

## *Mycobacterium avium* subspecies *paratuberculosis* infection and autoimmune condition: investigation of family members

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**Infezione da *Mycobacterium avium* subspecies *paratuberculosis* e autoimmunità: studio su componenti di una stessa famiglia**

### SUMMARY

*Mycobacterium avium* subspecies *paratuberculosis* (MAP) causes Johne's disease, a chronic granulomatous intestinal condition which affects ruminants, including cattle, sheep, goats, and farmed deer. In more recent studies water and milk supplies have both been suggested as vehicles of MAP transmission between cattle and humans. Recently our group observed immune responses to MAP in an Italian patient with Hashimoto's thyroiditis and Melkersson-Rosenthal syndrome. The focus of our work was evaluate the hypothesis of a possible MAP infection in other members of the same family.

### INTRODUCTION

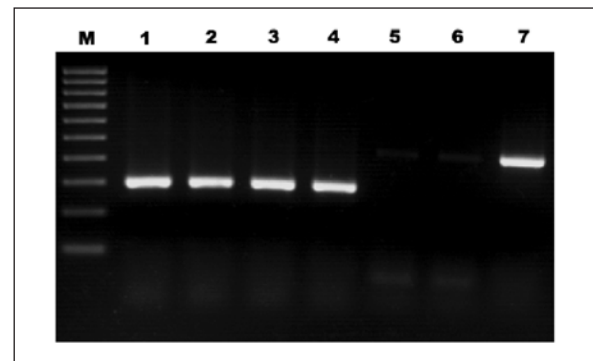
Recently our group observed immune responses to *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in an Italian patient with Hashimoto's thyroiditis and Melkersson-Rosenthal syndrome (2). MAP causes Johne's disease, a chronic granulomatous intestinal condition which affects ruminants, including cattle, sheep, goats, and farmed deer (7). In more recent studies water and milk supplies have both been suggested as vehicles of MAP transmission between cattle and humans. Several, risk of human exposure to MAP exist, and the primary focus has mainly been on dairy products. Milk may be contaminated directly within the udder or indirectly as a result of fecal contamination (10). This bacterium has attracted considerable interest owing to the rapidly growing body of scientific evidence suggesting that human infection with this micro-organism may be one of the causative agents in Crohn's disease (7). There is still much to be learned about MAP and the diseases that it may cause in humans. Recent findings by Sechi and Dow link diabetes to Crohn's disease, since they found MAP bacteremia in autoimmune diabetes (3, 12, 13). Consumption of cow's milk early in life is a recognized risk factor in the development of these diseases, and environmental micro-organisms are thought to trigger autoimmune responses in genetically susceptible individuals (3).

In the Italian patient with Hashimoto's thyroiditis and Melkersson-Rosenthal syndrome, the examination of the anamnesis, of the patient's family history and lifestyle lead us to hypothesize that there was the possibility of a MAP infection in other members of the same family. The family lives, from the childhood, in a farm with cattle, in an Italian region in which the MAP infection in animal farm is widespread. For a long period of the life, family members drank cow's milk produced directly from their farm and actually continue the diet based on dairy products. On general examination, two of the brothers of the case reported above, referred to suffer for many years of bowel disorders and following laboratory investigation positive levels of thyroid anti-thyroglobulin antibodies (1323 mU/ml; normal range < 40 UI/mL) and anti-thyroperoxidase antibodies (>1000 mU/ml; normal range < 35 mU/ml) were detected.

Based on the observations deduced from family clinical picture and laboratory tests, we hypothesized the existence of MAP infection in the two brothers.

We performed Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) to reveal the presence of viable MAP using mRNA extracted from peripheral blood mononuclear cells of these subjects, amplifying IS900 sequences which are

unique to MAP. The positive MAP control was ATCC 19698 DNA, and a band co-migrating with the ATCC 19698 DNA at the predicted amplicon size was considered positive. Bands were purified and sequenced (San Raffaele Biomedical Science Park, Milan, Italy). The sequence identity of the final amplicon was compared with the Genebank accession group EF514830 sequence for MAP IS900 using the International Nucleotide Sequence Database (8) and sequence alignment analyses. The PCR data (Figure) show bands that co-migrates with the positive control at the predicted 298 bp amplicon size. The sequenced DNA of the representative sample bands showed 100% identity with MAP IS900.



**Figure.** Agarose gel electrophoresis of amplified IS900 fragments. M, molecular marker. Lane 1, *Mycobacterium avium* subspecies *paratuberculosis* positive control. Lane 2, 3, 4, three family members. Lane 5, distilled water negative control. Lane 6, *Mycobacterium avium* subspecies *paratuberculosis* negative control subject. RT-PCR of internal control GADPH (lane 7).

### DISCUSSION

There is current evidence that a percentage of healthy individuals have MAP DNA in their blood, the significance of which remains to be determined (6).

However, there are specific states of immune dysfunction or genetic predisposition that could promote mycobacterial infections.

Identification of MAP in the blood of these three family members could be more than a coincidence.

It is common knowledge that human pathogens often express proteins with a high antigenic potential and strong homolo-

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gies with human proteins.

Evolutionary pressures based upon the necessity to escape host specific immune responses may have determined this phenomenon, known as “molecular mimicry”.

It is reasonable to assume that certain individuals can develop abnormal immune responses upon contact with an antigen that mimics a self-protein.

The human heat shock protein (Hsp) 60, for example, with a high degree of homology with Hsp65 of MAP, shares a sequence homology with a wide range of autoantigens including those of Hashimoto's thyroiditis (5).

Epitope mimicry is widely thought to be the mechanism for the induction of autoimmune disease.

The theory is that an infectious agent (parasite, bacteria, yeast or virus) displays epitopes immunologically resembling host determinants and due to the minor antigenic differences between the two, the pathogen's epitope is able to induce an immune response that breaks tolerance to the host epitope.

The cross-reactive T or B cell is then able to induce a pathogenic autoimmune response that leads to disease (9).

### CONCLUSION

Founding MAP infection in family members with high titles of anti-thyroid antibodies could be according to the hypothesis that molecular mimicry is one of the mechanism by which autoimmune diseases can occur in association with infectious agents.

Up to date, the currently available evidence is insufficient to confirm or disprove that *Mycobacterium paratuberculosis* is a causative agent of human autoimmune diseases.

Prospective studies are needed to test this hypothesis over a wider range of possible etiologies that include combination of infectious, immunological and genetic factors.

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