Mycobacterium avium Subspecies *paratuberculosis* Bacteremia in Type 1 Diabetes Mellitus: An Infectious Trigger?

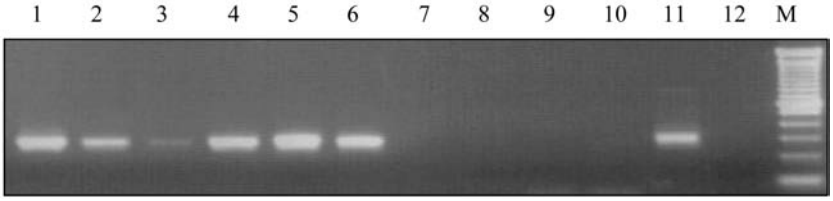

Figure 1. Representative PCR results showing amplification (or otherwise) of 298-bp genomic fragment corresponding to IS900 in diabetic patients *(lanes 1–8). Lanes 9* and *10,* Control subjects without type 1 diabetes mellitus. *Lanes 11* and *12, Mycobacterium avium* subspecies *paratuberculosis*–positive and *M. avium* subspecies *paratuberculosis*–negative control subjects, respectively. M, molecular marker (100-bp ladder).

To the Editor—*Mycobacterium avium* subspecies *paratuberculosis* (MAP) is the established cause of paratuberculosis in ruminants (i.e., Johne disease). The bacterium is shed in the milk of infected cows and survives pasteurization. Recently, an association between MAP and Crohn disease has been suggested, wherein MAP has been found to persist in a cell wall–deficient form, escaping clearance by the host immune system [1, 2].

Type 1 (insulin-dependent) diabetes mellitus (T1DM) reflects the interactions of polygenic traits with ill-defined environmental factors, and it is unknown what initiates and maintains autoimmunity to self-antigens expressed in the pancreatic islets of Langerhans [3]. Consumption of cow's milk early in life has been a recognized risk factor in the development of this disease, and environmental microorganisms are thought to trigger autoimmune responses in genetically susceptible individuals [4]. MAP bacilli have recently been hypothesized to trigger molecular mimicry and killing of pancreatic islet cells by the immune system.

We attempted to test the association of MAP with T1DM in an area of endemicity, such as Sardinia, by testing patients with T1DM for the presence of MAP bacilli in peripheral blood. A total of 96 participants, composed of 46 patients with T1DM and 50 healthy control subjects, were tested for the presence of MAP-specific IS900 signature (figure 1) using total DNA extracted from PBMCs. Informed consent from patients, as well as other necessary clearances, were procured before blood samples were obtained. PCR was performed to detect MAP DNA, as described elsewhere [2]. IS900 fragment identity was confirmed by sequencing (GenBank accession group 1517012) and by BLAST analysis against sequences in the National Center for Biotechnology Information database [5].

A total of 29 (63%) of 46 blood samples from diabetic patients were found to be positive for MAP, whereas only 8 (16%) of the 50 samples from healthy control subjects were positive for MAP. Although a majority of diabetic patients with positive PCR results had a family history of diabetes or other genetic and/or autoimmune disorders, 14 diabetic individuals with positive PCR results did not have any history of diabetes or other autoimmune diseases in their families. Although the differences in the outcome of PCR positivity were statistically validated and found to be significant ($\chi^2 = 10.07$; $P \leq .01$), it is our understanding that MAP-positive control subjects might possibly be representing either the genetic resistance or the asymptomatic forms of variable duration that generally precede the clinical presentation of T1DM.

Genetic evidence suggests that there are specific states of immune dysfunction that promote both T1DM and mycobacterial infection [6, 7]. Deficiency of vitamin D has been implicated as a risk factor for T1DM. Interestingly, vitamin D is also implicated in limiting mycobacterial infection by upregulating expression of an antimicrobial peptide [8].

The island of Sardinia has a high incidence of Crohn disease and other autoimmune diseases, such as T1DM, with a very high prevalence of MAP in Sardinian patients with Crohn disease [2, 3]. Because MAP is present in almost one-half of the sheep herds tested in Sardinia, it is probably endemically contaminating water, milk, and animal feed [9].

Finding evidence of MAP bacteremia in patients with T1DM is a novel observation that might provide an important foundation in establishing an infectious etiology for T1DM. These results also might possibly have implications for countries that have the greatest livestock populations and high incidence of MAP concurrent with the highest numbers of patients with T1DM. Although it is tempting to suggest that MAP could be a potential cause of autoimmune responses associated with T1DM in Sardinia, we believe that a large-scale study involving patients from different genetic and geographic backgrounds might further validate our findings.

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Leonardo A. Sechi,[1] Daniela Paccagnini,[1] Sara Salza,[1] Adolfo Pacifico,[1] Niyaz Ahmed,[2] and Stefania Zanetti[1]

[1]Dipartimento di Scienze Biomediche, Università di Sassari, Sassari, Italy; and [2]Pathogen Evolution Group, Centre for DNA Fingerprinting and Diagnostics, Hyderabad, India

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Reprints or correspondence: Dr. Leonardo A. Sechi, Dipartimento di Scienze Biomediche, Università di Sassari, Viale San Pietro 43b, 07100 Sassari, Italy (sechila@uniss.it).

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Untangling the Immunological Implications of Nadir on CD4+ Cell Recovery during Suppressive Highly Active Antiretroviral Therapy

To the Editor—Moore et al. [1] recently described a significant difference in $CD4^+$ cell recovery following HIV RNA suppression among patients receiving HAART according to nadir $CD4^+$ cell count, with individuals whose nadir $CD4^+$ cell counts were <200 cells/μL not achieving a protective $CD4^+$ cell count.

A lack of $CD4^+$ cell rescue despite complete HAART-driven viremia suppression represents an everyday dilemma for HIV/AIDS clinicians [2]. Invariably, $CD4^+$ cell count nadir proved to be a major and reliable determinant of suboptimal $CD4^+$ cell recovery and clinical outcome [1–8]. However, the mere quantification of $CD4^+$ cell count nadir fails to qualitatively estimate the ultimate immunological mechanism(s) hindering $CD4^+$ cell rescue. Only by detailing the immunological holes in nadir-driven T cell homeostasis will it be possible to devise the most efficacious treatment approach.

By limiting immune recovery assessment to total $CD4^+$ cell count in a large patient cohort, Moore et al. [1], although gaining statistical power, miss an insight into the immunological role of $CD4^+$ cell count nadir. On the contrary, we feel that stringently focused studies are needed that address the time course of specific immune pathways by nadir $CD4^+$ cell count strata. It would be interesting to know whether the authors have found distinct dynamics in immunephenotype and other immunologic parameters according to $CD4^+$ cell count ranges.

Given their major regulatory role in T cell homeostasis, common γ-chain cytokines, including IL-2, IL-7, IL-15, and IL-21 [9], might be ideal markers of the direction of immune recovery. Thus, although Moore et al. [1] recommend HAART initiation at a $CD4^+$ cell count >350 cells/μL for the most robust immune recovery, we advocate a broad immunologic investigation of regulatory cytokine networks to obtain an indication of pretherapy immune damage and $CD4^+$ cell rescue potential, as well as possible clinical relapses.

As an example of the exploitation of γ-chain cytokine kinetics, we would like to share our experience with the IL-7 and IL-7R system [10] in a cohort of 18 antiretroviral-naive, HIV-positive patients with advanced infection (nadir $CD4^+$ cell count, <150 cells/μL) whom we observed prospectively for the first 12 months of HAART. These patients displayed different virological and immunological responses to HAART: 12 patients had concordant responses (HIV RNA level, $\leq$50 copies/mL; $CD4^+$ cell count, $\geq$200 cells/μL), and 6 patients had discordant responses (HIV RNA level, $\leq$50 copies/mL; $CD4^+$ cell count, $\leq$200 cells/μL). Despite having a higher median baseline plasma IL-7 level than that for patients with concordant responses, patients with discordant responses had a tendency toward reduced $CD4^+$ cell count and lower IL-7Rα availability, which, by indicating free IL-7 mainly resulting from receptor downmodulation rather than compensatory production, also rules out a functional potential on T cell homeostasis. In fact, during HAART, despite a progressive reduction in IL-7, only patients with concordant responses displayed a substantial increase in $CD4^+$ cell and IL-7Rα expression. These findings, by clearly pointing to opposite IL-7 and IL-7R dynamics, also indicate that a lack of IL-7 and IL-7R regulatory control over $CD4^+$ cell homeostasis could be the basis of discordant responses, which cautions against IL-7–based treatment.

In conclusion, it is time to investigate the early integration of classic quantitative determinations of $CD4^+$ cell count nadir proposed by Moore et al. [1] with additional qualitative measures of $CD4^+$ cell count nadir–associated immune imbalances. The effort should be to highlight the immunological role of $CD4^+$ cell count nadir, not only as a quantitative reflection of T cell depletion, but mainly as evidence of more-complex T cell qualitative impairment. The intriguing hypothesis that a lack of IL-7– and IL-7R–mediated regulatory function is behind discordant responses in antiretroviral-naive patients with advanced infection clearly indicates the complexity of the immunopathogenetic mechanisms of $CD4^+$ cell immune recovery. The possibility of monitoring major T cell homeostasis regulators, such as γ-chain cytokines, by possibly disclosing upstream breakdown offers the appealing prospect of targeted immune interventions.

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