REVIEW

Title page

The association of nontuberculous mycobacteria with immune-mediated chronic inflammatory and autoimmune diseases: a call for action

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Abstract

Background
The immunomodulators are capable of triggering chronic inflammatory and autoimmune disorders, often dubbed as “diseases of civilization”. Considerable evidence has been accumulated in recent years regarding the aetiological participation of nontuberculous mycobacteria in several serious chronic inflammatory and autoimmune diseases.

Main body
The derivatives of the peptidoglycans of mycobacterial cell walls act as immunomodulators after ingestion or inhalation. During the last decades, human exposure to nontuberculous bacteria has increased, and an increase in the incidence of serious chronic inflammatory and autoimmune diseases has generally followed. Exposure to mycobacteria has considerably increased with urbanisation, mainly with use of municipal water distribution systems. Increased exposure burden is also caused by changes in cattle farming, by the move to global marketing of cattle infected with paratuberculosis and by global marketing of powdered infant formula produced from cow milk frequently containing high numbers of *Mycobacterium avium* subsp*. paratuberculosis*. The most vulnerable group for exposure to mycobacterial triggers of chronic inflammatory and autoimmune disorders are undoubtedly new-borns and infants with immature immune systems. Dairy products and water are currently not tested for mycobacteria.

Conclusion
The pathogenic activity of mycobacteria is mediated through an impairment of a variety of elements of innate and adaptive immunity. Clinical disease is not a regular outcome of all exposures, as it presumably depends on the quantity of mycobacteria consumed, the stage of life and frequency of exposure, prior sensitisations and on the genetic predisposition. The first step in correcting the problem must be official statements by health and food hygiene authorities that human exposure to mycobacterial triggers poses a definite risk with respect to immune-mediated chronic inflammatory and autoimmune diseases. Mycobacteria should be subject to compulsory food hygienic inspection of powdered infant formulas. Self-imposed voluntary inspection would be highly desirable for milk, ice cream, drinking water (both tap and bottled) and beef from cows slaughtered at ages higher than 30 months. Interest of consumers of milk, ice cream, and beef hamburgers, in products with limited mycobacterial counts, would provide the necessary pressure on the producers to reduce the public exposure to mycobacterial triggers.

Keywords
Trigger, Chronic inflammatory diseases, Immunomodulator, Paratuberculosis, Food safety, Water

List of abbreviations

CD	Crohn's disease
CE	cell equivalent
CFA	Complete Freund Adjuvant
cfu	colony forming units
CNS	central nervous system
HSP	heat shock protein
IBD	inflammatory bowel disease
LPS	lipopolysaccharide
M. spp	Mycobacterium spp.
MDP	muramyl dipeptid
MAP	*Mycobacterium avium* subsp*. paratuberculosis*
MB	mycobacteria
MS	multiple sclerosis
NTM	nontuberculous mycobacteria
PD	Parkinson disease
T1D	type 1 diabetes mellitus

Mycobacteria – sophisticated and treacherous microorganisms with multifaceted pathogenesis

Mycobacteria have been found in 8,000-year-old human bones, and the diseases traditionally associated with these bacteria, tuberculosis and leprosy, have had a catastrophic impact on human health up to the 20th century. Nontuberculous mycobacteria have been so far considered saprophytes or at the most opportunistic pathogens (Table 1). The history of mycobacterial involvement in delayed-type hypersensitivity goes back to 1882, when Robert Koch described the tuberculin reaction. In the 1940s, Landsteiner and Chase [1] proved that the reaction was mediated by the cellular arm of the immune system. Both live and inactivated mycobacteria act as immunomodulators, and they do so after ingestion or inhalation. These activities are properties of the derivatives of the peptidoglycans of mycobacterial cell walls. The N-acetyl muramyl-dipeptide was designated as the smallest adjuvant molecule already in 1974 [2]. N-glycolyl MDP, a constituent of mycobacteria, is more potent and efficacious at inducing innate responses and T cell-mediated immunity than N-acetyl MDP derived from most bacteria [3,4]. In accordance with these properties of mycobacteria, in addition to being conventional pathogens, the importance of nontuberculous mycobacteria as immunomodulatory environmental pollutants has emerged in the last two decades [5-7].

Table 1

During the last decades, human exposure to nontuberculous bacteria has increased dramatically, and interestingly, an increase in the incidence of serious chronic inflammatory and autoimmune diseases has generally followed [8,9]. The public is generally surrounded by, and exposed to mycobacteria, and human activities contribute to their environmental build-up [10,11]. Exposure to mycobacteria has considerably increased as a consequence of the phenomena associated with urbanisation, e.g., use of municipal water distribution systems, increase of airborne dust, the use of indoor and outdoor recreational swimming pools and the practice of baby swimming. Increased exposure burden is caused by changes in the technologies used in cattle farming, by the move to global marketing of cattle infected with paratuberculosis and by global marketing of powdered infant formula produced from cow milk frequently containing high numbers of *Mycobacterium avium* subsp*. paratuberculosis* (MAP). It is this powdered infant formula that harbours an especially great potential to negatively affect public health in the long term [12,13]. In addition, the widespread consumption of fast food prepared from infected ground beef can also be part of this mycobacterial assault on public health [14]. Inhalation of aerosol contaminated with mycobacteria in showers, whirlpools and indoor swimming pools represents yet another path of human exposure. A further increase in the mycobacterial burden can be expected from the application of hydroponic and aeroponic technologies in high-capacity growing systems.

The clear chronological association between the worldwide build-up of mycobacteria in the environment and the appearance of chronic inflammatory and autoimmune diseases has been well documented. The most plausible mechanism of mycobacterial pathogenesis of these disorders involves the immunomodulatory substances known to be released by both live and dead

mycobacteria. Such immunomodulatory triggers have only been widely studied during the last 15 years, but it is already obvious that human technological and environmental developments are the driving force behind the sharp rise in immune-mediated chronic inflammatory and autoimmune diseases (Table 2).

Table 2

Environmental factors play a big role in introducing ambiguity to the classification of diseases, whether through pollutants, physical factors, socioeconomic status or other factors. Consequently, the same aetiological factor can initiate different pathways of pathogenesis due to host differences, which in turn induce different clinical manifestations of the disease, or even diseases considered to be entirely distinct nosological entities. Frequently reported co-morbidities, together with clustering of the affected subjects indicate the involvement of the same or similar trigger factor. While Alzheimer's disease is not listed in Table 2, a frequent association of mycobacterioses and dementia has been noted [15-20]. Similarly, a recently published case report has described neutrophilic urticarial dermatosis concurrent with inflammatory bowel disease and systemic lupus erythematosus [21]. However, interpretations of clinical observations of various chronic inflammatory and autoimmune disorders and their analyses often do not take into consideration the new and changing paradigms of mycobacterial immunopathogenetic capacity [7]. Most authors, however, suspect the involvement of unknown environmental contributing factors in the causality of this class of disorders. Mycobacterial immunomodulatory triggers fulfil the criteria for the missing "environmental contributing factor" very well.

The well-documented relatively recent worldwide build-up of nontuberculous bacteria, and their marked capacity to trigger a variety of immunopathological processes, has, however, not yet fully permeated the awareness of medical practitioners, medical educators, various related research fields and that of the general public. Powerful evidence regarding the correlation between the significant environmental increase in mycobacteria from bovine infections with *M. paratuberculosis* and the increase in the incidence of Crohn's disease has been well documented in Iceland and in the Czech Republic [7]. Based on the knowledge of the historical events leading to the spread of *M. paratuberculosis,* it can be projected that the long-term environmental burden from these mycobacteria will almost certainly continue to increase worldwide.

Important characteristics of mycobacteria that lead to high rates of human exposure

Mycobacteria are perfectly equipped for survival in water and soil, due to their remarkable properties, like the ability to grow in nutritionally poor environments, resistance to higher temperatures and common disinfectants as well as their ability to replicate over an unusually large range of temperatures [11,22]. Mycobacteria grow well in the biofilms created within household plumbing and also within amoebas [23-26]. In addition they can be found in host macrophages [27]. Furthermore, their survival

is aided by their abilities to form spores [28] and to change the structure of their cell membranes [29]. Mycobacteria easily enter into aerosol created during showering or by surf breaks. Mycobacteria are, however, also able to “jump out” of still waters, by first accumulating in water-air interfaces and then being propelled into the air with the release of air bubbles [30]. Mycobacteria survive in river water and also in sediments for many months and even years [31,32]. Mycobacterial ecology is described in detail in a monograph published in 2009 [33] and in many review papers [10,31,34-37].

Thus, nontuberculous bacteria not only act as conventional pathogens in people and animals (Table 1), but perhaps more importantly also release immunomodulatory substances (Table 3) causing chronic inflammatory and autoimmune diseases (Table 4).

Table 3

Table 4

The immunomodulators are capable of triggering chronic inflammatory and autoimmune disorders, often dubbed as “diseases of civilization”, the aetiology of which, however, remains widely debated among researchers. The tissues affected, and the clinical symptoms exhibited, often vary widely, and clinical symptoms often manifest only with a delay of several years [7,38,39]. Considerable evidence has been accumulated in recent years regarding the aetiological participation of nontuberculous mycobacteria in several serious diseases like Crohn’s disease, Type 1 diabetes, multiple sclerosis, asthma, psoriasis, etc. The degree of clinical manifestation of these chronic inflammatory diseases is variable, as it likely depends on the number of mycobacterial cells entering the host, the route of exposure, previous priming, the length of action in the host, the host age, genetic factors, general immune competence as well as on other factors.

Human exposure may occur orally with food or water, or by inhalation in dusty environments or during showering or swimming in indoors pools or heavily contaminated rivers or lakes. Generally, the exposed individuals are unaware of the danger, because there are no immediate symptoms of acute disease. After a period of latency, any subsequent symptoms of chronic inflammatory or autoimmune disorders that may develop are difficult to associate with the past exposure, regardless of whether the exposure was short and substantial, or cumulative over an extended period of time. The most vulnerable group for exposure to mycobacterial triggers of chronic inflammatory and autoimmune disorders are undoubtedly new-borns and infants with immature immune systems. This mainly occurs in those children who are not breastfed, and whose dried formula or the water used for the preparation of such formula, may contain high numbers of mycobacteria.

Mycobacterium avium subsp*. parat*uberculosis and other mycobacterial species have been linked to several immune-mediated chronic inflammatory and autoimmune diseases. Presumably, mycobacterial constituents act as triggers of these diseases in conjunction with certain host genetic factors or functionally altered leukocytes. This results in interference in certain metabolic pathways,

which then leads to pathological processes in respective target tissues. It is important to keep in mind that this aetiological function can be accomplished even by exposure to dead mycobacteria. In the absence of trials involving long-term or even lifetime follow-up of humans deliberately exposed to mycobacteria, which would be clearly ethically impermissible, the best experimental evidence for the immunopathogenic activity of *Mycobacterium avium* subsp*. paratuberculosis* in humans with respect to Crohn's disease comes from Golan et al. [40]. This report significantly extended the previous evidence of MAP detection in breast milk, blood and intestinal tissues of patients with Crohn's disease [41] by determining that in the human small intestine xenograft model, MAP actively invades epithelial goblet cells and induces tissue damage and inflammation.

Non-tuberculous mycobacteria are widely present in the environment

The sources of human exposure to nontuberculous mycobacteria are water (Table 5), aerosols, food (Table 6) and soil [42,43].

Table 5

Table 6

So far, attention has been primarily directed towards speciation of isolates obtained by sample cultivation in liquid or solid media. However, current methods of reproducible determination of the total count of living and dead mycobacteria are not suitable for routine screening. Moreover, in the course of establishing the counts of mycobacteria in water and food according to the colony forming units, the number of the bacterial cells is significantly underestimated (Table 7).

Table 7

Taken together, the above observations demonstrate that any finding of mycobacteria in water or food has to be acknowledged as a risk, irrespective of the viability or the number of cells present.

Worldwide failure of mycobacterial control

Non-tuberculous mycobacteria have generally only been considered to be opportunistic pathogens of humans. In addition, pasteurisation of milk and heat treatment of meat during food preparation have been considered sufficient measures for devitalisation of these bacteria. However, there are serious problems with this rationale, as many studies have shown that approximately 2% of samples of pasteurised retail milk contained cultivable live bacteria. More importantly, even the bacteria that are killed by milk pasteurisation or heat treatment of meat are not rendered harmless, as they can still act as triggers of immunologically mediated chronic inflammatory and autoimmune disorders in people.

Dairy products and water are currently not tested for mycobacteria, even though they do pose a great risk. Paratuberculosis in cattle is currently generally not considered to be very important, because in infected animals it may be subclinical for years, and it generally does not cause mass mortality. Calves get infected from their mothers or from the contaminated environment, and the disease appears often only after two or three years. Diarrhoea is the main clinical symptom, but initially it does not cause concern for the farmers. Loss of body condition and cachexia develop only after months or years. During the course of the infection cows shed large numbers of MAP in milk and faeces (up to 10^4 per millilitre of milk and 10^9 per gram of faeces). One infected cow daily sheds up to $4x10^{13}$ MAP cells in faeces and millions in milk. Beef can also contain mycobacteria (up to 10^4 per gram). The problem is compounded by the fact that paratuberculosis in cattle has undergone huge global spread during the last decades due to the very active trade of cows. The bacteria cannot be detected during the latent phases of the infection, and serological detection of carriers is not reliable. Therefore, movement of animals among farms is tolerated, and international trade is not regulated with respect to the spread of paratuberculosis. Currently, in many countries, up to 90% of farms are affected. Paratuberculosis affects not only cattle, but also buffaloes, camels, sheep and goats, and the milk and meat of these animals also poses a risk to the public [44].

It also needs to be pointed out that the above-mentioned immunological triggers can be released not only from *Mycobacterium avium* subsp*. paratuberculosis*, but also from other non-tuberculous mycobacteria commonly present in the environment. Up to 10,000 mycobacteria can be present in 1 ml of tap water and up to 8 million in one package of powdered infant milk. The health of infants may also be endangered if such water is used for formula, food preparation or bathing. Once again, mycobacteria are not subject to control; thus, exposure of formula-fed babies can may occur not only through dried milk powder, but also from the water used to prepare the formula and later in the life from vegetable-based meals.

Interactions between environmental factors and mucosal tissues in the early neonatal period and infancy may be critical in directing and controlling the expression of disease-specific responses in later life [45]. Powdered infant formula has been the subject of intensive research since 1991, undoubtedly due to the huge increase in production and globally marketing of this food product which saves the lives of new-borns and infants who cannot be breastfed. Formulas are made from milk with unknown numbers of live and dead MAP, unless the manufacturer controls for this bacterial contaminant. There are no regulatory measures to keep MAP under control. This situation has the potential to negatively affect the health of infants in the long term, worldwide. The danger for the health of children consuming such milk, the main constituents of infant formulas, stems from the presence of triggers of several serious chronic diseases. The benefits of breast colostrum and milk in protection against infectious agents have been well-studied and are indisputable. However, bottle-fed children are not protected from the triggers, as indicated by their higher incidence of chronic inflammatory and autoimmune disorders. This evidence is highly suggestive of an aetiological association between formula and this class of diseases.

Importantly, the level of human exposure has been dramatically increasing in the last decades due to the global spread of paratuberculosis in cattle, small ruminants, buffaloes and camels. High levels of mycobacteria are present at certain stages of bovine paratuberculosis in milk and meat of clinically healthy animals. Such products are still marketed without control of mycobacteria contamination, although their impact on public health is obvious. The production of mycobacteria-free or mycobacteria-low infant powdered milk should be regarded as a priority. Human exposure to nontuberculous mycobacteria has been intensified not only through the globalisation of foodstuffs and animal trade, but also through the increasing density of urban inhabitation accompanied by the municipal distribution of water and by global climate change. Further increases in global warming and urbanisation are expected [46,47].

Mycobacteria and the so-called "diseases of civilization"

Both the world-wide trend for human migration from rural to urban areas and the development of new technologies in the dairy cattle industry have had a negative impact on human health worldwide, due to the alarming rise in the incidence of non-tuberculous mycobacteria. The accumulating evidence from the published research data regarding the threat to public health of these bacteria, formerly considered innocuous, has been ignored for more than 50 years. The preponderance of the evidence strongly suggests that these ubiquitous mycobacteria are the triggers responsible for the alarming global rise in the incidence of chronic immune-mediated inflammatory and autoimmune diseases. In this respect, Type 1 diabetes, Crohn's disease, multiple sclerosis, asthma, psoriasis, rheumatoid arthritis and others, have been subject to extensive studies. In spite of the fact that the triggers for the development of these chronic inflammatory and autoimmune diseases are bacterial, the aetiology, pathogenesis, diagnosis and therapy of these conditions are vastly different from common infectious diseases. The pathogenic activity of mycobacteria is indirect, and is mediated through an impairment of a variety of elements of innate and adaptive immunity. Conversely, confirmation of the presence of mycobacteria, whether by culture or through the presence of their DNA, may not be associated with any disease, or disease may become clinically manifested only after several years.

The impact of civilisation factors, also called "westernisation of lifestyle", on human exposure to mycobacteria can be summarised as follows:

- More people are dependent on municipal water supplies
- Environment and foodstuffs are important sources of mycobacteria due to the paratuberculosis pandemic in animals
- Pre-term born babies are mostly fed by formula, and more of these infants survive this critical period of life
- More people practice jogging and swimming in polluted environments
- Even some current technological progress like cattle slurry spraying [48,49] and the use of air blowers for cleaning of sidewalks and public spaces [50] contribute to human exposure to mycobacteria via the respiratory route

Conclusions

The published data present convincing evidence of the following:

- Nontuberculous mycobacteria are opportunistic pathogens, particularly for people with compromised immunity (infants, seniors, transplant recipients, persons infected with HIV and other immunosuppressive infections and non-infectious immunosuppressive conditions like certain cancers)
- Mycobacteria release immuno-modulatory triggers, causing chronic inflammatory and autoimmune disorders
- Mycobacteria enter the hosts orally (food, water), by inhalation, via damaged skin, by contaminated catheters and endoscopic instruments, during tooth extraction or loss, during percutaneous procedures under ultrasound examinations, during ultrasound examinations in the vicinity of injury or surgical incision or during tattooing, etc.
- *M. avium* subsp*. paratuberculosis* can be shed in high numbers in the milk of cows with subclinical paratuberculosis, and they have also been found in dairy products and beef
- Nontuberculous mycobacteria are rather commonly detected in drinking water, rivers, swimming pools, municipal water distribution systems and even in bottled mineral and table waters
- Mycobacteria survive for a long time not only in the environment, but also in some disinfection solutions, in gels used for ultrasound examination or in tattoo ink

Starting in the 1960s, publications on nontuberculous mycobacteria have appeared in the Web of Science database (Clarivate Analytics) with increasing frequency. Among the most active authors are J. Kazda (50 publications, 1964–2005), J. O. Falkinham (118 publications, 1976–2017) and I. Pavlik (177 publications, 1994–2017). The number of annual publications retrieved with the keyword “paratuberculosis” ranged between one and 16 between 1945 and 1989, but it increased to more than 200 publications annually in the 2006 to 2017 period. Search results for “Crohn” retrieved less than ten papers per year from 1945 to 1960 and less than 1,000 from 1961 to 1995. However, there were 3,000 to 4,000 publications annually from 2011 to 2017 [51](updated by KH).

The impact of mycobacteria on human chronic inflammatory diseases can only be studied indirectly, as experimental studies are ethically unacceptable. Nevertheless, many rigorous, well-executed studies are available, and based on empirical data many hypotheses have been proposed. Unfortunately, the interpretation of these studies is often done without full awareness of the complementary and compounding nature of these results, just because they come from seemingly unrelated disciplines. The disregard for the pathogenic potential of mycobacteria has reached such proportions that even a comprehensive review regarding Crohn’s disease and mycobacterioses [52] does not mention MAP and its immunopathogenic capacity. The well-documented connection between chronic inflammatory and autoimmune diseases on one side, and mycobacterial triggers on the other, offers in our view a plausible interpretation of the current known facts. (Table 8).

Table 8

The aim of this commentary is to bring the attention of the reader to the global risk posed by mycobacteria. In particular, we call for focus on baby formula, in which triggers released from the mycobacteria present can significantly influence the development of the baby’s immune responsiveness. As documented in papers cited in this commentary, in spite of the accumulating evidence in this regard for over 100 years, the association with chronic inflammatory and autoimmune

disorders has become buried in squabbles over proposed competing hypotheses, and, regrettably, it remains woefully underestimated.

Ample evidence for the important role of mycobacterial triggers associated with chronic inflammatory diseases, particularly in the cell-mediated arm of the response, comes from the analysis of many publications regarding Complete Freund Adjuvant (CFA), intensively used in research since the late 1950s. It is the mycobacterial component that makes the discussion of CFA relevant for the purpose of this report. In addition to its action as a powerful immune adjuvant, there has been a dawning realisation that CFA also causes serious side effects by acting on the same pathways of the immune response. As a result, CFA has been banned for use in human immunisation [53] because of these serious and debilitating side effects [54]. The unacceptable negative effects on the recipients of the CFA were unambiguously linked to the inactivated mycobacterial content, which prompted the search for mycobacterial subunits that would retain the potent immunostimulatory activity of the bacteria but lack the undesirable toxic side effects. The minimal structure needed for the immunomodulatory effect of CFA was identified as N-acetyl muramyl-lalanyl-D-isoglutamine [2]. However, even this structure was still too toxic for humans [53]. Synthetic N-glycolyl MDP, the constituent of mycobacteria, is more potent and efficacious at inducing innate responses and T cell-mediated immunity than N-acetyl MDP derived from most bacteria [3,4]. Some mechanisms exerted by immunomodulators are listed in Table 5. This gives cause for alarm, especially as oral and respiratory exposure of humans and particularly children to mycobacteria is rapidly increasing but still unacknowledged as a very probable serious health risk.

Immunomodulatory triggers (Table 4) may impact either leukocyte activities or target tissues, or they may interfere with host metabolic pathways. The net effect of the triggers is undoubtedly influenced by the quantity of mycobacteria and the length of exposure, by whether previous sensitisation has taken place, by the stage of the host's immune development and host genetic factors. Epigenetic factors have also been found to be play a role [55]. The fact that triggers participate in the aetiology of diseases with diverse symptomatologies can be explained by their variable natures, the route of exposure, the affected metabolic pathways and the state of target host tissues. By far the most information in this respect has been obtained on triggers present in the mycobacterial cell wall. Consequently, the global rise in the incidence of bovine paratuberculosis is closely paralleled by a dramatic increase in the prevalence of chronic inflammatory and autoimmune diseases, often dubbed "diseases of civilization". However, the already established critical role of mycobacteria in the causation of chronic inflammatory and autoimmune disorders is still underestimated by some.

In conclusion, there are large amount of research data strongly suggesting that mycobacteria in food and water are dangerous, and that it does not matter if they are consumed live or dead. However, clinical disease is not a regular outcome of all exposures, as it presumably depends on the quantity of mycobacteria consumed, the stage of life and frequency of exposure, prior sensitisations and on the genetic predisposition of the affected person. This makes the causative association of mycobacteria with inflammatory and autoimmune conditions clinically less obvious.

Recommendations

The first step in correcting the problem must be official statements by health and food hygiene authorities that human exposure to mycobacterial triggers poses a definite risk with respect to immune-mediated chronic inflammatory and autoimmune diseases. In the European Union, such an official document should be requested by the European Commission from the European Food Safety Authority as the Scientific Opinion of EFSA and should be followed by European Commission Regulation. Similar legal procedures should proceed globally to manage the global threat of powdered infant milk; such initiatives would gradually decrease the risk posed by exposure.

Various approaches to lowering the risk associated with human exposure to mycobacterial triggers of chronic inflammatory and autoimmune diseases are summarised in Table 9. In our view, only the requirement for “MAP-free” powdered infant milk requires strict regulations. Other commodities would not require introduction of regulations, but voluntary testing may be suggested to manufacturers of dairy products, ground beef, fruits and vegetables and bottled drinking water, as a means of product quality promotion. Public swimming pool water, water of spa facilities, hospitals and dental clinics should be tested at the expense of the owners of businesses. In all cases where the products or water are designated “Mycobacteria-free” or “Mycobacteria-low” (lower than 500 cells equivalent) there should be regulations in place enabling monitoring of the declared status, associated with enforcement of sanctions for false labelling and deception of consumers.

Table 9

The public has to have access to general information and to answers to the important questions (Table 9). The responsibility for public awareness of the importance of mycobacteria as participants in the causation of chronic diseases lies firstly with the public health authorities, family physicians and serious investigative media but also with the communities affected by these disorders.

Mycobacteria should be subject to compulsory food hygienic inspection of powdered infant formulas. Self-imposed voluntary inspection would be highly desirable for milk, ice cream, drinking water (both tap and bottled) and beef from cows slaughtered at ages higher than 30 months. The expenditures needed should be easily recoverable if such products were labelled “MAP-free” or “Mycobacteria-low”. This would, undoubtedly, motivate cattle farmers to cull their mycobacteria-shedding animals. Continuous monitoring of MAP in milk samples collected before transfer from farm refrigeration tanks to transport tanks would enable early detection of increases in MAP shedding. Testing of pooled samples would make early identification of a new shedder possible, and the immediate elimination of the shedders should facilitate production of “MAP-free” or “MAP-low” milk within a reasonable period of time.

Furthermore, it would be prudent to monitor the mycobacterial content of water used in hospitals, in hydrotherapy and in wellness facilities. Swimming pools, especially those where baby swimming is practiced, should be encouraged to monitor mycobacterial content in the water and air. Furthermore, commercial services regarding mycobacterial contamination should be available to households interested in such testing. This service depends on the development of a simple sampling procedure, methods of postal delivery of samples to the testing laboratories and by financially viable testing. All results should be available for evaluation and publication. It is hoped that the data accumulated by such testing will then facilitate more research regarding the association of mycobacteria with various chronic inflammatory and autoimmune disorders. Furthermore, it is hoped that the results of such testing will also provide the needed incentive for the manufacture and distribution of affordable microbial filters.

Women suffering with Crohn's disease shed mycobacteria in milk [56]. Therefore, breast milk of mothers with Crohn's disease should be tested for mycobacteria throughout the nursing period, and interruption of breastfeeding in cases of shedding would be a prudent in order to prevent the exposure of babies and to safeguard the development of proper immune responsiveness.

Agencies supporting research should make it a priority to encourage the screening of the mycobacterial contamination of milk, water, food items and the establishment of a single national database for all laboratories. The certified methods of monitoring must be simple, aimed at detecting MAP in milk and dairy products and *Mycobacterium* spp. in other commodities and validated to reliably determine when an arbitrary count limit, e.g., 500 or 1,000 cell equivalents per gram or millilitre, is exceeded.

While it is not possible to completely eliminate human exposure to mycobacteria, this does not mean that the substantial body of important research findings pointing to the potential of nontuberculous mycobacteria in inducing chronic inflammatory and autoimmune diseases should continue to be ignored. A basic measure that can, and should be taken now, is a risk awareness campaign, so that people can avoid unnecessary exposure to large numbers of mycobacteria, which after ingestion or inhalation release triggers of disease affecting millions of people. It is undeniable that the increasing numbers of patients affected with chronic inflammatory and autoimmune disorders could be reduced if a global public campaign to raise awareness of this connection were conducted. Individuals could then take their own measures to minimise their risk of developing these disorders. Consequently, they could influence the development of proper public health policies by lobbying health experts and lawmakers.

A call for Action (Table 10) will be published on the internet. Relevant servers can share this activity, which could start a massive information campaign. Supporters are welcome.

Table 10

Table 1
Non-tuberculous mycobacteria are known opportunistic pathogens (selected references: year of publication, number in Reference list, see more in the paper**)**

DISORDERS ASSOCIATED WITH INGESTION OR INHALATION OF NONTUBERCULOUS MYCOBACTERIA

SKIN AND SOFT TISSUES INFECTIONS

2000	[57]	M. avium in bath tub heating unit, familial cluster (father and two children)
2014	[58]	A review

CERVICAL LYMPHADENITIS IN CHILDREN

1998	[59]	M. paratuberculosis cervical lymphadenitis, followed by terminal ileitis similar to Crohn's disease
2014	[60]	Atypical mycobacteriosis

OSTEOMYELITIS

2015	[61]	Severe consequences and a poor prognosis

PULMONARY DISEASES

1998	[62]	Endemic granulomatous pneumonitis "Lifeguard lung" (M. abscessus):
2014	[63]	Pulmonary infections are most commonly due to M. avium complex (MAC), M. kansasii, and M. abscessus
2015	[64]	Pulmonary Infection with NTM: A Review
2016	[65]	Two types of NTM lung diseases reported; fibrocavitary and nodular bronchiectatic forms
2016	[66]	M. abscessus is responsible for lung diseases and healthcare-associated extrapulmonary infections
2016	[67]	Respiratory diseases and increased mortality associated with pulmonary NTM infection
2017	[68]	Pulmonary disease caused by NTM is steadily increasing worldwide
2017	[69]	NTM disease is becoming more prevalent
2017	[70]	Complication of cystíc fibrosis
2018	[71]	Pulmonary NTM infections has increased in recent decades (pleurisy, pneumothorax)

DISEASES ASSOCIATED WITH USE OF MYCOBACTERIALLY CONTAMINATED MEDICAL TOOLS

REVIEW ARTICLE

2017	[72]	Mycobacterial outbreaks in healthcare settings have been underrecognized.

CATHETERS

2016	[73]	Ventriculoperitoneal shunt placement complicated with M. abscessus
2017	[74]	M. fortuitum empyema associated with an infected pleural catheter

COSMETIC MEDICINE

2016	[75]	Seeking cosmetic surgery in the developing world (medical tourism)
2017	[76]	M. chelonae infection after abdominal liposuction and gluteal fat injection

BRONCHOSCOPY RISK

2010	[77]	Water and biofilm samples collected from the bronchoscopy preparation laboratory

RESPIRATORY INFECTION

2016	[78]	Contaminated nebulizers

ULTRASOUND TRANSMISSION GEL

2016	[79]	M. massiliense infections associated with ultrasound transmission gel, one death in a neonate

HEATER-COOLER UNIT USED IN CARDIOSURGERY

2016	[80]	M. chimaera detected in the water tanks of the HCUs and in exhaust air
2016	[81]	UK, Birmingham, M.chimeara in water from heater-cooler units of cardiopulmonary bypass equipment, 3000 cfu/l
2017	[82]	M. chimaera infections following cardiac surgery
2017	[83]	M. chimaera infection should be considered after surgery with cardiopulmonary bypass; disseminated infection or sternal wound infection
2017	[84]	Many HCUs are contaminated with M. chimaera and complex biofilms

TATOOING

2012	[85]	M. chelonae associated with skin and soft-tissue infections
2013	[86]	regulation and use of tattoo inks should be considered
2013	[87]	MB isolated from 71/142 (50%) cases, M. chelonae identified in 48/71 (67.6%) isolates
2013	[88]	M. chelonae cultured from the ink
2014	[89]	INTM infections happen in tattoos with increasing frequency through contaminated ink or water or water used to dilute inks

Table 2

Non-tuberculous mycobacteria are suspected triggers of immune mediated chronic inflammatory and autoimmune diseases (selected references: year of publication, number in Reference list, see more in the paper**)**

CROHN'S DISEASE		
1983	[90]	Possible association with no or very short periods of breast-feeding *).
1993	[91]	Negative association of breast-feeding with Crohn's disease *).
2000	[56]	MAP role in CD pathogenesis supported.
2000	[92]	Crohn's disease is heterogeneous disorder of mutifactorial etiology in which hereditary (genetic) and environmental (microbial, behaviour) factors interact to produce the disease.
2001	[93]	Breastfeeding decreases the risk of several chronic inflammatory diseases *)
2004	[94]	Breastfeeding is associated with lower risks of Crohn disease *)
2004	[95]	In the affluent parts of the world there has been a steady and simultaneous increase of at least three groups of disease: (1) allergies, (2) inflammatory bowel diseases (IBD; e.g. Crohn's disease and ulcerative colitis) and (3) autoimmunity (e.g. type 1 diabetes and multiple sclerosis).
2004	[96]	Involvement of commensal microflora and its components with strong immunoactivating properties (e.g. LPS, peptidoglycans, superantigens, bacterial DNA, HSP) in aetiopathogenetic mechanism of various complex, multifactorial and multigenic diseases, including inflammatory bowel diseases, periodontal disease, rheumatoid arthritis, atherosclerosis, allergy, multiorgan failure, colon cancer has been recently suggested.
2005	[12]	Infant dried milk can be a source of mycobacterial triggers.
2006	[97]	Incidences of Crohn's disease and ulcerative colitis were observed to be highest among persons of high socioeconomic status, persons with the lowest rates of enteric infection, and persons with the highest rates of multiple sclerosis.
2007	[98]	Exposure to bacterial antigens and other environmental factors in combination with a genetic susceptibility was implicated in the aetiology of inflammatory bowel disease; preterm birth and other perinatal circumstances were associated with the development of IBD
2009	[99]	Cluster of Crohn's disease possibly linked to fully treated drinking water
2009	[39]	Intracellular infection with the primary pathogen causes an immune dysregulation and a specific chronic enteric neuropathy with loss of mucosal integrity
2009	[100]	Industrialized status and affluence are the common denominators.
2011	[13]	MAP in concentrations from 48 to 32 500 cells per gram of powdered infant milk were found in 18 out of 51 investigated samples (35%).
2014	[101]	The majority of the studies definitively support the role of MAP in at least 30%-50% of CD patients
2014	[102]	The microbiome influences mucosal immune networks
2015	[103]	Crohn's disease and ulcerative colitis are increased in patients with asthma and chronic obstructive pulmonary disease (COPD) compared to the general population (27% and 55% higher than in the general population of Quebec).
2015	[104]	Fifteen percent of patients with Crohn's Disease have an affected family member and twin studies for CD have shown 50% concordance in monozygotic twins compared to <10% in izygotics; The evidence that genetic factors contribute in small part to disease pathogenesis confirms the important role of microbial and environmental factors. Epigenetic factors can mediate interactions between environment and genome.
2015	[105]	The increased incidence of the diseases to which MAP has been related: Blau syndrome, type 1 diabetes, Hashimoto thyroiditis, and multiple sclerosis.
2017	[106]	A possible transmission of MAP from animal-derived products to humans
TYPE 1 DIABETES		
2005	[107]	Breastfeeding reduced incidence of Type-1 diabetes *)
2006	[108]	Exposure to cow's milk early in Life is a recognized risk factor in the development of T1DM.
2015	[109]	Cow's milk intake may increase risk of islet autoimmunity and progression to T1D
2015	[110]	Breast-feeding for 12 months predicts a lower risk of type 1 diabetes *).
2016	[111]	Cow's milk consumption is associated with an increased risk of type 1 diabetes.
2016	[112]	MAP has been previously associated to T1D as a putative environmental agent triggering or accelerating the disease.
2017	[113]	A rare case of multiple autoimmune syndrom presenting with psoriasis, vitiligo, and Crohn's disease suggest that tumor necrosis factor-alpha may be associated with the pathogenesis of all three conditions.
2017	[114]	MAP/proinsulin detected among patients, with the highest prevalence in the 32-41year- old T1D-like LADA subgroup, supports a hypothesis of MAP contribution in the development of autoimmunity.
2017	[115]	The environmental risk factors might be involved in T1D pathogenesis, as they might initiate autoimmunity or accelerate and precipitate an already ongoing beta cell destruction.
MULTIPLE SCLEROSIS		
2001	[93]	Protective effects of breastfeeding decreases the risk of several chronic inflammatory diseases *).
2008	[116]	Children with inflammatory demyelination, CNS injury, and T1D exhibited heightened T-cell reactivities to self-antigens.
2016	[117]	MAP could act as a risk factor or a triggering agent of MS.
2017	[118]	Further evidence for a possible association between MAP and MS.
2017	[119]	Heat shock proteins and recognition by innate immunity, and toll-like receptor signalling-mediated are responses to mycobacterium exposure.
RHEUMATOID ARTHRITIS		
2001	[93]	Protective effects of breastfeeding decreases the risk of several chronic inflammatory diseases *).
2016	[120]	Nontuberculous mycobacterial diseases were observed with higher frequency in patients with rheumatoid arthritis, compared to the patients of the control group.

ASTHMA
2015 [121] Infants at risk of asthma exhibited transient gut microbial dysbiosis during the first 100 days of life.
2017 [122] Intake of fastfood was positively associated with a higher prevalence of wheeze in adolescents

SARCOIDOSIS
2006 [123] A potential role of mycobacteria in sarcoidosis.
2008 [124] Environmental agents (particularly of infectious origin) capable of inducing granulomatous inflammation develop the disease, suggesting that a particular trigger results in overtly recognizable phenotypes only when the appropriate genetic trait also occurs.
2016 [125] Molecular modelling revealed specific T-cell receptor-HLA-DRB1*03-peptide interactions, with a previously identified, sarcoidosis-associated vimentin peptide.

HYPERSENSITIVITY PNEUMONITIS, HOT TUB LUNG
2013 [126] Diffuse granulomatous lung disease caused by inhalation of water aerosol containing non-tuberculous mycobacteria.
2016 [127] NTM isolation and pulmonary disease are reported to rise in frequency

2017 [128] A type of hypersensitivity pneumonitis caused by inhalational exposure to the Mycobacterium avium complex.
2018 [129] Pathogenesis is attributed to a combination of immune complex-mediated and delayed hypersensitivity reactions to the inciting agent, mostly mycobacteria.

THYROIDITIS
2012 [130] MAP HSP65 acts as a trigger of autoimmune diseases
2017 [131] IS900 PCR: 28/76 (36.8%) positive patients of thyroiditis

ATOPIC DISEASES
2003 [132] Breastfeeding seems to protect from the development of atopic disease *)

AUTISM
2011 [133] MAP triggers autism by stimulating antibodies through molecular mimicry to its heat shock protein HSP65, that cross react with myelin basic protein.

PARKINSON DISEASE
2014 [134] Genetic defects associated with PD also result in a permissive environment for MAP infection--ineffective xenophagy.

LUPUS ERYTHEMATOSUS
2017 [16] Systemic lupus erythematosus is significantly associated with dementia.

BLAU SYNDROM
2003 [135] NOD2 (CARD15) is involved in the predisposition to Crohn's disease and Blau syndrome. In addition, biochemical evidence has unraveled the role of NOD1 (Card4) and NOD2 (Card15) as intracellular sensors of bacterial peptidoglycan.

*) In other words: formula feeding has an opposite effect, obviously due to the mycobacterial triggers [7]

Table 3

Some triggers and conditions associated with the immune mediated diseases (selected references: year of publication, number in Reference list)

MURAMYLDIPEPTIDE AND COMPONENTS OF THE CELL WALL
1975 [136]
1994 [137]
2003 [138,139]
2005 [140,141]
2006 [142]
2009 [143]

MYCOBACTERIAL CELL-WALL LIPID MONOMYCOLOYL GLYCEROL
2016 [144]

HEAT-SHOCK PROTEINS, MOLECULAR MIMICRY
2009 [145]
2011 [133]

PROINFLAMMATORY CYTOKINES AND OTHER PROINFLAMMATORY MOLECULES
2003 [138]
2011 [146]

PARTICIPATION OF GENE POLYMORFISM
2003 [138]
2016 [147,148]

Table 4

Actions of the triggers (selected references: year of publication, number in Reference list**)**

Muropeptides trigger intracellular signaling cascades, leading to altered gene expression and activation of the immune response muramyldipeptide/components of the cell wall
2006 [142]
2017 [149]

Activation of CARD15 by components of the bacterial wall and further activation of NFkappaB, a proinflammatory molecule
2003 [138]

Peptidoglycan-derived muramyl dipeptide (MDP) activates innate immunity via the host sensor NOD2.
2003 [139]
2009 [4]
2014 [7]
2015 [3]
2016 [150,151]
2017 [152]
2017 [153]

Crohn's disease-specific mutated NOD2/CARD15 causes an impaired epithelial barrier
2006 [154]

Failure of response to the penetration of bacteria and other bowel contents through the intestinal mucosal barrier
2008 [155]

Muramyl dipeptide induced little TNF alpha or interleukin 1 beta, but strong interleukin-8 secretion
2005 [141]

Immune dysregulation and a specific chronic enteric neuropathy with loss of mucosal integrity
2009 [39]

The crosstalk between autophagy and inflammation
[156]

Innate immunity and pathogenesis
2008 [155,157]
2009 [158]
2009 [159]
2013 [160]

Gamma delta T cells have both innate and adaptive characteristics and functions
2009 [161]

Defects in innate immune sensing of intracellular bacteria and the handling of these organisms through autophagy
2010 [162]

Molecular mimicry is the proposed etiopathology by which MAP stimulates autoantibodies associated with several chronic diseases
2012 [130]
2016 [163]

Molecular mimicry between MAP Heat shock protein 65 K (Hsp65) and human Glutamic Acid Decarboxylase 65 K (GAD65) may be the trigger for T1DM
2013 [164]

MAP epitopes as antigens stimulating beta-cell autoimmunity (T1DM)
2016 [165,166]

Induction of Th1 and Th17 responses
2016 [144]

Table 5

Mycobacteria in water (selected references: year of publication, number in Reference list, see more in the paper**)**

REVIEWS		
1984	[167]	
2004	[37]	
2009	[10,35]	
2011	[168]	
2012	[36]	
2015	[22,169,170]	
2016	[171,172]	
2017	[173]	
DRINKING AND TAPWATER		**Location, frequency and maximal numbers of NTB found**
1991	[174]	Germany, in 82% of samples, 4.5 x 10(5) cfu/l
1995	[175]	Germany, Berlin, in 42% of samples, 70% of sites
1996	[176]	Czech Rep., Olomouc , M. gordonae in 20% of samples
1999	[177]	USA, MB in 35% water and in 54% of ice samples
1999	[178]	USA, Los Angeles, MB recovered from 12/13(92%), 45/55 (82%) homes, 31/31 (100%) commercial buildings and 15/15 (100%) of hospitals
2001	[179]	refrigenerated fountain in a hospital
2001	[180]	USA, MB cultured from 15% from 528 samples, up to 700,000 cfu/l
2002	[181]	Taiwan, MB (PCR) in 10/49 samples
2002	[182]	France, MB cultured from 104/144 (72%) of samples, >600 cfu/l
2004	[183]	Finland, MB cultured from water 80%, 140 cfu/l, from deposits 3.9x10(5) cfu/g
2005	[184]	Portugal, 92.5% samples from the Lisbon water distribution system
2006	[185]	USA, point-of-use sites in public or commercial buildings are persistently colonized
2007	[186]	Korea, 50/150 samples of tap water from hospitals
2009	[187]	100-fold above background water contents
2009	[188]	MB cultured from 21/49 (43%) cold and 32/44 (73%) warm water samples
2010	[189]	USA, MAP DNA in 88% of drinking water samples, <500 target copies per liter
2010	[190]	Italy, MAP cultured from 62% of water samples, 300 cfu/l
2011	[191]	USA, MB in 109/394 (28%) samples
2011	[192]	Australia, M. lentiflavum cultured from 13/206 (6.3%) drinking water sites
2012	[193]	Mexico Cïty, MB isolated from 36/69 (52%) of potable water samples
2013	[194]	Czech Rep., MAP DNA in 76.7% of samples (reservoir and household sediments
2013	[195]	Australia, Brisbane, MB cultured from 76/189 (40.2%) sites of municipal water distribution system in summer and from 160/195 (82%) sites in winter
2014	[43]	UK, MB DNA detected in 28/30 (93%) samples from showers, 10(10) CE/l
2014	[196]	Czech Rep., MB cultured from 94/396 (23.7%) of water samples
2016	[197]	Iran, MB isolated from 71/148 (48%) samples of hospital water samples
2016	[198]	MB by qPCR in 78% of 134 roof-harvested rainwater tank samples water 1.1 x 10(5) CE/l for M. avium, 6.6 x 10(5) CE/l for M. intracellulare
2016	[199]	USA, MB in drinking water from water treatment plants (in 36% of treated water samples)
2016	[200]	Canary Island, MB (PCR) in 47.4% of 135 household potable water samples
2016	[201]	Czech Rep., Brno, MB in wastewater treatment plant effluent, 3,8 x 10(4) CE/ml
2016	[202]	MB in biofilms of domestic shower hoses
2016	[203]	France, MB in water from 2 lakes, more in surface microlayer than in water column
2017	[204]	diversity of the genus Mycobacterium was screened in treated municipal wastewater by pyrosequencing
2017	[205]	Iran, Kermanshah, MB cultured and typed in 35/110 (32%) of drinking water samples
2017	[206]	New children's hospital: 6 months after plumbing system was flushed and disinfected, an increased incidence of rapidly growing mycobacteria was detected in clinical cultures
BOTTLED TABLE WATER		
1997	[207]	Greece, MB in 23/150 (15.6%) of bottled table water (>1000 cfu/l in 4%)
2009	[208]	growth of MB on a PET bottle wall in an oligotrophic environment
2015	[209]	Brazil, Sao Paulo, MB cultured from bottled mineral water
SWIMMIG POOLS		
1999	[210]	Finland, MB in 5 of 7 (71%)
RIVER AND LAKE WATER AND SEDIMENTS		
2005	[31]	UK, River Taff, MAP IS900 in 31/96 (32.3%) samples collected for one year
2005	[211]	Northern Ireland, MAP in 15/90 (8%) of untreated water samples
2006	[34]	UK, River Tiwi, MAP IS900 in 48/70 (68.8%) samples collected for nine months
2014	[43]	UK, Cardiff, MAP DNA detected in 1/5 aerosol samples above the river Taff
2014	[212]	Czech Rep., MB in pond sediments and plants
2017	[213]	China, Beijing, M. avium one of the most abundant species in urban recreational water
FISH TANK WATER AND BIOFILMS		
2010	[214]	high numbers of colonies recovered from filters and swabs, 21,000 cfu/filter

Table 6

Mycobacteria in foodstuffs (selected references: year of publication, number in Reference list, see more in the paper**)**

2000	[215]	USA, MB cultured from 25/121 (20.6%) samples of food collected from supermarkets
2002	[216]	Switzerland, MAP IS900 in 1384 (19.7%) of bulk-tank milk samples
2005	[217]	Czech Rep., MAP cultured from 4/244 (1,6%) of commercially pasteurized cow's milk, and from 13/66 (19.7%) raw milk from subclinically infected dairy cows
2011	[218]	General data
2011	[219]	USA, food manufacturing practices fail to reliably kill MAP, actions to limit human exposure to MAP needed
2013	[220]	Czech Rep., MB DNA in 14/23 (60.9%) of retailed and in 21/23 (91.3%) frozen fresh water fish
2015	[221]	Mexico City, MB cultured from ready-to-eat salads (7/100) and sprouts (12/100)
2016	[222]	Mexico City, MB cultured and typed from 6/102 (6%) of resh-squeezed orange juice samples
2016	[223]	Czech Rep., MB cultured from 17/178 (9.6%) samples of raw and frozen fruits and vegetables; MB using qPCR were present in almost all 178 samples, 10(4) CE/g
2016	[224]	Viable MAP detected by phage-PCR in 9, by culture in 3 and by direct PCR in 7 of 32 samples of powdered infant formula (13%, 9% and 22%, respectively)
2017	[225]	Australia, MAP detected by qPCR in 6/122 (4.9%) samples of powdered infant formula, < 10 MAP cells/1.5 g, no viable MAP in 122 samples
2017	[226]	Spain, MB DNA in 15% of 138 dairy products and 2% of 119 meat products purchased from the main supermarket chains
2017	[227]	USA, viable MAP in calf milk replacer (similar to powdered infant formula)

Table 7

Factors affecting accuracy of the mycobacterial counts in tested samples

- The actual number of live mycobacteria in samples is actually 10 to 100-fold higher than the number of colonies.
- Mycobacteria in milk are not equally distributed between the cells, solid particles, cream and whey.
- The counts of mycobacteria in household plumbing can vary according to the employed method of sample collection, they can differ for cold and hot water, and in addition the counts may not be reproducible.
- In rivers, distribution of mycobacteria can significantly vary between the sediment, water column, and the surface layer.
- Immunomodulatory substances are released even from the dead mycobacteria.

Table 8

Frequently presented hypothesis and their alternative interpretation

Non-tuberculous mycobacteria act as conventional pathogens.
Live non-tuberculous mycobacteria may cause inflammation, but triggers released from dead mycobacteria pose evidently another type of risk.
***M. avium* subsp. *paratuberculosis* cannot be a cause of Crohn's disease, because it is not found in the intestines of all affected patients, and it can be detected in the intestines of healthy individuals.**
All consumers can be orally or by inhalation exposed by live or dead mycobacteria. Chronic inflammatory diseases, Crohn's disease included, develop more often in hosts genetically predisposed or primed earlier, hence, mycobacteria or their DNA can be found both in groups with clinical manifestations and in healthy controls. On the other hand, mycobacteria need not be cultured in all clinical cases.
Breast milk protects against immuno-mediated inflammatory and autoimmune diseases
When the epidemiological studies use well selected cohorts, it is found that formula fed infants get sick more often. Milk of cows with subclinical paratuberculosis often contains large amounts of mycobacteria, releasing triggers of immune-mediated disorders. These triggers expose children at the critical and vulnerable period of maturation of the immune system. The acquired sensitization may manifest itself in disease with considerable delay, or even in adulthood. No evidence is available that breast milk could eliminate these triggers. However, it makes more sense that the association between breastfeeding and the reduced incidence of chronic inflammatory diseases might be related to no or limited exposure to triggers, if powered infant milk and tap water are not ingested by the babies.
Hygienic hypothesis assumes insufficient environmental microbial exposure of children that would presumably down modulate inflammatory and autoimmune responses. In this respect correlation of the incidence of these diseases with access to hot water in urban areas, and generally improved hygiene, are often mentioned
Mycobacteria are present in tap water from communal sources and from hot water boilers. This water increases opportunities of exposure of children not only by its use in preparation of formulas, but also during bathing and showering. Increased exposure is also during "baby swimming". Apart from the oral exposure, inhalation of aerosol exposure during showering or in indoors swimming pools can also occur.
Cold chain hypothesis proposes an association between Crohn's disease and the use of refrigerators and freezers
While it is possible that some psychrophilic bacteria participate also as triggers of chronic inflammatory diseases, their impact is obviously overestimated. More likely, household refrigeration, increasing incidence of bovine paratuberculosis, human migration to urban setting, use of tap water for cooking, bathing and showering, and swimming in indoors swimming pools act in concert.
Crohn's disease is basically a genetic disease, activated by unknown environmental factor/s, stress or by another disease
Dramatic increase of Crohn's disease in different countries and continents, in people of various ethnic and genetic backgrounds, has proven that the phenomenon is undoubtedly caused by the triggers that had spread globally during a relatively short period. This is incompatible with the proposed role of host genetics as the primary cause of these disorders. Regarding the environmental co-factors, currently no other factors, except mycobacteria, are known to have the necessary prerequisite to affect such geographically and genetically diverse populations in such a short time. Bovine paratuberculosis, baby formula and tap water from the municipal distribution system have the potential to do it.
The increase of chronic inflammatory diseases is simply a result of the availability of more sensitive diagnostic procedures
The increase of the incidence of inflammatory diseases has been reported concurrently in countries with significant differences in the level of diagnostic capabilities. Hence, the sensitivity of diagnosis can't be the determining factor.
Crohn's disease is caused by other concurrent diseases
Polymorbidity is common in chronic inflammatory and autoimmune diseases (Table 3). This is compatible with the exposure to immune-modulatory triggers leading to damage of various target tissues. All different disease entities with different clinical symptoms, but very likely with similar pathogenesis, are outcomes of the consequences of the mycobacterial triggers, rather than a result of induction by different disease entities.

Table 9

Recommended measures to prevent human exposure to nontuberculous mycobacteria

A Regulatory measure urgently needed
Obligatory testing of powdered infant milk for MAP

B Data collection by the national veterinary inspection should be used for evaluation of pathological findings
Paratuberculosis in slaughtered cattle more than 30 months old (farm code, the number and age of the animal)
Mycobacterioses in slaughtered swine (farm code, the number of affected heads and livers in animals slaughtered in one day)

C Voluntary testing by the commercial producers, accompanied by "MAP free" declaration
Dairy farms: MAP in bulk-tank milk continuously tested; if MAP count elevates, analyses of the pooled milk samples are followed by immediate culling of the shedder
Milk processing industries: MAP in truck-tanker milk
Ice cream producers: MAP in milk purchased
Minced beef producers: MAP in final products
Bottlers of table and mineral water and beverages: M. spp. in water reservoirs (before processing)

D On-demand commercial testing for mycobacteria
MAP in breast milk of mothers suffering from Crohn's disease (breast feeding interruption is recommended after positive testing)
M. spp. in household tap water (the use of filtration unit after positive testing is recommended *)
M. spp. in swimming pools water and in air of indoor pools (clients should be informed about control)

E Knowledge dissemination regarding the risks of the triggers **)
A booklet: General information *)
Leaflets: Why Infant dry milk not tested for MAP should be avoided *)
Why household water filtration and exchange of shower heads is recommended *)
Why baby swimming in pools, not in the long term tested, should be avoided *)

F Non-governmental support expected
Screening for MAP and M. spp. in foodstuffs and water, followed by publication of the results (e. g. type and percentage of samples with more than 1000 cells per millilitre or gram)
Research grants for development of rapid and inexpensive analytical methods for M. spp. and MAP
Grants for establishment of laboratories for water and foodstuffs analysis
Grants for establishment and maintenance of databases and for evaluation of the data

*) mainly in families with infants up to 1 year age of in families with patients suffering from any immune mediated chronic inflammatory or autoimmune disease
**) with participation of family physicians, specialists and the general public.

Table 10

Call for Action

- Relevant programs of of high schools and universities should provide information regarding the risks associated with mycobacterial triggers of chronic immune mediated and autoimmune diseases.
- Food and water of preterm born babies, and those babies that are not breast fed for other reasons, must be controlled for absence of mycobacteria above permitted counts.
- Interest of consumers of milk, ice cream, and beef hamburgers, in products with limited mycobacterial counts, will provide the necessary pressure on the producers to reduce the public exposure to mycobacterial triggers.
- Food producers should voluntarily control relevant ingredients, particularly milk and ground beef, and they should be required to include the results on labels.
- Operators of public swimming pools and hydrotherapy facilities should also contribute to reduction of exposure.

For more information visit www.centaur.vri.cz or www.upvav.cz

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