Arthritis Research & Therapy

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Exploring the association between *Mycobacterium avium* subspecies *paratuberculosis* infection and rheumatoid arthritis: an immunological perspective

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Abstract

Background *Mycobacterium avium* subspecies *Paratuberculosis* (MAP) is a bacterium known to cause Johne's disease in ruminants and has been implicated in several autoimmune diseases. This study aimed to investigate the potential association between MAP infection and Rheumatoid Arthritis (RA).

Methods A total of 119 patients with RA and 120 healthy controls (HCs) were enrolled in the study. The participants were outpatient attendees at a rheumatology specialist's clinic, selected according to the 2010 ACR/EULAR Classification Criteria for RA. Their serum samples were analyzed for antibodies against two peptides, MAP_4027$_{18–32}$ and IRF5$_{424-434}$, using an indirect enzyme-linked immunosorbent assay (ELISA).

Results A significant difference was found in the levels of anti-MAP antibodies between RA patients and HCs. RA patients were more likely to have anti-MAP_4027$_{18–32}$ antibodies (44.5%) vs. 10.8% in HCs. Among RA patients, treatment group patients had more antibodies (51.6%) against MAP_4027$_{18–32}$ than no-treatment group patients (36.4%), but this difference was not statistically significant. The antigen IRF5$_{424-434}$ showed the highest antibody seroreactivity, being present in a higher percentage of RA patients (60.5%) compared to HCs (8.3%). This difference was statistically significant. There was a moderate correlation between IRF5$_{424-434}$ and its MAP_4027$_{18-32}$ homolog.

Conclusions The study findings suggest that anti-MAP antibodies are more prevalent in RA patients compared to healthy controls, potentially implicating MAP in the development of RA. The strong immunological response to the antigen IRF5$_{424-434}$ warrants further investigation. Although the difference in antibody levels between previously diagnosed and newly diagnosed RA patients was not statistically significant, the overall higher prevalence of these antibodies in the RA cohort supports the hypothesis of MAP's involvement.

Keywords *Mycobacterium avium* subsp. *Paratuberculosis*, Arthritis, Rheumatoid, Molecular Mimicry

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Introduction

Mycobacterium avium subsp. *Paratuberculosis* (MAP) a gram-positive bacterium with a lipid-rich cell wall, thrives intracellularly. Its resilience poses risks to livestock and animal husbandry [1–3]. Paratuberculosis, caused by MAP, has a worldwide distribution, with significant regional variations in prevalence. A survey across 48 countries revealed that over 20% of livestock herds were infected in about half of these regions [3]. In Europe, between-herd seroprevalence in cattle ranges from 38 to 68%, while in North America, a 2007 study found MAP-positive cows in 68.1% of dairy herds in major U.S. states [4]. In Asia, Indian studies reported MAP prevalence in bovines ranging from 23.1 to 29%, with higher rates in large ruminants [5]. Meanwhile, in Switzerland, approximately 60 bovine cases are diagnosed annually, though the true prevalence is likely underestimated due to limited diagnostic measures [4].

MAP can indeed be transmitted from animals to humans through various routes [6–8]. In humans, MAP has the potential to provoke autoimmune reactions through a mechanism known as molecular mimicry. In this process, MAP antigens closely resemble the body's own tissue antigens, which may lead the immune system to mistakenly attack the body's cells. This inappropriate immune response is facilitated by MAP's ability to persist within the host's macrophages, leading to altered antigen presentation and T-cell activation. The immune system's failure to distinguish between MAP antigens and the body's own antigens can result in chronic inflammation and autoimmune conditions. Examples of such diseases include Crohn's disease (CD) [9], Hashimoto's thyroiditis (HT) [10], type 1 diabetes (T1D) [11], sarcoidosis [12], multiple sclerosis (MS) [13], and rheumatoid arthritis (RA) [14]. RA is a chronic inflammatory autoimmune disorder that primarily affects the joints but can also involve other parts of the body, such as the skin, blood vessels, heart, lungs, and muscles [15]. The prevalence of RA varies globally, typically ranging between 0.3% and 1% in the general population, with an annual incidence of approximately 40 new cases per 100,000 individuals [16]. The disease more commonly affects women, with a male to female ratio of about 1:3. RA can develop at any age, but it most frequently begins in individuals between the ages of 30 and 60 [17]. The etiology and pathogenesis of RA are indeed complex, involving a combination of genetic susceptibility and environmental factors. Known risk factors include smoking, which is the most significant environmental risk, as well as other potential triggers such as air pollution, occupational exposures, dietary factors, and microbial agents. Also, Genetic variations in genes like CARD15 and SLC11A1 influence an individual's immune response to MAP infection, potentially contributing to autoimmune diseases [18]. However, the exact cause of RA remains elusive, and it is the subject of ongoing research and debate [19]. As previously mentioned, MAP could be one of the potential environmental factors that trigger the onset of RA [20]. Upon transmission to humans and colonization within the host, through various pathways, including the consumption of contaminated dairy products, exposure to infected livestock, and environmental sources such as water supplies [21].This parasitic organism may alter the host's immune system through a process known as molecular mimicry. This involves the presentation of peptide sequences that resemble those of host cells, thereby blurring the distinction between self and non-self. In fact, it has been demonstrated that peptides derived from this bacterium can be cross-recognized by antibodies that target self-epitopes [22]. Research has indeed shown that interferon regulatory factors (IRFs) are associated with autoimmunity and play a crucial role in inflammation [23]. IRF5 is a transcription factor that plays a critical role in regulating inflammatory and immune responses [24]. Recent studies have highlighted that IRF5 is involved in both chronic and acute inflammation and is implicated in the development of various autoimmune disorders, including RA, Inflammatory Bowel Disease (IBD), Systemic Lupus Erythematosus (SLE), and Sjögren Syndrome (SS) [25, 26]. Polymorphisms in the IRF5 gene have been associated with an increased risk of developing autoimmune conditions [27].

While the direct interaction between IRF5 and MAP is not extensively documented, the dysregulation of IRF5 in response to infections is known to potentially exacerbate autoimmune responses [28, 29].

In our previous study, we investigated the molecular detection of the IS900 gene of MAP in RA patients and HCs. The study revealed that MAP detection was significantly higher in RA patients (31.9%) compared to HCs (12.5%). A notable observation was the higher frequency of MAP detection in newly diagnosed RA patients who had not yet received medication. Additionally, indirect ELISA tests identified antibodies against $MAP3865c_{125-133}$ and $ZnT8_{178-186}$ in RA patients, highlighting a potential link between MAP infection and RA pathogenesis [14].

Building on these findings, the current study extends our investigation to explore the immunological responses to MAP_4027_{18-32} and $IRF5_{424-434}$, peptides selected for their structural similarity and relevance to the molecular mimicry hypothesis. This targeted approach aims to further elucidate the role of MAP in RA development.

A 2023 study by Asgari et al. examined MAP's involvement in MS, identifying significant correlations between MAP peptides and human autoantigens, particularly IRF5. This study underscored MAP's potential as a trigger for autoimmune responses due to cross-reactivity

mechanisms. These insights informed our choice of peptides in the present study, emphasizing their relevance to autoimmune conditions like RA [13].

In light of these findings, we have investigated the humoral response to a peptide analogous to MAP_4027$_{18–32}$ and human interferon regulatory factor 5 (IRF5$_{424–434}$). The objective of this study is to examine the potential link between MAP infection and RA.

Materials and methods

Participant characteristics and ethical considerations

Peripheral blood was collected from 239 study participants, which included 119 RA patients (22 males and 97 females with a median age of 50.30 years) and 120 healthy controls (HCs) (18 males and 102 females with a median age of 50.28 years), were recruited from outpatient attendees at a rheumatology specialist's clinic in Gorgan, Iran. The recruitment period spanned from October 2021 to May 2022. The RA patients were divided into two categories: (1) no-treatment group patients who had not previously received medication for RA, and (2) treatment group patients who had been undergoing treatment with various RA medications, such as steroids, disease-modifying anti-rheumatic drugs (DMARDs), the anti-tumor necrosis factor-alpha medication adalimumab, and non-steroidal anti-inflammatory drugs (NSAIDs). Patient selection was based 2010 classification criteria for rheumatoid arthritis to the collaborative effort of the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR), as established by the American College of Rheumatology, with outpatients being referred to a rheumatology specialist's office [30]. The control group consisted of 120 healthy individuals referred to diagnostic laboratories for non-autoimmune-related medical assessments. These individuals underwent rigorous evaluation to confirm the absence of autoimmune conditions, which included clinical assessments and laboratory tests for markers such as rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibodies. While these controls were primarily selected as healthy, their referral to hospital labs was for reasons unrelated to autoimmunity, ensuring no confounding factors related to RA diagnosis. Information about all participants is provided in Table 1.

Table 1 Demographic, clinical and laboratory characteristics of RA participants and HCs

			Patient ($n=119$)	Control ($n=120$)
MAP-4027 Positive	Age (mean)		49.73 ± 14.25	50.85 ± 9.92
	Sex	Female	46 (86.8%)	13 (100%)
		Male	7 (13.2%)	0
	RF	Positive	4 (78.8%)	0
		Negative	11 (21.2%)	13 (100%)
	anti-CCP	Positive	34 (66.7%)	0
		Negative	17 (33.3%)	13 (100%)
MAP-4027 Negative	Age		50.9 ± 14.14	50.21 ± 11.65
	Sex	Female	50 (78.1%)	89 (83.2%)
		Male	14 (21.9%)	18 (16.8%)
	RF	Positive	37 (69.8%)	0
		Negative	16 (30.2%)	107 (100%)
	anti-CCP	Positive	34 (66.7%)	0
		Negative	17 (33.3%)	13 (100%)
IRF5 Positive	Age		50.32 ± 13.87	50.35 ± 11.55
	Sex	Female	59 (81.9%)	10 (100%)
		Male	13 (18.1%)	0
	RF	Positive	27 (64.3%)	0
		Negative	15 (35.7%)	110 (100%)
	anti-CCP	Positive	28 (68.3%)	0
		Negative	13 (31.7%)	110 (100%)
IRF5 Negative	Age		50.27 ± 14.62	50.35 ± 11.55
	Sex	Female	38 (80.9%)	92 (83.6%)
		Male	9 (19.1%)	18 (16.4%)
	RF	Positive	27 (64.3%)	0
		Negative	15 (35.7%)	110 (100%)
	anti-CCP	Positive	28 (68.3%)	0
		Negative	13 (31.1%)	110 (100%)

The study's ethical aspects were reviewed and endorsed by the ethics committee of Golestan University of Medical Sciences. It adheres to the ethical principles set forth in the Declaration of Helsinki (Ethics code: IR.GOUMS.REC.1401.042).

Serum preparation

We collected whole blood using venipuncture needles and vacuum-sealed tubes. After collection, we allowed the blood to clot for approximately 20 min at room temperature. Next, we centrifuged the blood tube at 1000–2000×g for 10 min in a refrigerated centrifuge. The resulting supernatant was divided into 1.5-microtube aliquots as serum, which we stored at – 20 °C for use in the indirect ELISA (Enzyme-Linked Immunosorbent Assay) test.

Peptide synthesis

The peptides, MAP_4027$_{18–32}$ (AVVPVLAYAAARL-LL) and IRF5$_{424–434}$ (VVPV—AARL-LLE), both achieved a synthesis purity level exceeding 95% (Biomatik, Canada). They were solubilized in dimethyl sulfoxide (DMSO) at a concentration of 10 mM and stored at – 70 °C.

ELISA procedures

- A 96-well flat-bottom plate (Jet Bio-Fill, China) was coated with peptides (MAP_4027$_{18–32}$ or IRF$_{5424–434}$) at a concentration of 10 µg/ml. The coating was done in 0.05 M carbonate-bicarbonate buffer (pH 9.6) and incubated overnight at 4 °C.

- The plate was washed with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBS-T). Blocking solution (5% gelatin) was added to each well and incubated at 37 °C for 1 h.
- After five washes, serum samples (diluted 1:100) were added to the wells and incubated at 37 °C for 1 h.
- Following five washes, rabbit anti-human IgG conjugated with horseradish peroxidase (HRP) (dilution 1:3500) was added.
- After 45 min on a shaker at room temperature, the wells were washed five times.
- A substrate solution (3,3',5,5'-tetramethylbenzidine, TMB) was added, and the plates were incubated at room temperature for 15 min.
- The reaction was stopped by adding 100 µL of 2 N H2SO4.
- Absorbance was measured at a wavelength of 450 nm using an ELISA plate reader.

In this test, the positive control consisted of the serum sample from a patient whose PCR and bacterial culture results from the blood sample were positive. The negative control, on the other hand, was rabbit blood serum.

Statical analysis

The statistical analysis was performed using SPSS software (version 19.0) and GraphPad Prism (version 9.0). Relevant tests were used to compare variables between the two groups. Non-parametric data were analyzed using the Mann–Whitney test. Categorical data were expressed as percentages and frequencies, while numerical variables were presented as mean ± SD. A significance level of $P < 0.05$ was considered statistically significant. The positivity cutoff for peptides was set within the range of 0.55. The correlation between variables was assessed using Pearson's correlation coefficient, which measures the linear relationship between two variables. The choice of Pearson's method ensures that the results are based on continuous and normally distributed data.

Results

Our research revealed a notable disparity in the detection and mean levels of anti-MAP antibodies between RA patients and the HCs. Specifically, anti-MAP_4027$_{18-32}$ antibodies were identified in 44.5% of RA patients (53 out of 119), in stark contrast to just 10.8% of the HCs (13 out of 120), marking a statistically significant difference ($p < 0.0001$).

In terms of serum, compared to the tested antigens, IRF5$_{424-434}$ showed more prevalence of antibodies than other antigen. These were found in 60.5% of RA patients (69 of 119) compared to 8.3% of HCs (10 of 120), again showing a statistically significant difference ($p < 0.0001$) (Fig. 1).

Upon examining the subgroups of RA patients, we observed that treatment group patients exhibited a higher average presence of antibodies against MAP_4027$_{18-32}$ (51.6%, 33 out of 64) compared to no-treatment group patients (36.4%, 20 out of 55); however, this difference was not statistically significant.

Additionally, antibodies against IRF5$_{424-434}$ were detected more frequently in treatment group patients (70.3%, 45 out of 64) than in no-treatment group patients (49.1%, 27 out of 55), and this difference was statistically significant ($p < 0.0001$).

We noted a significant disparity in the heightened reactivity to at least one of the assessed epitopes between subjects with RA and HCs, with 65.5% of RA subjects showing reactivity compared to 13.3% of HCs, a difference that was statistically significant ($p < 0.0001$).

Finally, our study found a moderate correlation between the presence of IRF5$_{424-434}$ and its MAP_4027$_{18-32}$ homolog (R2 = 0.4213) (Fig. 2). This suggests that these two antigens may be linked in some way, which could have implications for understanding the role of MAP in RA.

Discussion

MAP is a significant pathogen, primarily responsible for causing diseases in livestock such as cows and sheep. It is also implicated in the development of human autoimmune diseases. MAP's resilience is remarkable, with the ability to survive processes like pasteurization and chlorination, thus posing a potential threat to public health.

Numerous studies have detected elevated antibody levels against several MAP proteins and corresponding human epitopes [13, 14, 22, 31–33]. This finding supports the theory that MAP infection might initiate and exacerbate diseases in genetically predisposed individuals through a mechanism known as molecular mimicry. This concept suggests that the immune system may confuse the body's own proteins with foreign pathogens due to the structural similarity between certain microbial epitopes and self-proteins. Consequently, this can lead to an autoimmune response where the immune system mistakenly targets the host's own proteins.

Our findings indicate that while some autoantibodies may be detectable prior to the onset of RA, their levels significantly increase with disease progression, suggesting they play a dual role: as potential early biomarkers for disease risk and as amplifiers of inflammation during disease progression. This observation highlights a complex relationship between autoantibody production, disease course, and treatment effects, offering insights into the evolving pathophysiology of RA.

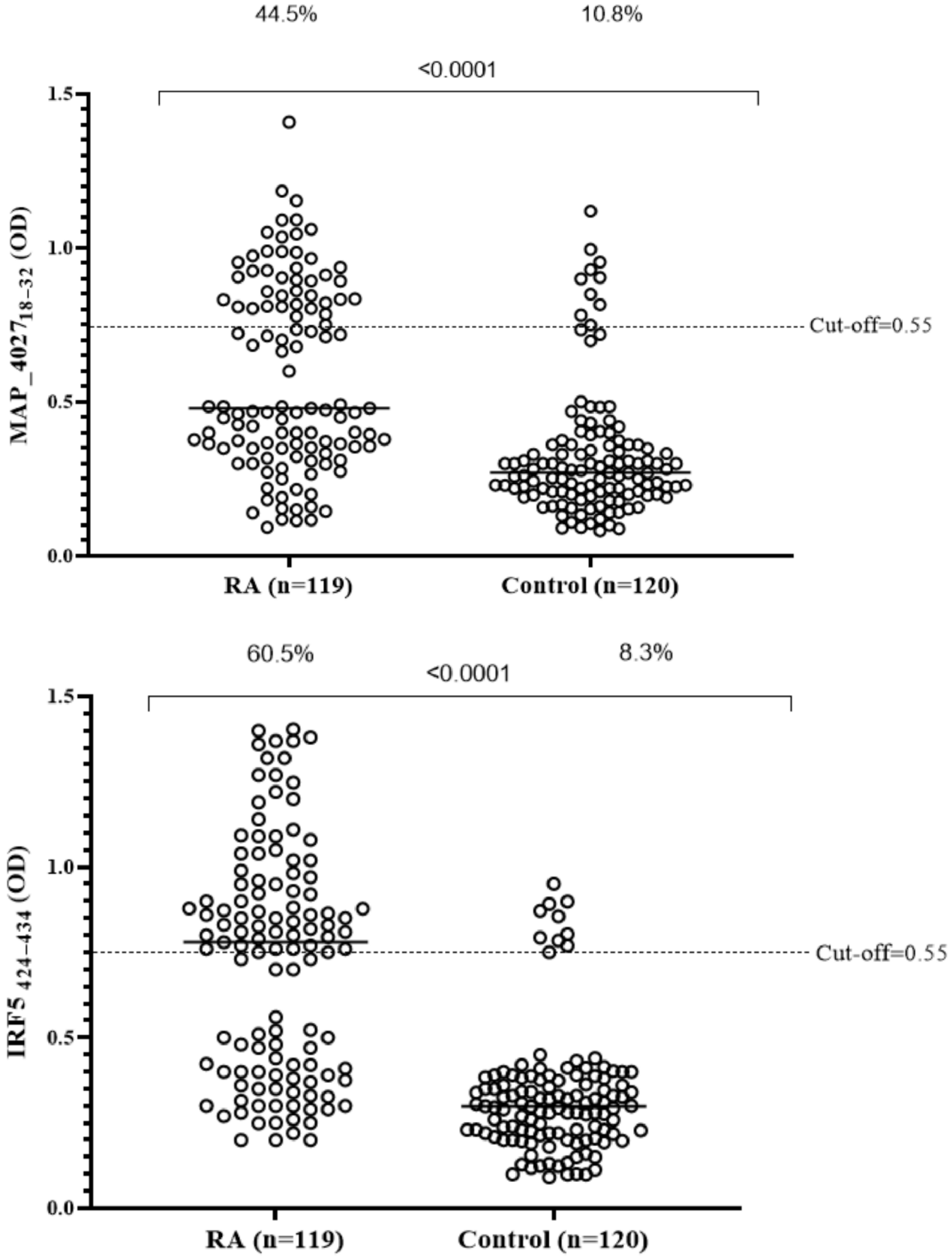

Fig. 1 (See legend on next page.)

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Fig. 1 The ELISA-based analysis assessed the antibody reactivity to MAP_4027$_{18–32}$ and its homologue IRF5$_{424–434}$ in subjects with RA and HCs. The dotted lines denote the positivity thresholds determined by ROC analysis. The Mann–Whitney test was used for the p-values and Fisher's exact test was used for calculating the percentages of positivity

Our study revealed that patients with RA produced a higher quantity of antibodies against the peptides MAP_4027$_{18–32}$ and IRF5$_{424–434}$ compared to the HCs group, with a statistically significant difference ($p < 0.0001$). In patients with previously diagnosed RA, the prevalence of antibodies against MAP_4027$_{18–32}$ and IRF5$_{424-434}$ was higher compared to patients with newly diagnosed RA.

This finding supports the hypothesis that molecular mimicry may play a role in the pathogenesis of RA [34]. In the context of molecular mimicry, the immune system recognizes both microbial antigens and self-antigens due to structural similarities. This cross-reaction may lead to autoimmunity, as the immune response inadvertently targets host tissues while fighting the pathogen [35]. Although molecular mimicry is intriguing, other factors contribute to the observed associations between MAP and RA [36]. Chronic MAP infection can trigger systemic inflammation, potentially exacerbating autoimmune responses [37]. Additionally, MAP may modulate immune responses, affecting the balance between proinflammatory and regulatory pathways [38]. Genetic predispositions and common environmental triggers could also play a role. It's essential to consider that the association may be coincidental, lacking a direct causal link [39].

In the context of RA, it is hypothesized that MAP infections could induce a specific humoral immune response targeting the host protein IRF5, a transcription factor that is part of the interferon regulatory factor family [40]. IRF5 plays a crucial role in regulating the Type I interferon pathway and orchestrating the immune response to infections [41]. Antibodies are generated through the humoral immune response. In this case, the MAP component acts as the antigen, which is recognized and bound by antibodies, marking it for destruction by other components of the immune system [42, 43]. The potential for autoimmune attacks resulting in synovial tissue degradation due to cross-reactive antigens remains a hypothesis. Our study does not provide direct evidence for this mechanism, and further experimental research is necessary to investigate these possibilities [44].

In a comparative case-control study, evaluated the immune response to protein tyrosine phosphatase A (PtpA) and protein kinase G (PknG) in RA patients compared to HCs [31]. The results showed significantly elevated antibody levels to MAP proteins in RA patients' sera, indicating prior exposure to MAP.

Our results show that antibody frequency is lower in newly diagnosed RA patients compared to those with longstanding RA. This has two implications: it may indicate that antibodies against MAP and IRF5 signal the onset of the disease in recent infections, and in established RA cases, other factors may have been influential initially, with MAP presence possibly affecting symptom severity or disease progression—although our study did not investigate this. Longitudinal studies would be useful to establish causality and to examine genetic predispositions and other environmental factors beyond MAP.

This study acknowledges several limitations that may impact data interpretation. First, the sample size, although adequate, may not fully represent the diverse demographics affected by RA, potentially limiting the generalizability of the findings. Second, the cross-sectional nature of the study precludes establishing a causal relationship between MAP infection and RA. Third, while this study uses serological evidence to demonstrate an association between exposure to MAP and RA, it does not consider other environmental or genetic factors that may play a role in disease pathogenesis. Furthermore, relying on the presence of antibodies as an indicator of MAP infection may not reflect the current infection status or the direct role of MAP in the development of RA. Finally, the data presented are based on the detection of antibodies against specific MAP-related peptides, which may not capture the full spectrum of immune responses to MAP or its molecular mimicry mechanisms in RA patients. Despite these limitations, this study investigated the potential association between MAP infection and RA, contributing to a broader understanding of the etiology of RA and the role of environmental factors in autoimmune diseases. In addition, the study does not consider other environmental or genetic factors that could influence RA pathogenesis. For instance, genetic predispositions such as specific HLA-DRB1 alleles have been strongly associated with RA [45]. Environmental factors like smoking, diet, and infections other than MAP could also play significant roles [46]. Smoking, for example, has been shown to increase the risk of RA, particularly in individuals with certain genetic backgrounds [47]. Additionally, other microbial agents and their interactions with the host immune system could contribute to the development and progression of RA. Including these factors in future research could provide a more comprehensive understanding of RA pathogenesis and help identify additional targets for prevention and treatment.

In selecting MAP_4027$_{18–32}$ for this study, we prioritized its structural similarity to the human IRF5$_{424–434}$ peptide. This similarity enables a targeted investigation into the role of molecular mimicry in RA pathogenesis. Furthermore, previous findings demonstrated significant

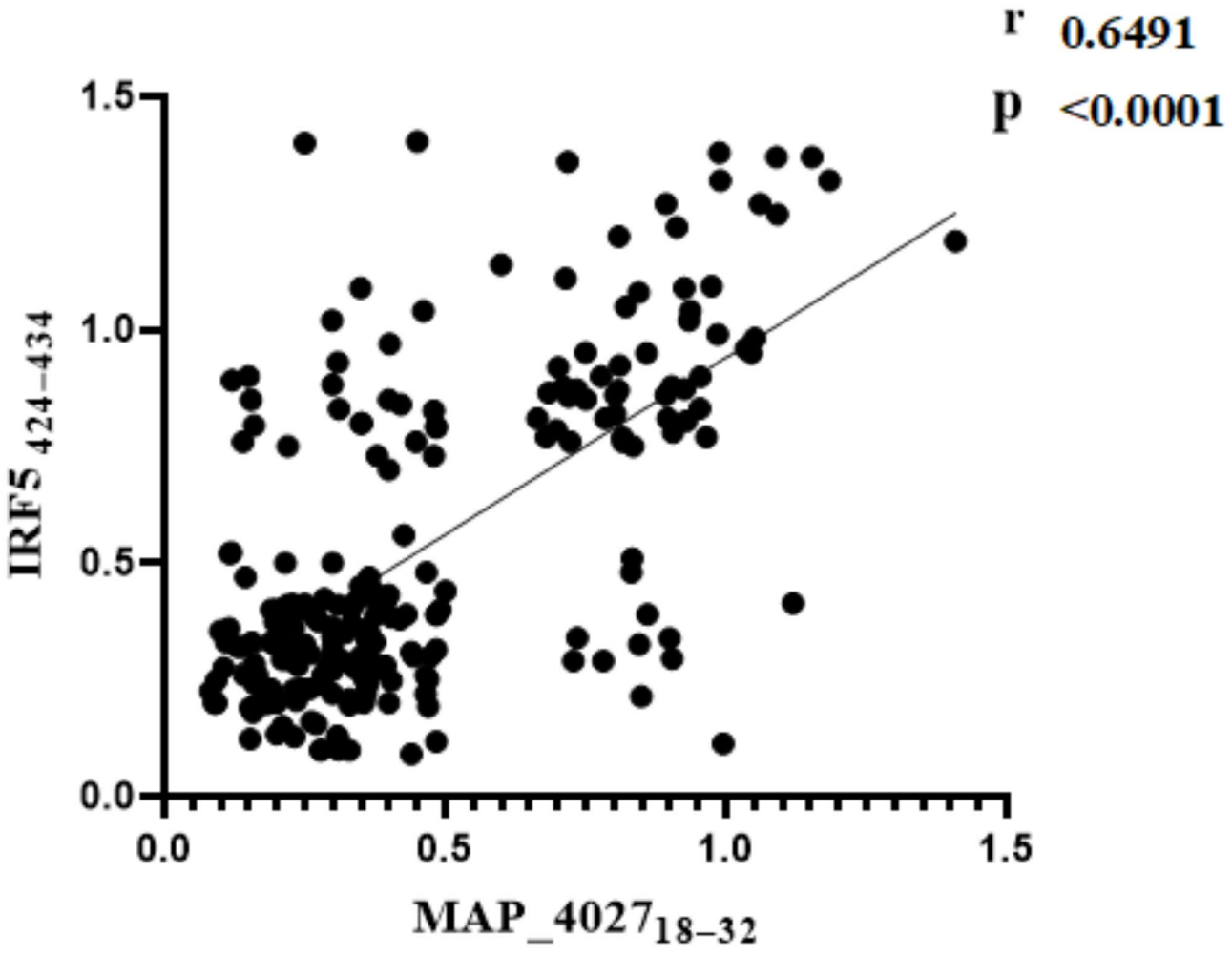

Fig. 2 Scatter plot expressing correlation between MAP_4027$_{18–32}$ and IRF5$_{424–434}$ derived peptides in RA and HCs groups

antibody seroreactivity to MAP_4027$_{18–32}$ in autoimmune disease cohorts, suggesting its importance in immune responses and its relevance for studying MAP-associated mechanisms.

Although MAP_4027$_{18–32}$ and MAP3865c$_{125–133}$ have both shown elevated antibody levels in RA patients compared to healthy controls, neither has been conclusively established as immunodominant among all possible MAP peptides. Immunodominance is determined by the capacity of an epitope to elicit a robust immune response across diverse human populations, a question requiring broader epitope mapping and additional studies. Our findings highlight their relevance in the context of RA, but further research is necessary to confirm their immunodominance.

A limitation of this study is the lack of direct comparison of autoantibody responses to MAP_402718–32 and MAP3865c125–133 in individuals with confirmed MAP infection versus uninfected individuals. While the significant antibody prevalence in RA patients relative to healthy controls suggests a MAP-related immune mechanism, future studies should include cohorts stratified by MAP infection status. This approach would enable a deeper understanding of the association between these peptides and autoimmune responses in MAP-infected individuals.

Conclusion

This study, along with prior research, indicates that MAP, an obligate intracellular bacterium responsible for Johne's disease in ruminants, could predispose individuals to RA and has been linked to various human diseases. The transmission of MAP from ruminants to humans is a significant concern, emphasizing the need for preventive measures. Vaccination of ruminants against MAP can decrease its prevalence in these animals, thus lowering human transmission risk. Additionally, avoiding traditional dairy products, which may contain MAP bacteria if not pasteurized, is advisable. Pasteurization effectively eliminates MAP and other pathogens, ensuring

safer dairy consumption. These findings highlight MAP's potential role in RA development and the importance of preventing its transmission from ruminants to humans.

Acknowledgements
The authors would like to thank the personnel of the Department of Microbiology and other project colleagues at Golestan University of Medical Sciences, Gorgan, Iran, for the technical services.

Author contributions
NA is the first author who performed all laboratory experiments and sample collection, collected and analyzed data, and drafted the manuscript. EG participated in the coordination and advised in all parts of the study. ST and MA are rheumatologists who diagnosed and validated patients with RA and provided the specimens from all cases. HRN participated in the design and setup of the ELISA test. SZ participated in the study design and coordination and supervised all study parts and drafted the manuscript. All authors read and approved the manuscript.

Funding
Golestan University of Medical Sciences supported this study (Project number: 112501).

Data availability
Data transparency is provided.

Declarations

Ethics approval and consent to participate
This project was approved by the Ethics Committee of Golestan University of Medical Sciences (No. IR.GOUMS. REC.1401.042).

Consent to participate
Informed consent to participate was obtained from all participants in the study. All participants received a comprehensive explanation of the study purpose, procedures, and their rights as research participants. They were informed that participation was voluntary and they could withdraw at any time without penalty. Signed consent forms were obtained from all participants prior to their involvement in the study. Both the patient and control groups were asked to complete a demographic questionnaire after providing their informed consent.

Clinical trial number
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 12 December 2024 / Accepted: 7 February 2025
Published online: 21 February 2025

References

1. Dow CT, Ellingson JL. Detection of Mycobacterium avium ss. Paratuberculosis in Blau Syndrome tissues. Autoimmune Dis. 2010;2011:127692.
2. Garvey M. Mycobacterium avium subspecies paratuberculosis: a possible causative agent in human morbidity and risk to public health safety. Open Vet J. 2018;8(2):172–81.
3. Whittington R, Donat K, Weber MF, Kelton D, Nielsen SS, Eisenberg S, et al. Control of paratuberculosis: who, why and how. A review of 48 countries. BMC Vet Res. 2019;15(1):198.
4. Ottardi M, Lechner I, Wang J, Schmitt S, Schneeberger M, Schmid RM, et al. Seroprevalence of Mycobacterium avium subsp. paratuberculosis in Swiss dairy herds and risk factors for a positive herd status and within-herd prevalence. Front Vet Sci. 2024;11:1409694.
5. Singh AV, Chauhan DS, Singh SV, Kumar V, Singh A, Yadav A, et al. Current status of Mycobacterium avium subspecies paratuberculosis infection in animals & humans in India: what needs to be done? Indian J Med Res. 2016;144(5):661–71.
6. Pickup RW, Rhodes G, Arnott S, Sidi-Boumedine K, Bull TJ, Weightman A, et al. Mycobacterium avium subsp. paratuberculosis in the catchment area and water of the River Taff in South Wales, United Kingdom, and its potential relationship to clustering of Crohn's disease cases in the city of Cardiff. Appl Environ Microbiol. 2005;71(4):2130–9.
7. Grewal SK, Rajeev S, Sreevatsan S, Michel FC. Jr. Persistence of Mycobacterium avium subsp. paratuberculosis and other zoonotic pathogens during simulated composting, manure packing, and liquid storage of dairy manure. Appl Environ Microbiol. 2006;72(1):565–74.
8. Hruska K, Bartos M, Kralik P, Pavlik I. Mycobacterium avium subsp. paratuberculosis in powdered infant milk: paratuberculosis in cattle - the public health problem to be solved. Vet Med. 2005;50(8):327–35.
9. Agrawal G, Aitken J, Hamblin H, Collins M, Borody TJ. Putting Crohn's on the MAP: five common questions on the contribution of Mycobacterium avium subspecies paratuberculosis to the pathophysiology of Crohn's Disease. Dig Dis Sci. 2021;66(2):348–58.
10. Moghadam M, Ghaemi EA, Akbari H, Razavi Nikoo H, Zamani S. Mycobacterium avium subsp. paratuberculosis and Hashimoto's thyroiditis: is MAP the trigger? Front Cell Infect Microbiol. 2022;12:972929.
11. Ozana V, Hruska K, Sechi LA. Neglected facts on Mycobacterium Avium subspecies paratuberculosis and type 1 diabetes. Int J Mol Sci. 2022;23(7).
12. Ekundayo TC, Okoh AI. Systematic Assessment of Mycobacterium avium subspecies paratuberculosis infections from 1911–2019: a growth analysis of Association with Human Autoimmune diseases. Microorganisms. 2020;8(8).
13. Asgari N, Ghaemi EA, Naeimi MH, Tahamtan A, Sechi LA, Zamani S. Cross-reactivity between Mycobacterium avium subspecies paratuberculosis 4027 peptide and human IRF5 may contribute to multiple sclerosis in Iranian patients. Heliyon. 2023;9(11):e22137.
14. Asgari N, Ghaemi EA, Tavasoli S, Aghaei M, Razavi Nikoo H, Sechi LA, et al. Detection of Mycobacterium avium subspecies paratuberculosis in the blood of patients with rheumatoid arthritis by using Serological and Molecular techniques. Curr Microbiol. 2023;80(11):359.
15. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. Lancet. 2010;376(9746):1094–108.
16. Krajewska-Włodarczyk M, Szeląg M, Batko B, Żuber Z, Orleański M, Podwójcic K, et al. Rheumatoid arthritis epidemiology: a nationwide study in Poland. Rheumatol Int. 2024;44(6):1155–63.
17. Pavlov-Dolijanovic S, Bogojevic M, Nozica-Radulovic T, Radunovic G, Mujovic N. Elderly-Onset Rheumatoid Arthritis: characteristics and treatment options. Medicina. 2023;59(10):1878.
18. Sechi LA, Dow CT. Mycobacterium avium ss. Paratuberculosis zoonosis - the Hundred Year War - Beyond Crohn's Disease. Front Immunol. 2015;6:96.
19. Kondo N, Kuroda T, Kobayashi D. Cytokine networks in the pathogenesis of rheumatoid arthritis. Int J Mol Sci. 2021;22(20).
20. Bo M, Jasemi S, Uras G, Erre GL, Passiu G, Sechi LA. Role of infections in the pathogenesis of rheumatoid arthritis: focus on Mycobacteria. Microorganisms. 2020;8(10).
21. McNees AL, Markesich D, Zayyani NR, Graham DY. Mycobacterium paratuberculosis as a cause of Crohn's disease. Expert Rev Gastroenterol Hepatol. 2015;9(12):1523–34.
22. Mameli G, Cocco E, Frau J, Marrosu MG, Sechi LA. Epstein Barr Virus and Mycobacterium avium subsp. paratuberculosis peptides are recognized in sera and cerebrospinal fluid of MS patients. Sci Rep. 2016;6:22401.
23. Matta B, Song S, Li D, Barnes BJ. Interferon regulatory factor signaling in autoimmune disease. Cytokine. 2017;98:15–26.
24. Byrne AJ, Weiss M, Mathie SA, Walker SA, Eames HL, Saliba D, et al. A critical role for IRF5 in regulating allergic airway inflammation. Mucosal Immunol. 2017;10(3):716–26.
25. Almuttaqi H, Udalova IA. Advances and challenges in targeting IRF5, a key regulator of inflammation. Febs j. 2019;286(9):1624–37.
26. Weiss M, Byrne AJ, Blazek K, Saliba DG, Pease JE, Perocheau D, et al. IRF5 controls both acute and chronic inflammation. Proc Natl Acad Sci U S A. 2015;112(35):11001–6.
27. Yanagimachi M, Naruto T, Miyamae T, Hara T, Kikuchi M, Hara R, et al. Association of IRF5 polymorphisms with susceptibility to macrophage activation syndrome in patients with juvenile idiopathic arthritis. J Rheumatol. 2011;38(4):769–74.

28. Ban T, Sato GR, Tamura T. Regulation and role of the transcription factor IRF5 in innate immune responses and systemic lupus erythematosus. Int Immunol. 2018;30(11):529–36.
29. Espeschit IF, Bastos DSS, Fonseca Junior A, Cardoso SA, Ferrari MLA, Moreira MAS. Mycobacterium avium subsp. paratuberculosis and Crohn's disease: characterization of the interaction with different aspects of the disease. Braz J Microbiol. 2023;54(2):1239–49.
30. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against Rheumatism collaborative initiative. Ann Rheum Dis. 2010;69(9):1580–8.
31. Hayashi F, Isobe N, Cossu D, Yokoyama K, Sakoda A, Matsushita T, et al. Elevated mycobacterium avium subsp. paratuberculosis (MAP) antibody titer in Japanese multiple sclerosis. J Neuroimmunol. 2021;360:577701.
32. Bo M, Niegowska M, Eames HL, Almuttaqi H, Arru G, Erre GL, et al. Antibody response to homologous epitopes of Epstein-Barr virus, Mycobacterium avium subsp. paratuberculosis and IRF5 in patients with different connective tissue diseases and in mouse model of antigen-induced arthritis. J Transl Autoimmun. 2020;3:100048.
33. Bo M, Erre GL, Niegowska M, Piras M, Taras L, Longu MG, et al. Interferon regulatory factor 5 is a potential target of autoimmune response triggered by Epstein-Barr virus and Mycobacterium avium subsp. paratuberculosis in rheumatoid arthritis: investigating a mechanism of molecular mimicry. Clin Exp Rheumatol. 2018;36(3):376–81.
34. Moten D, Teneva I, Apostolova D, Batsalova T, Dzhambazov B. Molecular Mimicry of the rheumatoid arthritis-related immunodominant T-Cell epitope within type II collagen (CII260-270) by the bacterial L-Asparaginase. Int J Mol Sci. 2022;23(16):9149.
35. Thaper D, Prabha V. Molecular Mimicry: unravelling the role of autoantibodies in Autoimmune diseases and Infertility. In: Sobti RC, Sobti A, editors. Biomedical Translational Research: technologies for improving Healthcare. Singapore: Springer Nature Singapore; 2022. pp. 395–406.
36. Suliman BA. Potential clinical implications of molecular mimicry-induced autoimmunity. Immun Inflamm Dis. 2024;12(2):e1178.
37. Rani PS, Sechi LA, Ahmed N. Mycobacterium avium subsp. paratuberculosis as a trigger of type-1 diabetes: destination Sardinia, or beyond? Gut Pathog. 2010;2(1):1.
38. Gupta SK, Wilson T, Maclean PH, Rehm BHA, Heiser A, Buddle BM, et al. Mycobacterium avium subsp. paratuberculosis antigens induce cellular immune responses in cattle without causing reactivity to tuberculin in the tuberculosis skin test. Front Immunol. 2022;13:1087015.
39. Waddell LA, Rajić A, Stärk KD, Mc ES. The zoonotic potential of Mycobacterium avium ssp. paratuberculosis: a systematic review and meta-analyses of the evidence. Epidemiol Infect. 2015;143(15):3135–57.
40. Jefferies CA. Regulating IRFs in IFN Driven Disease. Front Immunol. 2019;10:325.
41. Romão VC, Fonseca JE. Etiology and risk factors for rheumatoid arthritis: a state-of-the-art review. Front Med. 2021;8:689698.
42. Marshall JS, Warrington R, Watson W, Kim HL. An introduction to immunology and immunopathology. Allergy, asthma, and clinical immunology. Official J Can Soc Allergy Clin Immunol. 2018;14(Suppl 2):49.
43. Cusick MF, Libbey JE, Fujinami RS. Molecular mimicry as a mechanism of autoimmune disease. Clin Rev Allergy Immunol. 2012;42(1):102–11.
44. Moten D, Teneva I, Apostolova D, Batsalova T, Dzhambazov B. Molecular Mimicry of the rheumatoid arthritis-related immunodominant T-Cell epitope within type II collagen (CII260-270) by the bacterial L-Asparaginase. Int J Mol Sci. 2022;23(16).
45. Inoue M, Nagafuchi Y, Ota M, Tsuchiya H, Tateishi S, Kanda H, et al. Carriers of HLA-DRB1*04:05 have a better clinical response to abatacept in rheumatoid arthritis. Sci Rep. 2023;13(1):15250.
46. Ahn J, Hayes RB. Environmental influences on the human microbiome and implications for noncommunicable disease. Annu Rev Public Health. 2021;42:277–92.
47. Ishikawa Y, Terao C. The impact of cigarette smoking on risk of rheumatoid arthritis: a narrative review. Cells. 2020;9(2):475.

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