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Title page:

Treatment associated inflammatory deterioration in tuberculous meningitis: unpicking the paradox

Editorial accompanying Marais et al, 'Inflammasome activation underlies central nervous system deterioration in HIV-associated tuberculosis'

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Ever since the advent of anti-tuberculosis chemotherapy in the late 1940s, physicians have recognized that in some patients the symptoms and signs of tuberculosis worsen after the start of treatment. These clinical manifestations have been called 'paradoxical reactions', because they occur despite effective anti-microbial therapy. Their pathogenesis is poorly understood, but they are broadly considered to be caused by excessive inflammation to dead and dying bacteria [1]. Paradoxical reactions are observed to occur more frequently in those with lymph node and disseminated tuberculosis and in those co-infected with HIV [2]. However, paradoxical reactions that occur in HIV co-infected individuals recently started on antiretroviral therapy (ART) have acquired a different moniker: immune reconstitution inflammatory syndrome (IRIS) [3]. IRIS reactions are more common in individuals with very low peripheral blood CT4+ T-cell counts and high plasma HIV loads, who are started on ART shortly after anti-tuberculosis chemotherapy. Despite the fundamental importance of ART to IRIS, paradoxical reactions and IRIS share many similarities. Both are paradoxical, in that they occur in the face of effective anti-microbial (and antiretroviral) treatment; both are believed to be the result of excessive inflammation; and both cause serious clinical consequences when they involve the brain in association with tuberculous meningitis (TBM).

In this issue of the *Journal of Infectious Diseases*, Marais and colleagues describe an elegant clinical investigation of the pathogenesis of TBM-associated central nervous system (CNS) IRIS. HIV-associated TBM kills around half of all sufferers, and CNS IRIS is a major contributor to the high mortality. The underlying inflammatory mechanisms leading to TBM-associated IRIS are largely unknown, but in the current and two earlier publications Marais and colleagues offer new and important insights [4, 5]. They prospectively followed 34 HIV-1 infected South African adults treated for drug-sensitive TBM with standard anti-tuberculosis chemotherapy and adjunctive prednisone (1.5mg/kg/day). Paired blood and cerebrospinal fluid (CSF) samples were taken at presentation, after 2 weeks of anti-

tuberculosis treatment and when ART was initiated, after 2 weeks of ART, and if CNS IRIS occurred. IRIS developed in 16 subjects (47%), a median of 14 days after starting ART, with symptoms and signs of worsening headache (n=12), new onset seizures (n=4), reduced consciousness (n=6) and paraparesis (n=3). Brain imaging showed that these manifestations were associated with tuberculoma development in six subjects, infarcts in four, and hydrocephalus in two. These findings are well-described complications of TBM in HIV-infected and uninfected individuals and can occur before or during anti-tuberculosis treatment.

Marais et al. conducted careful longitudinal analyses of paired blood and CSF samples, describing the relationships between the development of IRIS and CSF leucocyte numbers and types, the concentrations of an array (>30) of blood and CSF inflammatory mediators, and, in their current article, blood transcriptional profiles. They showed that TBM-associated CNS IRIS has an inflammatory signature characterized by neutrophil and inflammasome-mediated pro-inflammatory responses. There were two particularly surprising and important observations. First, despite their and many others' observations that TBM-associated inflammation is highly compartmentalized to the brain, the inflammatory signatures of neurological IRIS were clearly detected by transcriptional profiling of the blood. Second, neurological IRIS was associated with marked neutrophil-dependent inflammatory activation (but not inflammasome activation) that was detectable before the start of anti-tuberculosis treatment and many weeks before the development of IRIS in both blood and CSF. These findings are important because they may help clinicians predict who will develop IRIS, but they also suggest early inflammatory responses to *Mycobacterium tuberculosis* infection, independent of those driven by anti-tuberculosis and anti-retroviral treatment, may determine much later treatment outcomes.

The observed link between neutrophil-dependent inflammation and TBM-associated IRIS is intriguing. The role of neutrophils in the pathogenesis of tuberculosis is controversial [6, 7]. Animal models of tuberculosis suggest neutrophils are protective against early infection [8], but may exacerbate pathology in established disease [7, 9]. Data from humans are limited. Higher blood neutrophil counts at tuberculosis diagnosis have been associated with reduced risk of death in tuberculosis infection [10] and slower sputum conversion to negative during therapy [11], and a neutrophil-driven, interferon (IFN)-inducible transcript signature in human whole blood has been correlated with the clinical severity of lung tuberculosis [12]. Some of the most interesting data, however, come from clinical studies of TBM.

TBM is a relatively rare form of tuberculosis but its severity, combined with an ability to characterize its inflammatory pathology and treatment response by serial CSF sampling and brain imaging, havemade it a fruitful model for studying tuberculosis pathogenesis. It has long been recognized that the absolute numbers of CSF neutrophils, and their proportions relative to other leucocytes, can vary substantially before and after starting anti-tuberculosis treatment [13]. In HIV-uninfected adults with TBM, paradoxical reactions and, in particular, the development of brain tuberculoma, are associated with increased CSF neutrophil numbers [14-16]. And in both HIV-infected and uninfected individuals higher numbers of CSF neutrophils are associated with an increased likelihood of culturing *M. tuberculosis* from the CSF [17, 18].

This last finding is especially pertinent given the observations of Marais and colleagues. *M. tuberculosis* load is hard to measure accurately from clinical specimens, especially in CSF where bacterial numbers are usually very low. But the ability to culture bacteria, and the time-to-positivity of the culture, can provide an indication of likely bacterial numbers. In those who developed IRIS, *M. tuberculosis* was cultured from CSF taken before and after 2 weeks of anti-tuberculosis treatment in 94% and 44% of patients respectively, compared to

35% and 0% in those without IRIS. Drug resistance did not explain these differences. When these observations are seen alongside those made in HIV-uninfected patients with TBM, the similarities are striking. Post-treatment inflammatory reactions, in both patient populations, appear linked to neutrophil-mediated inflammation, high bacterial loads and, possibly, slow bacterial killing in the CSF.

Where do these new data leave clinicians and researchers? The inflammatory 'signature' of TBM-associated CNS IRIS appears to be present long before it actually develops, indicating the potential for clinical risk stratification. Marais et al. found that IRIS was associated with an array of increased CSF inflammatory mediators before the start of anti-tuberculosis treatment, including pro-inflammatory cytokines, chemokines, matrix metalloproteinases (MMPs), and various neutrophil-associated mediators [4, 5]. The best predictors of IRIS, however, appeared to be absolute CSF neutrophil counts, IFN-γ and TNF-α concentrations before the start of anti-tuberculosis treatment, and CSF neutrophil counts and S100A8/A9 concentrations after two weeks of ART. S100A8/A9 (or calprotectin) is a heterodimer of two calcium-binding proteins S100A8 and S100A9, which promotes recruitment of neutrophils and monocytes by inducing production of proinflammatory cytokines and chemokines [19, 20]. S100A8/A9-producing neutrophils have been found within inflammatory lung granulomas of patients with active tuberculosis, and serum S100A8/A9 concentrations positively correlate with neutrophil numbers in peripheral blood and lung pathology [21]. Whether S100A8/A9 concentrations can be used as a potential biomarker of inflammation, disease severity, and IRIS/paradoxical reaction risk needs further investigation.

A pre-treatment inflammatory signature that predicts subsequent IRIS also introduces the possibility that human genetic variants may predispose some patients to IRIS-associated hyper-inflammatory responses to *M. tuberculosis* infection. If true, it may enable additional risk stratification. A recent investigation from India reported that the severity of IRIS in all

forms of tuberculosis was associated with polymorphisms in the gene encoding Leukotriene A4 hydrolase (LTA4H) [16]. The biological plausibility of this observation is supported by a previous study in which *LTA4H* variants were linked to pre-treatment CSF inflammatory phenotype, dexamethasone responsiveness, and survival in HIV-uninfected Vietnamese adults with TBM [22].

Finally, it must be hoped that greater understanding of the inflammatory mechanisms underpinning IRIS and other paradoxical reactions will lead to improved prevention and treatment. As Marais and colleagues discovered, adjunctive corticosteroids did not appear to prevent IRIS in their cohort (at the doses given), although others have shown they quicken symptom resolution in the treatment of non-CNS tuberculosis-associated IRIS [23]. Alternative agents with the potential to target neutrophil-mediated inflammation may be more effective than corticosteroids. Animal models of tuberculosis have suggested a number of widely available drugs, approved for other indications, that could be re-purposed as neutrophil-directed therapies. For example, roflumilast is a type 4 phosphodiesterase inhibitor approved to treat chronic obstructive pulmonary disease, but in a murine tuberculosis model it blocked neutrophil recruitment, reduced pro-inflammatory cytokines production and, in combination with isoniazid, resulted in a 0.5-log10 reduction in lung bacillary load compared to isoniazid monotherapy [24]. Doxycycline is an approved broad-spectrum antibiotic and MMP inhibitor that, given the strong links between CSF MMP-9 concentrations and IRIS and survival from TBM [25, 26], may also have a role in treatment [27]. Lastly, the non-steroidal anti-inflammatory drug ibuprofen was found to ameliorate neutrophil mediated inflammation and resulted in a one-log reduction in pulmonary mycobacterial load and increased survival of mice infected with *M. tuberculosis* [28]. All these agents have the potential to move rapidly into clinical trials in humans and highlight the value of meticulous clinical studies of the type exemplified by Marias and colleagues. Unpicking the paradox of treatment-

associated inflammatory deterioration in TBM promises to lead to its better prevention and treatment.

References:

1. Bell LC, Breen R, Miller RF, Noursadeghi M, Lipman M. Paradoxical reactions and immune reconstitution inflammatory syndrome in tuberculosis. International journal of infectious diseases. **2015**; 32:39-45.

2. Brown CS, Smith CJ, Breen RA, et al. Determinants of treatment-related paradoxical reactions during anti-tuberculosis therapy: a case control study. BMC infectious diseases **2016**; 16:479.

3. Lai RP, Meintjes G, Wilkinson RJ. HIV-1 tuberculosis-associated immune reconstitution inflammatory syndrome. Seminars in immunopathology **2016**; 38:185-98.

4. Marais S, Meintjes G, Pepper DJ, et al. Frequency, severity, and prediction of tuberculous meningitis immune reconstitution inflammatory syndrome. Clinical infectious diseases. **2013**; 56:450-60.

5. Marais S, Wilkinson KA, Lesosky M, et al. Neutrophil-associated central nervous system inflammation in tuberculous meningitis immune reconstitution inflammatory syndrome. Clinical infectious diseases. **2014**; 59:1638-47.

6. Dallenga T, Schaible UE. Neutrophils in tuberculosis--first line of defence or booster of disease and targets for host-directed therapy? Pathogens and disease **2016**; 74.

7. Lowe DM, Redford PS, Wilkinson RJ, O'Garra A, Martineau AR. Neutrophils in tuberculosis: friend or foe? Trends in immunology **2012**; 33:14-25.

8. Sugawara I, Udagawa T, Yamada H. Rat neutrophils prevent the development of tuberculosis. Infection and immunity **2004**; 72:1804-6.

9. Nandi B, Behar SM. Regulation of neutrophils by interferon-gamma limits lung inflammation during tuberculosis infection. The Journal of experimental medicine **2011**; 208:2251-62.

10. Lowe DM, Bandara AK, Packe GE, et al. Neutrophilia independently predicts death in tuberculosis. The European respiratory journal **2013**; 42:1752-7.

11. Brahmbhatt S, Black GF, Carroll NM, et al. Immune markers measured before treatment predict outcome of intensive phase tuberculosis therapy. Clinical and experimental immunology **2006**; 146:243-52.

12. Berry MP, Graham CM, McNab FW, et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature **2010**; 466:973-7.

13. Teoh R, O'Mahony G, Yeung VT. Polymorphonuclear pleocytosis in the cerebrospinal fluid during chemotherapy for tuberculous meningitis. Journal of neurology **1986**; 233:237-41.

14. Thwaites GE, Macmullen-Price J, Tran TH, et al. Serial MRI to determine the effect of dexamethasone on the cerebral pathology of tuberculous meningitis: an observational study. The Lancet Neurology **2007**; 6:230-6.

15. Garg RK, Malhotra HS, Kumar N. Paradoxical reaction in HIV negative tuberculous meningitis. Journal of the neurological sciences **2014**; 340:26-36.

16. Singh AK, Malhotra HS, Garg RK, et al. Paradoxical reaction in tuberculous meningitis: presentation, predictors and impact on prognosis. BMC infectious diseases **2016**; 16:306.

17. Thwaites GE, Chau TT, Farrar JJ. Improving the bacteriological diagnosis of tuberculous meningitis. Journal of clinical microbiology **2004**; 42:378-9.

18. Puccioni-Sohler M, Brandao CO. Factors associated to the positive cerebrospinal fluid culture in the tuberculous meningitis. Arquivos de neuro-psiquiatria **2007**; 65:48-53.

19. Gebhardt C, Nemeth J, Angel P, Hess J. S100A8 and S100A9 in inflammation and cancer. Biochemical pharmacology **2006**; 72:1622-31.

20. Leanderson T, Liberg D, Ivars F. S100A9 as a Pharmacological Target Molecule in Inflammation and Cancer. Endocrine, metabolic & immune disorders drug targets **2015**; 15:97-104.

21. Gopal R, Monin L, Torres D, et al. S100A8/A9 proteins mediate neutrophilic inflammation and lung pathology during tuberculosis. American journal of respiratory and critical care medicine **2013**; 188:1137-46.

22. Tobin DM, Roca FJ, Oh SF, et al. Host genotype-specific therapies can optimize the inflammatory response to mycobacterial infections. Cell **2012**; 148:434-46.

23. Meintjes G, Wilkinson RJ, Morroni C, et al. Randomized placebo-controlled trial of prednisone for paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome. Aids **2010**; 24:2381-90.

24. Maiga MC, Ahidjo BA, Maiga M, Bishai WR. Roflumilast, a Type 4 Phosphodiesterase Inhibitor, Shows Promising Adjunctive, Host-Directed Therapeutic Activity in a Mouse Model of Tuberculosis. Antimicrobial agents and chemotherapy **2015**; 59:7888-90.

25. Green JA, Tran CT, Farrar JJ, et al. Dexamethasone, cerebrospinal fluid matrix metalloproteinase concentrations and clinical outcomes in tuberculous meningitis. PloS one **2009**; 4:e7277.

26. Price NM, Farrar J, Tran TT, Nguyen TH, Tran TH, Friedland JS. Identification of a matrix-degrading phenotype in human tuberculosis in vitro and in vivo. Journal of immunology **2001**; 166:4223-30.

27. Ong CW, Elkington PT, Brilha S, et al. Neutrophil-Derived MMP-8 Drives AMPK-Dependent Matrix Destruction in Human Pulmonary Tuberculosis. PLoS pathogens **2015**; 11:e1004917.

28. Vilaplana C, Marzo E, Tapia G, Diaz J, Garcia V, Cardona PJ. Ibuprofen therapy resulted in significantly decreased tissue bacillary loads and increased survival in a new murine experimental model of active tuberculosis. The Journal of infectious diseases **2013**; 208:199-202.