Mycobacterium avium infections in children

Johanna Thegerström



Department of Clinical and Experimental Medicine (IKE), Division of Clinical Immunology, Faculty of Health Sciences, Linköping University SE-581 83 Linköping, Sweden

> Department of Clinical Physiology, Kalmar County Hospital, SE-391 85 Kalmar

> > Linköping and Kalmar 2009

Front cover: Child and cage bird. Bookmark.

Printed by:

LiU-Tryck, Linköping, Sweden 2009

Distributed by:

Department of Clinical and Experimental Medicine (IKE) Division of Clinical Immunology Faculty of Health Sciences Linköping University SE-581 83 Linköping, Sweden

ISBN: 978-91-7393-623-1

ISSN: 0345-0082

"Mais.... chanter,

Rêver, rire, passer, être seul, être libre...

Travailler sans souci de gloire ou de fortune,
A tel voyage, auquel on pense, dans la lune!
N'écrire jamais rien qui de soi ne sortît,
Et modeste d'ailleurs, se dire: mon petit,
Sois satisfait des fleurs, des fruits, même des feuilles,
Si c'est dans ton jardin à toi que tu les cueilles!
Puis, s'il advient d'un peu triompher, par hasard,
Ne pas être obligé d'en rien rendre à César,
Vis-à-vis de soi-même en garder le mérite,
Bref, dédaignant d'être le lierre parasite,
Lors même qu'on n'est pas le chêne ou le tilleul,
Ne pas monter bien haut peut-être, mais tout seul!"

Cyrano de Bergerac Edmond Rostand, 1897.

TABLE OF CONTENTS

Abbreviation	ns	8
Abstract		9
Sammanfatt	ning	10
ORIGINAL PA	PERS	11
INTRODUCTION	DN	13
N	Mycobacteria	13
1	Non-tuberculous mycobacteria in mammals and birds	16
E	Epidemiology	19
(Clinical features of Mycobacterium avium infections	21
٦	The host defence against M. avium infection	28
AIMS		37
MATERIAL AN	ID METHOD	39
RESULTS AN	D DISCUSSION	45
ļ	s <i>M. avium</i> infection in humans a zoonosis?	45
I	nfection by ingestion of water?	47
(Clinical features	52
1	mmunological considerations	55
CONCLUDING	REMARKS	59
ACKNOWLED	GEMENTS/ TACK	60
REFERENCE	LIST	63
Original pa	PERS	77

Abbreviations

AFB Acid-fast bacteria

AFR Acid-fast rods

AIDS acquired immune deficiency syndrome

BCG Bacille Calmette -Guérin

DC dendritic cell

HIV human immunodeficiency virus

GPL glycopeptidolipid

IFN interferonIL interleukin

IS insertion sequence

MAC Mycobacterium avium complex

MHC major histocompatibility complex

NK cell natural killer cell

NTM non-tuberculous mycobacteria

PBMC peripheral blood mononuclear cell

PBS phosphate buffered saline PPD purified protein derivative

RFLP restriction fragment length polymorphism

STAT signal transducer and activator of transcription

TB tuberculosis

Th T helper

TNF tumor necrosis factor

TLR Toll like receptor

Abstract

Mycobacterium avium belongs to a group of over 130 species of non-tuberculous mycobacteria (NTM) or environmental mycobacteria. The subspecies Mycobacterium avium avium was originally described as the causative agent of bird tuberculosis, but was later found to cause disease also in humans. Small children display a special form of infection that is seldom detected in other age groups. It manifests as a chronic lymphadenitis usually in the head and neck region. The incidence rate is approximately 1-5/100,000 children/year. However, exposure to this bacterium is high as judged by sensitin skin test studies. Even if a lot of persons are infected with M. avium, a majority of them do not develop disease and the bacterium is therefore considered to be of low virulence, causing disease mainly in immunocompromised persons. Children with M. avium lymphadenitis, however, usually do not have any known deficiencies in the immune system.

This thesis elucidates why small children are prone to develop disease by *M. avium*. Investigation of a possible zoonotic spread of this bacterium to children involved analysis and comparison of different strains isolated from birds and other animals and from children, using the restriction fragment length polymorphism (RFLP) method on insertion sequence IS *1245*, resulting in the finding that the children were infected exclusively with the new proposed subspecies *M. avium hominissuis*. Animals in general and birds in particular were infected with the subspecies *M. avium avium* (using the more narrow definition). Moreover, when investigating the immunological response of human peripheral blood mononuclear cells (PBMCs) to stimulation with *M. avium hominissuis* and *M. avium avium*, respectively, it was found that the former subspecies induced lower IFN-γ and IL-17 than the latter, but higher levels of Il-10, which might contribute to explain the higher pathogenicity of *M. avium hominissuis* in humans.

Through studies of the geographical distribution of cases of *M. avium* infection in children in Sweden and the seasonal variation of the disease, a fluctuation of the incidence over the year was detected, with higher numbers of cases in the autumn months and lower numbers in the late spring. There was a higher incidence rate in children living close to water than in those living in the inland or in the urban areas. Therefore, outdoor natural water is the most probable source of infection in children with *M. avium* lymphadenitis.

Through a descriptive clinical retrospective study, complete surgical removal of the affected lymph node was found to lead to better results than treatment by incision and drainage of abscess or expectation only.

Finally there might be several explanations as to why an individual develops disease after infection with *M. avium*, such as, exposure, bacterial virulence factors or possible specific deficiencies of the immune system of the host or a combination of these factors. Which are the more important factors regarding children with *M. avium* lymphadenitis is still an open question.

Sammanfattning

Mycobacterium avium tillhör gruppen icke-tuberkulösa mykobakterier eller miljömykobakterier som innehåller mer än 130 olika species. Subgruppen Mycobacterium avium avium beskrevs först som den bakterie som orsakar fågeltuberkulos, men senare beskrevs även att den kunde orsaka sjukdom hos människa. Små barn får en speciell form av denna infektion som sällan ses i andra åldersgrupper. Den yttrar sig i form av en kronisk lymfkörtelinflammation främst i huvud-hals regionen. Den årliga incidensen ligger kring 1-5/100,000 barn/år, vilket gör den till en ovanlig sjukdom. Exponeringen för M. avium är hög, vilket visas av hudtester med sensitin. Huvuddelen av de personer som exponeras blir dock inte sjuka. M. avium anses därför ha låg virulens och orsaka sjukdom ffa hos immunosupprimerade individer. Barn med M. avium lymfadenit brukar däremot inte ha några påvisbara brister i sitt immunsystem.

Arbetena i denna avhandling har, men hjälp av olika tillvägagångssätt, syftat till att försöka klargöra varför vissa små barn är benägna att utveckla sjukdom när de smittas med *M. avium*. Jag undersökte om det kunde röra sig om en möjlig zoonotisk spridning genom att analysera och jämföra olika bakteriestammar som isolerats från fåglar eller andra djur och från barn, med restriction fragment length polymorphism (RFLP) på insertions-sekvens IS 1245. Barnen var uteslutande infekterade med den nya föreslagna subgruppen *M. avium hominissuis* medan djuren i allmänhet och fåglarna i synnerhet var infekterade med subgruppen *M. avium avium* (i den strängare bemärkelsen av termen). Dessutom visade det sig när jag undersökte dessa två subgruppers inverkan på immunförsvaret genom att stimulera perifera mononukleära celler från blod, att *M. avium hominissuis* inducerade lägre halter IFN-γ och IL-17 och högre halter IL-10 än *M. avium avium*, vilket kan bidra till att förklara varför *M. avium hominissuis* är mer patogen för människor.

Jag fann en årstidsberoende fluktuation av incidensen med fler fall under höstmånaderna och färre fall på senvåren. Vidare var incidensen högre hos barn som bodde nära vatten jämfört med dem som bodde i inlandet eller i storstäderna. Vatten ute i naturen är därför den mest sannolika smittkällan för barn som drabbas av *M. avium* lymfadenit. En klinisk, retrospektiv studie visade att operation med borttagande av den infekterade körteln gav bättre resultat än om man bara inciderade och dränerade området eller avvaktade spontanläkning.

Slutligen kan det finnas flera förklaringar till varför en individ drabbas av *M. avium* infektion: exponering, virulensfaktorer hos olika bakteriestammar eller specifika brister i människans immunförsvar, eller en kombination av dessa faktorer. Vilket som är av störst betydelse för barn som drabbas av *M. avium* infektion är ännu oklart.

ORIGINAL PAPERS IN THIS THESIS

This thesis is based on the following papers, which will be referred to by their Roman numerals.

Paper I: *Mycobacterium avium* with the bird type IS*1245* RFLP profile is commonly found in wild and domestic animals, but rarely in humans. Thegerström J, Marklund BI, Hoffner S, Axelsson-Olsson D, Kauppinen J, Olsen B. *Scand J Infect Dis.* 2005; 37(1):15-20.

Paper II: *Mycobacterium avium* lymphadenopathy among children, Sweden. Thegerström J, Romanus V, Friman V, Brudin L, Haemig PD, Olsen B. *Emerg Infect Dis.* 2008; 14(4):661-3.

Paper III: Clinical features and incidence of *Mycobacterium avium* infections in **children.** Thegerström J, Friman V, Nylén O, Romanus V, Olsen B. *Scand J Infect Dis.* 2008; 40(6-7):481-6.

Paper IV: Mycobacterium avium avium and Mycobacterium avium hominissuis give different cytokine responses after in vitro stimulation of human blood mononuclear cells.

Thegerström J, Jönsson B, Brudin L, Olsen B, Wold A, Ernerudh J, Friman V. Manuscript.

INTRODUCTION

Mycobacteria

Mycobacteria are aerobic, non-motile acid-fast rods (AFR) about 1-10 µm long. They have wax-like cell walls which are composed of several glycolipids and long chain fatty acids (mycolic acids). The thick cell wall is responsible for the acid-fastness which means that the bacteria do not decolorize with acidified alcohol after staining. A classical staining method of mycobacteria is the Ziehl-Neelsen staining.

Identification

Mycobacteria are identified by a combination of phenotypic and genotypic tests. In addition to growth rate, also pigmentation, colony morphology and specific growth requirements are used. For example, *M. avium* is a slow-growing, non-chromogenic mycobacterium (non-pigmented in both light and dark) with four different colony types.

Genotypic tests include different methods to detect species-specific insertion sequence (IS) elements (see further under Methods), non-IS-based polymerase chain reaction (PCR) differentiation methods and sequence-based classification by study of the ribosomal operon or housekeeping genes, such as, the 65-kDa heat shock protein (hsp65) gene. In taxonomic studies, the gene encoding the 16S rRNA is the primary target (Turenne CY et al. 2007). The genome sequence of the reference strain *M. avium* 104 from the blood of an AIDS patient (a representative of the *M. avium hominissuis* strains, see below) has been available since 2003 (The Institute for Genomic Research (TIGR), http://www.tigr.org/).

Today different genetic test kits based on PCR amplification exist, and at least rapid detection of *M. tuberculosis* is done routinely on clinical samples. For *M. avium* there are similar tests available based on the detection of rRNA with separate probes for *M. avium* and *M. intracellulare*, such as, the AccuProbe test (GenProbe, Inc., San Diego, CA).

Non-tuberculous mycobacteria (NTM)

M. avium is part of the group called non-tuberculous mycobacteria (NTM), also called environmental mycobacteria, atypical mycobacteria or mycobacteria other than tuberculosis (MOTT) in the literature. This group contains about 130 different species, whereof about 60 are potentially pathogenic to humans (Jarzembowski JA and Young MB 2008). They are distinguished from *Mycobacterium tuberculosis* and *Mycobacterium leprae* not so much by their ability to cause serious disease in humans, but rather differences in natural habitats and contagiousness. NTM are environmental mycobacteria or animal pathogens with no reported transmission from man to man.

Mycobacterium avium complex (MAC)

Together with *M. intracellulare*, *M. avium* forms the *Mycobacterium avium* complex (MAC) which is a commonly used term in the literature. Genotypic studies clearly divide *M*.

avium and M. intracellulare into two distinct species. Sometimes the species M. scrofulaceum is (wrongly) added to this group which is then called MAIS.

The highly antigenic, typable serovar-specific glycopeptidolipids (ssGPLs) that are exposed at the surface of the thick lipid-rich asymmetrical bilayered cell wall of *M. avium* allow the classification of MAC by seroagglutination reactions, originally described by Schaefer (Yoder WD and Schaefer WB 1971) or by using thin-layer chromatography in 28 different serovar types. Serovars 1-6, 8-11 and 21 are assigned to *M. avium*.

Mycobacterium avium and its subspecies

M. avium in turn consists of the subspecies *M. avium* subsp. *avium*, *M. avium* subsp. *paratuberculosis* (causative agent of Johne's disease in cattle) and *M. avium* subsp. *silvaticum* (Wood-pigeon bacilli) (Thorel MF et al. 1990).

Based on restriction fragment length polymorphism (RFLP) studies of insertion sequences IS1245 and IS901 together with observed host specificity, growth temperature differences and differences in the 16S-23S internal transcribed spacer (ITS) sequence, *M. avium* subsp. *avium* has further been proposed to be divided into two groups: (1) *M. avium avium* which is restricted to strains containing the insertion sequence IS901 and showing the typical bird-type/three-band profile on IS1245 RFLP and which is most often isolated from birds, and (2) *M. avium hominissuis* which is proposed as a new subspecies for the strains isolated mainly from humans and pigs, showing multiband profiles on RFLP IS1245 and containing no IS901 (Mijs W et al. 2002). *M. avium avium* is mostly associated with serotypes 1, 2 and 3, but the same serotypes can be represented across the two subgroups.

In our studies we focus on the difference between these last two subspecies and use the denominations *M. avium avium* and *M. avium hominissuis*. When the distinction is not known, *M. avium*, MAC or NTM are used.

It is questioned whether *M. avium* subsp. *silvaticum* is really a unique subspecies as it has many common characteristics with *M. avium avium*. No genetic or genomic studies have revealed a distinction between the two subspecies. In several studies where only molecular methods are employed for strain classification and not phenotypic studies, strains classified as IS901⁺ might as well be *M. avium silvaticum* as *M. avium avium* (Turenne CY et al. 2007).

Phenotypical characteristics of M. avium

Growth rate

There are rapid-growing and slow-growing mycobacteria, and *M. avium* belongs to the latter group. The slow-growing mycobacteria take longer than seven days to form colonies owing to the possession of only one copy of the genes encoding the 16S rRNA cistrons, whereas the rapid-growing mycobacteria have two sets of these genes. The synthesis of long-chain mycolic acids contained in the impermeable, lipid-rich cell wall also costs a lot of energy and contributes to slow growth. The slow growth rate might be an advantage in so much

that it gives more time for adaptation to stressful environments and time to accumulate resistance mutations for ribosomal targeting antibiotics (Primm TP et al. 2004).

Adaptation of M. avium to the environment

M. avium and many other NTM are very hardy bacteria that can adapt to different environments. M. avium grows at a wide range of pH values, especially at acidic pH and over a temperature range of 10-45°C. It tolerates high salt concentrations and survives in ocean water. It can utilize a wide range of carbon and nitrogen sources or down-regulate its metabolism at starvation conditions (Falkinham JO III 2002). This is an organized metabolic shutdown with differential gene regulation and metabolic pathway rearrangements during an adaptive phase leading to a metabolic dormancy persistence state. With return of nutrients the bacteria are able to respond rapidly and return to a growth-focused state (Archuleta RJ et al. 2005).

Biofilm formation

Different oxidative stress responses in *M. avium* also lead to biofilm formation, which is another characteristic of some NTM, including *M. avium*, important for their survival (Geier H et al. 2008). Biofilms permit the bacteria to persist in drinking water distribution systems, for example. The glycopeptidolipids in the cell walls of the bacteria are thought to be of importance for the different sliding motility and biofilm formation capacity of different MAC strains (Yamazaki Y et al. 2006). *M. intracellulare* is more often isolated from biofilms than *M. avium* (Falkinham JO III et al. 2001).

Resistance to antimicrobial agents

The impermeable cell wall of *M. avium* and other mycobacteria renders them innately resistant to a wide range of antimicrobial agents, including antibiotics and disinfectants. *M. avium* is approximately 1000 times more resistant to chlorine than is *Escherichia coli*, the standard for drinking water disinfection in the US (Taylor RH et al. 2000).

Virulance and pathogenicity

M. avium strains can exhibit different colony morphologies; rough, smooth-transparent and smooth-opaque. In the 1970s and onward, studies have been made on the relative virulence of the different colony morphologies. Divergent conclusions have been drawn (Schorey JS and Sweet L 2008). Rough isolates seem to be the most virulent to chickens and mice, and smooth-transparent isolates seem to be more virulent than smooth-opaque ones (Schaefer WB et al. 1970). The authors speculated already at this time that it had to do with different surface properties of the bacteria. This was further suspected when the macrophage induced gene (mig) in M. avium was found to be correlated with virulence (Plum G et al. 1997, Meyer M et al. 1998), since the mig gene turned out to be involved in the metabolism of fatty acids (Morsczeck C et al. 2001), an important component of the cell walls of M. avium. The ser gene cluster was also identified, which encodes for the synthesis of specific oligosaccharides of the glycopeptidolipids (GPLs) of M. avium strains (Belisle JT et al.

1993). Some rough colony *M. avium* strains probably have altered, or lack, glycosylation of the lipopeptide core of the GPLs.

Total lipid fractions of bacteria, purified GPLs and lipoglycans (different forms of lipoarabinomannans (LAM); in the case of *M. avium*: ManLAM) have been tested for their immunomodulatory properties with divergent results (Schorey JS and Sweet L 2008). Several studies have observed pro-inflammatory responses in human macrophages induced by GPLs, but responses seem to be dependent on slight structural modifications (acetylation and methylation patterns) in the carbohydrates of the GPLs (Sweet L et al. 2008). The response is mediated through Toll like receptor 2 (TLR2) and the intracellular signalling pathway is dependent on the myeloid differentiation primary-response protein 88 (MyD88) and activation of transcription factor NF-κB (Sweet L and Schorey JS 2006). It has also been shown that some *M. avium* lipids or GPLs inhibit cytokine secretion or T cell proliferative responses (Horgen L et al. 2000, Kano H et al 2005).

One study links the presence of IS901 with virulence for birds. Attenuation of virulence (for pullets) of IS901-positive strains was associated with multiple *in vitro* subculture, polyclonal infection or human passage (Dvorska L et al. 2003).

Also, some particularly virulent *M. avium hominissuis* strains have been identified in AIDS patients. Another study found a MAC strain in a HIV-negative patient with pulmonary disease, where progressive disease correlated with bacterial persistence in macrophages and high bacterial load and inflammation in mice (Tateishi Y et al. 2009).

Non-tuberculous mycobacteria in mammals and birds

Non-tuberculous mycobacteria in humans

It was first a general belief that all NTM or environmental mycobacteria, including the causative agent of avian tuberculosis, were non-pathogenic to humans.

The first case of human disease due to what was supposedly *M. avium* was an isolation from sputum in a patient with underlying chronic lung disease, reported in 1943 (Feldman WH et al. 1943). In 1948 and 1949 the first cases of avian tuberculous lymphadenitis in children were reported, and in 1956 the first descriptive study of this new kind of scrofula in children was published (Prissick FH and Masson AM. 1956). The causative mycobacterium was proposed to be named *Mycobacterium scrofulaceum* in 1957. In other studies the bacteria had been termed *Nocardia intracellularis* or Battey bacilli, and not until 1967 was it clear that these agents were all phenotypically very similar to *M. avium* (Runyon EH. 1967). Several studies extending from the 1950s and onward describe the disease in children.

In the 1980s *M. avium* was further noted as a consequence of the emerging AIDS epidemic, where *M. avium* was found to be the etiologic agent in disseminated mycobacterial disease.

Strains of the *Mycobacterium avium* complex are probably the third or the fourth most common cause of mycobacterial disease in man. *M. tuberculosis* and *M. leprae* are

historically and still today the most common (and obligate) human pathogens. Recently *M. ulcerans* has emerged as a mycobacterium causing a cutaneous infection known as Buruli ulcer in immunocompetent individuals with increasing incidences in tropical developing countries (Ashford DA et al. 2001).

As shown by sensitin studies (Edwards LB et al. 1969, Baily GV 1979) and studies of antibody levels to *M. avium* (Fairchok MP et al. 1995), exposure rates in humans are as high as 70-95% in some areas. Nevertheless very few immunocompetent individuals develop disease. Instead they might have asymptomatic respiratory or intestinal colonization (Inderlied CB et al. 1993).

Humans are almost exclusively infected with the IS1245 RFLP multiband profile strains called *M. avium hominissuis*, whereas there are only a few rare cases of findings of *M. avium avium* in humans. The latter are mostly isolates from sputum, and the clinical relevance might therefore be difficult to evaluate. An exception is a small group of six HIV positive patients in the French Caribbean islands and Guiana that showed a 2-band pattern on IS1245 RFLP analysis, which might represent *M. avium avium* strains (Legrand E et al. 2000). To our knowledge no child with *M. avium* lymphadenitis has been reported in the literature to be infected with a *M. avium avium* strain.

Mycobacteriosis in birds

Mycobacterial disease in birds was first described in 1890 (Maffucci A. 1894), and the causative agent, found to be a distinct species, was termed *Mycobacterium avium* in 1901 (Chester FD. 1901).

A few decades ago mycobacteriosis in poultry was a huge problem in the industry, but is now rare thanks to successful measures of strict hygiene practices to minimise contact with faeces and soil, and the identification and elimination of infected birds.

Even if *M. avium* got its name as the etiologic agent in avian tuberculosis, new molecular techniques for species identification in pet birds with mycobacterial disease have revealed a high prevalence of non-culturable mycobacteria in pet birds, primarily *M. genavese*. This slow-growing non-tuberculous mycobacterium is the most commonly isolated species in pet birds in recent studies (71%), *M. avium* being only the second most common species isolated (17%) (Hoop RK et al.1996, Manarolla G et al. 2009). The incidence of mycobacteriosis in pet birds is estimated to be 0.5-14% in post-mortem surveys (Lennox AM. 2007).

M. avium is more commonly identified in aviaries at zoos and in wild birds than in companion psittacine birds. Outbreaks among small flocks of birds have occurred (Kauppinen et al. 2001 and Dvorska L et al. 2007) causing concern mainly for losses of valuable or endangered species from collections or breeding programmes.

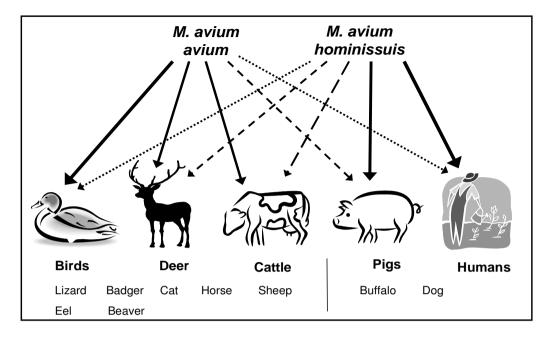


Figure 1. The respective hosts of M. avium avium and M. avium hominissuis. Birds, deer, (cattle), pigs and humans are the main groups with numerous isolations of M. avium mentioned in the literature. Below are noted host species with occasional findings of M. avium avium or M. avium hominissuis.

A thick, black arrow indicates that the subspecies constitutes the majority of findings in the host, and a weaker, broken arrow signifies that the subspecies constitutes a lesser proportion of or only occasional findings.

Susceptibility between bird orders varies greatly but mycobacteriosis has been reported in them all. Studies of incidence in wild birds depend on dead birds sent to a laboratory for necropsy. There might be a selection as to which kinds of birds are found and sent for examination (a dead predatory bird being of more interest than a dead gull, for example) thereby perhaps skewing the incidence rates in different bird species. Anseriformes (ducks, geese and swans) are considered the most susceptible in most studies (Tell LA et al. 2001).

Birds are almost exclusively infected with *M. avium avium* strains, with only occasional findings of *M. avium hominissuis* strains, particularly in birds that live in captivity or near humans.

Mycobacteriosis in pigs

Pigs are infected with *M. avium*, and it appears to be an increasing problem. For example, the prevalence of infected lymph nodes in slaughtered pigs in Finland has increased ten-fold the last decade (Tirkkonen T et al. 2007). Both *M. avium avium* and *M. avium hominissuis* are found in pigs, but the distribution between the two groups varies from one study to another. Some studies show none or only single isolates of *M. avium avium* and instead a

majority of *M. avium hominissuis* strains (Johansen TB et al. 2007, Norway, Tirkkonen et al. 2007, Finland, Oliveira et al. 2003, Brazil, O'Grady D et al. 2000, Ireland and Komiju et al. 1999, the Netherlands). Other studies from New Zealand (Collins DM et al. 1997), Sweden (Thegerström J et al. 2005) and Germany (Möbius et al. 2006) found 70%, 46% and 55%, respectively, of *M. avium avium* strains in pigs.

Epidemiology Possible sources of infection

Mycobacteria in water and other environmental sources

Water

The connection between *M. avium* and water is based, on one hand, on the frequent isolation of these bacteria from different water sources in combination with experimental evidence of the ability of *M. avium* to grow in natural and drinking waters at a wide range of temperatures (10-45°C), pH (4-7) and salt concentrations (up to 2%), using a wide range of carbon and nitrogen sources for growth or surviving by oligotrophy, and on the other hand, on studies using sensitin skin testing of the healthy population that show a higher proportion of skin test positive persons in coastal areas (Edwards LB et al. 1969, Larsson LO et al. 1991, Dascalopoulos et al. 1995). The ability of *M. avium* to grow inside amoebas (Steinert M et al. 1998) and protozoas and even gain virulence in doing so (Cirillo JD et al. 1997) also shows that the bacteria are adapted to aquatic environments. Moreover the ability of *M. avium* to resist chlorine and other disinfectants results in its selection in drinking water (Falkinham JO III. 2002).

Some studies show high yields of *M. avium* in water samples, while others find very few isolates of *M. avium* but instead a multitude of other NTM of different species and often a big proportion of non-identifiable strains. It might have to do with different isolation techniques (centrifugation or filtration) and identification techniques, or to geographic variations of different species. In older studies *M. avium*, *M. intracellulare* and sometimes also *M. scrofulaceum* are grouped together, because there were no means to distinguish one species from another reliably at the time. These studies sometimes specify the serovars of the *M. avium* strains recovered, but no study specifies whether they are *M. avium avium* strains or *M. avium hominissuis* strains.

Goslee and Wolinsky tested 321 water samples and found 27% positive yields of NTM in natural waters, 21% in drinking waters and 50% in waters that had been in contact with animals. 21% of positive cultures belonged to the MAIS complex (Goslee S and Wolinsky E 1976). Another study showed 65% recovery of MAIS in water samples from brown-water coastal swamps in the southeastern US (Kirschner RA et al. 1992). In Finland NTM were found in 100% of samples from brook water from 53 drainage areas characterized by boreal coniferous forests and numerous peatlands (Iivanainen E et al. 1993).

Studies of water distribution systems have shown 3% *M. avium* in the United States (Falkinham III et al. 2001), 0% *M. avium* in spite of 72% positive NTM samples in Paris, France (Le Dantec et al. 2002) and <1% *M. avium* despite high numbers of positive NTM samples from the Han River and tap water in Korea (Lee ES et al. 2008).

An epidemiologic, multinational study conducted in the US, Finland, Zaire and Kenya found similar levels of MAC in environmental samples (28%) in the different countries, but lower levels of MAC in water supply systems in Kenya (0%) than in the other countries (30%). Yields were greater in rivers and streams with moving waters (58%) compared to lakes and ponds with still water (12%) (von Reyn CF et al. 1993). Recirculating hot water systems in hospitals in the US have been shown to be persistently colonized with the same type of *M. avium* strain over long periods of time (von Reyn CF et al. 1994).

Food

Different alimentary products have been proposed as a source of infection to humans. *M. avium* isolates have been found in broccoli, spinach, different types of lettuce, mushrooms and leeks, and one isolate was identical to a clinical isolate (Yoder et al. 1999). One can question, however, whether it was the vegetable itself or the water it was rinsed in that permitted detection of *M. avium*. There is also a reported association with hard cheese (Horsburgh CR Jr et al. 1994) that has not been found in other studies (Reed C et al. 2006).

Soil

In a population-based survey where *M. avium* contact or infection was measured by positive reaction to *M. avium* sensitin skin test, occupational exposure to soil was identified as a risk factor (Reed C et al. 2006). In a study on HIV-positive patients with *M. avium* infection, *M. avium* was found in 27% of soil samples from potted plants in the patient's home, and some isolates were similar but not identical to the patient's strains (Yajko DM et al. 1995).

Epidemiology in animals

Birds

An outbreak of mycobacteriosis in a flock of 38 captive water birds in a zoological garden in the Czech Republic was shown to be due to *M. avium avium* infection of the same RFLP IS901 type. However, *M. avium hominissuis* was also isolated from 60% of the infected birds, but was not correlated to tuberculous lesions to the same extent and was also found in faecal samples. Both *M. avium avium* and *M. avium hominissuis* were found in environmental samples of diverse origin in the surroundings (water, soil, sand, webs and faeces), but the exact source of the infection could not be found. In this study *M. avium hominissuis* seemed to have more the characteristics of colonizing agents than pathogenic bacteria to the birds (Dvorska L et al. 2007).

A study of ducks, geese and swans in a collection of wildfowl in Great Britain found that birds with the feeding habits of diving or dabbling had higher incidences of mycobacteriosis than grazing birds and the explanation given was that divers and dabblers obviously were exposed to water to a greater extent and grazers were able to avoid visually contaminated patches of grass and also that their food was exposed to the sterilizing effect of ultraviolet irradiation. Interestingly, dabblers were the only few birds that showed primary pulmonary lesions, and that might be due to the production and inhalation of aerosols when dabbling in the surface waters. Another hypothesis concerning perching ducks that were highly susceptible to mycobacteriosis was that in captivity they were brought to live on the ground to a greater extent than in the wild where they have arboreal habitats, where mycobacterial immunity might be of lesser importance (Cromie RL et al. 1991).

A high prevalence of mycobacteriosis in predatory birds was found in the Netherlands 1975-1985. Some species within this group had higher incidences, for example, buzzards and falcons. These species fight their prey on the ground and often contract local injuries that might be infected, in contrast to accipiters that fight in the air with little injury (Smit T et al. 1987).

Pigs

Matlova L et al. (2004) isolated several *M. avium hominissuis* strains of identical genotype and serotype in pig lymph nodes and in sawdust used in two pig farms in the Czech Republic, and found one pig isolate and two sawdust isolates with identical RFLP IS*1245* profiles.

Wood shavings and peat have also been implied as sources of infection in pigs as well as soil, feed and compost.

Clinical features of *Mycobacterium avium* infections

Healthy children

The most common manifestation of *M. avium* infections in children is a subacute or chronic lymphadenitis in the cervical region. The first description of infection with nonchromogenic mycobacteria in children was made in 1956 (Prissic FH and Masson AM 1956). Several clinical studies and reviews have been made since the 1960s, and clinical descriptions are grossly concordant with one another (Margileth AM et al. 1984, Joshi W et al. 1989, Wolinsky E et al. 1995, Albright JT 2003, Haverkamp MH et al. 2004, Vu TT et al. 2005, Lindeboom JA et al. 2007, Cohen YH et al. 2008, Thegerström J et al. 2008).

Etiologic agent

M. avium as the principal etiologic agent in mycobacterial lymphadenitis in children is true in developed countries, where the prevalence of tuberculous lymphadenitis has decreased. In the developing world infections caused by *M. tuberculosis* are still predominant.

Before 1978 the most common NTM agent reported was *M. scrofulaceum*, but after this date a shift took place in several countries in favour of the *M. avium* complex (Wolinsky E et al. 1995). The principal explanation for this shift has been hypothesized to be the selection of *M. avium* in chlorinated water due to its greater resistance (Falkinham III 2002). MAC accounts for 50-90% of positive cultures from NTM lymph nodes in children in different studies. Most clinical studies of mycobacterial cervical lymphadenitis in children include all NTM species.

In some recent studies *M. haemophilum* seem to have risen in the proportion of isolated NTM to an almost equal level as *M. avium*, especially in older children (Lindeboom et al. 2005, the Netherlands, Cohen YH et al. 2008, Israel). This might be due to different culture and isolation methods and better isolation rates or to a true emergence of this new species in these countries.

Clinical features and diagnosis

Median age of children developing disease is between two and three years in most studies. The disease is uncommon after seven years of age. This is explained either by erupting teeth and oral exploratory behaviour in young ages, and/or an immature immune system at lower ages.

Most studies find a slight predominance of the disease in girls (about 60%) but a few studies find no difference in the incidence in boys and girls (Joshi W et al. 1989 and Lindeboom JA et al. 2007).

About 90% of the engaged lymph nodes are located in the head and neck region (jugulodigastric, submandibular and preauricular lymph node stations). The disease is unilateral in 90-95% of cases, even if several nodes on the same side might be affected. Sometimes the parotid gland is infected. More uncommon locations are inguinal, axillary and mediastinal lymph nodes.

The classical description is that of a perfectly healthy child with a painless enlarged lymph node at the side of the neck, although some studies describe fever or other systemic signs in about 25% of cases (Haverkamp MH et al. 2004, Vu TT et al. 2005, Thegerström J et al. 2008).

Thinning of the overlying skin and a bluish discoloration is common after a few weeks' duration of disease (50-85% of cases). About 40-50% of the children have cold abscesses, and about 5-20% have spontaneous fistulas at presentation (Joshi W et al. 1989, Gill MJ et al. 1987, Haverkamp MH et al. 2004, Vuu TT et al. 2005, Lindeboom JA et al. 2007).

In a study from Israel where no interventions were done and the children were followed by observation alone, giving a picture of the natural history of the disease, all children but three out of 92 had spontaneous drainage of purulent material from the engaged lymph node that lasted for three to eight weeks. After six months 71% of children had healed, and the remaining children were well after 9-12 months (Zeharia A et al. 2008).

Most studies show a delay of about 6-11 weeks from the onset of disease as noticed by a parent at home and the referral to hospital for diagnostic and therapeutic measures (Lindeboom JA et al. 2007, Thegerström J et al. 2008).

No standard for the diagnostic requirements for this disease exists. Therefore study populations differ in different studies. Cultures are reported to be positive in between 31 and 88% of cases (Albright JT 2003). Cultures can be performed on material from fine needle aspirations in a great proportion of cases (Tunkel D and Romaneschi KB 1995). In our material 59% of fine needle aspirations yielded positive cultures (Thegerström J et al. 2008). Cultures from biopsy or from extirpated lymph nodes have higher yields though. Some studies report that it is more difficult to obtain positive cultures from lymph nodes that have been affected a long time (Joshi W et al. 1989, Wolinsky E et al. 1995).

Several studies include non-culture verified cases where diagnosis instead is based on a combination of one or several of the following findings: typical clinical signs, positive purified protein derivative (PPD) and/or sensitin reactions, AFR on microscopic examination but negative culture, and typical histopathological changes in biopsy material.

Differential diagnoses include first of all pyogenic adenitis that is the most common cause of a swollen lymph node in the neck region. The child usually has systemic symptoms, and the lymph node is tender and warm with surrounding oedema in contrast to NTM lymphadenitis. Cat scratch disease, toxoplasmosis or tuberculous lymphadenitis are other alternative diagnoses. In the case of tuberculous adenitis the child usually has a known contact with an index case in the surroundings and shows evidence of intrathoracal disease on a chest x-ray. Congenital cysts and tumors are non-infectious differential diagnoses.

Treatment

When tuberculosis in children was still quite common in the Western countries, treatment of mycobacterial lymphadenitis in a child was always started with anti-tuberculous agents before a culture had proven a NTM etiology. However, anti-tuberculous agents have low efficacy against *M. avium* and other NTM due to high levels of resistance to the drugs used.

Studies from the 1970s and 80s demonstrated the overwhelming predominance of NTM over *M. tuberculosis* as the causative agent in cervical lymphadenitis in children (9:1, Lai KK et al. 1984). Several studies reported the good outcome of surgical excision with about 80-95% cure rates (Schaad UB et al. 1979, Harris BH et al. 1982, Margileth AM et al. 1984, Wolinsky E et al. 1995). Together this led to a different approach with surgical removal of the affected lymph nodes as the treatment of choice. Medical treatment was still chosen for special cases where surgery was considered difficult or when surgery failed, or when tuberculosis could not be ruled out.

In the 1990s positive reports were published on the efficacy of the new group of macrolides in the treatment of *M. avium* infections in AIDS patients (Shafran SD et al. 1996) and case

reports suggested that particularly clarithromycin could be effective in treating NTM lymphadenitis (Green PA et al. 1993, Tessier M-H et al. 1994).

In 2001-2004 a prospective randomized nationwide trial was conducted in the Netherlands, comparing medical treatment with clarithromycin and rifabutin in a group of 50 children, with surgical excision of the involved lymph nodes in a second group of 50 children. Surgical excision showed a significantly higher success rate (96%) than antibiotic therapy (66%) (Lindeboom JA et al. 2007).

Histopathological findings

Descriptions of histopathology differ in different studies. Some authors find no distinctly different features of *M. avium* lesions from lesions caused by *M. tuberculosis*. Most authors describe some sort of granulomatous necrotizising inflammation with epitheloid cells and sometimes giant cells. Sometimes granulomas are described as caseating (Margileth AM et al. 1984), dimorphic (granulomatous and pyogenic inflammation, Wolinsky E et al. 1995) or non-caseating with micro abscesses (Albright JT 2003). Acid-fast bacilli (AFB) are usually scant in number and located in the periphery of the necrotic zone. Neutrophil polymorphs are found scattered throughout the necrotic foci.

A problem in most studies is that lesions from different NTM are not distinguished from one another, but described as one group. Another problem is that the lesions probably look different depending on the time from infection. Dimorphic granulomas were found early in infection, caseating granulomas at about eight weeks post-infection and calcified granulomas in very old lesions of 12 months (Wolinsky E et al. 1995).

Features that might be characteristic of NTM are irregular granulomas with stellate or serpiginous necrosis, lack of significant caseation and distribution of neutrophil polymorphs in the central areas of necrosis (Pinder SE and Colville A 1993, Evans MJ 1998).

In patients with specific immune deficiencies predisposing them to mycobacterial infections, two types of granulomas have been described in *M. bovis* Bacille Calmette-Guérin (BCG) infections (Emile J-F et al. 1997). Type I is called tuberculoid and consists of well-defined granulomas with epiteloid and multinucleated giant cells, few AFB, surrounded by lymphocytes and fibrosis, and occasionally with central caseous necrosis. Type II is called lepromatous-like and consists of ill-defined, poorly differentiated granulomas with few giant cells and lymphocytes, but widespread presence of macrophages loaded with high numbers of AFB. Type I is correlated with survival of patients, and Type II with death of patients. Single descriptions of lesions in disseminated or severe *M. avium* infections seem to give a similar histopathologic picture as the Type II (Margileth AM et al. 1984)

Children with immune deficiencies

Primary and acquired immune deficiencies/syndromes

While healthy children are seldom affected, children with immunodeficiencies quite often suffer from NTM-associated diseases. Children with severe combined immunodeficiencies (SCID), NF-kB essential modulator (NEMO) deficiency, chronic granulomatous disease (CGD) or with HIV infection might develop disseminated disease by NTM. Children that are treated with immunosuppressive drugs, including steroids, are also at increased risk, but infection does not seem to be very common or else not diagnosed very often. For example, only a few cases of NTM infection in pediatric hematopoietic stem cell transplant recipients have been described with gastrointestinal involvement and disseminated disease (Nicholson O et al. 2006).

Specific immune deficiencies

During the last 15 years a number of molecular defects have been recognized that are associated with NTM disease. These deficiencies have also given important clues as to the protective immune response against NTM (see below under "The host defence against *M. avium* infection").

There is a clinical syndrome of Mendelian susceptibility to poorly virulent mycobacterial species caused by different mutations in five different genes encoding for the p40 subunit of IL-12 (and Il-23), the receptors IL-12R β 1, IFN- γ R1, IFN- γ R2 and the signal transducer and activator of transcription type 1 (STAT1). There are recessive and dominant, complete and partial variants of these mutations. Complete IFN- γ R1 deficiency is the most severe condition and often fatal. The more severe deficiencies cause disease early in childhood, and the milder forms might go undetected until adulthood. The clinical manifestations are disseminated disease or recurrent infections with unusual localisations due to BCG or NTM, sometimes Salmonella and occasionally severe viral infections. Salmonellosis is more common in patients with deficiencies in the IL-12/IL-12R pathway than in patients with IFN- γ R deficiencies. More limited mycobacterial infections and especially osteomyelitis are associated with the partially dominant IFN- γ R1 deficiency with good prognosis. Mutations in the gene for STAT-1 have similar effects as deficiencies in the IFN- γ receptors (Ottenhoff TH et al. 2002, Tran DQ 2005, Haverkamp MH et al. 2006).

BCG vaccination and NTM

The incidence of NTM diseases in children increased in Sweden from 0.06/100 000 population to 5.7/100 000 after discontinuation of the general BCG vaccination program in 1975 (Romanus V et al. 1995) indicating that BCG might have a protective effect against NTM in addition to tuberculosis.

It has also been discussed whether exposure to NTM might decrease the effect of BCG vaccination in areas where a majority of the population are exposed to NTM, as in many

countries with subtropical or tropical climate; This question arose after reports from the so-called Chingelput BCG-trial (Baily GV. Tuberculosis Prevention Trial, Madras, 1979 and 1980) which is the largest controlled field trial of BCG to date.

The study started in the early 1970s and involved >360 000 persons >10 years old in rural villages in southern India. Persons were randomly allocated to receive either of two BCG vaccines or a placebo. Every 2.5 years a follow-up was performed and tuberculosis diagnosed. At the fifth follow-up the investigators found that the efficacies of BCG vaccines were equal to that of the placebo. The environmental NTM exposure rate in the area where the study was conducted was 95% (as measured by sensitin skin tests).

Experimental studies in mice showed that NTM had equal protective effect to *M. tuberculosis* challenge as the BCG vaccination. Therefore, one might interpret the Chingelput trial in a different way. Because of the high exposure to NTM, there was no real placebo group since these persons had an equal degree of protection as the BCG-vaccinated groups. The high number of clinical cases of tuberculosis was explained by the fact that most infections were exogenous re-infections that BCG does not protect against and were not endogenous reactivations (Smith D 2000).

NTM disease in adults

MAC cervical lymphadenitis is seldom seen in adults. In one study 5/154 cases were >12 years old (Tai KK et al. 1984).

Pulmonary disease

Pulmonary disease caused by *M. avium* or other NTM is very rarely found in children except in children with cystic fibrosis (CF). Predisposing factors in adults are smoking and/or a chronic pulmonary disease, such as, pneumoconiosis, chronic obstructive pulmonary disease or black lung in the elderly, even though a new group of patients, primarily women, without risk factors also present with this disease (Prince DS et al. 1989). It can appear as an apical fibrocavitary lesion or nodular bronchiectasis and as solitary or multiple pulmonary nodules (Ramirez J et al. 2008). Disease is quite indistinguishable from tuberculosis with non-specific symptoms, such as, fever, weight loss, non-productive cough, dyspnoea, sweats, fatigues and haemoptysis (Ashford DA 2001). Pulmonary symptoms, chest radiographic findings and two positive cultures from sputum are consistent with MAC pulmonary disease (Kasperbauer SH and Daley CL 2008). *M. intracellulare* is a more common etiologic agent than *M. avium*. Treatment is with a combination of four different anti-tuberculous drugs. Hypersensitivity pneumonitis in adults coupled to some sort of occupational exposure of aerosols is also associated with MAC (Shelton BG et al. 1999).

MAC disease in AIDS patients

NTM infections in patients with AIDS are almost always caused by *M. avium* and more seldom by *M. intracellulare* or other NTM (Good RC 1985). Also some serotypes (4 and 8) are more often isolated than others, and isolates are often closely related on RFLP or by other genetic molecular epidemiologic tools, suggesting either a common environmental

source of infection or a number of strains that are particularly virulent to AIDS patients or might act synergistically with HIV (Grange JM et al. 1990). As many as 40% of patients with advanced AIDS develop disseminated infection (Horsburgh CR 1991). CD4+ counts <100 is a risk factor. The disease is rarely localized to lungs or lymph nodes. Clinical features are fever, night sweats and cachexia and sometimes severe diarrhoea (Young et al. 1988). Organisms are found in the liver, bone marrow, peripheral blood, intestinal tract and faeces.

NTM disease in animals

Birds

Antemortem diagnostic methods of NTM in birds are not satisfactory, and the relevance of the occurrence of *M. avium* in a faecal sample in a healthy bird is questionable as it might only reflect environmental non-pathogenic mycobacteria just "passing through". For want of anything better, the diagnosis in birds should be based on post-mortem macro and microscopic findings of mycobacteriosis.

The clinical signs of disease in birds are quite non-specific, the most consistent symptom being weight-loss. Sudden death in an apparently healthy bird is not uncommon. Abdominal distention, diarrhoea, lameness and ocular or skin infiltrations are other symptoms (Tell LA et al. 2001).

Port of entry of the bacteria is the gastrointestinal tract after ingestion. As a result the most commonly affected organs are intestines, liver and spleen. Lesions in bone marrow, lung, ovaries/testes and in kidneys occur. Typically there are large numbers of AFR in the granulomatous tissue. Lesions can be tuberculoid or non-tuberculoid. Enlarged liver and spleen are common, sometimes with distinct granulomas of varying size, sometimes with more diffuse histiocytic infiltration. Lesions are only occasionally characterized by classical tuberculous lesions, and thereby "bird tuberculosis" is more correctly called mycobacteriosis in birds (Tell LA et al. 2001).

Immunological factors in birds

There are a number of theories as to why certain bird species are more susceptible to *M. avium* disease and others less. There are both epidemiologic and genetic explanations.

One study found different susceptibilities to mycobacteriosis in birds from the Ardeideae and Threskiornithidae families during an outbreak of disease, despite that the birds from the two families shared the same environment and rearing conditions (Dvorska L et al. 2007). Lower incidences of disease in eider ducks than in mergansers, scoters and goldeneyes that all belong to the sea ducks and share similar life-styles have been reported (Cromie RL et al. 1991). Finally lower prevalence of infection in white morphs of the ring-neck doves (36%) was observed than in the non-white morphs (78%) (Saggese MD et al. 2008). These reports show that there are probable genetic factors in birds that make them more or less susceptible to infection with NTM.

Pigs

In pigs the disease seems to be restricted to the gastrointestinal tract with enlarged lymph nodes along intestines and sometimes also engagement of the liver and spleen, but no spread to other organs (in contrast to birds). Pigs seldom show symptoms, and the disease is discovered at examination after slaughter.

The host defence against *M. avium* infection

First line of defence

The mycobacteria infect the host by the intestinal or respiratory route and in exceptional cases by wounds penetrating the skin. The immune cells that first react to the bacteria are part of the innate immune system, which might be more important in mycobacterial infection than thought initially. The cells involved in the innate immune system respond mostly in a relatively unspecific way to "danger signals". However, the innate response and the acquired, more "specific" response are intertwined, and the cells regulate and stimulate each other in a dynamic way along the course of the infection.

The innate immune response

The innate immune response against *M. avium* is mediated by dendritic cells (DCs), monocytes, macrophages, neutrophils and natural killer (NK) cells. It has been demonstrated that *M. avium* signals through the Toll like cell receptor 2 (TLR2) (Lien E et al. 1999) and that TNF and IL-12 are important cytokines secreted by antigen presenting cells (APC) early in infection, even though *M. avium* seems to give less IL-12 than BCG (Demangel C et al. 2002). These cytokines stimulate anti-*M. avium* activity in macrophages and NK cells. These two cell types act in concert in the innate response against mycobacteria. IL-12 is also important in the induction and maturation of antigen-specific CD4+ T cells that will produce IFN-γ (Trinchieri G 2003). NK cells are important producers of IFN-γ early in infection (Smith D et al. 1997).

The acquired immune response

Specific CD4+ T lymphocytes protect against *M. avium* infection (Petrofsky M and Bermudez LE 2005). CD4+ T helper (Th) cells are roughly divided into different subsets according to their cytokine profile and to their contribution against different microbes (Zhu J and Paul WE 2008). Th1 cells are induced by IL-12 and typically produce IFN-γ and are important in the defence against intra-cellular microbes, whereas Th2 cells are induced by and typically produce IL-4 and are needed in the defence against parasites. Recently, Th17 cells, named after their production of IL-17, were added to the Th family. Although their role is far from clear, they seem important in the defence against worms and certain bacteria. It has been clearly shown that Th1 immunity is essential to the control of *M. avium* infection (Danelishvilli L and Bermudez LE. 2003), which is further supported by the findings of NTM infections in patients with defects in Th1-immunity, i.e. molecular defects

in the IL-12/ IFN- γ axis. Especially IFN- γ is essential for protection, shown both in the mouse model and in humans. The role for CD8+ T cells in *M. avium* infection is less established than for *M. tuberculosis* and CD8+ T cells have been shown to undergo apoptosis early in infection with *M. avium*. $\gamma\delta$ T lymphocytes are increased in number during *M. avium* infection (Danelishvilli L and Bermudez LE 2003) .

Cytokines of importance during *M. avium* infection

TNF

TNF is secreted early in mycobacterial infection by neutrophils, macrophages and NK cells, stimulating anti-mycobacterial activity in macrophages (Danelishvilli L and Bermudez LE 2003). TNF is also involved in the trapping of mycobacteria by apoptosis (Fratazzi C et al. 1999).

Mice that lack the TNF receptor have more severe disease as well as disorganized granuloma when infected with *M. avium* (Ehlers S et al. 1999). Patients that are treated with infliximab (a TNF blocker used in the treatment of different rheumatologic diseases and of Crohn's disease) have an increased risk of reactivation of tuberculosis (Gardam MA et al. 2003) and a few cases with NTM infections have also been reported (Salvana EM et al. 2007).

However, the protective role of TNF in *M. avium* infection seems to be more modest and transient than that of IFN-γ (Appelberg R et al. 1994). Levels of TNF decrease later in the course of infection (Danelishvilli L and Bermudez LE 2003).

TNF is negatively regulated by IL-10 (Couper KN et al. 2008).

IL-12

II-12 is considered to have a major role in the defence against *M. avium* (Bermudez LE et al. 1995) mainly by activating Th1 and IFN-γ producing cells, i.e. T cells and NK cells (Saunders BM et al. 1995). IFN-γ in turn, exerts a positive feed-back control on macrophages by stimulating their II-12 production. Monocytes/macrophages, neutrophils and DCs also produce IL-12 early in infection (Trinchieri G 2003).

The exclusive role of IL-12 in priming naïve CD4+ T cells to develop into Th1-committed T cells has been questioned. This might instead be more dependent on other TLR signalling pathways, and IL-12 might be more important in the expansion and fixing of already Th1 committed T cells (Trinchieri G 2003).

Some studies intriguingly indicate that IL-12 also stimulates IL-10 production at the same time as IFN- γ (Gerosa F et al. 1996). Il-10 is a potent inhibitor of IL-12 (Couper KN et al. 2008) and the induction of IL-10 could be an early start of down-regulatory pathways. IL-12p70 is formed by two subunits, p35 and p40. Another cytokine, IL-23, is formed by the p40 subunit together with a p19 subunit. The role of the Th1 inducing capacity of IL-12 might have been over-interpreted in some studies since the use of antibodies specific for the p40 subunit does not discriminate between the effects of IL-12 and IL-23 (Trinchieri G 2003). Moreover, the p40 subunit homodimer, Il-12p80, has also been suggested to have an active role, partly as a negative regulator by competitive binding to the receptor IL-12R β 1,

Cytokine	Sources	Stage	Effects	Comment	References
IL-10	DCs, Macrophages, T-cells	Early and late	Immuno-regulatory. Supresses TNF, IL-12p70 and IL-17. Inhibits macrophages and DCs. Down-regulates Th1 (and Th2).	To induce high IL-10 levels might be a way for <i>M. avium</i> to reduce protective Th1 inflammatory response in the host.	Couper KN et al. 2008.
INF	Neutrophils, macrophages, NK-cells	Early	Induces anti- <i>M. avium</i> activity in macrophages. Is involved in apoptosis of infected cells.	TNF has a transient role in the protection against M. avium.	Danelishvilli L and Bermudez LE 2003
IL-12p70	Macrophages, neutrophils, DCs	Early	Induces production of IFN- γ . Is involved in the priming, expansion and fixing of Th1 committed T-cells.	IL-12p70 consists of the subunits p40 and p35. Roles of the homodimer, IL-12p80 (p40 and p40), and of IL-23 (p40 and p19)?	Trinchieri G 2003 Ottenhoff TH et al. 2002
IFN-γ	NK-cells Th1 cells	Early Late	Stimulates anti- <i>M. avium</i> acitivity in macrophages/limiting <i>M. avium</i> growth. Role in granuloma formation. Stimulates II-12p70, TNF and NO. Involved in regulation of IL-17.	IFN- γ is the most important cytokine in the defence against M . avium.	Danelishvilli L and Bermudez LE 2003 Cooper A et al. 2002 Cruz A et al.2006
IL-17	γδ-T-cells Th17 cells	Early and late	Stimulates neutrophil-mediated inflammation. Enhances expression of β-defensins. Stimulates fusion of DCs and formation of giant cells.	Probably has a role both in the protection against mycobacteria and in the immunopathology/ granuloma formation.	Matsuzaki G and Umemura M 2007. Cooper AM and Khader SA 2008

Table 1. Cytokines of importance in the defence against M. avium.

partly as an agonistic factor in promoting migration of bacterially stimulated DCs (Cooper AM and Khader SA 2007).

IL-10

IL-10 is produced in M. avium infections. This cytokine has an immunoregulatory function for the immune system in limiting the pathology of highly virulent infections. Its role is complicated as too much IL-10 at the wrong time during an infection with a pathogen of low-to-moderate virulence might inhibit the proinflammatory response, resulting in worsened or uncontrolled infection (Couper KN et al. 2008). The total effects of IL-10 are inhibition of macrophages and DC functions and down-regulation of both Th1 and Th2 responses, with inhibition of the production of IL-1 α and β , IL-6, IL-12, IL-18 and TNF. It is also interesting to note that some proinflammatory cytokines, such as, IL-12 and IL-17, directly induce IL-10, thus allowing a self-regulation of their inflammatory response (Couper KN et al. 2008).

Transgenic mice overexpressing IL-10 are more susceptible to mycobacterial infections, and IL-10 knockout mice are less susceptible to the infection (Roque S et al. 2007).

IL-10 blocks apoptosis in surrounding macrophages and other cells by suppressing TNF (Feng CG et al. 2002).

MAC strains (isolated from human sputa) were reported to induce lager amounts of IL-10 from stimulated peripheral blood mononuclear cells (PBMCs) than *M. tuberculosis* strains (Ueda W et al. 1998).

IFN-

IFN- γ mediates its effects through two receptors, IFN- γ R1 and IFN- γ R2, which activate the STAT-1 intracellular signalling pathway. IFN- γ is necessary for control of mycobacterial infection. Early in infection IFN- γ is produced by NK cells (Smith D et al. 1997) and later by activated T cells. IFN- γ together with TNF stimulate anti-mycobacterial activity in macrophages (Danelishvilli L and Bermudez LE 2003).

Lack of IFN- γ leads to a disorganized granulomatous response to mycobacterial infection both in mice (Cooper A et al. 2002) and in humans (Emile JF et al. 1997). Therefore IFN- γ seems to play a role not only in limiting bacterial growth, but also by limiting the damaging immunopathology in chronic mycobacterial infection. This might be mediated through nitric oxide (NO) that is produced by macrophages in response to IFN- γ . NO impedes the adherence and transmigration of monocytes and granulocytes, and limits lymphocyte function and inflammatory response to *M. avium* which results in an immuosuppressive effect (Cooper A et al. 2002).

IFN- γ seems to be involved in the regulation of IL-17 production (Cruz A et al. 2006).

IL-17

IL-17, a cytokine that seems to be important in the pathogenesis of autoimmune diseases, being involved in tissue destruction by inflammatory cells, has recently been increasingly noted for its implication also in the protection against infections (Matsuzaki G and Umemura M 2007).

Studies of the importance of this cytokine in the defence against M. tuberculosis and in experimental infections with M. bovis BCG reveal that indeed it has an important role both in the killing of bacteria and in granuloma formation (Scriba TJ et al. 2008, Umemura M et al. 2007). In mycobacterial infections TCR $\gamma\delta$ T cells seem to be the main producers of IL-17 (Lockhart E et al. 2006) and it has been shown that $\gamma\delta$ T lymphocytes are increased in number also during M. avium infection (Danelishvilli L and Bermudez LE 2003). II-23, which is a cytokine that shares a common subunit, p40, with IL-12p70, stimulates the production of IL-17. IL12/23 knock-out mice (deficient in the p40 subunit) were more susceptible to mycobacteria than mice deficient in p35 (the other subunit of IL-12p70) suggesting the importance of both the IL-12p70/INF- γ axis and the IL-23/IL-17 axis in mycobacterial infections (Cooper AM and Khader SA 2008).

IL-17 stimulates G-CSF and IL-8 production that induces neutrophil-mediated inflammation, and it also enhances expression of anti-bacterial peptides, β-defensins (Matsuzaki G and Umemura M 2007).

IFN- γ has been shown to inhibit IL-17 production. This is done by IFN- γ stimulation of IL-12p70 production (thus lowering the IL-23 production by competition) and up-regulation of IL-12R β 2 resulting in expansion of IFN- γ -producing T-cells at the expense of Th17 cells (Cruz A et al. 2006). This inhibition is probably a necessary regulatory pathway for the host, since experiments in IFN- γ deficient mice show persisting increase in IL-17 leading to more neutrophils in granulomas and a destructive granulomatous response in BCG infections. It has also been shown that IL-17 stimulates fusion of DCs with formation of giant cells in the Langerhans cell histiocytosis model (Coury F et al. 2008). Giant cells are another important characteristic of tuberculous granulomas.

Cells of importance during M. avium infection

Neutrophils

It is known that neutrophils might kill *M. avium*. In mice, neutropenia during the first one to two weeks of infection (but not later in the course of infection) increased the bacterial loads in tissues, indicating a role in the early phase of infection. Neutrophils probably also have a role in granuloma formation (Danelishvilli L and Bermudez LE 2003).

Dendritic cells (DCs)

DCs encounter the bacteria early in infection on mucous membranes and in the skin. TLR2 on DCs reacts to diverse "danger signals", i.e. *M. avium* antigens, activates the cell that processes antigens and presents bacterial peptides on MHC I and II receptors on its surface. At the same time the DCs increase the production and presentation of T cell co-stimulatory molecules on the surface leading to stimulation of T cells. DCs are therefore a link between the innate and the acquired immune system. Through changes of chemokine receptors and adhesion molecules in contact with mycobacteria, DCs are then, when activated, able to leave the periphery and travel to local lymph nodes where they fulfil their function as antigen presenting cells (APC) (Lewinwohn DA et al. 2004).

Mycobacteria secrete huge amounts of lipids within the phagosome in macrophages. These lipids are transported around in the different cell compartments and are also secreted by exocytosis and might be taken up by surrounding cells, such as DCs. In contrast to MHC I and II that are molecules presenting bacterial protein products to other cells, the CD1 molecules, which are also expressed on DCs, are able to present mycobacterial glycolipids to so-called CD1-restricted T cells (i.e. CD1-controlled NK T cells and some CD1-dependent CD8+ cells) (Schaible UE and Kaufmann SH 2000).

In *M. tuberculosis* infection it is shown that DCs produce IL-12 that further stimulates a Th1 response (Romani L et al. 1997). However, in experimental infection with BCG (in mice), DCs were shown to produce IL-10 that was shown to down-regulate the IL-12p70 production of neighbouring DCs. Interestingly, IL-10 has also been shown to decrease the migratory ability of the DCs (probably via the down-regulation of IL-12p80, as shown by Khader SA et al. 2006) during BCG and TB infections in mice (Demangel C et al. 2002, Cooper AM and Khader SA 2007).

Macrophages

Macrophages constitute the main localization for *M. avium* infection where the bacteria are able to survive in phagosomes that are not acidified or fused with lysosomes due to the inhibition by the bacteria themselves (Danelishvilli L and Bermudez LE 2003). Macrophages exert anti-mycobacterial activity through the increase of bactericidal proteins and superoxide anion production which impairs the mycobacteria from replicating within the phagosome.

They can also trap the mycobacteria by undergoing apoptosis, which is an important defence mechanism since it contains the bacteria within apoptotic bodies and prevents the release of bacteria and spread of infection. Apoptosis in macrophages is probably TLR2-mediated (Quesniaux V et al. 2004). Macrophages are stimulated by cytokines secreted by neutrophils, NK cells and T cells and by autocrine production. TNF and IFN-y are the most important stimulators. Optimal macrophage activation requires direct contact with NK cells. The macrophage in turn is an important producer of IL-12 (Danelishvilli L and Bermudez LE 2003).

Figure 2. M. avium within cells. (Image: Dr. Edwin P. Ewing, Jr.)

Natural killer (NK) cells

NK cells have been shown to have an important role in the defence against M. avium infection. They are stimulated by IL-12 and IL-18 from macrophages and from neutrophils. In turn they stimulate anti-mycobacterial activity in macrophages by the release of cytokines, mainly TNF and IFN- γ and by direct cell-to-cell contact with macrophages (Danelishvilli L and Bermudez LE 2003).

CD4+ T helper cells

T cell depletion studies have shown that CD4+ T cells, coupled with reduced expression of IFN- γ and TNF, are required for control of *M. avium* infection in mice (Appelberg R et al.1994). Thus, T cells regulate macrophages and DCs by cytokine production that induces anti-mycobacterial activity, but also by direct cell-to-cell contact, where the CD40 ligand on T cells binds to CD40 molecules and activates the antigen-presenting cells leading to inhibited growth of *M. avium* (Hayashi T et al.1999).

Manipulation of the immune defence by the mycobacteria

Blocking of phagolysosome fusion

M. avium infects primarily monocytes or macrophages where it lives and replicates in non-acidified vacuoles that do not mature and do not fuse with lysosomes. Less is known about if and how *M. avium* blocks the phagosome-lysosome fusion, but one study suggests that the transcription factor NF-κB, which is blocked early in *M. avium* infection, is of importance in regulating genes involved in membrane trafficking and lysosomal enzymes (Gutierrez MG et al. 2008). The "hiding" in phagosomes is a way for the bacteria to escape the immune system.

Inhibition of apoptosis

Some mycobacteria seem to be able to inhibit apoptosis of infected cells, perhaps by inducing IL-10 that inhibits the effect of TNF on apoptosis (Feng CG et al. 2002).

Suppression of cytokine production and manipulation of cytokine responses

M. avium secretes large amounts of lipids within the macrophage. Soluble preparations of M. avium glycopeptidolipids have been shown to inhibit cytokine production (Horgen L et al. 2000), which is in accordance with observations that the bacterium itself seems to have properties that lower the response of both TNF and IFN- γ .

One way of manipulating the cytokine response, shown in the case of M. avium at least for IFN- γ , is to induce the production of suppressors of cytokine signalling (SOCS) that suppress the phosphorylation of the JAK/STAT1 signalling pathway, thereby impairing the effects of IFN- γ (Vázquez N et al. 2006).

Differences in the immune response in M. tuberculosis and M. avium infection

M. tuberculosis stimulates both TLR2 and TLR 4, whereas *M. avium* stimulates only TLR2 (Quesniaux V et al. 2004).

CD8+ T cells undergo apoptosis early in the infection with *M. avium* (Roger PM and Bermudez LE 2001) whereas they are important cells in the immune defence against *M. tuberculosis* (Danelishvilli L and Bermudez LE 2003).

Differences in the immune system between children and adults that may be of importance in mycobacterial disease

Although immunological studies might be difficult to carry out in young children, because quite large volumes of blood for testing are necessary for many immunological methods, there is evidence of impairment in both the innate and the adaptive immune system in children that might be of importance in mycobacterial disease.

There seem to be deficiencies in the macrophage function in young children. They also have poor DC functioning leading to poor T cell priming. In many congenital infections (syphilis, rubella and cytomegalovirus) infants have absent or reduced CD4+ T cell responses. In general, children seem to be biased towards development of Th2 responses to antigens (Lewinsohn DA et al. 2004). However, vaccination with BCG at birth is able to induce a Th1-type T cell priming response in infants, giving rise to PPD specific CD4+ T cells that persists up to one year of age (Vekemans J et al. 2001). About 60% of children who are vaccinated with BCG at birth develop a positive delayed T cell hypersensitivity (DTH) response (i.e. skin induration at PPD testing) which reflects, but is not strictly correlated with, T cell immunity. However the response wanes rapidly.

Newer immunological methods that require less amounts of blood will permit further studies on the immune system in young children.

See simplified scheme of immunological pathways, figure 3.

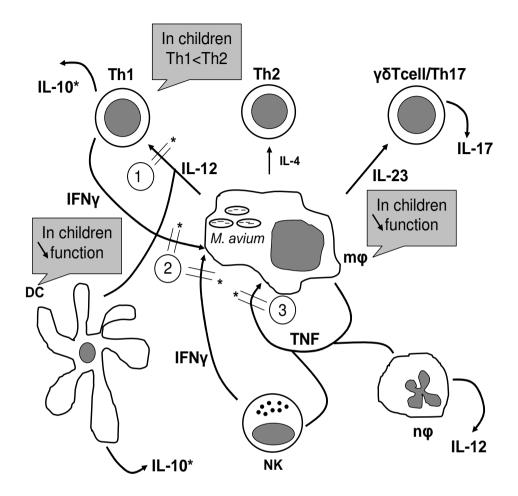


Figure 3. Immunological pathways of importance in the defence against M. avium infections. Children have deficiencies in macrophage and DC functions, and are biased towards development of Th2 responses, which probably render them more susceptible to mycobacterial disease. Note that this is a simplified scheme. The role of Th17 is so far unsettled. While Th17 often has been associated with CD4 Th cells, in the context of mycobacteria -T cells have been identified as important producers of this type of response.

- * IL-10 inhibits the effects of TNF and IL-12 (and indirectly of IFN-).
- **DC** dendritic cell, **NK** Natural killer cell, **Th** T helper cell, $\mathbf{n}\boldsymbol{\varphi}$ neutrophil, $\mathbf{m}\boldsymbol{\varphi}$ macrophage.
- Mutations in the IL-12 receptor genes or in the p40 subunit cause increased susceptibility to M. avium and other intracellular bacteria.
- Mutations in the IFN- receptor genes or in STAT1 (the intracellular signalling pathway of IFN-) cause increased susceptibility to M. avium and other intracellular bacteria.
- TNF blockers (for example infliximab) increase the risk for TB infection and there are case reports of NTM disease.

AIMS

- To determine whether children with *M. avium* lymphadenitis are infected with the same type of strains as birds and other animals as an indication of possible zoonotic spread of the disease.
- To establish whether there is a seasonal variation in the incidence of *M. avium* disease in children.
- To study the geographical distribution of *M. avium* lymphadenitis in children in relation to proximity to water, climate and urbanisation to clarify the epidemiological importance of environmental factors in the spread of these bacteria.
- To describe the clinical features, treatment and outcome of *M. avium* lymphadenitis in children in Sweden.
- To assess possible immunological differences between *M. avium avium* and *M. avium hominissuis* strains after in vitro stimulation of peripheral blood mononuclear cells by quantification of induced cytokine levels, as a means to establish the role of interactions between the bacteria and the immune system in the host specificity of these two subspecies.

MATERIAL AND METHOD

Patient groups

In all clinical and epidemiological studies (papers II and III) only children <7 years old with culture verified *M. avium* infection were included. The seventh birthday was chosen as the upper limit since the disease is uncommon above that age, and we wanted as homogenous a group as possible.

For calculation of annual incidence of *M. avium* disease in children and incidence in child populations living in different ecologic and geographic areas (cultivation zones and water categories, see paper II) the material consisted of 186 cases that had been reported to the Swedish Institute for Infectious Disease Control (SMI) between 1998 and 2003. One child with lung infection following aspiration of a foreign body was excluded. Additionally two children whose home addresses were unknown were excluded in the epidemiological studies. In total 183 children were included in these studies.

For the study of seasonal variation and clinical features of *M. avium* lymphadenitis in children (papers II and III) we aimed at obtaining medical records from the above 186 children. One of the seven ethics committees in Sweden (Örebro) did not allow us to copy the children's records, and therefore, 11 cases from this area were excluded. Another ethics committee in Lund approved the study, but wanted a special arrangement with opt-out information to the patients' families. (Opt-out information means either personal information about the study or general information, such as, an announcement in a newspaper that is supposed to reach the patient's families, so that they can contact the researcher if they do not want to take part in the study. No written consent is required.) Collection from this area in Sweden proved to be more difficult since some clinics did not have resources or time to fulfil this stipulation. We obtained 128 patient records (whereof one was excluded because of lung infection from a foreign body). In addition we added 35 records of previously known cases that had occurred between the years 1983 and 1997. In total, 162 children were included in these studies.

The children's medical records contained variable amounts of clinical information. All 162 records contained at least information on the age of the child and the month of onset of the disease (defined as the month a caretaker discovered an enlarged lymph node).

Strains

In all we have analysed 193 strains of *M. avium* with IS*1245* RFLP. The strains were obtained from SMI and from the clinical microbiology laboratories at Karolinska University Hospital in Stockholm, Sahlgrenska University Hospital in Göteborg, Linköping University Hospital, Malmö University Hospital and Umeå University Hospital. Further, nine isolates were obtained from Dr Kauppinen in Finland (Kauppinen J et al. 2001).

In paper I, 105 strains were analysed, 32 that had been isolated from children, 28 that had been isolated from HIV-positive and HIV-negative adults, and 45 that had been isolated

from different animals. In paper IV, 21 strains were used, nine that had been isolated from animals, one that had been isolated from an HIV-negative adult and 11 that had been isolated from children (see Figure 4).

The remaining results of the RFLP IS 1245 analyses are yet unpublished but were reported in a poster and published as an abstract at the Seventh International Conference on the Pathogenesis of Mycobacterial Infections, 2008, Saltsjöbaden, Stockholm, Sweden, and are not shown in this thesis.

Methods

Restriction fragment length polymorphism (RFLP)

This method analyses the genomic DNA using restriction endonucleases that cut the bacterial DNA into fragments. Separation of DNA fragments by agarose gel electrophoresis and transfer of DNA by Southern blot to a membrane, precede hybridization with specific probes for insertion elements and detection with chemoluminiscence methods which give different band patterns (fingerprints) for each strain examined.

The bacteria contain different insertion elements belonging to the family of insertion sequences (IS) and transposons. Species-specific insertion sequences can be used for species identification. Within a species, different strains might contain varying numbers of the insertion sequences making it a valuable tool in epidemiological studies.

IS6110 is used for the standardized fingerprinting of *M. tuberculosis* (van Embden JD et al. 1993). In the MAC group, IS900 is specific for *M. avium* subsp. *paratuberculosis* and IS1141 is present in *M. intracellulare*. IS1245, IS1311 and IS901 are present in *M. avium* subsp. *avium*.

IS1245, IS1311 and IS901

IS1245 is a 1414 bp-long insertion element that shares 85% sequence identity with IS1311 at the DNA level. A 427 bp probe (produced by PCR) has been used in hybridization experiments of IS1245. It was first shown in 1993 that only mycobacteria included in the *M. avium* group (*M. avium* subsp. *avium*, *M. avium* subsp. *paratuberculosis* and *M. avium* subsp. *silvaticum*) contained this element (Guerrero C et al. 1995). Several studies reported comparable results to the pulsed field gel electrophoresis (PFGE) method, and the IS1245 RFLP method was considered a useful tool in epidemiological studies. In 1998 a proposal for standardization of the method was published (van Soolingen D et al. 1998). IS1245 was shown to be quite stable, both in vivo and in vitro over time except for small one or two band changes (Bauer J and Bengård Anderson Å 1999). It was noted, however, that some hybridization bands appeared weaker than others, and this was hypothesized to be due to cross-hybridization with IS1311.

Host	Material	Year	RFLP IS 1245 profile
Pig	Liver	1986	
Horse	?	1987	
Buzzard	liver	1986	
Cow	LN	1986	1 11
Human	Sputum	1983	
Pig	Liver	1986	11
Pheasant	Granuloma	a 1986	
Pig	Liver	1986	
Cat	?	1990	
Pig	Liver+LN	1986	
Child	LN	2002	
Child	LN	2002	
Child	LN	2002	
Child	LN	1990	
Child	LN	1997	
Child	LN	2001	
Child	LN	1997	
Child	LN	2002	·
Child	LN	1984	
Child	LN	1998	
Child	LN	1983	

Figure 4. Restriction fragment length polymorphism (RFLP) IS1245 profiles and origin of 21 M. avium isolates. The first 10 isolates show the typical bird-type/three-band profile characteristic of M. avium avium. The three bands represent in reality one copy of IS1245 and two copies of IS1311 that are hybridized because of cross-reaction with the IS1245 probe. The following 11 isolates show multiband profiles characteristic of M. avium hominissuis. LN – Lymph node.

These are the 21 strains used in the immunological experiments (see paper IV).

A typical three-band pattern, called the bird type profile, was described in IS1245 RFLP in strains mainly isolated from birds and associated with serotypes 1, 2 and 3 (Ritacco V et al. 1998). Previously, IS901 had been found in a subgroup of *M. avium* strains specifically associated to animal pathogenicity (Kunze ZM et al. 1992). It was shown that the IS901 containing strains all exhibited the bird-type/three-band profile on IS1245 RFLP (Ritacco V et al. 1998). Isolates from humans and many porcine isolates instead contained multiple bands on IS1245 RFLP. This led to the suggestion to restrict the designation *M. avium avium* to the bird-type/three-band profile strains and the designation *M. avium hominissuis* for the human/porcine multiband profile strains (Mijs W et al. 2002) (For examples of the different RFLP IS1245 profiles, see figure 4).

There seemed to be a discrepancy and sometimes confusion in the literature about the presence and copy number of IS1245 and IS1311 in the different *M. avium* subspecies. When a shorter probe to IS1245 (175 bp) that shared only 75% homology with the IS1311 probe (198 bp) was used, it was shown that the classical three-band pattern of IS1245 in *M. avium avium* instead consisted of one copy of IS1245 and two copies of IS1311 (Johansen TB et al. 2005). It was also shown by this more specific probe that *M. avium paratuberculosis* did not contain any IS1245, but displayed seven bands with IS1311.

In our studies we have performed IS1245 RFLP according to the standardized method with the long IS1245 probe (van Soolingen D et al. 1998). Initially we also performed IS1311 RFLP on some isolates (unpublished work) and noticed the common faint bands due to cross hybridization. In the first study (paper I) we used an external marker (a reference strain) for standardization. For epidemiological purposes, the results were well interpretable when it came to distinguishing the bird-type/three-band profile from the multiband profile and making gross comparisons of multiband profile strains. In later experiments, however (see Thegerström J, abstract at the Seventh International Conference on the Pathogenesis of Mycobacterial Infections, 2008, Saltsjöbaden, Stockholm and paper IV) we instead used an internal size marker which permitted an even more precise standardization, which is preferable when it comes to comparing the strains containing a high number of bands (as in the children's isolates) to one another.

Immunological experiments

Preparation of bacteria for stimulation tests

The *M. avium* strains were grown on Middlebrook 7H10 agar for about four weeks and harvested in Dubecco's endotoxin-free PBS (because endotoxins might influence the stimulation of peripheral blood mononuclear cells (PBMCs) and the release of cytokines). Bacteria were killed by UV-irradiation. It had been shown experimentally that at least one hour irradiation time is required (Jönsson B. unpublished work). Bacterial concentration was adjusted, and suspensions of bacteria were stored in -70°C until used. Inactivated bacteria were re-inoculated on Middlebrook 7H10 agar and growth was checked after eight weeks.

Isolation of peripheral blood mononuclear cells (PBMC) and stimulation tests

This method has been elaborated and used for a long time at the Department of Clinical Microbiology, Sahlgrenska University Hospital, Göteborg (Hessle CC et al. 2005) and is described in detail in paper IV.

Cytokine measurement by multiplex bead array analysis

This technique combines the principle of a sandwich immunoassay with the fluorescent-bead-based luminex technique. It is a relatively new technique, and its advantages are that it allows the analysis of several cytokines in a single sample and allows the determination of cytokine levels with good precision within a wide range.

Based on the principles of flow cytometry, microspheres (beads) are lined up one by one, and two Luminex lasers read both the type of cytokine, recognized on each bead's special dye mixture colour, as well as the amount of cytokine bound to the bead, measured by the intensity of Phycoerythrin luminescence that is proportional to the amount of cytokine in pg/mL, using the standard curve.

In our experiments (paper IV), there were about 2700 individual analyses of cytokine levels to be done. The cytokine levels had very different ranges, from extremely low as in IL12p70 (just above detectable levels) to very high as in IFN- γ and in IL-10 (in particular for the Gram negative control). This meant that we had to do dilutions and multiple analyses, requiring a great amount of work.

RESULTS AND DISCUSSION

An obvious problem in studying *M. avium* and in reviewing the literature of both epidemiological and immunological studies is the vast array of phenotypes expressed within the *M. avium* group, and the uncertainties and changes in taxonomy within the species. The interpretation of those studies, where the relatively recent separation of *M. avium avium* and *M. avium hominissuis* was not yet implemented, is particularly difficult. Seemingly contradictory results in different studies of *M. avium* might be explained by the use of very different strains in the experiments (Turenne CY et al. 2007).

The strength of our set of studies is that we have consequently endeavoured to include and study as clearly defined and homogenous groups of bacteria or patients as possible. In the epidemiological studies we include only children with *M. avium* infection in contrast to many others that study NTM lymphadenitis in children as one group. Thanks to the RFLP IS 1245 studies we are well acquainted with our isolates and their subspecies. In the immunological study the specific aim was to compare the effects on the immune system of two well-defined groups within *M. avium*, namely, *M. avium avium* and *M. avium hominissuis*.

Epidemiology (papers I, II and III) Is *M. avium* infection in humans a zoonosis?

Infection by contact with birds or other animals (paper I)?

Pet birds

The earlier belief that children are infected through pet birds stems partly from case reports and partly from epidemiological studies. The presence of pet birds in the homes of healthy schoolchildren reacting to *M. avium* or *M. scrofulaceum* sensitins was significantly higher than in those of sensitin-negative children (Lind A et al. 1991). In our material of 162 reviewed medical records of children with *M. avium* lymphadenitis, we could not find any indication of this (Paper III; Thegerström J et al. 2008), but as it was a retrospective study, we could not be sure that all the children had been asked about bird or animal contacts or if so, that this was noted in their records.

In connection with a recent prospective Dutch nationwide multi-centre study with the objective to study the treatment of NTM cervicofacial lymphadenitis in children (Lindeboom JA et al. 2007) it was noted not least in the press a concern over "bird tuberculosis". The researchers investigated this further (Bruijnesteijn van Coppenraet et al. 2008); it was noted through a questionnaire for epidemiological purposes that 8 out of 34 children were in households that kept pet birds, but the presence of pet birds in the general population was not known. On IS 1245 RFLP studies all isolates from children showed multiband profiles, confirming infection with *M. avium hominissuis*. No *M. avium* could be isolated from bird materials collected from the patient's pet birds. The researchers conclude that the disease in children is not related to "bird tuberculosis".

A remote possibility remains that pet birds all the same occasionally would be colonized with *M. avium hominissuis* and pass this on to children, but since *M. avium hominissuis* very rarely has been isolated in pet birds this is highly unlikely.

Wild birds

The hypothesis that children were infected through wild birds or other animals is based on the fact that only small children develop the particular form of infection with *M. avium* lymphadenitis in the head and neck region, leading to the speculation that the children are exposed to *M. avium* in a way adults are not. This form of the disease almost does not exist in other age groups. The hypothesis is that small children by their behaviour are exposed to different materials heavily contaminated by bird faeces, in putting things they find on the ground in their mouths or by cuddling with animals.

In paper I (Thegerström J et al. 2005) we wanted to investigate the possibility that children were infected with similar strains as birds or other animals. Using the RFLP IS 1245 method we typed 105 isolates. All 32 isolates from children showed multiband profiles characteristic of *M. avium hominissuis*, and 12 of 14 isolates from birds showed the typical bird-type/three-band profile characteristic of *M. avium avium*. Two birds were infected with multiband strains. These isolates were provided by Dr Kauppinen in Finland from farmraised lesser white fronted geese. It shows that particularly birds in captivity or living in an environment close to human activities are able to pick up *M. avium hominissuis*, probably from the surrounding environment.

Pigs

The high prevalence of *M. avium hominissuis* isolates among pigs and close genetic relatedness between porcine and human *M. avium hominissuis* isolates on RFLP IS*1245* studies have led to the speculations either that pigs could be a source of infection to humans by contaminated meat or that pigs and humans share a common environmental source of infection. One study found that 61% of human isolates and 59% of porcine isolates were at least 75% alike on RFLP IS*1245* (Komijn et al. 1999). The human isolates that are compared to the pig isolates are in many studies sputum isolates, which seem to give the best match with porcine isolates. Isolates from blood in AIDS patients are also used for comparison, but rarely isolates from children. In our study (Thegerström J et al. 2005) none of the 13 *M. avium hominissuis* isolates from pigs clustered with any of the 32 isolates from lymph nodes in children. One of the isolates from an HIV-positive patient clustered with two porcine isolates in our material (80% resemblance). In Norway one human isolate from a lymph node (probably a child) was identical to a porcine isolate in the same geographical area (Johansen TB et al. 2006).

A slightly higher number of NTM cases in children than expected was found in a region of the Netherlands which was particularly rich in pig farms, leading to the speculation of a connection (Haverkamp et al. 2004). In our epidemiological study of the geographical distribution of *M. avium* cases in children in Sweden (paper II, Thegerström et al. 2008) we found no relation to pig farms, even if we did not examine this explicitly. Pig farms in

Sweden are mostly located in the southern part (cultivation zone 1-2, see Figure 5) and in inland areas, whereas we found the highest incidence of disease in the coastal areas and in an area in the north of Sweden (cultivation zone 5, see Figure 5).

An interesting question is why there is such a great variation in the proportions of *M. avium avium* and *M. avium hominissuis* in pigs among different geographic areas? Studies from Germany, Sweden and New Zealand found an equal proportion or domination of *M. avium avium* in pigs, whereas studies from Norway, Finland, Ireland and the Netherlands found no or only single such isolates. Is it due to different animal farming traditions, different feedstuff or bedding? Or is it due to sampling from farms of different sizes and varying degrees of industrialization? Could it be due to true differences in the prevalence of strains in the environment? It seems unlikely that the type of strains present in the environment should vary naturally to this degree in so closely neighbouring countries with similar climates and geography.

Infection by ingestion of water?

Identical matches by molecular methods

To establish a causal connection with high probability between a water source and disease in a patient, one has to show that isolates from patient and environmental source are identical with some molecular genetic method. A few cases have been demonstrated. Identical isolates on pulsed field gel electrophoresis (PFGE) were found in three AIDS patients and two hospital water samples, in two AIDS patients and three hospital water samples and in one AIDS patient and one river water sample in the US. The remaining 86% of patients had unique strains on PFGE that did not correlate with environmental samples (von Reyn CF et al. 1994).

Another study found only 1/88 clinical isolates that was identical with a drinking water sample on PFGE in the US (Hilborn et al. 2008).

By large-restriction-fragment (LRF) pattern analyses three *M. avium* sputum isolates from patients showed identical matches with hospital waters or in one case with tap water at home and a water reservoir (Aronson T et al. 1999).

Considering the great variation of *M. avium* strains present in the environment and the many possible water sources at different times for each patient, these cases, although few in number, do show that drinking water or hot water from showers are possible sources of infection with *M. avium*, at least in AIDS patients and possibly in adults with pulmonary disease even if exposure by aerosols is more natural to suspect in those cases.

No matches between environmental isolates (either natural or drinking waters) and isolates from children with *M. avium* lymphadenitis have been made.

Epidemiological data in favour of the water hypothesis (Paper II)

In paper II (Thegerström J et al. 2008) we conducted an epidemiological study where we sought to establish a correlation between *M. avium* disease in children and living close to

water, as this had only been indirectly implied earlier when referring to studies of sensitin reactivity in healthy populations. Larsson and co-workers showed in the late 1980s and early 1990s that 25 % of non-BCG-vaccinated schoolchildren in an urban coastal area in Sweden reacted to *M. avium* sensitin, whereas only 10% of a comparable group in an inland rural area was positive (Lind A et al. 1991, Larsson LO et al. 1993). In a healthy population of four to five year old children in the same urban coastal area as above, 8% reacted to *M. avium* sensitin (Larsson LO et al. 1991).

Study design

Knowing the reported number of cases of M. avium culture verified infections in children <7 years old during 1998-2003 and attributing a water category to each patient according to the home addresses, we calculated the incidence of the disease in the child population living (1) within a five km distance from the Baltic or the Western Sea (Coastal water category) or (2) within a five km distance from one of the three big lakes in Sweden (Lake Vänern, Vättern and Mälaren) alternatively within two km of a small lake or a river (Fresh water category) or (3) not close to water (Inland category) or (4) in one of the three largest cities in Sweden (Stockholm, Göteborg and Malmö; Urban category). Similarly we calculated the incidence in different cultivation zones (1-8, see Figure 5). We also determined the month of onset of disease and described a seasonal variation.

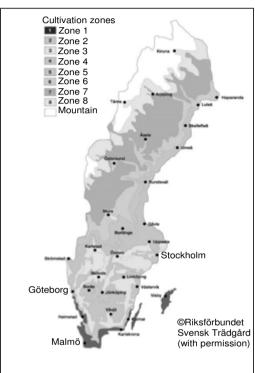


Figure 5. Map of Sweden, with the different cultivation zones (1-8 and mountain).

Results

The lowest incidences of *M. avium* lymphadenitis in children were found in the inland population and in the urban population. The incidence in the population living along the coast was comparable to the mean incidence of the country, but when adjusting for the low incidences in the cities, which all three are located by the sea, the incidence in the coastal areas was significantly higher compared to the inland areas. The population living along the western coast (salt water) and the population living along the eastern coast (brackish water in the Baltic Sea) had similar incidences. The highest incidence was found in the population living close to fresh water (lakes or rivers) (see Figure 6).

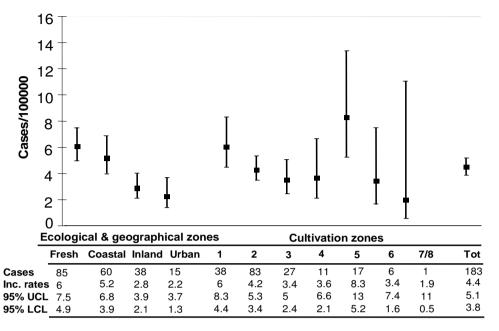


Figure 6. Number of cases, incidence rates (cases/100000 children/year) and 95% confidence intervals of M. avium disease in children grouped according to ecologic, geographic and cultivation zones, Sweden, 1998-2003.

Freshwater, coastal water, inland, urban (Stockholm, Göteborg and Malmö, the 3 largest cities in Sweden) areas and the different cultivation zones (1-8, zone 1 being the warmest) are depicted. UCL, upper confidence limit; LCL, lower confidence limit.

We also found a highly significant seasonal variation of the disease with a peak in October and a nadir in April. This differs from infectious diseases transmitted man-to-man which peak during the winter months and to some extent from diseases transmitted by vectors, like Lyme disease that peaks during the summer months.

Seasonal variation

Our interpretation of the seasonal variation is that it is due to a combination of changing temperature and nutritional conditions in the environment throughout the year and changing human activities in different seasons. Previous studies have shown either predominance of the disease in winter or no seasonal variation at all (O'Brien DP et al. 2000, Engback HC et al. 1981, Gill MJ et al. 1987). An Australian study showed a tendency of seasonal variation with lower incidence in autumn (Joshi W et al. 1989). However, this referred to admission date and not when a caretaker first noted an enlarged lymph node as we used in our study. Taking into account the delay between discovery of disease and admission at hospital (about two or three months) the lowest incidence would be in summer or spring also in the Australian study.

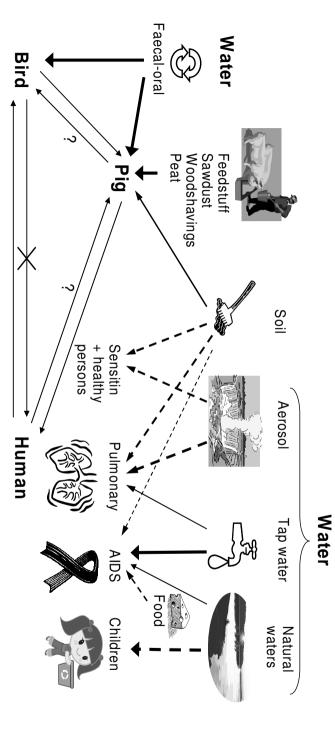
Cultivation zones

Our hypothesis was that the seasonal variation was directly caused by temperature variation during the year. According to the Swedish Meteorological and Hydrological Institute (SMHI, http://www.smhi.se/) the meteorological winter in the north of Sweden is approximately four months longer than in the south. In winter, with temperatures below 0°C, the ground frost and the icing would lower the amount of infectious bacteria in soil and water, and consequently decrease the incidence of M. avium infection during the corresponding period. If this was true, we could expect to find a difference of timing in the seasonal variation between the south and coastal Sweden, corresponding to the cultivation zones 1-3, and the north and inland of Sweden, corresponding to the cultivation zones 4-7. We would expect an earlier drop in incidence during fall in the north as well as a later rise in incidence in spring. However, we could not verify this in our results as the time for nadir of incidence was shifted by at most 30 days in zones 4-7 and the incidence curves of both regions peaked in October and was equally prolonged in January and February before dropping. One could also have expected a more flattened sinusoidal curve in the warmer region given that the climate conditions are more even during the year, but our results show the opposite. The length of winter, therefore, does not seem to be of much importance for the seasonal variation. However, because of compensation after the vernal equinox of more light in the North, the meteorological beginning of spring differs approximately 45 days between the north and the south and that of summer does not differ more than 21 days between the north and the south (SMHI), which is more consistent with our results over seasonal variation in the various cultivation zones.

Spring means better temperature and nutritional conditions for growth of bacteria in nature. *M. avium* probably is able to start to grow when temperature reaches above 10°C (Falkinham JO III 2002). It is known that *M. avium* can enter a metabolic state of dormancy in response to starvation and recover rapidly when conditions improve again (Archuleta RJ et al. 2005). Our interpretation of the seasonal variation and the lack of differences between the cultivation zones, is that it is due to a combination of changing temperature and nutritional conditions in the environment throughout the year, with a rapid resurge of *M. avium* in nature in the spring and early summer months, which is when children most probably start to get infected. Spring and summer are also the seasons where children play outside to a greater extent and are therefore more exposed to water and soil in nature. The incidence curve starts to rise before the bathing season, though, and is prolonged beyond it.

Drinking water

The pronounced seasonal variation of the disease contradicts spread by drinking water since the conditions in man-made water systems are kept quite constant during the year and children are exposed to it all year round, as opposed to natural waters that are directly affected by the changes in climate. We also show more disease in children living in areas close to lakes and rivers and a lower incidence of *M. avium* disease in the city areas where drinking water is more chlorinated, which should allow selection of mycobacteria and one would therefore expect higher incidences of disease in cities if infection was spread by drinking water. In most reviews of NTM infections, it is considered a rural disease. In a



water (drinking water or hot water) in AIDS patients, and aerosols or dust in pulmonary patients and in the cases of sensitin skin birds share findings of M. avium avium strains but no certain transmission between the species has been shown. possible as some identical matches of isolates have been found (especially with sputum isolates and with AIDS patients). Pigs and sensitin skin tests). Spread from birds to humans is unlikely as no identical matches of isolates by molecular methods have been groups (children with lymphadenitis, patients with AIDS, patients with pulmonary disease and healthy persons with positive test conversion in healthy persons. Water plays an essential role in the spread of M. avium. The main sources of infection are probably natural waters in children, tap found except occasional findings of M. avium avium in humans (mainly in sputum isolates). Spread between pigs and humans is **Figure 5.** Possible environmental sources and transmission pathways of M. avium in birds, pigs and different human patient

there is other epidemiological evidence in favour of the theory of spread Black arrows indicate there is some sort of molecular epidemiological evidence for the theory of spread and broken arrows signify study of 86 children in Australia, most patients (79%) were city dwellers though (Joshi W et al. 1986).

Aerosols

If the route of infection among these children was by inhaling aerosols, we would expect to find the highest incidence of *M. avium* infection in the coastal areas or along rivers as large moving surface waters produce aerosols. Cities along the coast should not be exempt from this.

Instead we found the highest incidence in populations living close to fresh water and a lower incidence in the cities located by the sea. Aerosols might explain why there is a discrepancy between the high proportion of positive sensitin skin test reactions in urban areas close to the sea (Lind A et al. 1991) and the low incidences of clinical disease in the same areas. The high prevalence of skin test positive children in populations close to the sea might be due to exposure to aerosols, but *M. avium* clinical disease in children is probably not due to exposure to aerosols.

Limitations of the study

Our analysis is based on the assumption that children get infected near their home. Evidently we do not know that; they could have been infected on vacation in any other part of the country or abroad. When evaluating the proportion of the child population living close to water, we also did not take into account the very smallest lakes or brooks which did not show on the 1:300000 scale map that we used. Sweden is a country rich in natural waters. Some misclassification might therefore have occurred, but it would have affected population and cases equally.

In conclusion

The low incidences both in inland areas and in urban areas strengthen each other as they show the same thing: that incidence of *M. avium* infection decreases with absence of close contact with natural waters. The high incidences in fresh water areas and coastal areas favour the hypothesis that *M. avium* infection in children with cervical lymphadenitis is acquired by ingestion of natural waters.

Clinical features (Paper III)

Clinical parameters in chronic cervical lymphadenitis caused by *M. avium* or other NTM in children, such as age, sex and clinical signs are quite consistent through several studies, including ours (Thegerström J et al. 2008).

Increased susceptibility in young children

A new finding was that small children (\leq 24 months) had more often systemic symptoms (10% of children) than older children (1%) and a tendency for a more rapid onset of disease.

We also reported that younger children tended to have a more pronounced seasonal variation in the onset of disease compared to older children (paper II, Thegerström J et al. 2008), interpreted as infection manifesting closer to time of inoculation. Together this led us to hypothesize that there is a specific influence of age on the maturity of the immune system in a way that is important in the defence against *M. avium* infection, rendering small children more vulnerable and resulting in (1) higher incidences of the disease in this age group, (2) more rapid onset of the disease after inoculation and (3) more systemic symptoms.

This finding is in line with the fact that young children develop disease from *M. tuberculosis* more frequently after exposure than older children and adults (73% versus 25%, Gedde-Dahl T 1952) and the fact that infants younger than 12 months develop meningeal and disseminated disease from *M. tuberculosis* more frequently than older children and adults (20% versus <1%, Starke JR et al. 1992).

Treatment

In accordance with most other retrospective studies, we found evidence of the superior outcome of treatment by surgical excision of the affected lymph node over that of expectation after fine needle aspiration or incision and drainage only (Thegerström J et al. 2008). This seems to be quite well documented in the literature (Haris BH et al. 1982, Margileth AM et al. 1984, Sigalet D et al. 1992, Wolinsky E 1995, Lindeboom JA et al. 2007). For example, one study demonstrated a cure rate with surgical excision of 92%, while that of incision/drainage was only 16% (Schaad UB et al. 1979).

How come then that only 32% of the patients in our study were treated with operation initially and 18% did not undergo excision at all, even later in the course of disease? It could be because of some reports that show spontaneous regress after needle aspiration (Alessi DP et al. 1988) and others warning for the complications of surgery, i.e. facial nerve damage, secondary wound infections and scars (Albright JT et al. 2003) that have influenced earnose-throat (ENT) physicians to be careful.

The results from the only prospective randomized clinical study comparing antibiotic therapy with clarithromycin and rifabutin (after needle aspiration for diagnosis) and surgical excision, showing a 33% difference in favour of surgical treatment (Lindeboom JA et al. 2007), should perhaps put an end to the doubts as to the superiority of surgery as the treatment of choice. However, there are some considerations. In the Dutch randomized study all children were operated at the same specialised center, probably giving an exceptionally good cure rate (96%) and only one case of permanent facial nerve damage. In our study we found seven cases of facial nerve damage (whether transient or permanent could not be determined), whereof five had been operated at smaller hospitals. It also remains that there will always be individual cases where surgery is not possible or inappropriate because of the location of the lesion. In these cases medical treatment might well be successful (66% success rate according to the study by Lindeboom et al. 2007).

Another baffling study that in part contradicts the above and probably will continue to maintain the doubts as to which is the best treatment for these children, is one conducted in Israel during 1990-2004, where parents of 92 children with NTM chronic cervical lymphadenitis were offered expectation and mentally prepared for a long course of disease in their child, and where indeed all children healed after a period of spontaneous drainage for three to eight weeks, most (71%) after six months, 98% after nine months and all after twelve months, with only a flat, skin coloured scar at follow-up two years later. According to the authors no parent asked for a plastic surgery revision of the scar (Zeharia A et al. 2008).

So, the question is, are we too active with these children, causing more harm than good and should we instead concentrate on supporting the parents mentally to put up with the child having this wound for six to twelve months?

The results of the Israeli study should probably be carefully interpreted. As the authors themselves point out, a randomized study is needed to further support this approach. Also one must take into account that the disease might be of different severity in different parts of the world, depending on the types of *M. avium* and other NTM strains present in the environment and their virulence and specific properties. For example, increasing numbers of NTM lymphadenitis in children coupled with more severe cases in 2000-2004 was recently reported in Canada (Vu TTV et al. 2005).

Summing up, referral on clinical suspicion or after fine needle aspiration to an experienced ENT surgeon who will perform a complete excision of the affected lymph nodes, after assessing the risks for facial nerve damage in each patient, will most probably benefit the child. The worst thing to do is probably to make an incision and apply drainage (which would easily be done if the disease is initially mistaken for a common pyogenic bacterial infection). If this is done and the child does not show rapid signs of improvement, it is probably advisable to make a surgical excision.

Limitations of study III

As in every retrospective study, we were dependent on the available information in the patient's medical records. The amount and quality of information varied a lot. Especially follow-up information was scarce. Some questions could not be answered, for example: did the children have pet animals at home or live near farms? Were the children BCG vaccinated? No mention of this in the record could be interpreted as a negative answer, but should not be taken for granted. Family history and hereditary factors were seldom mentioned; for example, if any member in the child's family had previously had NTM disease, TB disease or complications of BCG vaccination as indications of underlying hereditary conditions.

Immunological considerations (papers IV and II)

Study design and results

We investigated the effect on the human immune system of ten isolates of *M. avium avium* from animals and one human adult, characterized by the bird-type/three-band profile on RFLP IS*1245* typing and of eleven isolates of *M. avium hominissuis* isolated from children with lymphadenitis, characterized by multiband profiles on RFLP IS*1245*. Peripheral blood mononuclear cells (PBMC) from six healthy blood donors were stimulated with inactivated mycobacteria *in vitro* followed by quantification of the cytokines IL-10, IL-12p70, TNF, IFNγ and IL-17 secreted in cell-free supernatants, by multiplex bead array analysis (Luminex).

M. avium hominissuis induced significantly more IL-10 and significantly less IFNγ and IL-17 than did *M. avium avium*. Both strains induced relatively high levels of IL-17.

Immunological reasons for host specificity

The results are in accordance with and might explain existing molecular epidemiological and clinical evidence that different subsets of *M. avium* strains infect humans and animals, respectively (Ritacco V et al. 1998, Thegerström J et al. 2005). We hypothesize that the human immune system might not allow one of the subspecies, *M. avium avium*, to establish infection in humans because of its ability to induce a protective and efficient Th1 inflammatory response without a dampening IL-10 anti-inflammatory response.

An alternative explanation for the relative host specificity of *M. avium avium* and *M. avium hominissuis* would be that the subspecies evolve in so very different ecological niches that only animals but not humans come in contact with *M. avium avium*. However, there is no real evidence of this as studies of environmental isolates of *M. avium* from different milieus rather show abundance of the mycobacteria in a wide range of different materials, even though RFLP studies of environmental isolates are scarce.

M. avium avium infects a great range of different mammals and birds, but very rarely humans, whereas M. avium hominissuis is capable of infecting humans, pigs and occasionally birds (see Figure 1). Little is actually known about the immune system in birds (Cromie RL et al. 2000) and susceptibility to M. avium avium infection also varies a great deal from one bird species to another (Tell LA et al. 2001). Taking our results into consideration the explanation for this relative host specificity would be that there are significant differences in the functions of the immune systems of humans and birds and probably between humans and other mammals; and that there are bacterial factors that interact differently with these immune functions.

The role of IL-10 in M. avium infection and inherent factors of bacterial strains

IL-10 seems to have a central role in directing the cytokine-dependent immune defence against M. avium in humans with its reported inhibitory effects on TNF, IL-12p70 and IFN- γ (Couper KN et al. 2008) further underscored by the IL-10 blocking experiments in our

study that showed increased levels of IL-12p70, TNF and IFN- γ when PBMCs were incubated with an anti-IL-10 antibody before stimulation with the bacteria.

Comparison of the stimulating effects of increased bacterial concentrations on secretion of IL-10 and IFN-γ demonstrate different reactions, where the *M. avium hominissuis* group showed an increase in IL-10 and decrease in IFN-γ with more bacteria, while in contrast the *M. avium avium* group showed the opposite, i.e. decrease in IL-10 and increase in IFN-γ. The fact that IL-10 levels do not react in a parallel way with increasing bacterial concentrations in the two groups indicates that the difference between subspecies is not merely about a weaker or stronger linear IL-10 response to the bacteria, but that there are properties inherent to the respective subspecies that actively stimulate or inhibit Il-10 production.

It becomes clear, however, that IL-10 alone cannot account for the differences between the groups. In the IL-10 blocking study, there was no difference in IL-10 levels between the groups (perhaps due to the lower bacterial concentration used and fewer blood donors). All the same, interestingly, the two groups behaved differently for IFN-γ, showing that (equally) decreasing levels of IL-10 seem to unveil inherent properties of *M. avium avium* to stimulate IFN-γ production more than *M. avium hominissuis*.

One hypothesis is that the differences between the subspecies might be explained by cell wall GPL contents and structure of the different strains, since GLPs have been shown to have immunomodulatory properties (Schorey JS and Sweet L 2008).

The role of IL-17

We noted relatively high levels of IL-17 in cultures of PBMCs stimulated with both groups of *M. avium*. *M. avium hominissuis* which is more pathogenic to humans gave lower levels of Il-17 than *M. avium avium* that do not seem to be able to establish infection in humans, supporting the postulated role for Il-17 in the immune defence in mycobacterial infections in general (Matsuzaki G and Umemura M 2007) and in *M. avium* infection in particular.

Interestingly, IL-17 levels, in contrast to levels of the other cytokines, decreased with II-10 blocking. This might be a direct effect of the IL-10 blocking, but alternatively could be an effect of the increasing IFN- γ levels, since IFN- γ has been shown to inhibit IL-17 production (Cruz A et al. 2006). This is probably a necessary regulatory pathway for the host to limit inflammation since experiments in IFN- γ deficient mice show persisting increase in IL-17 leading to more neutrophils in granulomas and a destructive granulomatous response at BCG-infection (Umemura M et al. 2007).

We speculate that the high levels of IFN- γ and IL-17 induced by *M. avium avium* help the host to clear this bacteria, whereas the lower levels induced by *M. avium hominissuis* allow infection to be established.

We also speculate that, in vivo, the relatively high levels of IL-17 induced by M. avium hominissuis infection, which might not be down-regulated sufficiently if IFN- γ levels

decrease later in the course of infection, contribute to pathogenicity by increased inflammation.

In children with *M. avium* lymphadenitis, histopathological studies show (Pinder SE and Colville A 1993) granulomatous inflammation with eosinophilic non-caseous or caseous necrosis, presence of neutrophils scattered throughout the necrotic foci (IL-17 recruits neutrophils to inflammatory sites. Cruz A et al. 2008) and frequent observations of Langhans' giant cells (IL-17 stimulates fusion of dendritic cells with formation of giant cells. Coury F et al. 2008). AFB are usually scant in number, and it might be difficult even to obtain a positive culture for diagnosis.

M. avium lymphadenitis is clinically characterized by discoloration and destruction of the overlying skin tissue, eventually leading to sinus formations. It is known that most of these infections do heal spontaneously but after several months' expectation and with tissue damage and scar formation (Wolinsky E et al. 1995). This suggests that these children are actually not so incapable of eliminating the mycobacteria and that the local inflammation and tissue destruction might be the greater problem.

These clinical and histopathological findings, together with our findings of IL-17 production after in vitro stimulation of PBMCs with *M. avium*, suggest that IL-17 might indeed play an important role in the pathogenesis of *M. avium* lymphadenitis in children.

Evidence of specific immune deficiencies in children with M. avium lymphadenitis

Many children are probably exposed to *M. avium hominissuis*, as judged by sensitin skin test studies (Lind A et al. 1991) but only very few display a clinical disease.

The affected children are otherwise healthy and not prone to other infections. Children with the now well-known hereditary conditions of Mendelian susceptibility to poorly virulent mycobacterial species, with mutations affecting the IL-12/ IFN- γ /STAT1-axis, display more severe or recurrent disease than the children with one episode of local *M. avium* infection only (Ottenhoff TH et al. 2002). Other, yet undiscovered, discreet deficiencies in the immune response, selectively affecting the pathogenesis of *M. avium* infection, might exist though.

The majority of children in our study were not investigated for immune deficiencies, even though four children had bilateral involvement and a few children suffered repeated relapses, which should lead to the suspicion that they might have an underlying immune weakness.

In our epidemiological study over the geographical distribution of *M. avium* cases in children in Sweden, we found an extremely high incidence of *M. avium* lymphadenitis in one of the colder cultivation zones (zone 5, see Figure 5 and 6). This comprises a few geographically close districts in the north of Sweden, where the population historically has been isolated and where some genetic diseases exist. As no climate or geographical factor

really could explain this, the higher susceptibility to *M. avium* disease in children in this area might speculatively be ascribed to genetic factors.

Another observation hinting in this direction was that among the 162 children with *M. avium* lymphadenitis, we found two brothers who got the disease in the same year and two other children with siblings who had suffered from NTM infection several years earlier. Considering an incidence of 4.5/100000 children/year, this is on the verge of defying chance even if the material is small.

One study indicates that children with M. avium lymphadenitis produce less IFN- γ than controls (Nylén O et al. 2000). It would be interesting to investigate whether they also produce reduced or excessive amounts of IL-17, since we found this cytokine to be abundantly produced at in vitro stimulation of PBMCs with M. avium. The default might as well lie in the combat against the mycobacteria, as in limiting the tissue destruction mediated by the immune defence.

Limitations of the study

There is a limitation in interpreting the differences in cytokine stimulation between *M. avium avium* and *M. avium hominissuis*. In the literature there is clear evidence that some *M. avium hominissuis* strains are more virulent than others. In selecting *M. avium hominissuis* strains from children with lymphadenitis, we might have chosen especially virulent strains of *M. avium hominissuis*. Would the difference between the two groups be as pronounced if we had chosen *M. avium hominissuis* strains from infected pigs or environmental strains? On the other hand there is also evidence that some *M. avium avium* strains are more virulent than others. Our *M. avium avium* strains constituted a more heterogeneous group since the isolates came from different hosts and some might not have had clinical significance (pig isolates and the isolate from the lungs of a human adult).

CONCLUDING REMARKS

The results from these studies show that children are not infected with *M. avium* from birds. Disease in children can therefore not be incriminated as a bird borne zoonotic disease, despite its name, *M. avium*. Infection is likely through a common source to pigs and humans.

The geographical distribution of cases and the seasonal variation in our epidemiological study point to natural outdoor waters as the most probable source of infection in children with *M. avium* lymphadenitis.

The clinical picture of *M. avium* lymphadenitis in children in Sweden is well in accordance with international studies. Our results support the common view that the disease should be treated by surgical removal of the affected tissue.

The age distribution of *M. avium* lymphadenitis in children and differences in clinical features between younger and older children show that immaturity of the immune system is a general predisposing factor. The low incidence of the disease, despite a high exposure rate and possible geographic and familiar clusterings, points in the direction that there might be additional, individual (genetic), predisposing factors.

Bacterial interactions with the immune system with resulting differences in the amounts of cytokines produced, and particularly the ability to induce a prominent Th1 response, might explain the relative host specificity of the subspecies *M. avium avium* and *M. avium hominissuis*. IL-17 might be an important cytokine in the control of infection and in the pathogenesis of the disease.

ACKNOWLEDGEMENTS / TACK

Jag vill börja med att tacka min huvudhandledare, **Björn Olsen**, för allt stöd, peppning och goda råd. För mig som hämmas av traditionella auktoriteter (präglad bland annat av skolsystemet i Frankrike), har din tillbakalutade, fria och flexibla handledarstil gjort mycket gott. Du har en förmåga att, utan fördomar, se kvaliteter hos olika sorters människor. Jag är glad att du såg mig och ville vara den vägvisare jag behövde för att våga mig ut på denna resa. Din entusiasm har många gånger skingrat mina tvivel och sporrat mig vidare.

Dessutom vill jag tacka mina två biträdande handledare, **Britt-Inger Marklund** och **Jan Ernerudh**. BIM, du tog dig an mig när jag var novis och du har lärt mig allt jag kan om labarbete och hur man "gullar" med mykobakterier för att de ska växa och må bra. Vi kämpade med RFLP-metoden och lyckades ju hyfsat till slut och hade roligt på köpet. Du är alltid glad och det smittar av sig. Senare fick vi mycket god hjälp av **Diana** som förädlade RFLPn ett steg till – många tack för dina gedigna insatser på labbet! Janne, du har funnits med nästan från början i bakgrunden och varit till god hjälp i det formella kring forskningsutbildningen. Det var roligt, nu när jag i 4:e arbetet äntligen nådde fram till immunologin, att få göra ett konkret arbete med dig och ta del av din gedigna kunskap. Jag vill också tacka dig för att du tog dig tid att läsa och ha synpunkter på ramen. Tack också till dina analytiker, **Petra** och **Marie**, som gjorde ett gigantiskt jobb med Luminex-analyserna.

När jag sedan på egen hand gav mig ut och iväg (ända till Göteborg) och in på att börja labba med helt nya metoder, fann jag att jag inte var helt bortkommen bakom pipetten, vilket främst är ett gott betyg till BIM. Utan **Bodil Jönssons** noggranna handledning hade det förstås ändå inte gått. Ett stort tack till henne och hennes kollegor på Agnes Wolds Mikrobiologiska avdelning på Sahlgrenska sjukhuset!

Mitt sista immunologiskt inriktade arbete har **Vanda Friman** tagit initiativ till. Vanda, du har funnits med som en intresserad samarbetspartner även under de kliniska delarna av mitt projekt. Din expertkunskap och ditt intresse för immunbrister hos barn har jag också haft användning av i min kliniska verksamhet. Tack också för att du tog väl hand om mig i Göteborg och till och med följde med på bisarra inslag i Göteborgs filmfestival som sammanföll med min vistelse där vintern 2007.

RFLP-analyserna har varit en följetong under en stor del av mitt avhandlingsarbete och har bland annat fört det goda med sig att det tog mig till smittskyddsinstitutet (SMI) i Stockholm. Där var **Sven Hoffner** tidigt inblandad och delade med sig av både kunnande och "gris-stammar". Hela Svens TB-avdelning på SMI har varit mycket gästfria och besöken där behagliga och trevliga. Speciellt stort tack till **Solomon** och senare **Ramona** som inte bara skapade utrymme åt mig på sitt rum, utan också hjälpte mig att få ordning på dataprogrammet där jag stoppade in mina bandmönster och stirrade ögonen blinda för att avgöra om ett band är ett band, är ett band?

I ett annat hus, ett stenkast längre upp i backen, i utkanten av KI-området, som jag återser med nostalgi eftersom det var där jag pluggade medicin på 90-talet, där fanns **Victoria Romanus** som med stor vänlighet lät mig ta del både av långa listor och av sitt gamla,

gedigna barn-material. Tack, Victoria, för god handledning på de epidemiologiska bitarna i mitt arbete!

Lars Brudin har på sista tiden blivit min chef och arbetskollega på Klinisk fysiologi i Kalmar. Tack för dina insatser i samtliga arbeten när det handlat om att strukturera upp mina råmaterial i snygga excel-tabeller och för hjälp och vägledning i Statistica. Du är oförtruten i din hjälpsamhet och intresse för forskning i allmänhet och statistik i synnerhet.

Jag vill också tacka **Eric**, **Lars** och **Pär**, mina tre chefer på Barnkliniken, samt **Annkristin**, min nuvarande chef på Klinisk fysiologi. Det är tufft att forska och samtidigt vara kliniker. Med er hjälp har det åtminstone varit möjligt tack vare bidrag till forskningstid. Tack för ert intresse och stöd!

Min tid har också finansierats av centrala medel från FoU-kommittén i Kalmar och mitt projekt av medel från FORSS, Forskningsrådet i sydöstra Sverige.

Jag vill slutligen tacka familj och vänner.

Tack, pappa. Du har varit min förebild i nästan allt, men framförallt har jag sporrats till och delat glädjen över intellektuella utmaningar med dig. Du har inspirerat mitt intresse för både vetenskap och humaniora.

Tack till mina bröder, Jakob och John. John, du har tagit vissa risker för att hjälpa mig med bakgrunds-researchen till denna avhandling. Du vet vad jag menar, mina läppar är förseglade – tack, det har varit till stor nytta!

Tack också till mina/våra vänner. Den "valda" familjen är också viktig! Speciellt tack till Annika och Gittan för att ni tar så bra hand om Alexander när vi ses. Han gillar er! Tack också till Emma och Bea för att vi får dela detta vanliga att vara föräldrar med er och detta ovanliga att vara två mammor.

Tack, Marie! Bakom varje framgångsrik forskare står en kvinna, en fru, sägs det. Så också i mitt fall, även om könsrollerna är satta ur spel i vår familj, som tur är. Tack för att du finns i mitt liv. Tack för det stöd och den extra tid du givit mig under arbetet med avhandlingen.

Tack, Alexander, för att du finns, full av liv och att du med obändig kraft och oändlig nyfikenhet upptäcker världen och ständigt kapar min uppmärksamhet och visar sådant som är verkligt viktigt. Mitt i detta tumult kommer snart en ny liten människa till vår familj. Vi väntar på dig och längtar efter att få lära känna dig!

REFERENCE LIST

Albright JT, Pransky SM. Nontuberculous mycobacterial infections of the head and neck. Pediatr Clin North Am. 2003 Apr;50(2):503-14. Review.

Alessi DP, Dudley JP. Atypical mycobacteria-induced cervical adenitis. Treatment by needle aspiration. Arch Otolaryngol Head Neck Surg. 1988 Jun;114(6):664-6.

Appelberg R, Castro AG, Pedrosa J, Silva RA, Orme IM, Minóprio P. Role of gamma interferon and tumor necrosis factor alpha during T-cell-independent and -dependent phases of Mycobacterium avium infection. Infect Immun. 1994 Sep;62(9):3962-71.

Archuleta RJ, Yvonne Hoppes P, Primm TP. Mycobacterium avium enters a state of metabolic dormancy in response to starvation. Tuberculosis (Edinb). 2005 May;85(3):147-58.

Aronson T, Holtzman A, Glover N, Boian M, Froman S, Berlin OG, Hill H, Stelma G Jr. Comparison of large restriction fragments of Mycobacterium avium isolates recovered from AIDS and non-AIDS patients with those of isolates from potable water. J Clin Microbiol. 1999 Apr;37(4):1008-12.

Ashford DA, Whitney E, Raghunathan P, Cosivi O. Epidemiology of selected mycobacteria that infect humans and other animals. Rev Sci Tech. 2001 Apr;20(1):325-37. Review.

Baily GV. Tuberculosis prevention Trial, Madras. Indian J Med Res. 1980 Jul;72 Suppl:1-74.

Bauer J, Andersen AB. Stability of insertion sequence IS1245, a marker for differentiation of Mycobacterium avium strains. J Clin Microbiol. 1999 Feb;37(2):442-4.

Bauer J, Andersen AB, Askgaard D, Giese SB, Larsen B. Typing of clinical Mycobacterium avium complex strains cultured during a 2-year period in Denmark by using IS1245. J Clin Microbiol. 1999 Mar;37(3):600-5.

Belisle JT, Klaczkiewicz K, Brennan PJ, Jacobs WR Jr, Inamine JM. Rough morphological variants of Mycobacterium avium. Characterization of genomic deletions resulting in the loss of glycopeptidolipid expression. J Biol Chem. 1993 May 15;268(14):10517-23.

Bermudez LE, Wu M, Young LS. Interleukin-12-stimulated natural killer cells can activate human macrophages to inhibit growth of Mycobacterium avium. Infect Immun. 1995 Oct;63(10):4099-104.

Bruijnesteijn van Coppenraet LE, de Haas PE, Lindeboom JA, Kuijper EJ, van Soolingen D. Lymphadenitis in children is caused by Mycobacterium avium hominissuis and not related to 'bird tuberculosis'. Eur J Clin Microbiol Infect Dis. 2008 Apr;27(4):293-9.

Cirillo JD, Falkow S, Tompkins LS, Bermudez LE. Interaction of Mycobacterium avium with environmental amoebae enhances virulence. Infect Immun. 1997 Sep;65(9):3759-67.

Cohen YH, Amir J, Ashkenazi S, Eidlitz-Markus T, Samra Z, Kaufmann L, Zeharia A. Mycobacterium haemophilum and lymphadenitis in immunocompetent children, Israel. Emerg Infect Dis. 2008 Sep;14(9):1437-9.

Collins DM, Cavaignac S, de Lisle GW. Use of four DNA insertion sequences to characterize strains of the Mycobacterium avium complex isolated from animals. Mol Cell Probes. 1997 Oct;11(5):373-80.

Cooper AM, Adams LB, Dalton DK, Appelberg R, Ehlers S. IFN-gamma and NO in mycobacterial disease: new jobs for old hands. Trends Microbiol. 2002 May;10(5):221-6.

Cooper AM, Khader SA. Il-12p40: an inherently agonistic cytokine. Trends Immunol. 2007 Jan;28(1):33-8.

Couper KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. J Immunol. 2008 May 1;180(9):5771-7. Review.

Coury F, Annels N, Rivollier A, Olsson S, Santoro A, Speziani C, Azocar O, Flacher M, Djebali S, Tebib J, Brytting M, Egeler RM, Rabourdin-Combe C, Henter JI, Arico M, Delprat C. Langerhans cell histiocytosis reveals a new IL-17A-dependent pathway of dendritic cell fusion. Nat Med. 2008 Jan;14(1):81-7.

Cromie RL, Brown MJ, Price DJ, Stanford JL. Susceptibility of captive wildfowl to avian tuberculosis: the importance of genetic and environmental factors. Tubercle. 1991 Jun;72(2):105-9.

Cruz A, Khader SA, Torrado E, Fraga A, Pearl JE, Pedrosa J, Cooper AM, Castro AG. Cutting edge: IFN-gamma regulates the induction and expansion of IL-17-producing CD4 T cells during mycobacterial infection. J Immunol. 2006 Aug 1;177(3):1416-20.

Danelishvilli L, Bermudez LE. Role of type I cytokines in host defense against Mycobacterium avium infection. Curr Pharm Des. 2003;9(1):61-5. Review.

Dascalopoulos GA, Loukas S, Constantopoulos SH. Wide geographic variations of sensitivity to MOTT sensitins in Greece. Eur Respir J. 1995 May;8(5):715-7.

Demangel C, Bertolino P, Britton WJ. Autocrine IL-10 impairs dendritic cell (DC)-derived immune responses to mycobacterial infection by suppressing DC trafficking to draining lymph nodes and local IL-12 production. Eur J Immunol. 2002 Apr;32(4):994-1002.

Dvorska L, Bull TJ, Bartos M, Matlova L, Svastova P, Weston RT, Kintr J, Parmova I, Van Soolingen D, Pavlik I. A standardised restriction fragment length polymorphism (RFLP) method

for typing Mycobacterium avium isolates links IS901 with virulence for birds. J Microbiol Methods. 2003 Oct:55(1):11-27.

Dvorska L, Matlova L, Ayele WY, Fischer OA, Amemori T, Weston RT, Alvarez J, Beran V, Moravkova M, Pavlik I. Avian tuberculosis in naturally infected captive water birds of the Ardeideae and Threskiornithidae families studied by serotyping, IS901 RFLP typing, and virulence for poultry. Vet Microbiol. 2007 Jan 31;119(2-4):366-74.

Edwards LD, Acquaviva FA, Livesay VT, Cross FW, Palmer CE. An atlas of sensitivity to tuberculin, PPD-B, and histoplasmin in the United States. Am Rev Respir Dis. 1969 Apr;99(4):Suppl:1-132.

Ehlers S, Benini J, Kutsch S, Endres R, Rietschel ET, Pfeffer K. Fatal granuloma necrosis without exacerbated mycobacterial growth in tumor necrosis factor receptor p55 gene-deficient mice intravenously infected with Mycobacterium avium. Infect Immun. 1999 Jul;67(7):3571-9.

Emile JF, Patey N, Altare F, Lamhamedi S, Jouanguy E, Boman F, Quillard J, Lecomte-Houcke M, Verola O, Mousnier JF, Dijoud F, Blanche S, Fischer A, Brousse N, Casanova JL. Correlation of granuloma structure with clinical outcome defines two types of idiopathic disseminated BCG infection. J Pathol. 1997 Jan;181(1):25-30. Review.

Engback HC, Vergmann B, Bentzon MW. [Cervical lymphadenitis in children cause by atypical mycobacteria and Mycobacterium avium] Ugeskr Laeger. 1981 Oct 19;143(43):2787-91. Danish.

Evans MJ, Smith NM, Thornton CM, Youngson GG, Gray ES. Atypical mycobacterial lymphadenitis in childhood--a clinicopathological study of 17 cases. J Clin Pathol. 1998 Dec;51(12):925-7. Review.

Falkinham JO 3rd. Nontuberculous mycobacteria in the environment. Clin Chest Med. 2002 Sep;23(3):529-51. Review.

Falkinham JO 3rd, Norton CD, LeChevallier MW. Factors influencing numbers of Mycobacterium avium, Mycobacterium intracellulare, and other Mycobacteria in drinking water distribution systems. Appl Environ Microbiol. 2001 Mar;67(3):1225-31.

Feng CG, Kullberg MC, Jankovic D, Cheever AW, Caspar P, Coffman RL, Sher A. Transgenic mice expressing human interleukin-10 in the antigen-presenting cell compartment show increased susceptibility to infection with Mycobacterium avium associated with decreased macrophage effector function and apoptosis. Infect Immun. 2002 Dec;70(12):6672-9.

Flórido M, Gonçalves AS, Silva RA, Ehlers S, Cooper AM, Appelberg R. Resistance of virulent Mycobacterium avium to gamma interferon-mediated antimicrobial activity suggests additional signals for induction of mycobacteriostasis. Infect Immun. 1999 Jul;67(7):3610-8.

Flórido M, Cooper AM, Appelberg R. Immunological basis of the development of necrotic lesions following Mycobacterium avium infection. Immunology. 2002 Aug;106(4):590-601.

Fratazzi C, Arbeit RD, Carini C, Balcewicz-Sablinska K, Keane J,Kornfeld H, Remold HG. Macrophage apoptosis in mycobacterial infections. J. Leukoc. Biol. 66: 763–764; 1999.

Gardam MA, Keystone EC, Menzies R, Manners S, Skamene E, Long R, Vinh DC. Antitumour necrosis factor agents and tuberculosis risk: mechanisms of action and clinical management. Lancet Infect Dis. 2003 Mar;3(3):148-55. Review.

Gedde-Dahl T. Tuberculous infection in the light of tuberculin matriculation. Am J Hyg. 1952 Sep;56(2):139-214.

Geier H, Mostowy S, Cangelosi GA, Behr MA, Ford TE. Autoinducer-2 triggers the oxidative stress response in Mycobacterium avium, leading to biofilm formation. Appl Environ Microbiol. 2008 Mar;74(6):1798-804.

Gerosa F, Paganin C, Peritt D, Paiola F, Scupoli MT, Aste-Amezaga M, Frank I, Trinchieri G. Interleukin-12 primes human CD4 and CD8 T cell clones for high production of both interferongamma and interleukin-10. J Exp Med. 1996 Jun 1;183(6):2559-69.

Gill MJ, Fanning EA, Chomyc S. Childhood lymphadenitis in a harsh northern climate due to atypical mycobacteria. Scand J Infect Dis. 1987;19(1):77-83.

Good RC. Opportunistic pathogens in the genus Mycobacterium. Annu Rev Microbiol. 1985;39:347-69. Review.

Goslee S, Wolinsky E. Water as a source of potentially pathogenic mycobacteria. Am Rev Respir Dis. 1976 Mar;113(3):287-92

Grange JM, Yates MD, Boughton E. The avian tubercle bacillus and its relatives. J Appl Bacteriol. 1990 May;68(5):411-31. Review.

Green PA, von Reyn CF, Smith RP Jr. Mycobacterium avium complex parotid lymphadenitis: successful therapy with clarithromycin and ethambutol. Pediatr Infect Dis J. 1993 Jul;12(7):615-7.

Guerrero C, Bernasconi C, Burki D, Bodmer T, Telenti A. A novel insertion element from Mycobacterium avium, IS1245, is a specific target for analysis of strain relatedness. J Clin Microbiol. 1995 Feb;33(2):304-7.

Gutierrez MG, Mishra BB, Jordao L, Elliott E, Anes E, Griffiths G. NF-kappa B activation controls phagolysosome fusion-mediated killing of mycobacteria by macrophages. J Immunol. 2008 Aug 15;181(4):2651-63.

Gutierrez MG, Gonzalez AP, Anes E, Griffiths G. Role of lipids in killing mycobacteria by macrophages: evidence for NF-kappaB-dependent and -independent killing induced by different lipids. Cell Microbiol. 2009 Mar;11(3):406-20.

Harris BH, Webb HW, Wilkinson AH Jr, Santelices AA. Mycobacterial lymphadenitis. J Pediatr Surg. 1982 Oct;17(5):589-90.

Haverkamp MH, Arend SM, Lindeboom JA, Hartwig NG, van Dissel JT. Nontuberculous mycobacterial infection in children: a 2-year prospective surveillance study in the Netherlands. Clin Infect Dis. 2004 Aug 15;39(4):450-6.

Haverkamp MH, van Dissel JT, Holland SM. Human host genetic factors in nontuberculous mycobacterial infection: lessons from single gene disorders affecting innate and adaptive immunity and lessons from molecular defects in interferon-gamma-dependent signaling. Microbes Infect. 2006 Apr;8(4):1157-66.

Hayashi T, Rao SP, Meylan PR, Kornbluth RS, Catanzaro A. Role of CD40 ligand in Mycobacterium avium infection. Infect Immun. 1999 Jul;67(7):3558-65.

Hessle CC, Andersson B, Wold AE. Gram-positive and Gram-negative bacteria elicit different patterns of pro-inflammatory cytokines in human monocytes. Cytokine. 2005 Jun 21;30(6):311-8.

Hilborn ED, Yakrus MA, Covert TC, Harris SI, Donnelly SF, Schmitt MT, Toney S, Bailey SA, Stelma GN Jr. Molecular comparison of Mycobacterium avium isolates from clinical and environmental sources. Appl Environ Microbiol. 2008 Aug;74(15):4966-8.

Hoop RK, Böttger EC, Pfyffer GE. Etiological agents of mycobacterioses in pet birds between 1986 and 1995. J Clin Microbiol. 1996 Apr;34(4):991-2.

Horgen L, Barrow EL, Barrow WW, Rastogi N. Exposure of human peripheral blood mononuclear cells to total lipids and serovar-specific glycopeptidolipids from Mycobacterium avium serovars 4 and 8 results in inhibition of TH1-type responses. Microb Pathog. 2000 Jul;29(1):9-16.

Horsburgh CR Jr. Mycobacterium avium complex infection in the acquired immunodeficiency syndrome. N Engl J Med. 1991 May 9;324(19):1332-8. Review

Horsburgh CR Jr, Chin DP, Yajko DM, Hopewell PC, Nassos PS, Elkin EP, Hadley WK, Stone EN, Simon EM, Gonzalez P, et al. J Infect Dis. Environmental risk factors for acquisition of Mycobacterium avium complex in persons with human immunodeficiency virus infection. 1994 Aug;170(2):362-7.

Inderlied CB, Kemper CA, Bermudez LE. The Mycobacterium avium complex. Clin Microbiol Rev. 1993 Jul;6(3):266-310. Review.

Iivanainen EK, Martikainen PJ, Väänänen PK, Katila ML. Environmental Factors Affecting the Occurrence of Mycobacteria in Brook Waters. Appl Environ Microbiol. 1993 Feb;59(2):398-404.

Jarzembowski JA, Young MB. Nontuberculous mycobacterial infections. Arch Pathol Lab Med. 2008 Aug;132(8):1333-41. Review

Johansen TB, Djønne B, Jensen MR, Olsen I. Distribution of IS1311 and IS1245 in Mycobacterium avium subspecies revisited. J Clin Microbiol. 2005 May;43(5):2500-2.

Johansen TB, Olsen I, Jensen MR, Dahle UR, Holstad G, Djønne B. New probes used for IS1245 and IS1311 restriction fragment length polymorphism of Mycobacterium avium subsp. avium and Mycobacterium avium subsp. hominissuis isolates of human and animal origin in Norway. BMC Microbiol. 2007 Mar 5;7:14.

Joshi W, Davidson PM, Jones PG, Campbell PE, Roberton DM. Non-tuberculous mycobacterial lymphadenitis in children. Eur J Pediatr. 1989 Aug;148(8):751-4.

Kano H, Doi T, Fujita Y, Takimoto H, Yano I, Kumazawa Y. Serotype-specific modulation of human monocyte functions by glycopeptidolipid (GPL) isolated from Mycobacterium avium complex. Biol Pharm Bull. 2005 Feb;28(2):335-9.

Kasperbauer SH, Daley CL. Diagnosis and treatment of infections due to Mycobacterium avium complex. Semin Respir Crit Care Med. 2008 Oct;29(5):569-76. Epub 2008 Sep 22. Review.

Kauppinen J, Hintikka E, Iivanainen E, Katila M. PCR-based typing of Mycobacterium avium isolates in an epidemic among farmed lesser white-fronted geese (Anser erythropus). Vet Microbiol. 2001 Jul 3;81(1):41-50.

Khader SA, Pearl JE, Sakamoto K, Gilmartin L, Bell GK, Jelley-Gibbs DM, Ghilardi N, deSauvage F, Cooper AM. IL-23 Compensates for the Absence of IL-12p70 and Is Essential for the IL-17 Response during Tuberculosis but Is Dispensable for Protection and Antigen-Specific IFN-γ Responses if IL-12p70 Is Available. The Journal of Immunology, 2005, 175: 788–795.

Khader SA, Partida-Sanchez S, Bell G, Jelley-Gibbs DM, Swain S, Pearl JE, Ghilardi N, Desauvage FJ, Lund FE, Cooper AM. Interleukin 12p40 is required for dendritic cell migration and T cell priming after Mycobacterium tuberculosis infection. J Exp Med. 2006 Jul 10;203(7):1805-15.

Kirschner RA Jr, Parker BC, Falkinham JO 3rd. Epidemiology of infection by nontuberculous mycobacteria. Mycobacterium avium, Mycobacterium intracellulare, and Mycobacterium scrofulaceum in acid, brown-water swamps of the southeastern United States and their association with environmental variables. Am Rev Respir Dis. 1992 Feb;145(2 Pt 1):271-5.

Komijn RE, de Haas PE, Schneider MM, Eger T, Nieuwenhuijs JH, van den Hoek RJ, Bakker D, van Zijd Erveld FG, van Soolingen D. Prevalence of Mycobacterium avium in slaughter pigs in The Netherlands and comparison of IS1245 restriction fragment length polymorphism patterns of porcine and human isolates. J Clin Microbiol. 1999 May:37(5):1254-9.

Kunze ZM, Portaels F, McFadden JJ. Biologically distinct subtypes of Mycobacterium avium differ in possession of insertion sequence IS901. J Clin Microbiol. 1992 Sep;30(9):2366-72.

Lai KK, Stottmeier KD, Sherman IH, McCabe WR. Mycobacterial cervical lymphadenopathy. Relation of etiologic agents to age. JAMA. 1984 Mar 9;251(10):1286-8.

Larsson LO, Skoogh BE, Bentzon MW, Magnusson M, Olofson J, Taranger J, Lind A. Sensitivity to sensitins and tuberculin in Swedish children. II. A study of preschool children. Tubercle. 1991 Mar;72(1):37-42.

Larsson LO, Bentzon MW, Lind A, Magnusson M, Sandegård G, Skoogh BE, Boëthius G. Sensitivity to sensitins and tuberculin in Swedish children. Part 5: A study of school children in an inland rural area. Tuber Lung Dis. 1993 Dec;74(6):371-6.

Le Dantec C, Duguet JP, Montiel A, Dumoutier N, Dubrou S, Vincent V. Occurrence of mycobacteria in water treatment lines and in water distribution systems. Appl Environ Microbiol. 2002 Nov;68(11):5318-25.

Lee ES, Lee MY, Han SH, Ka JO. Occurrence and molecular differentiation of environmental mycobacteria in surface waters. J Microbiol Biotechnol. 2008 Jul;18(7):1207-15.

Legrand E, Sola C, Verdol B, Rastogi N. Genetic diversity of mycobacterium avium recovered from AIDS patients in the caribbean as studied by a consensus IS1245-RFLP method and pulsed-field gel electrophoresis. Res Microbiol. 2000 May;151(4):271-83.

Lennox AM. Mycobacteriosis in companion psittacine birds: a review. J Avian Med Surg. 2007 Sep;21(3):181-7. Review.

Lewinsohn DA, Gennaro ML, Scholvinck L, Lewinsohn DM. Tuberculosis immunology in children: diagnostic and therapeutic challenges and opportunities. Int J Tuberc Lung Dis. 2004 May;8(5):658-74. Review.

Lind A, Larsson LO, Bentzon MW, Magnusson M, Olofson J, Sjögren I, Strannegard IL, Skoogh BE. Sensitivity to sensitins and tuberculin in Swedish children. I. A study of schoolchildren in an urban area. Tubercle. 1991 Mar;72(1):29-36.

Lindeboom JA, Kuijper EJ, Bruijnesteijn van Coppenraet ES, Lindeboom R, Prins JM. Surgical excision versus antibiotic treatment for nontuberculous mycobacterial cervicofacial

lymphadenitis in children: a multicenter, randomized, controlled trial. Clin Infect Dis. 2007 Apr 15;44(8):1057-64.

Lockhart E, Green AM, Flynn JL. IL-17 production is dominated by gammadelta T cells rather than CD4 T cells during Mycobacterium tuberculosis infection. J Immunol. 2006 Oct 1;177(7):4662-9.

Maffucci A. II. Experimental Researches upon the Products of the Tubercle Bacillus. Ann Surg. 1894 Nov;20(5):556-60.

Manarolla G, Liandris E, Pisoni G, Sassera D, Grilli G, Gallazzi D, Sironi G, Moroni P, Piccinini R, Rampin T. Avian mycobacteriosis in companion birds: 20-year survey. Vet Microbiol. 2009 Feb 2;133(4):323-7.

Margileth AM, Chandra R, Altman RP. Chronic lymphadenopathy due to mycobacterial infection. Clinical features, diagnosis, histopathology, and management. Am J Dis Child. 1984 Oct;138(10):917-22.

Matlova L, Dvorska L, Palecek K, Maurenc L, Bartos M, Pavlik I. Impact of sawdust and wood shavings in bedding on pig tuberculous lesions in lymph nodes, and IS1245 RFLP analysis of Mycobacterium avium subsp. hominissuis of serotypes 6 and 8 isolated from pigs and environment. Vet Microbiol. 2004 Sep 8;102(3-4):227-36.

Matsuzaki G, Umemura M. Interleukin-17 as an effector molecule of innate and acquired immunity against infections. Microbiol Immunol. 2007;51(12):1139-47. Review.

Mijs W, de Haas P, Rossau R, Van der Laan T, Rigouts L, Portaels F, van Soolingen D. Molecular evidence to support a proposal to reserve the designation Mycobacterium avium subsp. avium for bird-type isolates and 'M. avium subsp. hominissuis' for the human/porcine type of M. avium. Int J Syst Evol Microbiol. 2002 Sep;52(Pt 5):1505-18.

Morsczeck C, Berger S, Plum G. The macrophage-induced gene (mig) of Mycobacterium avium encodes a medium-chain acyl-coenzyme A synthetase. Biochim Biophys Acta. 2001 Oct 31;1521(1-3):59-65.

Möbius P, Lentzsch P, Moser I, Naumann L, Martin G, Köhler H. Comparative macrorestriction and RFLP analysis of Mycobacterium avium subsp. avium and Mycobacterium avium subsp. hominissuis isolates from man, pig, and cattle. Vet Microbiol. 2006 Oct 31;117(2-4):284-91.

Nicholson O, Feja K, LaRussa P, George D, Unal E, Della Latta P, Cairo M, Saiman L. Nontuberculous mycobacterial infections in pediatric hematopoietic stem cell transplant recipients: case report and review of the literature. Pediatr Infect Dis J. 2006 Mar;25(3):263-7. Review.

Nylén O, Berg-Kelly K, Andersson B. Cervical lymph node infections with non-tuberculous mycobacteria in preschool children: interferon gamma deficiency as a possible cause of clinical infection. Acta Paediatr. 2000 Nov;89(11):1322-5.

O'Brien DP, Currie BJ, Krause VL. Nontuberculous mycobacterial disease in northern Australia: a case series and review of the literature. Clin Infect Dis. 2000 Oct;31(4):958-67.

O'Grady D, Flynn O, Costello E, Quigley F, Gogarty A, McGuirk J, O'Rourke J, Gibbons N. Restriction fragment length polymorphism analysis of Mycobacterium avium isolates from animal and human sources. Int J Tuberc Lung Dis. 2000 Mar;4(3):278-81.

Oliveira RS, Sircili MP, Oliveira EM, Balian SC, Ferreira-Neto JS, Leão SC. Identification of Mycobacterium avium genotypes with distinctive traits by combination of IS1245-based restriction fragment length polymorphism and restriction analysis of hsp65. J Clin Microbiol. 2003 Jan;41(1):44-9.

Ottenhoff TH, Verreck FA, Lichtenauer-Kaligis EG, Hoeve MA, Sanal O, van Dissel JT. Genetics, cytokines and human infectious disease: lessons from weakly pathogenic mycobacteria and salmonellae. Nat Genet. 2002 Sep;32(1):97-105. Review.

Petrofsky M, Bermudez LE. CD4+ T cells but Not CD8+ or gammadelta+ lymphocytes are required for host protection against Mycobacterium avium infection and dissemination through the intestinal route. Infect Immun. 2005 May;73(5):2621-7.

Pinder SE, Colville A. Mycobacterial cervical lymphadenitis in children: can histological assessment help differentiate infections caused by non-tuberculous mycobacteria from Mycobacterium tuberculosis? Histopathology. 1993 Jan;22(1):59-64.

Primm TP, Lucero CA, Falkinham JO 3rd. Health impacts of environmental mycobacteria. Clin Microbiol Rev. 2004 Jan;17(1):98-106. Review.

Plum G, Brenden M, Clark-Curtiss JE, Pulverer G. Cloning, sequencing, and expression of the mig gene of Mycobacterium avium, which codes for a secreted macrophage-induced protein. Infect Immun. 1997 Nov;65(11):4548-57.

Prince DS, Peterson DD, Steiner RM, Gottlieb JE, Scott R, Israel HL, Figueroa WG, Fish JE. Infection with Mycobacterium avium complex in patients without predisposing conditions. N Engl J Med. 1989 Sep 28;321(13):863-8.

Prissick FH, Masson AM. Cervical lymphadenitis in children caused by chromogenic Mycobacteria. Can Med Assoc J. 1956 Nov 15;75(10):798-803.

Quesniaux V, Fremond C, Jacobs M, Parida S, Nicolle D, Yeremeev V, Bihl F, Erard F, Botha T, Drennan M, Soler MN, Le Bert M, Schnyder B, Ryffel B. Toll-like receptor pathways in the immune responses to mycobacteria. Microbes Infect. 2004 Aug;6(10):946-59. Review.

Ramirez J, Mason C, Ali J, Lopez FA. Mycobacterium avium complex pulmonary disease: management options in HIV-negative patients. J La State Med Soc. 2008 Sep-Oct;160(5):248-54; quiz 254, 293.

Reed C, von Reyn CF, Chamblee S, Ellerbrock TV, Johnson JW, Marsh BJ, Johnson LS, Trenschel RJ, Horsburgh CR Jr. Environmental risk factors for infection with Mycobacterium avium complex. Am J Epidemiol. 2006 Jul 1;164(1):32-40.

Ritacco V, Kremer K, van der Laan T, Pijnenburg JE, de Haas PE, van Soolingen D. Use of IS901 and IS1245 in RFLP typing of Mycobacterium avium complex: relatedness among serovar reference strains, human and animal isolates. Int J Tuberc Lung Dis. 1998 Mar;2(3):242-51.

Romani L, Puccetti P, Bistoni F. Interleukin-12 in infectious diseases. Clin Microbiol Rev. 1997 Oct;10(4):611-36. Review.

Romanus V, Hallander HO, Wåhlén P, Olinder-Nielsen AM, Magnusson PH, Juhlin I. Atypical mycobacteria in extrapulmonary disease among children. Incidence in Sweden from 1969 to 1990, related to changing BCG-vaccination coverage. Tuber Lung Dis. 1995 Aug;76(4):300-10.

Roque S, Nobrega C, Appelberg R, Correia-Neves M. IL-10 underlies distinct susceptibility of BALB/c and C57BL/6 mice to Mycobacterium avium infection and influences efficacy of antibiotic therapy. J Immunol. 2007 Jun 15;178(12):8028-35.

Roger PM, Bermudez LE. Infection of mice with Mycobacterium avium primes CD8+ lymphocytes for apoptosis upon exposure to macrophages. Clin Immunol. 2001 Jun:99(3):378-86.

Runyon EH. Mycobacterium intracellulare. Am Rev Respir Dis. 1967 May;95(5):861-5.

Saggese MD, Tizard I, Phalen DN. Mycobacteriosis in naturally infected ring-neck doves (Streptopelia risoria): investigation of the association between feather colour and susceptibility to infection, disease and lesions type. Avian Pathol. 2008 Aug;37(4):443-50.

Salvana EM, Cooper GS, Salata RA. Mycobacterium other than tuberculosis (MOTT) infection: an emerging disease in infliximab-treated patients. J Infect. 2007 Dec;55(6):484-7.

Saunders BM, Zhan Y, Cheers C. Endogenous interleukin-12 is involved in resistance of mice to Mycobacterium avium complex infection. Infect Immun. 1995 Oct:63(10):4011-5.

Scriba TJ, Kalsdorf B, Abrahams DA, Isaacs F, Hofmeister J, Black G, Hassan HY, Wilkinson RJ, Walzl G, Gelderbloem SJ, Mahomed H, Hussey GD, Hanekom WA. Distinct,

specific IL-17- and IL-22-producing CD4+ T cell subsets contribute to the human antimycobacterial immune response. J Immunol. 2008 Feb 1;180(3):1962-70.

Schaad UB, Votteler TP, McCracken GH Jr, Nelson JD. Management of atypical mycobacterial lymphadenitis in childhood: a review based on 380 cases. J Pediatr. 1979 Sep;95(3):356-60.

Schaefer WB, Davis CL, Cohn ML. Pathogenicity of transparent, opaque, and rough variants of Mycobacterium avium in chickens and mice. Am Rev Respir Dis. 1970 Oct;102(4):499-506.

Schaible UE, Kaufmann SH. CD1 and CD1-restricted T cells in infections with intracellular bacteria. Trends Microbiol. 2000 Sep;8(9):419-25. Review.

Schorey JS, Sweet L. The mycobacterial glycopeptidolipids: structure, function, and their role in pathogenesis. Glycobiology. 2008 Nov;18(11):832-41. Epub 2008 Aug 22. Review.

Shafran SD, Singer J, Zarowny DP, Phillips P, Salit I, Walmsley SL, Fong IW, Gill MJ, Rachlis AR, Lalonde RG, Fanning MM, Tsoukas CM. A comparison of two regimens for the treatment of Mycobacterium avium complex bacteremia in AIDS: rifabutin, ethambutol, and clarithromycin versus rifampin, ethambutol, clofazimine, and ciprofloxacin. Canadian HIV Trials Network Protocol 010 Study Group. N Engl J Med. 1996 Aug 8;335(6):377-83.

Shelton BG, Flanders WD, Morris GK. Mycobacterium sp. as a possible cause of hypersensitivity pneumonitis in machine workers. Emerg Infect Dis. 1999 Mar-Apr;5(2):270-3.

Sigalet D, Lees G, Fanning A. Atypical tuberculosis in the pediatric patient: implications for the pediatric surgeon. J Pediatr Surg. 1992 Nov;27(11):1381-4.

Smit T, Eger A, Haagsma J, Bakhuizen T. Avian tuberculosis in wild birds in the Netherlands. J Wildl Dis. 1987 Jul;23(3):485-7.

Smith D, Hänsch H, Bancroft G, Ehlers S. T-cell-independent granuloma formation in response to Mycobacterium avium: role of tumour necrosis factor-alpha and interferon-gamma. Immunology. 1997 Dec;92(4):413-21.

Smith D, Wiegeshaus E, Balasubramanian V. An analysis of some hypotheses related to the Chingelput bacille Calmette-Guérin trial. Clin Infect Dis. 2000 Sep;31 Suppl 3:S77-80.

Starke JR, Jacobs RF, Jereb J. Resurgence of tuberculosis in children. J Pediatr. 1992 Jun: 120(6):839-55. Review.

Steinert M, Birkness K, White E, Fields B, Quinn F. Mycobacterium avium bacilli grow saprozoically in coculture with Acanthamoeba polyphaga and survive within cyst walls. Appl Environ Microbiol. 1998 Jun;64(6):2256-61

Sweet L, Schorey JS. Glycopeptidolipids from Mycobacterium avium promote macrophage activation in a TLR2- and MyD88-dependent manner. J Leukoc Biol. 2006 Aug;80(2):415-23.

Sweet L, Zhang W, Torres-Fewell H, Serianni A, Boggess W, Schorey J. Mycobacterium avium glycopeptidolipids require specific acetylation and methylation patterns for signaling through toll-like receptor 2. J Biol Chem. 2008 Nov 28;283(48):33221-31.

Tateishi Y, Hirayama Y, Ozeki Y, Nishiuchi Y, Yoshimura M, Kang J, Shibata A, Hirata K, Kitada S, Maekura R, Ogura H, Kobayashi K, Matsumoto S. Virulence of Mycobacterium avium complex strains isolated from immunocompetent patients. Microb Pathog. 2009 Jan;46(1):6-12.

Taylor RH, Falkinham JO 3rd, Norton CD, LeChevallier MW. Chlorine, chlorine, chlorine dioxide, and ozone susceptibility of Mycobacterium avium. Appl Environ Microbiol. 2000 Apr;66(4):1702-5.

Tell LA, Woods L, Cromie RL. Mycobacteriosis in birds. Rev Sci Tech. 2001 Apr;20(1):180-203. Review.

Tessier MH, Amoric JC, Méchinaud F, Dubesset D, Litoux P, Stalder JF. Clarithromycin for atypical mycobacterial lymphadenitis in non-immunocompromised children. Lancet. 1994 Dec 24-31;344(8939-8940):1778.

Thegerström J, Marklund BI, Hoffner S, Axelsson-Olsson D, Kauppinen J, Olsen B. Mycobacterium avium with the bird type IS1245 RFLP profile is commonly found in wild and domestic animals, but rarely in humans. Scand J Infect Dis. 2005;37(1):15-20.

Thegerström J, Friman V, Nylén O, Romanus V, Olsen B. Clinical features and incidence of Mycobacterium avium infections in children. Scand J Infect Dis. 2008;40(6-7):481-6.

Thegerström J, Romanus V, Friman V, Brudin L, Haemig PD, Olsen B. Mycobacterium avium lymphadenopathy among children, Sweden. Emerg Infect Dis. 2008 Apr;14(4):661-3.

Tirkkonen T, Pakarinen J, Moisander AM, Mäkinen J, Soini H, Ali-Vehmas T. High genetic relatedness among Mycobacterium avium strains isolated from pigs and humans revealed by comparative IS1245 RFLP analysis. Vet Microbiol. 2007 Nov 15;125(1-2):175-81.

Tran DQ. Susceptibility to mycobacterial infections due to interferon-gamma and interleukin-12 pathway defects. Allergy Asthma Proc. 2005 Sep-Oct;26(5):418-21.

Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nat Rev Immunol. 2003 Feb;3(2):133-46. Review.

Tunkel DE, Romaneschi KB. Surgical treatment of cervicofacial nontuberculous mycobacterial adenitis in children. Laryngoscope. 1995 Oct;105(10):1024-8.

Tunkel DE, Baroody FM, Sherman ME. Fine-needle aspiration biopsy of cervicofacial masses in children. Arch Otolaryngol Head Neck Surg. 1995 May;121(5):533-6.

Turenne CY, Wallace R Jr, Behr MA. Mycobacterium avium in the postgenomic era. Clin Microbiol Rev. 2007 Apr;20(2):205-29. Review.

Ueda W, Fujiwara H, Tsuyuguchi I, Kuroki T, Yano I. Increased production of interleukin-10 by human blood monocytes stimulated with Mycobacterium avium-intracellulare complex. Kansenshogaku Zasshi. 1998 Jul;72(7):753-60.

Umemura M, Yahagi A, Hamada S, Begum MD, Watanabe H, Kawakami K, Suda T, Sudo K, Nakae S, Iwakura Y, Matsuzaki G. IL-17-mediated regulation of innate and acquired immune response against pulmonary Mycobacterium bovis bacille Calmette-Guerin infection. J Immunol. 2007 Mar 15;178(6):3786-96.

van Embden JD, Cave MD, Crawford JT, Dale JW, Eisenach KD, Gicquel B, Hermans P, Martin C, McAdam R, Shinnick TM, et al. Strain identification of Mycobacterium tuberculosis by DNA fingerprinting: recommendations for a standardized methodology. J Clin Microbiol. 1993 Feb;31(2):406-9.

van Soolingen D, Bauer J, Ritacco V, Leão SC, Pavlik I, Vincent V, Rastogi N, Gori A, Bodmer T, Garzelli C, Garcia MJ. IS1245 restriction fragment length polymorphism typing of Mycobacterium avium isolates: proposal for standardization. J Clin Microbiol. 1998 Oct:36(10):3051-4.

Vázquez N, Greenwell-Wild T, Rekka S, Orenstein JM, Wahl SM. Mycobacterium avium-induced SOCS contributes to resistance to IFN-gamma-mediated mycobactericidal activity in human macrophages. J Leukoc Biol. 2006 Nov;80(5):1136-44.

von Reyn CF, Maslow JN, Barber TW, Falkinham JO 3rd, Arbeit RD. Persistent colonisation of potable water as a source of Mycobacterium avium infection in AIDS. Lancet. 1994 May 7;343(8906):1137-41.

von Reyn CF, Waddell RD, Eaton T, Arbeit RD, Maslow JN, Barber TW, Brindle RJ, Gilks CF, Lumio J, Lähdevirta J, et al. Isolation of Mycobacterium avium complex from water in the United States, Finland, Zaire, and Kenya. von Reyn CF, Waddell RD, Eaton T, Arbeit RD, Maslow JN, Barber TW, Brindle RJ, Gilks CF, Lumio J, Lähdevirta J, et al.J Clin Microbiol. 1993 Dec;31(12):3227-30.

Wolinsky E. Mycobacterial lymphadenitis in children: a prospective study of 105 nontuberculous cases with long-term follow-up. Clin Infect Dis. 1995 Apr;20(4):954-63.

Vu TT, Daniel SJ, Quach C. Nontuberculous mycobacteria in children: a changing pattern. J Otolaryngol. 2005 Jun;34 Suppl 1:S40-4.

Vekemans J, Amedei A, Ota MO, D'Elios MM, Goetghebuer T, Ismaili J, Newport MJ, Del Prete G, Goldman M, McAdam KP, Marchant A. Neonatal bacillus Calmette-Guérin vaccination induces adult-like IFN-gamma production by CD4+ T lymphocytes. Eur J Immunol. 2001 May;31(5):1531-5.

Yamazaki Y, Danelishvili L, Wu M, Hidaka E, Katsuyama T, Stang B, Petrofsky M, Bildfell R, Bermudez LE. The ability to form biofilm influences Mycobacterium avium invasion and translocation of bronchial epithelial cells. Cell Microbiol. 2006 May;8(5):806-14.

Yajko DM, Chin DP, Gonzalez PC, Nassos PS, Hopewell PC, Reingold AL, Horsburgh CR Jr, Yakrus MA, Ostroff SM, Hadley WK. Mycobacterium avium complex in water, food, and soil samples collected from the environment of HIV-infected individuals. J Acquir Immune Defic Syndr Hum Retrovirol. 1995 Jun 1;9(2):176-82.

Young LS. Mycobacterium avium complex infection. J Infect Dis. 1988 May;157(5):863-7. Review.

Yoder S, Argueta C, Holtzman A, Aronson T, Berlin OG, Tomasek P, Glover N, Froman S, Stelma G Jr. PCR comparison of Mycobacterium avium isolates obtained from patients and foods. Appl Environ Microbiol. 1999 Jun;65(6):2650-3.

Yoder WD, Schaefer WB. Comparison of the seroagglutination test with the pathogenicity test in the chicken for the identification of Mycobacterium avium and Mycobacterium intracellulare. Am Rev Respir Dis. 1971 Feb;103(2):173-8.

Zeharia A, Eidlitz-Markus T, Haimi-Cohen Y, Samra Z, Kaufman L, Amir J. Management of nontuberculous mycobacteria-induced cervical lymphadenitis with observation alone. Pediatr Infect Dis J. 2008 Oct;27(10):920-2.