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REVIEW OF THE OCCURRENCE OF MYCOBACTIN DEPENDENCE AMONG MYCOBACTERIA SPECIES

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Résumé

FRÉQUENCE DE LA MYCOBACTINE DÉPENDANCE CHEZ LES DIFFÉRENTES ESPÈCES DE MYCOBACTÉRIES. — La plupart des mycobactéries sont capables de synthétiser la mycobactine. Mais, Mycobacterium paratuberculosis, M. avium atypique comme les mycobactéries isolées de pigeons ramiers et certaines souches de M. avium typique en primo-culture, ne possèdent pas cette aptitude. Elles exigent l'adjonction de mycobactine aux milieux de culture pour se développer in vitro.

Mycobacteria were early recognized to form a compact group of organisms, linked in the first place by their property of «acid-fastness». Among these organisms were various pathogens and some saprophytes. Most of these species of mycobacteria could be grown on laboratory media, but others could not. One was the bacillus found in the lesions of leprosy, and the other was the organism characteristic of chronic enteritis in cattle known as Johne's disease and called Mycobacterium paratuberculosis. M. paratuberculosis is an organism first observed by Johne and Frothingham (1895). Twort and Ingram (1913) attempted unsuccessfully to grow M. paratuberculosis by supplementing the medium with extracts of cattle tissues. They postulated that failure to cultivate the organism «must be due to the absence of some necessary foodstuff» and that the missing substance would most likely be found in tubercle bacilli. They showed that when dried killed human tubercle bacilli were added to egg medium, a good growth of M. paratuberculosis developed. They were also able to obtain growth using other mycobacteria but also using extracts prepared with organic solvents. Twort rightfully concluded that all the organisms in this group of mycobacteria needed an «essential substance» that was vital to their growth. Most mycobacteria were able to make this substance for themselves, but M. paratuberculosis lacked this capacity.

Francis et al. (1953) suggested the name of Mycobactin for a growth factor, present in acid-fast bacteria, which is essential for the growth of M. paratuberculosis.

Francis et al. (1953) argued that since a growth factor for M. paratuberculosis was produced by M. tuberculosis and seemed highly specific for the mycobacteria, its structure, if known, should provide a highly suitable model for the synthesis of compounds having a specific action against the mycobacteria. First it was necessary to isolate the growth factor in a pure form. For this purpose, the non-pathogenic and easily grown organism M. phlei was chosen. This proved a fortunate choice in several ways. On suitable media it produces relatively large amounts of growth factor; it also happens to be the only organism from which a crystalline product with growth promoting action for M. paratuberculosis has yet been obtained.

Mycobactin P, isolated in 1946 (in Snow,
strains from the wood-pigeon (Columba palumbus domestic and wild animals in several laboratories: presence of mycobactin, have been isolated from bacteria, that on primary culture grew only in the tuberculosis. Numerous strains of other mycobacteria were originally supposed to be chemically identical, but at least nine mycobactins are now known to exist, all possess a similar basic molecular pattern but are distinguished by variations in details of structure. The relationship between classification of the species and the chemical structure of the mycobactins may provide in the future a useful taxonomic tool (Snow, 1970).

The role of mycobactin

The acquisition of iron is essential for the growth of a microorganism in vitro or in vivo. As this element is usually present either in vitro in an insoluble form or in vivo linked to an organic molecule of the host, microorganisms are obliged to extract iron for themselves under most growth conditions.

Mycobacteria differ from other bacteria in that they elaborate more than one type of iron-binding compound. So far, three compounds of different properties have been described: mycobactin (Snow, 1970), salicylic acid (Ratledge and Winder, 1962), and exochelins (Macham and Ratledge, 1975). The exochelins are proposed as the functional extracellular iron-binding agents of BCG and other mycobacteria, the role of mycobactin being confined to that of a cell wall iron transporter (Macham et al., 1975). The mycobactin is a lipid soluble iron-binding compound which is located in the boundary layers of the organism where it transports iron across the thick lipoidal layers of the mycobacterial cell (Ratledge and Marshall, 1972). Iron is released from the carrier by conversion of the ferric ion to a ferrous ion by a ferrimycobactin oxidoreductase (NAD(P)H) (Brown and Ratledge, 1975) and the ferrous iron is then available for intracellular purposes.

The strains

The addition of mycobactin as a growth factor to various media has long been practised for the primary isolation of Mycobacterium paratuberculosis. Numerous strains of other mycobacteria, that on primary culture grew only in the presence of mycobactin, have been isolated from domestic and wild animals in several laboratories: Mc Diarmid (1962) described the isolation of such strains from the wood-pigeon (Columba palumbus L.). Rankin and Mc Diarmid (1968) referred to similar strains from various species of free-living British deer. Jorgensen and Clausen (1978) isolated from a roe-deer with generalized tuberculosis-like lesions a mycobacterium hitherto found only in wood-pigeons. And Thorel and Desmettre (1982) performed a comparative study of mycobactin-dependent strains isolated from wood-pigeon with M. avium and M. paratuberculosis.

On the other hand, using a medium containing mycobactin, Matthews (1969) showed that the number of isolates of mycobacteria from the cervical lymph nodes of pigs could be increased by 14.5%. From observations over several years, Matthews et al. (1978) estimate that a high proportion of virulent M. avium strains show the characteristic of mycobactin-dependence when media are inoculated with small numbers of viable units. Large inocula presumably contain sufficient mycobactin associated with the organisms to enable growth. These observations explain the increase in the proportion of mycobacterial strains isolated from the cervical lymph nodes of pigs (Matthews, 1969). Various degrees of mycobactin-dependence exist within the M. avium group of mycobacteria and that characteristic is not confined to M. paratuberculosis.

In the light of present knowledge Matthews (1969) recommends the use of media containing mycobactin in a concentration of at least 3.0 µg per ml for the primary isolation of M. avium. This observation may be of interest in relation to the primary isolation of other species of mycobacteria.

According to these reports, M. paratuberculosis, M. avium atypical like wood-pigeon mycobacteria and some strains of M. avium typical for the primary isolation, require mycobactin for growth in the laboratory.

The diseases

These strains can cause enteritis as well as generalized tuberculosis-like lesions:

Paratuberculosis is a contagious and enzootic disease of ruminants caused by the multiplication of a specific bacterium, M. paratuberculosis (Johnne's bacillus) in the mucous membrane of the intestine.

Under natural conditions, the disease in cattle spreads by ingestion of M. paratuberculosis from the contaminated environment. The disease persists in breeding stocks after the introduction of infected animals. A potential source of infection of calves is milk contaminated with the faeces of diseased cattle.

Clinically, the disease in bovines is characterized by chronic diarrhoea and emaciation. In sheep and goats, there may be simple intestinal catarrh but if diarrhoea develops, the animals usually die within a few days or weeks.
The occurrence of tuberculous infections in the wood-pigeon (*Columbus palumbus* L.), other than that associated with typical *Mycobacterium avium* has been recorded (Christiansen et al., 1946; McDiarmid, 1948, 1962; Solty and Wise, 1967). Previous authors have either failed to isolate the causal organism, or scanty growth has been obtained after prolonged cultivation on media usually employed for the isolation of *M. paratuberculosis*.

In the last few years, we have had the opportunity to observe lesions in the livers and spleens of wood-pigeons that resembled the lesions of avian tuberculosis; large numbers of acid-fast bacilli were found on smears made from these lesions, but culturing of the bacteria was not successful when media currently used in tuberculosis bacteriology were employed.

The strains isolated from the wood-pigeons formed a relatively homogeneous group, which could be distinguished from *M. avium* but were closely related to *M. paratuberculosis*.

Matthews and McDiarmid (1979) and Collins et al. (1983) showed that when chickens were injected intravenously with 10⁶ viable units of a wood-pigeon strain they died within 10 weeks. The lesions were typical of *M. avium* infection. Rabbits inoculated intravenously with 10⁸ viable units were killed at 20 to 23 weeks and lesions present in the lungs were those usually associated with *M. avium* infection.

In addition, mice inoculated intraperitoneally with 10⁴ viable units showed evidence of generalized infection with enlargement of spleen.

Matthews and McDiarmid (1979), Collins et al. (1983) and Thorel et al. (1983) inoculated three to four week-old calves intravenously with 10² to 10⁹ viable units. That dose was expected to cause pneumonia and death usually associated with *M. avium* in calves as originally described in the royal Commission report (Griffith, 1911). However the animals showed clinical signs of Johne’s Disease with excretion of acid-fast organisms in the faeces.

The wood-pigeon mycobacteria behave like *M. avium* in chickens and like *M. paratuberculosis* in calves.

Also, Jorgensen and Clausen (1976) described a mycobacteriosis with generalized tuberculosis-like lesions in a roe-deer caused by wood-pigeon mycobacteria.

On the other hand we must report that recently Chiodini et al. (1983) in Rhode Island Hospital isolated a mycobactin-dependent *Mycobacterium* species from a patient with Crohn’s disease. A segment of diseased ileum was cultured for mycobacteria by a concentration method on Herrold’s egg yolk medium with mycobactin. After 3-1/2 months incubation, a small mucoid colony was isolated. The organism recovered was an acid-fast, mycobactin-dependent *Mycobacterium* species which was different in growth characteristics and biochemical testing from other recognized pathogenic mycobacteria. The isolate resembles *M. paratuberculosis* and *M. avium*, but has features which distinguish it from both.

In another clinical case Thayer et al. (1983) have found *M. paratuberculosis* antibodies in Crohn’s disease. Studies currently in progress will attempt to determine the significance of this finding in understanding this inflammatory bowel disease.

**Conclusions**

A few species such as *M. paratuberculosis*, the wood-pigeon mycobacteria and some strains of *M. avium* cannot produce enough mycobactin for their own use when first being adapted from in vivo growth to in vitro growth on complex medium. The mycobactin-dependent strains usually can use the ferric mycobactin from any other species as their iron source, although several strains of *M. paratuberculosis* have been found which grow much faster with some mycobactins than others during primary isolation.

Meanwhile, mycobactin-dependence is a relative characteristic. After primary culture, mycobactin-dependence may be circumvented in several ways. *M. paratuberculosis* and wood-pigeon mycobacteria can grow on media without mycobactin, particularly that of Watson-Reid. It is thought that the low pH of this medium (5.5) and the formation of growth-stimulating compounds during autoclaving are the factors that circumvent the need of mycobactin. The mycobactin requirement of *M. paratuberculosis* has been also shown to be circumvented by incorporating 1 % (weight/volume) ferric ammonium citrate into serum or egg yolk medium.

Mycobactin-dependence has long been used as a taxonomic characteristic for *M. paratuberculosis*. However, it also has been known that occasionally a strain of *M. paratuberculosis* which required mycobactin for its primary isolation became mycobactin-independent. According to Merkal et al. (1981) it seemed likely that even *M. paratuberculosis* has the genetic information for production of mycobactin, but because of its usual habitat within macrophages its production of mycobactin was repressed. To test this, a rapidly growing culture of mycobactin-independent *M. paratuberculosis* was inoculated onto iron-deficient medium; when growth had stopped, the culture was assayed for mycobactin production. As anticipated, a mycobactin was produced. Since it is produced by the Johne’s bacillus, it has been named mycobactin J.

Recent results have demonstrated that, compa-
red with the heterologous mycobactin P, the homologous mycobactin J enhances the growth rate of *M. paratuberculosis* from infected tissues and increases the proportion of organisms which develop into colonies. Numerous strains of *M. avium* have been found to be mycobactin-dependent on primary isolation. It is possible that the use of mycobactin homologous to *M. avium* or to wood-pigeon mycobacteria would enhance the success rate of primary isolations from both lesions and environmental specimens.

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Summary

Most mycobacteria are able to make mycobactin for themselves. But, *Mycobacterium paratuberculosis*, *M. avium* atypical like wood-pigeon mycobacteria and some strains of *M. avium* typical for the primary isolation, lack this capacity and require mycobactin for growth in the laboratory.

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