesis are important and permanent tasks for universities, TV, radio broadcasting, newspapers and magazines, associations of medical and veterinary specialists, extension services, patients associations and other societies. Guidelines and information for consumers, farmers, food inspectors, food producers, veterinarians, medical practitioners, employees in risky professions like lifeguards, whirlpool and hot tub staff for self-education and optional preventive measures must be available.

Examples of how to decrease the risk are listed in Table 6. Nobody can have objections to breastfeeding, a simple prevention of the possible interaction

of mycobacteria with the innate immune systems of infants. However, mothers suffering from Crohn's disease should be informed that the shedding of MAP in their milk is possible, so formula feeding of their child should in these cases be preferred or the testing of breast milk for MAP should be recommended. Responsible parents should determine if tap water from household plumbing is safe for their infant and themselves. Mycobacteria-free water should be used for cooking and drinking. If the source of water is contaminated in the bathroom, a showerhead can be changed for a new one and the inhalation of mycobacteria can be avoided by bathing instead of showering. Evidently, reliable and cheap methods for MAP testing must be available.

A greater part of the mycobacterial load which reaches consumers is associated with MAP. The production of MAP, not milk, must be reduced. Therefore, paratuberculosis should be notified in the simplest and cheapest way. Farmers have to be motivated to control the disease. All cattle slaughtered according to the usual regulations can be inspected by a veterinarian or by an authorised person, who can provide the national database with ID numbers of animals with clinically proven paratuberculosis at slaughter in a simple and inexpensive way. It would be useful if official data on Crohn's disease incidence and data on the length of breastfeeding would also be available. However, it is necessary to keep in mind that the interpretation of such data will be possible only after 10 to 30 years.

The interest of consumers in finding MAP-free formula and MAP-free or low in MAP dairy products on the market should motivate farmers and the dairy industry to meet these requirements. Food safety and health authorities can support this aim by establishing maximum acceptable concentrations of mycobacteria based on DNA determination in bottled water, milk for dairies, ground beef, water in swimming pools and public hot tubs. The food industry should be encouraged to label products as mycobacteria-controlled.

Farmers must be informed that every animal purchased from another herd poses a risk. New-born calves should be separated immediately after delivery and fed with MAP-free colostrum

and milk. Cows shedding MAP must be culled or separated without delay. A voluntary endeavor of farmers and industry should be supported by governments and by international agreements. Research projects oriented towards the fast and cheap determination of mycobacteria, the obtaining of reliable data about communal water contamination, the development of technologies on how to prevent water contamination or how to decrease cell concentration would be appreciated. The most important field of research is the pathogenesis of chronic human inflammatory illnesses, which should result in new treatments for Crohn's disease and an improvement in the quality of life of patients, and last but not least in lower costs for treatment. It was not possible to acknowledge and cite in this review most of the important articles. The selected papers, published in the years 2013 to 2014, retrieved from the Web of Science (Thomson Reuters) are listed in the Table 7.

11. What should be borne in mind

Long-lasting and complicated problems usually have no simple and fast solutions. As regards MAP and mycobacteria in water no directions have been violated, because no directions on how to decrease the risk for consumers have ever been accepted. The collection of information and their presentation as a "superhypothesis" on MDP participation in the pathogenesis of Crohn's disease should result in simple legal measures which can be realised and sanctioned. More efforts should be made by farmers and food producers and consumers themselves. However, there is no reason for panic. Mankind and mycobacteria have lived together for thousands of years and this will continue. The balance has simply been changed by the introduction of new technologies and lifestyles and now must be turned back in favour of people. Scientific information must be respected by scientists themselves and must be disseminated to the people in charge in order to bring about change. Certainly, many questions remain to be answered. However, the decision makers should hear one strong and unanimous voice.

Table 1. Players on the scene posing a risk of triggering the chronic inflammatory diseases by mycobacteria (only breast feeding has a protective effect as documented for some of the diseases listed in Table 2)

Genome-wide association (<i>NOD2</i> and other genes)
Formula feeding
Beef
Milk and dairy products
Pork
Fruits and vegetables
Mineral and bottled water
Indoor swimming pools
Baby swimming
Air, aerosols
Communal water
Home plumbing
Home showering and bathing
Level of hygiene
Rural residency
Municipal residency
Social position
Immigration
Cattle, sheep and goats breeding
Animal wastes, manure, liquid dung, fertilization of fields
Pasture
Outdoor swimming in rivers and lakes
Biogas stations residues
Human tuberculosis
Leprosy
Other sources of MDP (infections with pathogen replication)

Table 2. Chronic inflammatory diseases with suspected links to previous exposure to mycobacteria or other infectious agents (examples only)

	Greenstein (2003)
	Carbone et al. (2005) MAP and others*
Crohn's disease	Lidar et al. (2009) viruses**
	Chiodini et al. (2012)
	Salem et al. 2013b, Salem et al. 2014
Multiple sclerosis	Compston and Coles (2008)
	Cossu et al. (2013b), Cossu et al. (2013c)
Type 1 diabetes mellitus	Dow (2006)
	Cossu et al. (2011), Cossu et al. (2013a)
Sarcoidosis	Reid and Chiodini (1993)
	El-Zaatari et al. (1996)
	Dubaniewicz et al. (2013)
Asthma	Nagel et al. (2010)
Chronic inflammation of joints and skin	Wenink et al. (2011)
Psoriasis	Rambukkana et al. (1993), Bay et al. (1998)
Spondylarthritis	Berthelot et al. (2013)
Blau syndrom	Dow and Ellingson (2010)
Autism	Dow (2011)
Respiratory disease	Richerson et al. (1982), Falkinham (2003), Prasse et al. (2013)

**Clostridium*, *Campylobacter jejuni*, *Campylobacter faecalis*, *Listeria monocytogenes*, *Brucella abortus*, *Yersinia pseudotuberculosis*, *Yersinia enterocolitica*, *Klebsiella* spp., *Chlamydia* spp., *Eubacterium* spp., *Peptostreptococcus* spp., *Bacteroides fragilis*, *Enterococcus faecalis*, and *Escherichia coli* (Carbone et al. 2005)

**mumps, measles, Epstein-Barr virus, rubella, cytomegalovirus (Lidar et al., 2009); however, little evidence of association with CD and many viruses have been confirmed recently (Wagner et al. 2013)

Table 3. A cow in numbers

	One cow	1000 cows
Body weight	600 kg	600 000 kg
Meat (beef) for consume (40%)	240 kg	240 000 kg
Protein in meat (20%)	48 kg	48 000 kg
Daily		
Milk yield	25 kg	25 000 kg
Feed consumption	80 kg	80 000 kg
Water consumption	100 l	100 000 l
Liquid dung (faeces and urine)	40 kg	40 000 kg
MAP cells in faeces (10^9 /g)	25^{12}	25^{15}
Annually		
Milk yield	9 500 kg	9 500 000 kg
Fat production (3.4%)	323 kg	323 000 kg
Protein production (3.2%)	304 kg	304 000 kg
Sugar (lactose) production (4.7%)	447 kg	447 000 kg
Minerals (0.72%)	68 kg	68 000 kg
Maximum MAP cells		
in milk	10 000/ml	
in infant formula	10 000/g	
in beef	10 000/g	
in faeces	1 000 000 000/g	
Dairy cows in the world (2010)		
Total cattle heads	264 566 000	
European Union	22 900 000	
USA	9 117 000	
Canada	959 100	
Japan	830 000	
Republic of Korea	241 000	
Milk production	596 560 884 000 kg	
European Union	134 157 000 kg	
USA	87 848 400 kg	
Japan	7 720 380 kg	
Canada	7 882 000 kg	
Republic of Korea	3 263 000 kg	
Beef production (2010)		
USA	11 789 000 kg	
European Union	7 920 000 kg	

Table 4. What should be drawn in the map as the different battle fields with mycobacteria

Dairy cattle farm or industrial unit

- 50% to 90% of dairy cattle herds infected in USA and Europe
- one infected cow today = 30% cattle infected in the same herd three years later
- MAP in milk and meat of infected cows

Milking hygiene

- MAP contamination of milk from faeces and the environment

Ultra heat treatment of milk is not fully effective in MAP devitalisation

MAP In infant formula

- MDP derived from live and dead MAP harms in the same way

Manure, liquid dung, slurry

- MAP is transported to field and pastures
- cattle faeces are on the market as fertilizer for gardens
- MAP from field dunghills contaminate pastures and watering places used for uninfected cattle

Biogas production units likely serve as bioreactors for MAP multiplication

- solid scraps used for soil fertilization

Pastures

- MAP is washed to surface waters, rivers and lakes
- farm and wild ruminants can be infected by MAP

Wild ruminants

- MAP reservoirs for cattle, sheep and goats
- venison from one infected deer consumed by restaurant guests poses smaller dose related risk for many people but a high risk if the whole infected deer or wild boar is consumed by members of the family of the hunter

Backyard poultry**Surface waters**

- risk for swimmers and nearby residents
- MAP persistence in sediments

Cooling towers**Sewage disposal plant****Slaughterhouses**

- intravital meat contamination
- surface contamination by content from intestines

Water industry

- water production plants
- municipal water distribution systems
- household plumbing, faucets, shower heads
- potable water reservoirs, small tanks or reservoirs used in emergency

Baby hygiene

- contaminated water used for cooking and showering

Swimming in open and inside pools

- infant and baby swimming

Hot tub and spas**Garden and potting soil**

- cattle and poultry slurry
- dry pellets available on the market

Hobbies and pets

- aquaria and terraria
- pigeons
- pet birds
- small ruminants

Milk and dairy products

- Infant formula feeding

Restaurants and fast foods

- ground beef, burgers, raw meet, bloody steaks
- water reservoirs in coffee machines
- self-service dispensers of beverages

Vegetables

- fertilizing
- hydroponics
- surface contamination

Bottled water and mineral water

Home dust

Street dust

- air blowers

Table 5. Publications on Crohn's disease hypotheses. The most important database Web of Science® (Thomson Reuters) of over 10 000 journals from over 45 different languages across the sciences offers information from more than 44 thousands papers about Crohn's, published from 1945. More than 3000 papers have been indexed in 2011 and 2012 (Figure 2)

Web of Science® Search results

Timespan = all years (from 1945 to 2013-02-06)

Topic = [Crohn* AND (hypothes* OR etiolog*)]

Results: 2486

AND Topic =

infection*	404
mycobact*	274
paratuberculosis	218
innate	168
NOD2	153
smoking	136
hygiene	94
"fatty acid*"	81
milk	60
lipid* OR cholesterol OR fosfolipid*	47
measles	43
helmint*	33
"vitamin D"	33
"infant feeding" OR formula	17
"environmental factor"	17
listeriosis OR Listeria	16
season*	11
Adherent Invasive Escherichia coli OR AIEC	10
"breast feeding"	9
mimicry	9
"Common Disease" OR "Common Variant"	9
[(social OR economical OR educational OR occupational) AND status]	8
"cold chain"	7
impaired AND killing*	4
"gall stone*"	4
metal	4
spheroplast	1

Table 6. Who should be in charge and what can be done

Every responsible parent

- breastfeeding as the first choice
- potable water for cooking and bathing the new-born and babies tested for mycobacteria
- People under higher risk (CD patients, their relatives)
- potable water for cooking and showering should be tested for mycobacteria
- should prefer MAP-free/low milk, dairy products and beef
- should avoid ground beef
- should avoid swimming in the catchment of cattle and sheep pastures
- should avoid swimming in indoor and outdoor pools if water is not tested for mycobacteria

Farmers

- tank milk samples and milk filters should be tested for MAP monthly
- new-born calves should be separated immediately and fed with MAP-free colostrum and milk
- heavy shedders should be immediately separated and culled

Dairy industry

- tank milk should be sorted according to MAP contamination
- MAP-free formula should be on the market
- milk and dairy products should be labelled as MAP low/free

Meat industry

- should support suppliers under PTB control

National, federal and EU regulatory authorities

- paratuberculosis in slaughtered animals should be a notifiable disease
- national register of herds infected with paratuberculosis should be open
- maximum acceptable concentration of mycobacteria per 100 ml of bottled water and milk for dairies
- maximum acceptable concentration of mycobacteria per 1 g of ground beef
- maximum acceptable concentration of mycobacteria per 100 ml of water in swimming pools and hot tubs

Universities

- more attention should be paid to mycobacteria in the undergraduate education of medical, veterinary, food safety, water industry, agriculture and environmental students
- extension services should be oriented to the control of paratuberculosis in cattle herds

Basic schools

- health risk associated with microorganisms in air, water, food and soil

Professional and patient's associations

- knowledge dissemination directed to gastroenterologists, gynaecologists, paediatricians, allergists, immunologists and practitioners

Research and development

- detection of MAP and *M. spp.* by rapid tests and by large scale laboratory tests at low costs
 - research on pathogenesis of CD and other MDP-triggered illnesses with the aim of focusing attention on innate immunity, priming of immunocompetent cells, directed apoptosis etc.
 - research on MAP replication in cattle with the aim of decreasing shedding
-

Table 7. Selected papers, published in the years 2013 to 2014, retrieved from the Web of Science® (Thomson Reuters®)

Selected publications from the years 2013 and 2014, which contain key information concerning the superhypothesis, are presented in this table with a few descriptive words (1st column), full or shortened abstract (2nd column) and the link to the References to the Table 7 (3rd column). The references to this table are listed behind the table, separately from the references to the text. The format imperfections, which exist in the Web of Science® database or are caused by transmission and copying of data between various databases are not corrected. The missing format of lower and upper indexes (e.g. Ig(2) instead of Ig₂ or 10(–6) instead of 10^{–6}) can be given as a typical example. Moreover, some abbreviations are not in capitals, hence DNA or MAP can appear as Dna or Map, respectively. US and British spelling was not unified. This presentation of published data is easy-to-navigate, supplies readers with more authentic information and minimises the misinterpretation of papers due to subjective wording by the authors of a review

MAP and CD	Title: Global warming' to <i>Mycobacterium avium</i> subspecies <i>paratuberculosis</i> There is a growing worldwide movement to investigate the relationship between Crohn's disease (CD) and microorganisms, especially for causality. Scientists and doctors are warming to this historical idea again, particularly with the advent of discoveries involving the gut microbiome, metagenomics and the revelations of the deficiencies of the innate immune system and autophagy. Looking back, early CD reports were already concerned with finding an infectious cause, which is being revisited by researchers around the globe. Conclusion: Current 'global warming' of scientific thought toward a microbial role in CD and seemingly unrelated therapeutic developments listed above augur well for patients with this chronic condition. The focus of therapy will now shift away from control of inflammation and toward control and eradication of the underlying pathogen/s, particularly MAP with restoration of defective immunity.	Agrawal et al. 2014
<ul style="list-style-type: none"> • history • autophagy • ntibiotics • competitive inhibition • therapeutic vaccination • microbiome modifying immunity 	NOD-like proteins (NLR) are a specialized group of intracellular receptors, which constitute an essential component of the host innate immune system. They were discovered more than a decade ago, but research on this particular class of microbial detectors is still ongoing to allow for a better understanding of the mechanisms, recognition of microorganisms, transmission of signals, and carrying out the activation of inflammatory signaling pathways. In this review, we discuss the construction of NOD1 and NOD2 receptors, their functions, and significance in the pathogenesis of inflammatory diseases in humans.	Antosz and Osiak 2013
<ul style="list-style-type: none"> • inflammatory diseases • pathogenesis 		
Spondylarthritis	Spondylarthritis is still viewed as a reaction to infectious agents, as opposed to an infection by persistent bacteria, for several reasons: (a) an infection is considered proven only when the organism can be cultured; (b) no studies have identified dormant bacteria in the tissues targeted by spondylarthritis; (c) the bacterial persistence hypothesis has no therapeutic implications at the time being, since antibiotics are effective neither on dormant bacteria nor on the manifestations of spondylarthritis; and (d) the high prevalence of borderline disorders combining features of spondylarthritis and of psoriatic arthritis, or even rheumatoid arthritis (RA), would indicate a role for dormant bacteria in these last two diseases. However, recent data on dormant bacteria have rekindled interest in the bacterial persistence hypothesis. Dormant bacteria cannot be cultured, because they express only a small group of genes, known as the regulon, which includes genes for transcription factors that block the expression of the usual bacterial genes. Certain forms of cell stress, such as molecule misfolding, promote the entry of bacteria into a state of dormancy, which induces the low-level release by the host cells of cytokines such as TNF. Whether HLA-B27 misfolding facilitates the persistence of dormant bacteria within spondylarthritis tissue targets	Berthelot et al. 2013
<ul style="list-style-type: none"> • dormant bacteria 		

<p>remains to be determined. If it does, then treatments that reactivate dormant bacteria might make these organisms susceptible to appropriate antibiotics and might therefore serve as useful adjuncts to nonsteroidal anti-inflammatory drugs and TNF alpha antagonists. TNF alpha antagonists rarely reactivate dormant bacteria, with the exception of Mycobacterium tuberculosis, which, together with metastatic cells, is the most extensively studied latency model to date. (C) 2012 Societe francaise de rhumatologie. Published by Elsevier Masson SAS.</p>	<p>Paratuberculosis (PTB) or Johne's disease is one of the most serious chronic debilitating diseases of ruminants worldwide that is caused by <i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> (MAP). MAP is a slow-growing bacterium that has very long latent periods, resulting in difficulties in diagnosing and controlling the disease, especially regarding the diagnosis of fecal shedders of MAP without any clinical signs. Based on this situation, attempts were made to identify biomarkers that show early responses to MAP infection in a macrophage cell line, RAW 264.7. In response to the infection with the bacterium, a lot of genes were turned on and/or off in the cells. Of the altered genes, three different categories were identified based on the time-dependent gene expression patterns. Those genes were considered as possible candidates for biomarkers of MAP infection after confirmation by quantitative RT-PCR analysis. To the best of our knowledge, this is the first attempt at discovering the host transcriptomic biomarkers of PTB, although further investigation will be required to determine whether these biomarker candidates are associated within the natural host.</p>	Cha et al. 2013
<p>Paratuberculosis</p> <ul style="list-style-type: none"> • MAP infected macrophages • biomarkers • early response 	<p>Molecular mimicry of host proteins is a common strategy adopted by bacterial pathogens to interfere with and exploit host processes. Despite the availability of pathogen genomes, few studies have attempted to predict virulence-associated mimicry relationships directly from genomic sequences. Here, we analyzed the proteomes of 62 pathogenic and 66 non-pathogenic bacterial species, and screened for the top pathogen-specific or pathogen-enriched sequence similarities to human proteins. The screen identified approximately 100 potential mimicry relationships including well-characterized examples among the top-scoring hits (e.g., RalF, internalin, yopH, and others), with about 1/3 of predicted relationships supported by existing literature. Examination of homology to virulence factors, statistically enriched functions, and comparison with literature indicated that the detected mimics target key host structures (e.g., extracellular matrix, ECM) and pathways (e.g., cell adhesion, lipid metabolism, and immune signaling). The top-scoring and most widespread mimicry pattern detected among pathogens consisted of elevated sequence similarities to ECM proteins including collagens and leucine-rich repeat proteins. Unexpectedly, analysis of the pathogen counterparts of these proteins revealed that they have evolved independently in different species of bacterial pathogens from separate repeat amplifications. Thus, our analysis provides evidence for two classes of mimics: complex proteins such as enzymes that have been acquired by eukaryote-to-pathogen horizontal transfer, and simpler repeat proteins that have independently evolved to mimic the host ECM. Ultimately, computational detection of pathogen-specific and pathogen-enriched similarities to host proteins provides insights into potentially novel mimicry-mediated virulence mechanisms of pathogenic bacteria.</p>	Doxey and McConkey 2013

<p>Sarcoidosis</p> <ul style="list-style-type: none"> • heat shock proteins • misfolding amyloid precursor protein • chronic low-grade exposure 	<p>In the light of the Matzinger's model of immune response, human heat shock proteins (HSPs) as main 'danger signals' (tissue damage-associated molecular patterns-DAMPs) or/and microbial HSPs as pathogen-associated molecular patterns (PAMPs) recognized by pattern recognition receptors (PRR), may induce sarcoid granuloma by both infectious and non-infectious factors in genetically different predisposed host. Regarding infectious causes of sarcoid models, low-virulence strains of, e.g. mycobacteria and propionibacteria recognized through changed PRR and persisting in altered host phagocytes, generate increased release of both human and microbial HSPs with their molecular and functional homology. High chronic spread of human and microbial HSPs altering cytokines, co-stimulatory molecules, and Tregs expression, apoptosis, oxidative stress, induces the autoimmunity, considered in sarcoidosis. Regarding non-infectious causes of sarcoidosis, human HSPs may be released at high levels during chronic low-grade exposure to misfolding amyloid precursor protein in stressed cells, phagocytosed metal fumes, pigments with/without aluminum in tattoos, and due to heat shock in firefighters. Therefore, human HSPs as DAMPs and/or microbial HSPs as PAMPs produced as a result of non-infectious and infectious factors may induce different models of sarcoidosis, depending on the genetic background of the host. The number/expression of PRRs/ligands may influence the occurrence of sarcoidosis in particular organs.</p>	Dubaniewicz 2013
<p>MAP</p> <ul style="list-style-type: none"> • salmonella vectored vaccine 	<p>Johnes disease (JD), caused by <i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> (MAP), occurs worldwide as chronic granulomatous enteritis of domestic and wild ruminants. To develop a cost effective vaccine, in a previous study we constructed an attenuated <i>Salmonella</i> strain that expressed a fusion product made up of partial fragments of MAP antigens (Ag85A, Ag85B and SOD) that imparted protection against challenge in a mouse model. In the current study we evaluated the differential immune response and protective efficacy of the Sal-Ag vaccine against challenge in a goat model as compared to the live attenuated vaccine MAP316F. PBMCs from goats vaccinated with Sal-Ag and challenged with MAP generated significantly lower levels of IFN-gamma, following in vitro stimulation with either Antigen-mix or PPD jhonin, than PBMC from MAP316F vaccinated animals. Flow cytometric analysis showed the increase in IFN-gamma correlated with a significantly higher level of proliferation of CD4, CD8 and gamma delta T cells and an increased expression of CD25 and CD45RO in MAP316F vaccinated animals as compared to control animals. Evaluation of a range of cytokines involved in Th1, Th2, Treg, and Th17 immune responses by quantitative PCR showed low levels of expression of Th1 (IFN-gamma, IL-2, IL-12) and proinflammatory cytokines (IL-6, IL-8, IL-18, TNF-alpha) in the Sal-Ag immunized group. Significant levels of Th2 and anti-inflammatory cytokines transcripts (IL-4, IL-10, IL-13, TGF-beta) were expressed but their level was low and with a pattern similar to the control group. Over all, Sal-Ag vaccine imparted partial protection that limited colonization in tissues of some animals upon challenge with wild type MAP but not to the level achieved with MAP316F. In conclusion, the data indicates that Sal-Ag vaccine induced only a low level of protective immunity that failed to limit the colonization of MAP in infected animals. Hence the Sal-Ag vaccine needs further refinement to increase its efficacy.</p>	Faisal et al. 2013
<p>Macrophages granuloma models</p> <ul style="list-style-type: none"> • bacteria-host interaction • dormant mycobacteria 	<p><i>Mycobacterium tuberculosis</i>, <i>Mycobacterium leprae</i>, <i>Mycobacterium bovis</i>, and <i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> can survive within host macrophages in a dormant state, encased within an organized aggregate of immune host cells called granuloma. Granulomas consist of uninfected macrophages, foamy macrophages, epithelioid cells, and T lymphocytes accumulated around infected macrophages. Within granulomas, activated macrophages can fuse to form multinucleated giant cells, also called giant Langhans cells. A rim of T lymphocytes surrounds the core, and a tight coat of fibro-</p>	Fitzgerald et al. 2014

<p>blast closes the structure. Several in vivo models have been used to study granuloma's structure and function, but recently developed in vitro models of granuloma show potential for closer observation of the early stages of host's responses to live mycobacteria. This paper reviews culture conditions that resulted in three-dimensional granulomas, formed by the adhesion of cell populations in peripheral blood mononuclear cells infected with mycobacteria. The similarities of these models to granulomas encountered in clinical specimens include cellular composition, granulomas' cytokine production, and cell surface antigens. A reliable in vitro dormancy model may serve as a useful platform to test whether drug candidates can kill dormant mycobacteria. Novel drugs that target dormancy-specific pathways may shorten the current long, difficult treatments necessary to cure mycobacterial diseases.</p>	<p>CD – associated fistulae</p> <ul style="list-style-type: none"> • TNF and MDP <p>Background: Intestinal fistulae represent a severe complication of Crohn's disease (CD). The authors have demonstrated that epithelial-to-mesenchymal transition plays a pivotal role in their pathogenesis. High levels of interleukin-13 and tumor necrosis factor (TNF) are detected in myofibroblast-like transitional cells covering the fistula tracts. Here, a functional role was investigated for the transcription factor Ets-1, TNF, and the bacterial wall component (muramyl dipeptide [MDP]) in the pathogenesis of CD-associated fistulae. Methods: Perianal fistulae from CD patients were analyzed by immunohistochemistry. Primary colonic lamina propria fibroblasts (CLPFs) were isolated from CD patients with or without fistulizing disease. Messenger RNA (mRNA) levels were assessed by real-time polymerase chain reaction in CLPF or HT29 intestinal epithelial cells (IECs) grown as monolayers or spheroids. Results: Strong expression of Ets-1 transcription factor was demonstrated in transitional cell covering the fistula tracts by immunohistochemistry. TNF induced mRNA expression of Ets-1 and 6-integrin in HT29 IEC and in CLPF from fistulizing CD patients. These effects could be fully blocked by administration of anti-TNF antibodies. In HT29 cells, TNF further induced mRNA levels of TNF and transforming growth factor beta by treatment for 24 hours. In fistula CLPF derived from CD patients, TNF induced expression of 6-integrin, TNF, and transforming growth factor beta. Of note, the bacterial wall component, MDP, induced mRNA levels of Ets-1, transforming growth factor beta, interleukin-13, SNAIL1, and 6-integrin in HT29 IEC monolayers and fistula CLPF by treatment for 24 hours. Conclusions: TNF and MDP induce the expression of factors associated with epithelial-to-mesenchymal transition and invasion in IEC and fistula CLPF. Our findings indicate that TNF and MDP might synergize in the pathogenesis of CD-associated fistulae.</p>	<p>MAP in milk</p> <ul style="list-style-type: none"> • homogenisation • HTST pasteurization <p>Mycobacterium avium ssp. paratuberculosis (MAP) can be present in cow milk and low numbers may survive high-temperature, short-time (HTST) pasteurization. Although HTST treatment leads to inactivation of at least 5 log(10) cycles, it might become necessary to enhance the efficacy of HTST by additional treatments such as homogenization if the debate about the role of MAP in Crohn's disease of humans concludes that MAP is a zoonotic agent. This study aimed to determine whether disrupting the clumps of MAP in milk by homogenization during the heat treatment process would enhance the inactivation of MAP. We used HTST pasteurization in a continuous-flow pilot-plant pasteurizer and evaluated the effect of upstream, downstream, and in-hold homogenization on inactivation of MAP at 72 degrees C with a holding time of 28 s was between 3.7 and 6.9 log(10) cycles, with an overall mean of 5.5 log(10) cycles. None of the 3 homogenization modes applied showed a statistically significant additional effect on the inactivation of MAP during HTST treatment.</p>
	<p>Frei et al. 2013</p>	<p>Hammer et al. 2014</p>

Chronic microbial exposure	Upon chronic microbial exposure and pattern-recognition receptor (PRR) stimulation, myeloid-derived cells undergo a distinct transcriptional program relative to acute PRR stimulation, with proinflammatory pathways being downregulated. However, other host-response pathways might be differentially regulated, and this concept has been relatively unexplored. Understanding mechanisms regulating chronic microbial exposure outcomes is important for conditions of ongoing infection or at mucosal surfaces, such as the intestine. The intracellular PRR nucleotide oligomerization domain 2 (Nod2) confers the highest genetic risk toward developing Crohn's disease (CD). We previously identified mechanisms mediating downregulation of proinflammatory pathways upon chronic Nod2 stimulation; here we sought to define how chronic Nod2 stimulation regulates bacterial killing. We find that, despite downregulating cytokine secretion upon restimulation through PRR and live bacteria, chronic Nod2 stimulation of human monocyte-derived macrophages enhances bacterial killing; this dual regulation is absent in CD Nod2-risk carriers. We show that chronic Nod2-mediated reprogramming of human monocyte-derived macrophages to a state of enhanced bacterial killing requires upregulated reactive oxygen/nitrogen species pathway function through increased p67phox/p47phox/nitric oxide synthase-2 expression; selectively knocking down each of these genes reverses the enhanced bacterial killing. Importantly, we find that, during chronic Nod2 stimulation, NLRP3/NLRP1 inflammasome-mediated caspase-1 activation with subsequent IL-1 secretion is essential for the subsequent bifurcation to downregulated proinflammatory cytokines and upregulated bacterial killing. Therefore, we identify mechanisms mediating the distinct inflammatory and microbicidal outcomes upon chronic stimulation of the CD-associated protein Nod2.	Hedl and Abraham 2013
CD and NOD2	IL-10 contributes to the maintenance of intestinal homeostasis via the regulation of inflammatory responses to enteric bacteria. Loss of IL-10 signaling results in spontaneous colitis in mice and early onset enterocolitis in humans. Nucleotide-binding oligomerization domain (NOD) 2 is an intracellular receptor of bacterial peptidoglycan products, and, although NOD2 mutations are associated with Crohn's disease, the precise role of NOD2 in the development of intestinal inflammation remains undefined. To determine the role of NOD2 in the development of colitis on the clinically relevant genetic background of IL-10-deficient signaling, we generated mice lacking IL-10 and NOD2 (IL-10(-/-)NOD(-/-)). Loss of NOD2 in IL-10(-/-) mice resulted in significant amelioration of chronic colitis, indicating that NOD2 signaling promotes the development of intestinal inflammation in IL-10(-/-) mice. Contrary to previous reports investigating immune function in NOD2(-/-) mice, T cell proliferative capacity and IL-2 production were not impaired, and immune polarization toward type 1 immunity was not affected. However, loss of NOD2 in IL-10-deficient macrophages reduced IL-6, TNF-alpha, and IL-12p40 production in response to bacterial stimulation. Further analysis of the intrinsic macrophage response before the onset of inflammation revealed that, in the absence of IL-10, synergistic signaling between various TLRs and NOD2 resulted in hyperresponsive, proinflammatory macrophages, thus providing the appropriate immune environment for the development of colitis. Data presented in this study demonstrate that NOD2 signaling contributes to intestinal inflammation that arises through loss of IL-10 and provides mechanistic insight into the development of colitis in inflammatory bowel disease patients with impaired IL-10 signaling.	Jamontt et al. 2013
Mycobacteria	Over the past several years, much has been revealed about the roles of autophagy and the mechanisms by which the autophagic pathway activates the host innate effector response against <i>Mycobacterium tuberculosis</i> (Mtb) infection. In response to invading mycobacteria, the host innate immune system not only recognizes pathogen motifs through innate	Jo 2013

	<p>receptors, it also produces appropriate effector proteins, including cytokines. These innate signals activate or regulate autophagic pathways during infection. It is now clear that vitamin D and functional vitamin D receptor signaling are critical in the activation of autophagic defenses against Mtb in human cells. Immunity-related GTPase family M proteins, including the cationic antimicrobial protein cathelicidin and autophagic receptor p62, participate in autophagic pathways that enhance antimicrobial activity against mycobacteria. Moreover, reactive oxygen species mediate antibacterial autophagy and successful antimicrobial responses during antibiotic chemotherapy. Recent work has also shown that pathogenic Mtb can be targeted by selective autophagy through an ESX-1 type VII secretion system. Here, we review the triggers, host factors, and intracellular pathways that regulate host autophagy and its impact on antimicrobial host defenses during mycobacterial infection.</p>	Johnson et al. 2014
Subclinical MAP infection	<p>We wanted to determine if augmented innate immune activation is associated with lesion development in a mycobacterial enhanced intestinal injury model. We evaluated the local immune response in a <i>Mycobacterium avium</i> paratuberculosis + dextran sulfate sodium (Map + DSS) model using BALB/c and severe combined immunodeficient (SCID) mice. Map + DSS BALB/c and SCID mice displayed a similar disease phenotype. Moreover, Map + DSS SCID mice had increased expression of interleukin 1 beta (IL-1 beta), tumor necrosis factor alpha (TNF-alpha), inducible nitric oxide synthase (iNOS) and increased numbers of F4/80 positive cells. Additionally, Map antigen is co-localized with iNOS and IL-1 beta positive cells. This suggests that subclinical Map infection promotes innate immune activation following injury to the intestinal epithelium.</p>	Johnson et al. 2013
Glycans and glycan-binding proteins	<p>Glycans and glycan-binding proteins are central to a properly functioning immune system. Perhaps the best known example of this is the selectin family of surface proteins that are primarily found on leukocytes, and which bind to endothelial glycans near sites of infection or inflammation and enable extravasation into tissues. In the past decade, however, several other immune pathways that are dependent on or sensitive to changes in glycan-mediated mechanisms have been revealed. These include antibody function, apoptosis, T helper (Th)1 versus Th2 skewing, T cell receptor signaling, and MHC class II antigen presentation. Here, we highlight how regulated changes in protein glycosylation both at the cell surface and on secreted glycoproteins can positively and negatively modulate the immune response.</p>	Jones et al. 2013
Inflammatory diseases	<p>Autophagy is a cellular mechanism for the sequestration and degradation of intracellular pathogens and compromised organelles, particularly damaged mitochondria. Autophagy also clears other cellular components, such as inflammasomes and cytokines, thus providing an important means of regulating inflammation. Defects in autophagy have been found by genetic association studies to confer susceptibility to several autoimmune and inflammatory disorders, particularly inflammatory bowel disease. Thus, the manipulation of autophagy in disease situations is of growing interest for therapeutic targeting; however, the involvement of autophagy in cellular homeostasis, in normal immune function and in inflammation is manifold. An appreciation of the intricacies of the contributions of this process to inflammation, and how these are altered by various immune and environmental stimuli, is essential for the understanding and interpretation of studies of inflammation and the design of therapeutics exploiting the manipulation of autophagy. This review focuses on the known roles of autophagy in the induction and maintenance of inflammation and on its role in the aetiology and regulation of inflammatory and autoimmune disorders.</p>	
• autophagy		
• cellular homeostasis		

<p>Paratuberculosis</p> <ul style="list-style-type: none"> • probiotics • <i>Lactobacillus animalis</i> • IFN-gamma • Interleukin-1 alpha • IL-12 • IL-10 • IL-6 • TNF-alpha 	<p>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 <i>Lactobacillus animalis</i> (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.</p>	<p>Karunasena et al. 2013</p>
<p>Microbiota</p> <ul style="list-style-type: none"> • host immune system • innate immune receptors • intestinal homeostasis 	<p>Human intestines harbor a diverse microbial community composed of a large number of bacteria and other microorganisms. These intestinal microbiota have evolved to achieve a symbiotic relationship with the host. In addition to aiding host metabolic pathways by breaking down foods and supplying nutrients to their host, microbiota play an important role in the development and maintenance of the host immune system. At the same time, the detection of microorganisms and their products by innate immune receptors such as TLRs (Toll-like receptors) and NLR (nucleotide-binding domain, leucine-rich repeats, or NOD-like receptor) proteins are critical for maintaining intestinal homeostasis by shaping microbial communities. This review summarizes recent progress about the role of TLRs and NLRs in the regulation of intestinal microbiota. Accumulating evidence suggest that intestinal microbiota have a large impact on both intestinal and systemic diseases. Therefore, understanding the mechanism of microbial regulation by TLRs and NLRs is important for the advancement of therapeutic interventions against digestive and other diseases.</p>	<p>Kobayashi 2013</p>
<p>Bacterial recognition</p> <ul style="list-style-type: none"> • innate immunity • host defense • innate immune sensors 	<p>Microbial challenges to the host initiate an array of defense processes through the activation of innate and adaptive immunity. Innate immunity consists of sensors or pattern-recognition receptors (PRRs) that are expressed on immune and non-immune cells and sense conserved pathogen-derived molecules or pathogen-associated molecular patterns (PAMPs) in various compartments of the host cells. Recognition of the PAMPs by PRRs triggers antimicrobial effector responses via the induction of proinflammatory cytokines and type I IFNs. Several families of PRRs, such as Toll-like receptors (TLRs),</p>	<p>Kumar et al. 2013</p>

<p>NOD-like receptors (NLRs), RIG-I-like receptors (RLRs), and DNA sensors and their respective PAMPs have been well studied in innate immunity and host defense. Here, we review the recent findings on bacterial recognition by TLRs and NLRs and the signaling pathways activated by these sensors.</p>	<p>Peripheral blood mononuclear cells of Crohn's disease (CD) patients with the common 1007fs mutation of the caspase recruitment domain-containing 15/nucleotide-binding oligomerization domain-containing 2 (CARD15/NOD2) gene show impaired nuclear factor kappa B (NF-kappa B) activation in response to muramyl dipeptide (MDP), as determined by Western blotting. We applied phospho-specific flow cytometry to examine NF-kappa B and p38 activation in whole blood monocytes of 16 CD patients with or without the 1007fs and previously described rare mutations of the CARD15 gene, and healthy reference subjects. Aliquots of whole blood were supplemented with MDP (0–1000 ng/mL), incubated for 10–40 min and processed for flow cytometry. Bacterial lipopolysaccharide (LPS) was used as a positive control agonist. We found that NF-kappa B and p38 phosphorylation induced by MDP was not detectable in monocytes of patients homozygous for the CARD15 1007fs mutation, while those induced by LPS were normal. We also determined MDP-induced NF-kappa B phosphorylation levels in nuclear extracts of mononuclear cells separated from blood using enzyme-linked immunosorbent assay (ELISA), and observed that the levels decreased in a 1007fs mutation-dose dependent manner. We conclude that phospho-specific whole blood flow cytometry provides a means to study phosphorylation of NF-kappa B and p38 in clinical samples and can be applied to screening of CD patients homozygous for the CARD15 1007fs mutation.</p>	<p>Kuuliala et al. 2013</p>
<p>MDP-sensing pathway defects</p> <ul style="list-style-type: none"> • blood monocytes • CARD15 1007fs mutation 	<p>Autoantibodies</p> <ul style="list-style-type: none"> • glycoprotein 2 • commonalities in the immunopathogenesis of the CD and PTB <p>Mycobacterium avium subspecies paratuberculosis (MAP) induces paratuberculosis (ptb) in ruminants and has clinical and histological features resembling Crohn's disease (CD). Pancreatic autoantibodies (PAB) targeting glycoprotein 2 (GP2) are specifically found in CD, but it is currently unknown whether these autoantibodies can be found in ruminants with ptb. IgG anti-MAP and anti-GP2 antibodies were tested by ELISA in 286 ruminants (212 sheep and 74 cattle). PAB testing was performed by indirect immunofluorescence (IF) using anti-sheep or anti-cattle specific antisera. PCR analysis confirmed the presence of MAP in anti-MAP positive samples. Anti-GP2 antibodies were more prevalent in anti-MAP antibody positive (26.9%) than in anti-MAP negative ruminants (8.7%, $p < 0.001$). Anti-GP2 antibodies were found in 16/70 (22.9%) anti-MAP positive sheep compared to 10/142 (7%, $p = 0.001$) anti-MAP antibody negative and in anti-MAP positive cattle than in negative counterparts (5/8 versus 8/66, $p = 0.003$). Absorbance values for anti-GP2 antibodies were higher in cattle than in sheep (mean 21 AU/mL \pm 25.45D versus 12.2 AU/mL \pm 23 SD, $p < 0.001$). There was no correlation between anti-GP2 and anti-MAP antibody concentrations. Anti-GP2 antibodies persisted up to 1/1000 and showed the characteristic IIF pancreatic pattern seen by anti-GP2 antibody positive CD samples. This is the first study to demonstrate the presence of CD-specific GP2-reactive pancreatic autoantibodies in MAP-infected ruminants. Our data suggest that CD and ptb are characterised by an antigen-driven loss of immunological tolerance to GP2, implying commonalities in the immunopathogenesis of the human and ruminant inflammatory bowel disorder.</p>	<p>Liaskos et al. 2013</p>

<p>NOD2 mutations and CD</p> <ul style="list-style-type: none"> • human peripheral B lymphocytes • MDP stimulation 	<p>Genetic and functional studies have associated variants in the NOD2/CARD15 gene with Crohn's disease. Aims This study aims to replicate the association of three common NOD2 mutations with Crohn's disease, study its effect on NOD2 expression in B cells and its interaction with other IBD-associated genes. Methods A total of 294 IBD patients (179 familial IBD, 115 sporadic IBD) and 298 unrelated healthy controls were from central Pennsylvania. NOD2 mutations were analyzed by primer-specific amplification, PCR based-RFLP, and validated with the ABI SNPlex(M) genotyping system. Gene-gene interaction was studied using a statistical model for epistasis analysis. Results Three common NOD2 mutations are associated with Crohn's disease ($p = 5.08 \times 10^{-7}$), 1.67×10^{-6}), and 1.87×10^{-2}) for 1007fs, R720W, and G908R, respectively), but not with ulcerative colitis ($p = 0.1046$, 0.1269, and 0.8929, respectively). For IBD overall, 1007fsinsC ($p = 4.4 \times 10^{-5}$) and R720W ($p = 9.24 \times 10^{-5}$) were associated with IBD, but not G908R ($p = 0.1198$). We discovered that NOD2 significant interactions of NOD2 with other IBD susceptibility genes IL23R, DLG5, and OCTN1. We discovered that NOD2 was expressed in both normal human peripheral blood B cells and in EBV-transformed B cell lines. Moreover, we further demonstrated that muramyl dipeptide (MDP) stimulation of B lymphocytes up-regulated expression of NF-kappa B-p50 mRNA. Conclusion NOD2 is expressed in peripheral B cells, and the up-regulation of NOD2 expression by MDP was significantly impaired by NOD2 mutations. The finding suggests a possible role of NOD2 in the immunological response in IBD pathogenesis.</p>	Lin et al. 2013
<p>Autophagy pathway</p> <ul style="list-style-type: none"> • innate immunity • cellular homeostasis 	<p>Autophagy (macroautophagy; "self-eating") is a degradation process, in which cytoplasmic content is engulfed and degraded by the lysosome. And, immunity is an important mechanism of the "self-defense" system. Autophagy has long been recognized as a stress response to nutrient deprivation. This will provide energy and anabolic building blocks to maintain cellular bioenergetic homeostasis. Thus, autophagy plays critical roles in regulating a wide variety of pathophysiological processes, including tumorigenesis, embryo development, tissue remodeling, and most recently, immunity. The latter shows that a self-eating (autophagy) process could regulate a self-defense (immune) system. In this review, we summarize the recent findings regarding the regulatory and mechanistic insights of the autophagy pathway in immunity.</p>	Liu et al. 2013
<p>MAP and CD</p> <ul style="list-style-type: none"> • cause or epiphenomenon 	<p>The origin of inflammatory bowel disease is unknown. Attempts have been made to isolate a microorganism that could explain the onset of inflammation, but no pathological agent has ever been identified. Johne's disease is a granulomatous chronic enteritis of cattle and sheep caused by <i>Mycobacterium avium</i> subspecies paratuberculosis (MAP) and shows some analogies with Crohn's disease (CD). Several studies have tried to clarify if MAP has a role in the etiology of CD. The present article provides an overview of the evidence in favor and against the "MAP-hypothesis", analyzing the methods commonly adopted to detect MAP and the role of antimycobacterial therapy in patients with inflammatory bowel disease. Studies were identified through the electronic database, MEDLINE, and were selected based on their relevance to the objective of the review. The presence of MAP was investigated using multiple diagnostic methods for MAP detection and in different tissue samples from patients affected by CD or ulcerative colitis and in healthy controls. On the basis of their studies, several authors support a close relationship between MAP and CD. Although increasing evidence of MAP detection in CD patients is unquestionable, a clear etiological link still needs to be proven.</p>	Liverani et al. 2014

<p>Crohn's disease</p> <ul style="list-style-type: none"> • NOD2 HSP70 • molecular chaperone • inflammation • bacterial cell wall fragments 	<p>Microbes are detected by the pathogen-associated molecular patterns through specific host pattern recognition receptors. Nucleotide-binding oligomerization domain-containing protein 2 (NOD2) is an intracellular pattern recognition receptor that recognizes fragments of the bacterial cell wall. NOD2 is important to human biology; when it is mutated it loses the ability to respond properly to bacterial cell wall fragments. To determine the mechanisms of misactivation in the NOD2 Crohn mutants, we developed a cell-based system to screen for protein-protein interactors of NOD2. We identified heat shock protein 70 (HSP70) as a protein interactor of both wild type and Crohn mutant NOD2. HSP70 has previously been linked to inflammation, especially in the regulation of anti-inflammatory molecules. Induced HSP70 expression in cells increased the response of NOD2 to bacterial cell wall fragments. In addition, an HSP70 inhibitor, KNK437, was capable of decreasing NOD2-mediated NF-kappa B activation in response to bacterial cell wall stimulation. We found HSP70 to regulate the half-life of NOD2, as increasing the HSP70 level in cells increased the half-life of NOD2, and down-regulating HSP70 decreased the half-life of NOD2. The expression levels of the Crohn-associated NOD2 variants were less compared with wild type. The overexpression of HSP70 significantly increased NOD2 levels as well as the signaling capacity of the mutants. Thus, our study shows that restoring the stability of the NOD2 Crohn mutants is sufficient for rescuing the ability of these mutations to signal the presence of a bacterial cell wall ligand.</p>	<p>Mohan and Grimes 2014</p>
<p>Crohn's disease</p> <ul style="list-style-type: none"> • oral biopsies • MAP molecular identification 	<p>Oral lesions may be found in patients with Crohn's disease (CD), in a percentage up to 20%. The aim of this study was to investigate a possible relationship between <i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> (MAP) and oral lesions in CD patients. 23 oral biopsies were examined performing IS900 Nested PCR; 9 of them were positive: 8 from CD patients and 1 from a control. Our purpose is to go on with this study, amplifying the number of subjects examined and testing subjects with oral lesions related to diseases other than CD to verify the specific association between MAP and oral lesions in CD patients.</p>	<p>Molicotti et al. 2013</p>
<p>Effector T-cell</p> <ul style="list-style-type: none"> • protective immune responses • in vivo imaging 	<p>T-cell responses are initiated within secondary lymphoid organs, and effector T-cells are released into the circulation where they home to inflamed tissues and mediate protective immune responses. Within non-lymphoid tissues, the types of cellular interactions and the dynamics that lead to clearance of infections are still relatively poorly understood. Here I review how imaging of effector T-cells within tissues has contributed to our understanding of immune responses, and examine some of the remaining questions that may benefit from in vivo imaging to reveal the intricacies of how immune cells function. A detailed understanding of the dynamics of T-cell responses within non-lymphoid tissues is important for the rational design of targeted therapies that influence key steps in disease progression.</p>	<p>Mueller 2013</p>
<p>MAP in cow's milk</p> <ul style="list-style-type: none"> • DNA methylase genotyping • detection of up to 10 CFU of MAP per ml of milk 	<p>Paratuberculosis is an infectious, chronic, and incurable disease that affects ruminants, caused by <i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i>. 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 <i>M. avium</i> subsp. <i>paratuberculosis</i> in patients with Crohn's disease has been described; it is of interest to rapidly detect <i>M. avium</i> subsp. <i>paratuberculosis</i> in milk for human consumption. IS900 insertion is used as a target for PCR amplification to identify the presence of <i>M. avium</i> subsp. <i>paratuberculosis</i> in biological samples. Two target sequences were selected: IS1 (155 bp) and IS2 (94 bp). These fragments have a 100% identity among all <i>M. avium</i> subsp. <i>paratuberculosis</i> strains sequenced.</p>	<p>Mundo et al. 2013</p>

	<p>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.</p>	Nakamura et al. 2014
<p>Dendritic cells</p> <ul style="list-style-type: none"> • MDP • endosomes • innate immune response 	<p>The detection of microbial pathogens involves the recognition of conserved microbial components by host cell sensors such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs). TLRs are membrane receptors that survey the extracellular environment for microbial infections, whereas NLRs are cytosolic complexes that detect microbial products that reach the cytosol. Upon detection, both sensor classes trigger innate inflammatory responses and allow the engagement of adaptive immunity(1,2). Endo-lysosomes are the entry sites for a variety of pathogens, and therefore the sites at which the immune system first senses their presence. Pathogens internalized by endocytosis are well known to activate TLRs 3 and 7-9 that are localized to endocytic compartments and detect ligands present in the endosomal lumen(3). Internalized pathogens also activate sensors in the cytosol such as NOD1 and NOD2 (ref. 2), indicating that endosomes also provide for the translocation of bacterial components across the endosomal membrane. Despite the fact that NOD2 is well understood to have a key role in regulating innate immune responses and that mutations at the NOD2 locus are a common risk factor in inflammatory bowel disease and possibly other chronic inflammatory states(4,5), little is known about how its ligands escape from endosomes. Here we show that two endo-lysosomal peptide transporters, SLC15A3 and SLC15A4, are preferentially expressed by dendritic cells, especially after TLR stimulation. The transporters mediate the egress of bacterially derived components, such as the NOD2 cognate ligand muramyl dipeptide (MDP)(6,7), and are selectively required for NOD2 responses to endosomally derived MDP. Enhanced expression of the transporters also generates endosomal membrane tubules characteristic of dendritic cells, which further enhanced the NOD2-dependent response to MDP. Finally, sensing required the recruitment of NOD2 and its effector kinase RIPK2 (refs 8, 9) to the endosomal membrane, possibly by forming a complex with SLC15A3 or SLC15A4. Thus, dendritic cell endosomes are specialized platforms for both the luminal and cytosolic sensing of pathogens.</p>	Naser et al. 2014
<p>CD and MAP</p> <ul style="list-style-type: none"> • the role of MAP supported in 30%–50% of CD patients 	<p>Crohn's disease (CD) is a chronic inflammatory condition that plagues millions all over the world. This debilitating bowel disease can start in early childhood and continue into late adulthood. Signs and symptoms are usually many and multiple tests are often required for the diagnosis and confirmation of this disease. However, little is still understood about the cause(s) of CD. As a result, several theories have been proposed over the years. One theory in particular is that Mycobacterium avium subspecies paratuberculosis (MAP) is intimately linked to the etiology of CD. This fastidious bacterium also known to cause Johne's disease in cattle has infected the intestines of animals for years. It is believed that due to the thick, waxy cell wall of MAP it is able to survive the process of pasteurization as well as chemical processes seen in irrigation purification systems. Subsequently meat, dairy products and water serve as key vehicles in the transmission of MAP infection to humans (from farm to fork) who have a genetic predisposition, thus leading to the development of CD. The</p>	

<p>challenges faced in culturing this bacterium from CD are many. Examples include its extreme slow growth, lack of cell wall, low abundance, and its mycobactin dependency. In this review article, data from 60 studies showing the detection and isolation of MAP by PCR and culture techniques have been reviewed. Although this review may not be 100% comprehensive of all studies, clearly the majority of the studies overwhelmingly and definitively support the role of MAP in at least 30%-50% of CD patients. It is very possible that lack of detection of MAP from some CD patients may be due to the absence of MAP role in these patients. The latter statement is conditional on utilization of methodology appropriate for detection of human MAP strains. Ultimately, stratification of CD and inflammatory bowel disease patients for the presence or absence of MAP is necessary for appropriate and effective treatment which may lead to a cure.</p>		
<p>NOD2 activation by MDP</p> <ul style="list-style-type: none"> • proinflammatory immune response 	<p>NOD2 activation by muramyl dipeptide causes a proinflammatory immune response in which the adaptor protein CARD9 works synergistically with NOD2 to drive p38 and c-Jun N-terminal kinase (JNK) signalling. To date the nature of the interaction between NOD2 and CARD9 remains undetermined. Here we show that this interaction is not mediated by the CARDs of NOD2 and CARD9 as previously suggested, but that NOD2 possesses two interaction sites for CARD9; one in the CARD-NACHT linker and one in the NACHT itself. Structured summary of protein interactions: NOD2 physically interacts with CARD9 by anti tag coimmunoprecipitation.</p>	Parkhouse et al. 2014
<p>NOD proteins</p> <ul style="list-style-type: none"> • regulators of inflammation • host defence pathways • autophagy 	<p>Entry of bacteria into host cells is an important virulence mechanism. Through peptidoglycan recognition, the nucleotide-binding oligomerization domain (NOD) proteins NOD1 and NOD2 enable detection of intracellular bacteria and promote their clearance through initiation of a pro-inflammatory transcriptional programme and other host defence pathways, including autophagy. Recent findings have expanded the scope of the cellular compartments monitored by NOD1 and NOD2 and have elucidated the signalling pathways that are triggered downstream of NOD activation. In vivo, NOD1 and NOD2 have complex roles, both during bacterial infection and at homeostasis. The association of alleles that encode constitutively active or constitutively inactive forms of NOD2 with different diseases highlights this complexity and indicates that a balanced level of NOD signalling is crucial for the maintenance of immune homeostasis.</p>	Philpott et al. 2014
<p>Inflammatory diseases</p> <ul style="list-style-type: none"> • shifts in microbiome composition • disruption of microbiome homeostasis • innate immune response 	<p>The human body is a superorganism in which thousands of microbial genomes continually interact with the human genome. A range of physical and neurological inflammatory diseases are now associated with shifts in microbiome composition. Seemingly disparate inflammatory conditions may arise from similar disruption of microbiome homeostasis. Intracellular pathogens long associated with inflammatory disease are able to slow the innate immune response by dysregulating activity of the VDR nuclear receptor. This facilitates the ability of other species to gradually accumulate in tissue and blood, where they generate proteins and metabolites that significantly interfere with the body's metabolic processes. The microbes that contribute to this dysfunction are often inherited from family members. Immunosuppressive therapies for inflammatory disease allow pathogens driving these processes to spread with greater ease. In contrast to immunosuppression, treatments that stimulate the immune system seem to allow for reversal of this pathogen-induced genomic dysregulation.</p>	Proal et al. 2014

<p>Intestinal tuberculosis and CD</p> <ul style="list-style-type: none"> • cytokine gene expression • innate immune activation • T-helper 1 polarisation 	<p>Intestinal tuberculosis (TB) and Crohn's disease closely resemble each other clinically and morphologically. Little is known of cytokine regulation in intestinal TB. OBJECTIVE: To compare cytokine gene expression in colonic mucosa and peripheral blood mononuclear cells (PBMC) in TB with that in Crohn's disease. METHODS: Biopsies were obtained from normal and ulcerated colonic mucosa of 12 intestinal TB and 11 Crohn's disease patients, and PBMC from 15 intestinal TB and 12 Crohn's disease patients and 11 healthy volunteers. RNA was extracted, and the expression of selected cytokines, chemokines and pattern recognition receptors quantified by reverse transcriptase real-time polymerase chain reaction using SYBR green. RESULTS: The mRNA expression of interleukin-8 (IL-8), induced protein-10, tumour necrosis factor-α, IL-23 p19 and IL-12 p40, and Toll-like receptors (TLR) 1 and 2 in the ulcerated mucosa was increased in both intestinal TB and Crohn's disease. Expression of growth-related oncogene-α was increased in intestinal TB, while expression of interferon-γ (IFN-γ) and TLR 4, 5 and 9 was increased in Crohn's disease. Expression of RANTES (regulated upon activation, normal T-cell expressed and secreted) was decreased in Crohn's disease. Secretion of IFN-γ or IL-10 from PBMC was not significantly altered in either disease. PBMC mRNA expression of IL-1 β, IL-6 and IL-8 mRNA was upregulated in Crohn's disease, while that of IL-17 was upregulated in intestinal TB. CONCLUSIONS: Cytokine gene expression patterns in intestinal mucosa and PBMC of intestinal TB were remarkably similar to Crohn's disease, and demonstrated innate immune activation and T-helper 1 polarisation.</p>	<p>Pugazhendhi et al. 2013</p>
<p>Bacterial sensor NOD2</p> <ul style="list-style-type: none"> • CD risk factors • <i>Bacteroides vulgatus</i> • multihit disease model • gene-microbe interactions 	<p>Nod2 has been extensively characterized as a bacterial sensor that induces an antimicrobial and inflammatory gene expression program. Therefore, it is unclear why Nod2 mutations that disrupt bacterial recognition are paradoxically among the highest risk factors for Crohn's disease, which involves an exaggerated immune response directed at intestinal bacteria. Here, we identified several abnormalities in the small-intestinal epithelium of Nod2(-/-) mice including inflammatory gene expression and goblet cell dysfunction, which were associated with excess interferon-γ production by intraepithelial lymphocytes and Myd88 activity. Remarkably, these abnormalities were dependent on the expansion of a common member of the intestinal microbiota <i>Bacteroides vulgatus</i>, which also mediated exacerbated inflammation in Nod2(-/-) mice upon small-intestinal injury. These results indicate that Nod2 prevents inflammatory pathologies by controlling the microbiota and support a multihit disease model involving specific gene-microbe interactions.</p>	<p>Ramanan et al. 2014</p>
<p>CD murine model</p> <ul style="list-style-type: none"> • peptidoglycan-Polysaccharide • CBA/J mice 	<p>The peptidoglycan-polysaccharide (PGPS) model using inbred rats closely mimics Crohn's disease. Our aim was to identify mouse strains that develop ileocolitis in response to bowel wall injection with PGPS. Mouse strains studied included NOD2 knockout animals, RICK/RIP2 knockout animals, and genetically inbred strains that are susceptible to inflammation. Mice underwent laparotomy with intramural injection of PGPS or human serum albumin in the terminal ileum, ileal Peyer's patches, and cecum. Gross abdominal score, cecal histologic score, and levels of pro-fibrotic factor mRNAs were determined 20 to 32 days after laparotomy. PGPS-injected wild-type and knockout mice with mutations in the NOD2 pathway had higher abdominal scores than human serum albumin-injected mice. The RICK knockout animals tended to have higher mean abdominal scores than the NOD2 knockout animals, but the differences were not significant. CBA/J mice were shown to have the most robust response to PGPS, demonstrating consistently higher abdominal scores than other strains. Animals killed on day 26 had an average gross abdominal score of 6.1 ± 1.5, compared with those on day 20 (3.0 ± 0.0) or day 32 (2.8 ± 0.9). PGPS-injected CBA/J mice studied 26 days after laparotomy developed the most robust inflammation and most closely mimicked the PGPS rat model and human Crohn's disease.</p>	<p>Reingold et al. 2013</p>

<p>Host-microbe crosstalk</p> <ul style="list-style-type: none"> • innate immunity • missing trigger factor • multitude of civilization disorders 	<p>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.</p>	Rosenstiel 2013
<p>Synthetic muramyl peptides</p> <ul style="list-style-type: none"> • immunomodulation • 36 analogs tested 	<p>Muramyl peptides (MPs) represent the building blocks of bacterial peptidoglycan, a critical component of bacterial cell walls. MPs are well characterized for their immunomodulatory properties, and numerous studies have delineated the role of MPs or synthetic MP analogs in host defense, adjuvanticity and inflammation. More recently, Nod1 and Nod2 have been identified as the host sensors for specific MPs, and, in particular, Nod2 was shown to detect muramyl dipeptide (MDP), a MP found in both Gram-positive and Gram-negative bacterial cell walls. Because mutations in Nod2 are associated with the etiology of Crohn's disease, there is a need to identify synthetic MP analogs that could potentiate Nod2-dependent immunity. Here, we analyzed the Nod2-activating property of 36 MP analogs that had been tested previously for their adjuvanticity and anti-infectious activity. Using a luciferase-based screen, we demonstrate that addition of a methyl group to the second amino acid of MDP generates a MDP derivative with enhanced Nod2-activating capacity. We further validated these results in murine macrophages, human dendritic cells and <i>in vivo</i>. These results offer a basis for the rational development of synthetic MPs that could be used in the treatment of inflammatory disorders that have been associated with Nod2 dysfunction, such as Crohn's disease.</p>	Rubino et al. 2013
<p>Autoimmune diseases</p> <ul style="list-style-type: none"> • pathogenesis • molecular modification pathway • hyper-immune-inflammatory response pathway • pathogen-driven autoimmune-mimicry 	<p>Autoimmunity causes pathological conditions resulting in autoimmune diseases (ADs). Although autoimmunity is a mystery, immunological dogma suggests that autoreactive cell reactivation (ACR) breaks self-tolerance and induces autoimmunity. Thus, ACR is a royal pathway for ADs. Cumulative evidence implicates environmental factors as secondary triggers of ADs in the genetically susceptible hosts. Infection is the most likely trigger. Although several mechanisms have been proposed to explain how infectious agents trigger ADs, ACR is assumed to be an essential pathway. Here, by showing some exemplary ADs, we propose two novel pathways, "molecular modification pathway" and "hyper-immune-inflammatory response pathway", which induce AD-like conditions directly by infectious agents without ACR. These AD-like conditions are actually not true "ADs" according to the current definition. Therefore, we define them as "pathogen-driven autoimmune-mimicry (PDAIM)". Confirming PDAIM will open perspectives in developing novel fundamental and non-immunosuppressive therapies for ADs. The idea should also provide novel insights into both the mechanisms of autoimmunity and the pathogenesis of ADs.</p>	Saeki and Ishihara 2014

<p>CD and MDP</p> <ul style="list-style-type: none"> • intestinal inflammatory response 	<p>Crohn's disease (CD) is one of main disease entities under the umbrella term chronic inflammatory bowel disease. The etiology of CD involves alterations in genetic, microbiological, and immunological factors. This review is devoted to the role of the bacterial wall compound muramyl dipeptide (MDP) for the activation of inflammatory pathways involved in the pathogenesis of CD. The importance of this molecule is underscored by the fact that (1) MDP, which is found in most Gram-negative and -positive bacteria, is able to trigger several immunological responses in the intestinal system, and (2) that alterations in several mediators of the MDP response including-but not restricted to-nucleotide oligomerization domain 2 (NOD2) are associated with CD. The normalization of MDP signaling is one of several important factors that influence the intestinal inflammatory response, a fact which emphasizes the pathogenic importance of MDP signaling for the pathogenesis of CD. The important aspects of NOD2 and non-NOD2 mediated effects of MDP for the development of CD are highlighted, as well as how alterations in these pathways might translate into the development of new therapeutic strategies.</p>	Salem et al. 2013
<p>CD and MDP</p> <ul style="list-style-type: none"> • innate immune responses • N-acetylated MDP • N-glycyl MDP • mycobacteria • human peripheral blood mononuclear cells • TNF alpha 	<p>Recognition of bacterial peptidoglycan-derived muramyl-dipeptide (MDP) by nucleotide oligomerization domain 2 (NOD2) induces crucial innate immune responses. Most bacteria carry the N-acetylated form of MDP (A-MDP) in their cell membranes, whereas N-glycyl MDP (G-MDP) is typical for mycobacteria. Experimental murine studies have reported G-MDP to have a greater NOD2-stimulating capacity than A-MDP. Since NOD2 polymorphisms are associated with Crohn's disease (CD), a link has been suggested between mycobacterial infections and CD. Thus, the aim was to investigate if NOD2 responses are dependent on type of MDP and further to determine the role of NOD2 gene variants for the bacterial recognition in CD. The response pattern to A-MDP, G-MDP, Mycobacterium segmatis (M. smegmatis; expressing mainly G-MDP) and M. segmatisDeltanahH (expressing A-MDP), Listeria monocytogenes (LM) (an A-MDP-containing bacteria), and Mycobacterium avium paratuberculosis (MAP) (a G-MDP-containing bacteria associated with CD) was investigated in human peripheral blood mononuclear cells (PBMCs). A-MDP and M. segmatisDeltanahH induced significantly higher tumor necrosis factor (TNF)-alpha protein levels in healthy wild-type NOD2 PBMCs compared with G-MDP and M. segmatis. NOD2 mutations resulted in a low TNF-alpha protein secretion following stimulation with LM. Contrary to this, TNF-alpha levels were unchanged upon MAP stimulation regardless of NOD2 genotype and MAP solely activated.</p>	Salem et al. 2014
<p>Autism spectrum disorders</p> <ul style="list-style-type: none"> • environmental factors • imbalanced immune response • maternal infection or autoimmune Diseases • neuropeptide imbalance 	<p>Autism spectrum disorders (ASD) comprise a group of neurodevelopmental abnormalities that begin in early childhood and are characterized by impairment of social communication and behavioral problems including restricted interests and repetitive behaviors. Several genes have been implicated in the pathogenesis of ASD, most of them are involved in neuronal synaptogenesis. A number of environmental factors and associated conditions such as gastrointestinal (GI) abnormalities and immune imbalance have been linked to the pathophysiology of ASD. According to the March 2012 report released by United States Centers for Disease Control and Prevention, the prevalence of ASD has sharply increased during the recent years and one out of 88 children suffers now from ASD symptoms. Although there is a strong genetic base for the disease, several associated factors could have a direct link to the pathogenesis of ASD or act as modifiers of the genes thus aggravating the initial problem. Many children suffering from ASD have GI problems such as abdominal pain, chronic diarrhea, constipation, vomiting, gastroesophageal reflux, and intestinal infections. A number of studies focusing on the intestinal mucosa, its permeability, abnormal gut development, leaky gut, and other GI problem raised many questions but</p>	Samsam et al. 2014

studies were somehow inconclusive and an expert panel of American Academy of Pediatrics has strongly recommended further investigation in these areas. GI tract has a direct connection with the immune system and an imbalanced immune response is usually seen in ASD children. Maternal infection or autoimmune diseases have been suspected. Activation of the immune system during early development may have deleterious effect on various organs including the nervous system. In this review we revisited briefly the GI and immune system abnormalities and neuropeptide imbalance and their role in the pathophysiology of ASD and discussed some future research directions.

Heat-killed mycobacteria	Shenderov et al. 2013
• T cell responses	Although adjuvants are critical vaccine components, their modes of action are poorly understood. In this study, we investigated the mechanisms by which the heat-killed mycobacteria in CFA promote Th17 CD4(+) T cell responses. We found that IL-17 secretion by CD4(+) T cells following CFA immunization requires MyD88 and IL-1 beta/IL-1R signaling.
• inflammasome activation	Through measurement of Ag-specific responses after adoptive transfer of OTII cells, we confirmed that MyD88-dependent signaling controls Th17 differentiation rather than simply production of IL-17. Additional experiments showed that CFA-induced Th17 differentiation involves IL-1 beta processing by the inflammasome, as mice lacking caspase-1, ASC, or NLRP3 exhibit partially defective responses after immunization. Biochemical fractionation studies further revealed that peptidoglycan is the major component of heat-killed mycobacteria responsible for inflammasome activation. By assaying IL1b transcripts in the injection site skin of CFA-immunized mice, we found that signaling through the adaptor molecule caspase activation and recruitment domain 9 (CARD9) plays a major role in triggering pro-IL-1 beta expression. Moreover, we demonstrated that recognition of the mycobacterial glycolipid trehalose dimycolate (cord factor) by the C-type lectin receptor mincle partially explains this CARD9 requirement. Importantly, purified peptidoglycan and cord factor administered in mineral oil synergized to recapitulate the Th17-promoting activity of CFA, and, as expected, this response was diminished in caspase-1 and CARD9-deficient mice. Taken together, these findings suggest a general strategy for the rational design of Th17-skewing adjuvants by combining agonists of the CARD9 pathway with inflammasome activators.

Adjuvant-induced arthritis	Shi et al. 2014
• synthetic peptides	Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease mediated by T cells. The aim of the present study was to investigate the therapeutic efficacy of synthetic peptides (HP-R1, HP-R2 and HP-R3), derived from the sequence of 65-kD mycobacterial heat shock protein (HSP), in the treatment of RA using adjuvant-induced arthritis (AA) animal model. Methods: AA was induced by a single intradermal injection Freund's complete adjuvant in male Lewis rats. At the first clinical sign of disease, rats were administered nasally by micropipette of peptides or phosphate buffer saline (PBS). Disease progression was monitored by measurement of body weight, arthritis score and paw swelling. The changes of histopathology were assessed by hematoxylin eosin staining. The serum levels of tumor necrosis factor (TNF) - alpha and interleukin (IL)-4 were measured by enzyme-linked immunosorbent assay (ELISA). Results: The peptides efficiently inhibited the footpad swelling and arthritic symptoms in AA rats. The synthetic peptides displayed significantly less inflammatory cellular infiltration and synovium hyperplasia than model controls. This effect was associated with a suppression of pro-inflammatory cytokine TNF-alpha production and an increase of anti-inflammatory cytokine IL-4 production after peptides treatment. Conclusions: These results suggest that the synthetic peptides derived from HSP65 induce highly effective protection against AA, which is mediated in part by down-regulation of inflammatory cytokines, and support the view that the synthetic peptides is a potential therapy for RA that may help to diminish both joint inflammation and destruction.

<p>Mycobacterial and mouse HSP70</p> <ul style="list-style-type: none"> • immunomodulation • dendritic cells 	<p>Previously, it has been shown that heat shock protein 70 (HSP70) can prevent inflammatory damage in experimental autoimmune disease models. Various possible underlying working mechanisms have been proposed. One possibility is that HSP70 induces a tolerogenic phenotype in dendritic cells (DCs) as a result of the direct interaction of the antigen with the DC. Tolerogenic DCs can induce antigen-specific regulatory T cells and dampen pathogenic T cell responses. We show that treatment of murine DCs with either mycobacterial (Mt) or mouse HSP70 and pulsed with the disease-inducing antigen induced suppression of proteoglycan-induced arthritis (PGIA), although mouse HSP70-treated DCs could ameliorate PGIA to a greater extent. In addition, while murine DCs treated with Mt- or mouse HSP70 had no significantly altered phenotype as compared to untreated DCs, HSP70-treated DCs pulsed with pOVA (ovalbumin peptide 323-339) induced a significantly increased production of IL-10 in pOVA-specific T cells. IL-10-producing T cells were earlier shown to be involved in Mt HSP70-induced suppression of PGIA. In conclusion, this study indicates that Mt- and mouse HSP70-treated BMDC can suppress PGIA via an IL-10-producing T cell-dependent manner.</p>	Spiering et al. 2013
<p>CD and MAP</p> <ul style="list-style-type: none"> • exacerbation of an already existing intestinal disease 	<p>Crohn's disease (CD) is a chronic inflammatory disorder of the human gastrointestinal tract. Although genetic, immunological, environmental, and bacterial factors have been implicated, the pathogenesis is incompletely understood. The histopathological appearance of CD strikingly resembles John's disease, a ruminant inflammatory bowel disease, caused by <i>Mycobacterium avium</i> ssp. <i>paratuberculosis</i> (MAP), but a causative role of MAP in CD has not been established. In this work, we hypothesized that MAP might exacerbate an already existing intestinal disease. Methods: We combined dextran sulfate sodium (DSS)-induced colitis with MAP infection in mice and monitored the immune response and bacterial count in different organs. Results: An increased size of liver and spleen was observed in DSS-treated and MAP-infected animals (DSS + MAP) as compared with DSS-treated uninfected (DSS + PBS) mice. Similarly, DSS treatment increased the number and size of MAP-induced liver granulomas and enhanced the MAP counts in enteric tissue. MAP infection in turn delayed the mucosal healing of DSS-induced tissue damage. Finally, high numbers of MAP were found in mesenteric fat tissue causing large granuloma and necrotic regions. Conclusions: Taken together, we present an in vivo model to study the role of MAP infection in CD. Our results confirm the hypothesis that MAP is able to exacerbate existing intestinal inflammation.</p>	Suwandi et al. 2014
<p>CD-associated mutations and polymorphisms</p> <ul style="list-style-type: none"> • immune deficiency • from apparent paradox to evolving paradigm 	<p>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.</p>	Vinh and Behr 2013

<p>Peptidoglycans</p> <ul style="list-style-type: none"> • enzymatic digestion • NOD2 recognition • host's immunomodulation process 	<p>Nucleotide oligomerization domain-containing protein 2 (Nod2), an innate immune receptor, recognizes bacterial cell-wall peptidoglycan (PGN), the minimum ligand of which is muramyl dipeptide (MDP). Enzymatic digestion of PGN appears to be important for Nod2 recognition. PGN is degraded by muramidase or glucosamidase through a process that produces two types of glycan sequence; glycans containing GlcNAc(14)MurNAc or MurNAc(14)GlcNAc. In this report, a range of disaccharide or tetrasaccharide fragments of each sequence were chemically synthesized, and their activities in stimulating human Nod2 (hNod2) were investigated. The results reveal that hNod2 recognitions is dependent on the glycan sequence, as demonstrated by comparing the activities of glycans with the same peptide moieties. (MurNAc(14)GlcNAc)2-containing structures exhibited stronger activity than those containing (GlcNAc(14)MurNAc)2. The results suggest that differences in the enzymatic degradation process affect the host's immunomodulation process.</p>	Wang et al. 2013
<p>Allergic asthma</p> <ul style="list-style-type: none"> • activation of eosinophils • bacterial infection mediated activation of NOD 	<p>Key intracytosolic pattern recognition receptors of innate immunity against bacterial infections are nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). We elucidated the NOD1 and NOD2-mediated activation of human eosinophils, the principal effector cells for allergic inflammation, upon interacting with human bronchial epithelial BEAS-2B cells in allergic asthma. Eosinophils constitutively expressed NOD1,2 but exhibited nonsignificant responses to release chemokines upon the stimulation by NOD1 ligand gamma-D-glutamyl-meso-diaminopimelic acid (iE-DAP) and NOD2 ligand muramyl dipeptide (MDP). However, iE-DAP and MDP could significantly upregulate cell surface expression of CD18 and intercellular adhesion molecule (ICAM)-1 on eosinophils and ICAM-1 on BEAS-2B cells, as well as induce chemokines CCL2 and CXCL8 release in the coculture system (all $P < 0.05$). Both eosinophils and BEAS-2B cells were the main source for CXCL8 and CCL2 release in the coculture system upon iE-DAP or MDP stimulation. Direct interaction between eosinophils and BEAS-2B cells is responsible for CCL2 release, and soluble mediators are implicated in CXCL8 release. ERK and NF-kappa B play regulatory roles for the expression of adhesion molecules and chemokines in coculture. Treatment with NOD1,2 ligand could induce the subepithelial fibrosis and significantly enhance the serum concentration of total IgE, chemokine CCL5 for eosinophils and T helper type 2 (Th2) cells and asthma Th2 cytokine IL-13 in bronchoalveolar lavage fluid of ovalbumin-sensitized allergic asthmatic mice (all $P < 0.05$). This study provides further evidence of bacterial infection-mediated activation of NOD1,2 in triggering allergic asthma via the activation of eosinophils interacting with bronchial epithelial cells at inflammatory airway.</p>	Wong et al. 2013
<p>Biosynthetic pathway of glycans</p> <ul style="list-style-type: none"> • epigenetics • environmental factor 	<p>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</p>	Zoldos et al. 2013

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.

CD and peptidoglycan recognition proteins	Inflammatory bowel disease (IBD) is a common disease, includes Crohn's disease (CD) and ulcerative colitis (UC), and is determined by altered gut bacterial populations and aberrant host immune response. Peptidoglycan recognition proteins (PGLYRP) are innate immunity bactericidal proteins expressed in the intestine. In mice, PGLYRPs modulate bacterial populations in the gut and sensitivity to experimentally induced UC. The role of PGLYRPs in humans with CD and/or UC has not been previously investigated. Here we tested the hypothesis that genetic variants in PGLYRP1, PGLYRP2, PGLYRP3 and PGLYRP4 genes associate with CD and/or UC and with gender and/or age of onset of disease in the patient population. We sequenced all PGLYRP exons in 372 CD patients, 77 UC patients, 265 population controls, 210 familial CD controls, and 24 familial UC controls, identified all polymorphisms in these populations, and analyzed the variants for significant association with CD and UC. We identified 16 polymorphisms in the four PGLYRP genes that significantly associated with CD, UC, and/or subgroups of patient populations. Of the 16, 5 significantly associated with both CD and UC, 6 with CD, and 5 with UC. 12 significant variants result in amino acid substitutions and based on structural modeling several of these missense variants may have structural and/or functional consequences for PGLYRP proteins. Our data demonstrate that genetic variants in PGLYRP genes associate with CD and UC and may provide a novel insight into the mechanism of pathogenesis of IBD.	Zulfiqar et al. 2013
• genetic variants		
• mechanism of pathogenesis		

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