Editorial

Host directed therapies (HDTs) and immune response signatures: insights into a role for interleukin-32

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The paradigm of *tuberculosis* (TB)

The clinical manifestations of recurrent episodes of TB reflect the complex interaction between *Mycobacterium tuberculosis* (*M.tb*) and the host immune response (1). There are an estimated 2 billion people in the world who have latent *M.tb* infection (LTBI) with no symptoms and signs of ill health. Co-evolution between *M.tb* and humans over centuries has established a delicate, balanced liaison between the host and pathogen (2). The clinical outcome of the encounter with *M.tb* in immune-competent individuals is apparently a success story: more than 90% of individuals exposed to *M.tb* (3) do not develop clinical TB. Therefore, TB provides a highly relevant paradigm of how optimally-activated and balanced host response networks tailor the outcome of an infection, which leads either to latent LTBI or to a life-threatening disease (4). Despite some advances in defining clinically relevant markers of immune protection against *M.tb* (5), the identification of host factors which enable protection to clinical TB remain elusive. Increasing evidence shows that biomarkers present in individuals with latent LTBI may represent components that orchestrate complex host anti-*M.tb* immune response networks and ultimately determine protection from expression of clinical TB disease. Contrary to this, *M.tb* infection can lead to intense immune and inflammatory responses which result in severe pulmonary pathology, eventually resulting in death. The paradigm that limiting overt inflammation in infectious diseases may result in benefit to the patient has been shown in settings of several infectious diseases, for example, the use of cytotoxic drugs such as etoposide and cyclosporine A, or limiting excessive inflammation and removing regulatory T cells (Tregs) in severe avian influenza A (H5N1) infection by (6), and use corticosteroids for the treatment of community—acquired pneumonia (7). The strength of inflammation is not only determined by the pathogen type [e.g., infection with the *M.tb* Beijing lineage that results in stronger pro-inflammatory responses (8)], but also by the host genetic background e.g., determined by SNPs associated with arachidonic acid metabolism, and pro-inflammatory cytokine receptors (8).

IL-32 as a key modulator of host-protective responses to *M.tb*

The degree of virulence of the infecting *M.tb* strain(s) is also shaped by environmental and socio-economic factors, geographical localisation and disease endemicity (9). At the cellular level, a fine-tuned interplay between the sentinel cells which first respond to primary *M.tb* infection and tissue-infiltrating lymphocytes is likely to determine the quality and efficacy of anti-*M.tb* immune response. The role of innate immune responses, including NK-T-cells (10), TCRγδ T-cells (11), innate lymphoid cells (12), MAIT cells (13) and neutrophils (14) to *M.tb* are not yet sufficiently defined to date. *M.tb* is not the only pathogen triggering these immune cells; other bacterial, viral or fungal pathogens along with physiological microflora (the 'microbiome') (15) trigger

inflammatory responses in healthy individuals and shape the host's encounter with pathogens.

Biosignatures of host-derived soluble factors are differentially-regulated in healthy, individuals harbouring LTBI, compared to those with active clinical TB disease, indicating that certain immune signalling molecules may confer protection against clinical disease (16). In support of this is Montoya and colleagues' findings that interleukin (IL)-32 may represent a biomarker for protection to TB (17). Although initially discovered as a factor facilitating tumour necrosis factor alpha (TNF-α) production (18), IL-32, in the context of human TB, can be regarded as an integral component of IL-15-induced signalling pathways. IL-15 and IL-32 together contribute to the host response network regulating progression of primary *M.tb* infection to full-blown clinical disease. The combination of IL-15- and IL-32-triggered effects result in increased gene expression involved in major histocompatibility complex class I (MHC-I)-dependent antigen processing, presentation, chemotaxis and lipid metabolism. This is exciting, since the quality and quantity of MHC class I-restricted targets may shape the adaptive ($CD8^+$) T-cell response, as reflected in detection of MHC class I and *M.tb*-epitope specific T cells in peripheral blood from patients with TB (19). IL-32 may also act on lipid metabolism in cells, a phenomenon which is currently insufficiently appreciated. For example, differential lipid metabolism i.e., inhibition of farnesyl pyrophosphate metabolism makes "stressed" cells (tumor or *M.tb*-infected) more "visible" to innate immune responses, particularly TCRγδ T-cells (20); and differential lipid cell membrane compositions affect T-cell memory formation and immune effector functions (21).

IL-15 has already been shown to be crucial for NK-cell maintenance in the periphery via induction of *Bcl*-2 expression (22). Exposure of hantavirus-infected endothelial cells expressing HLA-E to NK cells in the presence of IL-15 resulted in improved cytotoxic killing of target cells (23), suggesting immune-stimulatory properties of IL-15 against intracellular pathogens. Furthermore, productive RSV infection of lung epithelial cells was shown to augment IL-15 production along with cell surface expression of MICA, an MHC class I chain-related protein (24). However, the effects of IL-15/IL-32 on other molecules involved in triggering innate immune responses such as HLA-G (inhibitory for NK cells, dendritic cells, $CD8^+$ T-cells; activation of Tregs) (25) or CD1a-d (for antigen recognition by invariant NK T cells, MAIT cells) (26,27) remain to be determined.

IL-32 appears to involve vitamin D and interferon gamma (IFN-γ)-mediated, interdependent pathways, in host protective response against *M.tb*, as reflected by increased gene expression of the vitamin D receptor (*CYP27b1*), antimicrobial peptides (*CAMP*, *DEFB4*) and complement proteins (*C3*). The IL-15/IL-32 axis may not only affect innate immune responses, but also set the stage for adaptive immune responses, including the direct interaction with immune effector cells: IL-15, unlike IL-2, preferentially activates and expands antigen-specific T cells with a central memory phenotype (28) and provides long-term survival of T cells due to reduced TRAIL-mediated apoptosis (29). IL-15 protects NKT cells from adverse, immune-suppressive effects associated with macrophages as shown in preclinical models (30). Furthermore, the recruitment of antigen-presenting cells and enhanced expression of genes such as granzyme H (*GZMH*) and perforin 1 (*PRF1*), which encode effector molecules involved in cytolysis of target cells support the notion that IL-15/IL-32 favourably impact on anti-*M.tb* $CD8^+$ T-cell responses. Humoral immune responses in TB have recently generated renewed interest (31); and upregulation of CXCL13 expression raises the possibility that IL-32 may also trigger humoral immune responses. CXCL13 is a potent chemoattractant involved in B-cell infiltration into tissues, *in situ* activation and natural antibody production, which has been shown to promote clearance of bacterial pathogens (e.g., *Streptococcus* species) in the peritoneal cavity of mice (32).

Interestingly, patients with active TB who had undergone 6 months of standard anti-TB treatment exhibited a similar pattern of IL-32 gene expression to those who had LTBI, indicating that IL-15-mediated host response networks contribute to successful *M.tb* control. This was consolidated by messenger RNA (mRNA) analysis from whole blood of individuals from geographically-distinct locations, showing that IL-32 is highly expressed among individuals with LTBI, strengthening the notion that IL-32 is biologically and clinically relevant in protective anti-*M.tb* responses.

Learning from IL-32: other host-derived markers as stimuli for anti-*M.tb* response networks

The discovery of the role of IL-32 in orchestrating host defence in TB is yet another piece of evidence that multiple cytokine networks are required for sustained control of *M.tb* infection, including the concerted action of cytokine-mediated signalling pathways [(i.e., IFN-γ (33), IL-1β (34), type 1 IFNs (34,35), TNF-α (36)] as well as acute response

mechanisms. Pattern recognition receptors (PRRs) such as TLR1/2 (37) and the newly-redefined aryl hydrocarbon receptor (38) eliciting targeted anti-*M.tb* immune responses, including vitamin D-mediated antimicrobial activity, pro-inflammatory cytokine production and T-cell activation point in the same direction: innate and adaptive immune responses are required for *M.tb* control.

Host-directed therapy for improved clinical management of TB

In individuals who progress to developing active clinical TB diseases, several biological host checkpoints may have been perturbed or sub-optimally activated. These include the expression of PD-1 on the surface of T cells (39), activation of Tregs (40) and removal of certain effector T-cell subsets via neutralization with soluble anti-TNF receptor (41). Thus appropriate stimulation of these pathways may achieve positive regulation of inflammatory responses that could be beneficial in alleviating TB-related immunopathology (42,43). Interventional therapeutic strategies using drugs approved by the United States Food and Drug Administration (FDA) for non-TB indications is currently the focus of their use as adjunctive therapy with anti-TB treatment for improving patient survival in multi-drug resistant TB. It was recently shown that activation of the IL-1β pathway with zileuton (5-lipooxygenase inhibitor used for treating asthma) during TB pathogenesis triggers expression of cyclooxygenase 2 (COX-2) which leads to prostaglandin E2 (PGE2) release (34). This helps to ameliorate excessive type 1 IFN-driven hyper-inflammation in the lung, restoring organ function while reducing *M.tb* burden. The antidiabetic drug metformin (which activates AMP-activated protein kinase) was shown to kill intracellular *M.tb* in monocytes and macrophages via induction of mitochondrial reactive oxygen species (44). Furthermore, metformin dampened the inflammatory response milieu in lungs of treated *M.tb*-infected mice while enhancing the efficacy of anti-TB drugs. Cellular therapy using infusions of autologous bone-marrow derived mesenchymal stromal cells into patients with drug-resistant TB is another avenue being pursued to treat *M.tb*-induced severe pulmonary inflammation (45), and regenerating anti-*M.tb* immune responses.

Interference with the MHC class I antigen presentation pathway, such as described for IL-15/32 (17), represents a viable target to favourably modulate anti-*M.tb* immune responses. Repurposed drugs, such as the histone deacetylase inhibitors valproic acid (VPA) and vorinostat, both licensed for neurological indications, unveiled highly encouraging observations in the context of human immunodeficiency virus (HIV) infection. Both drugs perpetrated reactivation of latent viral reservoirs in CD4 T cells and increased virus susceptibility to immune attack as well as anti-retroviral treatment, subsequently warranting their first large-scale clinical trial in HIV-infected individuals (46). VPA also induces the upregulation of MHC class I antigen presentation (47), which may facilitate tailored $CD8^+$ T cell activity against *M.tb*-infected cells.

As the role and function of IL-32 is unravelled, it may turn out to play a critical role in balancing anti-*M.tb* responses, and limiting off-target collateral tissue damage consequential to abberant anti-*M.tb* cellular and humoral responses. IL-32 may also embody the 'missing link' between innate and adaptive immune responses that either tips the balance between damaging or protective immune responses. IL-32 could turn out to be an important clinically relevant marker for protective immunity, and/or may serve as a viable target for host-directed therapy, thus providing a promising therapeutic target in precision medicine in infectious diseases, particularly in TB.

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References

1. Gomes MG, Aguas R, Lopes JS, et al. How host heterogeneity governs tuberculosis reinfection? Proc Biol Sci 2012;279:2473-8.
2. Casali N, Nikolayevskyy V, Balabanova Y, et al. Evolution and transmission of drug-resistant tuberculosis in a Russian population. Nat Genet 2014;46:279-86.
3. WHO. Global tuberculosis report 2014. World Health Organization, Geneva, 2014. Accessed on Nov 12, 2014. Available online: http://www.who.int/tb/publications/global_report/en/
4. Zumla A, Atun R, Maeurer M, et al. Viewpoint: Scientific dogmas, paradoxes and mysteries of latent Mycobacterium tuberculosis infection. Trop Med Int Health 2011;16:79-83.
5. Wallis RS, Kim P, Cole S, et al. Tuberculosis biomarkers discovery: developments, needs, and challenges. Lancet Infect Dis 2013;13:362-72.
6. Henter JI, Chow CB, Leung CW,Cytotoxic therapy for severe avian influenza A (H5N1) infection. Lancet

2006;367:870-3.
7. Blum CA, Nigro N, Briel M, et al. Adjunct prednisone therapy for patients with community-acquired pneumonia: a multicentre, double-blind, randomised, placebo-controlled trial. Lancet 2015. [Epub ahead of print].
8. Parwati I, van Crevel R, van Soolingen D. Possible underlying mechanisms for successful emergence of the Mycobacterium tuberculosis Beijing genotype strains. Lancet Infect Dis 2010;10:103-11.
9. Narasimhan P, Wood J, Macintyre CR, et al. Risk factors for tuberculosis. Pulm Med 2013;2013:828939.
10. Kee SJ, Kwon YS, Park YW, et al. Dysfunction of natural killer T cells in patients with active Mycobacterium tuberculosis infection. Infect Immun 2012;80:2100-8.
11. Boom WH. Gammadelta T cells and Mycobacterium tuberculosis. Microbes Infect 1999;1:187-95.
12. Spits H, Cupedo T. Innate lymphoid cells: emerging insights in development, lineage relationships, and function. Annu Rev Immunol 2012;30:647-75.
13. Gold MC, Cerri S, Smyk-Pearson S, et al. Human mucosal associated invariant T cells detect bacterially infected cells. PLoS Biol 2010;8:e1000407.
14. Morris D, Nguyen T, Kim J, et al. An elucidation of neutrophil functions against Mycobacterium tuberculosis infection. Clin Dev Immunol 2013;2013:959650.
15. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 2009;9:313-23.
16. Maertzdorf J, Repsilber D, Parida SK, et al. Human gene expression profiles of susceptibility and resistance in tuberculosis. Genes Immun 2011;12:15-22.
17. Montoya D, Inkeles MS, Liu PT, et al. IL-32 is a molecular marker of a host defense network in human tuberculosis. Sci Transl Med 2014;6:250ra114.
18. Kim SH, Han SY, Azam T, et al. Interleukin-32: a cytokine and inducer of TNFalpha. Immunity 2005;22:131-42.
19. Axelsson-Robertson R, Loxton AG, Walzl G, et al. A broad profile of co-dominant epitopes shapes the peripheral Mycobacterium tuberculosis specific CD8+ T-cell immune response in South African patients with active tuberculosis. PLoS One 2013;8:e58309.
20. Gu S, Nawrocka W, Adams EJ. Sensing of Pyrophosphate Metabolites by Vγ9Vδ2 T Cells. Front Immunol 2015;5:688.
21. O'Sullivan D, van der Windt GJ, Huang SC, et al. Memory CD8(+) T cells use cell-intrinsic lipolysis to support the metabolic programming necessary for development. Immunity 2014;41:75-88.
22. Ranson T, Vosshenrich CA, Corcuff E, et al. IL-15 is an essential mediator of peripheral NK-cell homeostasis. Blood 2003;101:4887-93.
23. Björkström NK, Lindgren T, Stoltz M, et al. Rapid expansion and long-term persistence of elevated NK cell numbers in humans infected with hantavirus. J Exp Med 2011;208:13-21.
24. Zdrenghea MT, Telcian AG, Laza-Stanca V, et al. RSV infection modulates IL-15 production and MICA levels in respiratory epithelial cells. Eur Respir J 2012;39:712-20.
25. Curigliano G, Criscitiello C, Gelao L, et al. Molecular pathways: human leukocyte antigen G (HLA-G). Clin Cancer Res 2013;19:5564-71.
26. Cohen NR, Garg S, Brenner MB. Antigen Presentation by CD1 Lipids, T Cells, and NKT Cells in Microbial Immunity. Adv Immunol 2009;102:1-94.
27. Van Rhijn I, Kasmar A, de Jong A, et al. A conserved human T cell population targets mycobacterial antigens presented by CD1b. Nat Immunol 2013;14:706-13.
28. Klebanoff CA, Gattinoni L, Torabi-Parizi P, et al. Central memory self/tumor-reactive CD8+ T cells confer superior antitumor immunity compared with effector memory T cells. Proc Natl Acad Sci U S A 2005;102:9571-6.
29. Oh S, Perera LP, Terabe M, et al. IL-15 as a mediator of CD4+ help for CD8+ T cell longevity and avoidance of TRAIL-mediated apoptosis. Proc Natl Acad Sci U S A 2008;105:5201-6.
30. Liu D, Song L, Wei J, et al. IL-15 protects NKT cells from inhibition by tumor-associated macrophages and enhances antimetastatic activity. J Clin Invest 2012;122:2221-33.
31. Chan J, Mehta S, Bharrhan S, et al. The role of B cells and humoral immunity in Mycobacterium tuberculosis infection. Semin Immunol 2014;26:588-600.
32. Ansel KM, Harris RB, Cyster JG. CXCL13 is required for B1 cell homing, natural antibody production, and body cavity immunity. Immunity 2002;16:67-76.
33. Berry MP, Graham CM, McNab FW, et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature 2010;466:973-7.
34. Mayer-Barber KD, Andrade BB, Oland SD, et al. Host-directed therapy of tuberculosis based on interleukin-1 and type I interferon crosstalk. Nature 2014;511:99-103.
35. Dorhoi A, Yeremeev V, Nouailles G, et al. Type I IFN signaling triggers immunopathology in tuberculosis-susceptible mice by modulating lung phagocyte dynamics. Eur J Immunol 2014;44:2380-93.
36. Roca FJ, Ramakrishnan L.TNF dually mediates resistance and susceptibility to mycobacteria via mitochondrial

reactive oxygen species. Cell 2013;153:521-34.
37. Yu X, Zeng J, Xie J. Navigating through the maze of TLR2 mediated signaling network for better mycobacterium infection control. Biochimie 2014;102:1-8.
38. Moura-Alves P, Faé K, Houthuys E, et al. AhR sensing of bacterial pigments regulates antibacterial defence. Nature 2014;512:387-92.
39. Pagán AJ, Ramakrishnan L. Immunity and Immunopathology in the Tuberculous Granuloma. Cold Spring Harb Perspect Med 2014. [Epub ahead of print].
40. Lim HJ, Park JS, Cho YJ, et al. CD4(+)FoxP3(+) T regulatory cells in drug-susceptible and multidrug-resistant tuberculosis. Tuberculosis (Edinb) 2013;93:523-8.
41. Cantini F, Nannini C, Niccoli L, et al. Guidance for the management of patients with latent tuberculosis infection requiring biologic therapy in rheumatology and dermatology clinical practice. Autoimmun Rev 2015. [Epub ahead of print].
42. Kaufmann SH, Lange C, Rao M, et al. Progress in tuberculosis vaccine development and host-directed therapies--a state of the art review. Lancet Respir Med 2014;2:301-20.
43. Zumla A, Rao M, Parida SK, et al. Inflammation and tuberculosis: host-directed therapies. J Intern Med 2015;277:373-87.
44. Singhal A, Jie L, Kumar P, et al. Metformin as adjunct antituberculosis therapy. Sci Transl Med 2014;6:263ra159.
45. Skrahin A, Ahmed RK, Ferrara G, et al. Autologous mesenchymal stromal cell infusion as adjunct treatment in patients with multidrug and extensively drug-resistant tuberculosis: an open-label phase 1 safety trial. Lancet Respir Med 2014;2:108-22.
46. Rasmussen TA, Tolstrup M, Winckelmann A, et al. Eliminating the latent HIV reservoir by reactivation strategies: advancing to clinical trials. Hum Vaccin Immunother 2013;9:790-9.
47. Khan AN, Gregorie CJ, Tomasi TB. Histone deacetylase inhibitors induce TAP, LMP, Tapasin genes and MHC class I antigen presentation by melanoma cells. Cancer Immunol Immunother 2008;57:647-54.

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