identify aberrancy of coagulation in patients with bleeding or thrombotic events on ECMO. Northrop and colleagues demonstrated that ECMO patients experienced fewer bleeding events, less exposure to blood products, and increased circuit life after implementation of a comprehensive anticoagulation laboratory protocol that included thromboelastography, stratified in accordance with patient stability and heparin dose (11).

This excellent prospective study by Dalton and colleagues adds to our understanding of the frequency and timing of bleeding and thrombotic complications and their associations with clinically important outcomes from ECMO in pediatric patients. With the establishment of a study group, the stage is now set for investigating whether bleeding and thrombotic-associated outcomes are alterable with standardized anticoagulation and patient care practices. Future studies must address the innumerable challenges around coagulation, anticoagulation, and blood–circuit interface in a diverse and increasingly complex population of ECMO patients (12). ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Shannon M. Buckvold, M.D.
John P. Kinsella, M.D.
University of Colorado School of Medicine
Children's Hospital Colorado
Aurora, Colorado

References

1. Paden ML, Conrad SA, Rycus PT, Thiagarajan RR; ELSO Registry. Extracorporeal Life Support Organization Registry Report 2012. *ASAIO J* 2013;59:202–210.
2. Zangrillo A, Landoni G, Biondi-Zoccai G, Greco M, Greco T, Frati G, Patroniti N, Antonelli M, Pesenti A, Pappalardo F. A meta-analysis of complications and mortality of extracorporeal membrane oxygenation. *Crit Care Resusc* 2013;15:172–178.
3. Sy E, Sklar MC, Lequier L, Fan E, Kanji HD. Anticoagulation practices and the prevalence of major bleeding, thromboembolic events, and mortality in venoarterial extracorporeal membrane oxygenation: A systematic review and meta-analysis. *J Crit Care* 2017;39:87–96.
4. Dalton HJ, Reeder R, Garcia-Filion P, Holubkov R, Berg RA, Zuppa A, Moler FW, Shanley T, Pollack MM, Newth C, et al.; Eunice Kennedy Shriver National Institute of Child Health and Human Development Collaborative Pediatric Critical Care Research Network. Factors associated with bleeding and thrombosis in children receiving extracorporeal membrane oxygenation. *Am J Respir Crit Care Med* 2017;196:762–771.
5. Monagle P, Chalmers E, Chan A, et al. Antithrombotic therapy in neonates and children: American College of Chest Physicians evidence-based clinical practice guidelines, 8th ed. *Chest* 2008;133:887S–968S.
6. Ignjatovic V, Mertyn E, Monagle P. The coagulation system in children: developmental and pathophysiological considerations. *Semin Thromb Hemost* 2011;37:723–729.
7. Male C, Johnston M, Sparling C, Brooker L, Andrew M, Massicotte P. The influence of developmental haemostasis on the laboratory diagnosis and management of haemostatic disorders during infancy and childhood. *Clin Lab Med* 1999;19:39–69.
8. Lequier L, Annich G, Al-Ibrahim O, et al. ELSO anticoagulation guideline [accessed 2017 Apr 9]. Available from: <https://www.else.org/portals/0/files/elsoanticoagulationguideline8-2014-table-contents.pdf>
9. Bembea MM, Schwartz JM, Shah N, Colantuoni E, Lehmann CU, Kickler T, Pronovost P, Strouse JJ. Anticoagulation monitoring during pediatric extracorporeal membrane oxygenation. *ASAIO J* 2013;59:63–68.
10. Bembea MM, Annich G, Rycus P, Oldenburg G, Berkowitz I, Pronovost P. Variability in anticoagulation management of patients on extracorporeal membrane oxygenation: an international survey. *Pediatr Crit Care Med* 2013;14:e77–e84.
11. Northrop MS, Sidonio RF, Phillips SE, Smith AH, Daphne HC, Pietsch JB, Bridges BC. The use of an extracorporeal membrane oxygenation anticoagulation laboratory protocol is associated with decreased blood product use, decreased hemorrhagic complications, and increased circuit life. *Pediatr Crit Care Med* 2015;16:66–74.
12. Zabrocki LA, Brogan TV, Statler KD, Poss WB, Rollins MD, Bratton SL. Extracorporeal membrane oxygenation for pediatric respiratory failure: Survival and predictors of mortality. *Crit Care Med* 2011;39:364–370.

Copyright © 2017 by the American Thoracic Society

An Expanding Role for Environmental Microbes in Shaping the Immune Response to Infection with *Mycobacterium tuberculosis*

In this issue of the *Journal* (pp. 772–781) (1), Scriba and colleagues have evaluated immunologic responsiveness to each peptide contained within a pool of peptides previously characterized on the basis of CD4⁺ T-cell recognition from humans infected with *Mycobacterium tuberculosis* (Mtb) (2, 3). The authors sought to evaluate the immune response in those previously treated for tuberculosis (TB) (PostTB), or those with latent Mtb infection who have not been diagnosed with TB. As those with a history of prior TB are at increased risk for reinfection and subsequent progression (4), the authors postulated that TB would be associated with an altered or diminished CD4 response, which in turn might be needed for protection following rechallenge with Mtb. Responsiveness was evaluated either through IFN- γ enzyme-linked immunospot assay, or

using flow cytometry. In either case, the overall response to the epitope pool was diminished in the PostTB cohort.

The authors then compared the PostTB cohort to those with active TB, and found that the cumulative response in those with PostTB was diminished compared with those with TB. The authors then sought to further dissect the diminished responsiveness in the PostTB cohort. Those epitopes associated with a greater than twofold decrease in immune reactivity in the PostTB cohort were categorized as “Type 2” epitopes. Rather unexpectedly, these Type 2 epitopes accounted for most of the diminished aggregate response. Furthermore, these diminished responses were most apparent in subjects treated “recently,” as defined by those who received therapy within the prior six years.

Following extensive exploration of alternate mechanistic explanations such as the binding of peptides to HLA-II and the function and phenotype of the responding cells, the authors turned their attention to the potential of the Type 2 epitopes to be shared with other

Originally Published in Press as DOI: 10.1164/rccm.201708-1599ED on August 25, 2017

bacteria, particularly environmental nontuberculous mycobacteria (NTM). While the Type 2 epitopes were found to be more similar to those found in NTM, they were unexpectedly found to have a high degree of similarity to those found in the microbiome. Consequently, authors postulated that therapy for TB could result in alterations in the microbiome, which in turn resulted in the selective loss of T cells reactive or cross-reactive to these epitopes.

At its core, the immune system must distinguish self from non-self to allow appropriate recognition of foreign antigens, while preventing unwanted autoimmunity. The recognition of protein antigens poses a particular challenge, as bacterial and viral proteins are comprised of the same amino acids as those of the host. As a result, sampling of the “peptidome” depends upon a diverse array of HLA-I and HLA-II alleles. The ability of both CD4⁺ and CD8⁺ T cells to recognize a peptide depends upon the expression of the T-cell receptor, with CD4⁺ T cells recognizing longer, 15–20aa, peptides in the context of HLA-II, and CD8⁺ T cells recognizing shorter, 9–11aa, peptides in the context of HLA-I. The T-cell receptor is a heterodimer comprised of an α and β chain, which through somatic recombination can generate extensive diversity and breadth of peptide recognition.

Following selection in the thymus, T cells emerge with broad specificity, but with limited capacity to respond to microbial infection. Following exposure to a peptide presented via the HLA molecule, clonal expansion ensues, resulting in a population of “memory” cells capable of both limiting viral and/or bacterial growth, and in responding rapidly to reexposure to the same antigen (5). As peptides may be similar between viruses or bacteria, the concept of “original antigenic sin” can be extended to T cells, in that cells that have previously encountered a similar peptide might expand preferentially following exposure to a related virus or bacteria (6, 7).

That NTM may contain many epitopes that are shared with Mtb is well known, as environmental exposures to NTM can lead to positive tuberculin skin test responses, ultimately requiring the development of the tuberculin skin test cutoffs used to balance the likelihood of exposure to Mtb versus NTM (8). Similarly, exposure to environmental NTMs may influence the immunogenicity of vaccination with bacillus Calmette-Guérin (BCG) (9), possibly accounting for the diminished efficacy of BCG in the tropical latitudes where exposures to environmental NTM may either diminish BCG immunogenicity or serve to elicit a similar degree of protection (10). Finally, antigenic similarity between environmental NTM and Mtb has been suggested as a plausible explanation for the hyperconservation of HLA-II epitopes observed in Mtb (11, 12).

What is unexpected in the report by Scriba and colleagues is the similarity that was observed between the Type 2 epitopes and those found in the microbiome. As the peptides used in this study have been previously shown to be preferentially recognized by subjects with infection with Mtb versus those that are not infected, this relationship is necessarily complex. That is, the observed CD4⁺ T-cell response, while specific for Mtb, must reflect an additive or reinforcing effect of these related epitopes.

Therapy for TB requires prolonged, poly-antimicrobial therapy. Furthermore, rifampin is a broad spectrum antibiotic. That these antibiotics could alter the microbiome is supported by several recent observations (13–15). However, as anti-TB therapy would be expected to reduce the prevalence of antigens derived from both Mtb and as well as those from the microbiome, it is unexpected

that the T-cell responses to peptides with similarity to bacteria found in the microbiome would be preferentially diminished.

The human T-cell response reflects exposure to a wide variety of exogenous antigens. Both the frequency and phenotype of these responses reflect the proximity and abundance of antigenic exposures, such that removal of an antigen would be expected to result in diminished T-cell responses over time. Conversely, the persistence of high-frequency T-cell responses in the setting of chronic infections such as Mtb and cytomegalovirus would imply the persistence of these antigens (16–18). As the Type I responses were not impacted by therapy, it would support the hypothesis that treatment for Mtb does not necessarily eradicate Mtb or its associated antigens. This possibility is supported by the recent observation that in some cases, TB patients who are clinically cured continue to have Mtb RNA in their sputum and/or bronchoalveolar lavage, suggesting microbial persistence (19). Conversely, the finding that the Type 2 epitopes are selectively diminished would imply that proteins within the microbiome are necessary for the maintenance of these responses. This would suggest that the ability of our immune system to recognize infection with Mtb could be profoundly shaped by the microbiome. For example, one implication of this study is that TB treatment can diminish the breadth of the anti-TB response, possibly limiting the ability of the host to resist reexposure to Mtb, or to see Mtb antigens in the appropriate context. In this regard, it would be interesting to know at a protein level how recognition of Mtb was altered.

Conversely, it is also possible that cross-reactivity with proteins found within the microbiome might contribute to the impact that BCG vaccination has on infections other than Mtb, such as malaria (20).

Through the use of modern nucleic acid amplification techniques, the full diversity of the microbiome has only recently become apparent (21). In this regard, it is also possible that NTMs may comprise a larger part of the microbiome than currently appreciated. This was suggested in a recent report on the oral microbiome (22). Nonetheless, what the report from Scriba and colleagues makes clear is that the immune response to infection with Mtb, and possibly BCG, must be seen in the context of exposures to other, immunologically related microbes. This environmental context almost certainly reflects the ethnic, socioeconomic, and geographic settings in which exposure to Mtb occurs, and must be considered in the design and evaluation of novel vaccines. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

David M. Lewinsohn, M.D., Ph.D.
Pulmonary and Critical Care Medicine
Oregon Health & Science University
Portland, Oregon
 and
The Portland VA Medical Center
Portland, Oregon

ORCID ID: 0000-0001-9906-9494 (D.M.L.).

References

1. Scriba TJ, Carpenter C, Carrasco Pro S, Sidney J, Musvosvi M, Rozot V, Seumois G, Rosales SL, Vijayanand P, Goletti D, *et al*. Differential

- recognition of *Mycobacterium tuberculosis*-specific epitopes as a function of tuberculosis disease history. *Am J Respir Crit Care Med* 2017;196:772–781.
2. Lindestam Arlehamn CS, Gerasimova A, Mele F, Henderson R, Swann J, Greenbaum JA, Kim Y, Sidney J, James EA, Taplitz R, *et al.* Memory T cells in latent *Mycobacterium tuberculosis* infection are directed against three antigenic islands and largely contained in a CXCR3+CCR6+ Th1 subset. *PLoS Pathog* 2013;9:e1003130.
 3. Lindestam Arlehamn CS, McKinney DM, Carpenter C, Paul S, Rozot V, Makgotlho E, Gregg Y, van Rooyen M, Ernst JD, Hatherill M, *et al.* A quantitative analysis of complexity of human pathogen-specific CD4 T cell responses in healthy *M. tuberculosis* infected South Africans. *PLoS Pathog* 2016;12:e1005760.
 4. Andrews JR, Noubary F, Walensky RP, Cerda R, Losina E, Horsburgh CR. Risk of progression to active tuberculosis following reinfection with *Mycobacterium tuberculosis*. *Clin Infect Dis* 2012;54:784–791.
 5. Farber DL, Yudanin NA, Restifo NP. Human memory T cells: generation, compartmentalization and homeostasis. *Nat Rev Immunol* 2014;14:24–35.
 6. Francis T. On the doctrine of original antigenic sin. *Proceedings of the American Philosophical Society* 1960;104:572–578.
 7. McMichael AJ. The original sin of killer T cells. *Nature* 1998;394:421–422.
 8. Edwards PQ, Edwards LB. Story of the tuberculin test from an epidemiologic viewpoint. *Am Rev Respir Dis* 1960; 81(1): 1–47.
 9. Weir RE, Black GF, Nazareth B, Floyd S, Stenson S, Stanley C, Branson K, Sichali L, Chaguluka SD, Donovan L, *et al.* The influence of previous exposure to environmental mycobacteria on the interferon-gamma response to bacille Calmette-Guérin vaccination in southern England and northern Malawi. *Clin Exp Immunol* 2006;146:390–399.
 10. Mangtani P, Abubakar I, Ariti C, Beynon R, Pimpin L, Fine PE, Rodrigues LC, Smith PG, Lipman M, Whiting PF, *et al.* Protection by BCG vaccine against tuberculosis: a systematic review of randomized controlled trials. *Clin Infect Dis* 2014;58:470–480.
 11. Comas I, Chakravarti J, Small PM, Galagan J, Niemann S, Kremer K, Ernst JD, Gagneux S. Human T cell epitopes of *Mycobacterium tuberculosis* are evolutionarily hyperconserved. *Nat Genet* 2010;42:498–503.
 12. Lindestam Arlehamn CS, Paul S, Mele F, Huang C, Greenbaum JA, Vita R, Sidney J, Peters B, Sallusto F, Sette A. Immunological consequences of intragenus conservation of *Mycobacterium tuberculosis* T-cell epitopes. *Proc Natl Acad Sci USA* 2015;112: E147–E155.
 13. Hong BY, Maulén NP, Adami AJ, Granados H, Balcells ME, Cervantes J. Microbiome changes during tuberculosis and antituberculous therapy. *Clin Microbiol Rev* 2016;29:915–926.
 14. Namasivayam S, Maiga M, Yuan W, Thovarai V, Costa DL, Mittereder LR, Wipperfman MF, Glickman MS, Dzutsev A, Trinchieri G, *et al.* Longitudinal profiling reveals a persistent intestinal dysbiosis triggered by conventional anti-tuberculosis therapy. *Microbiome* 2017;5:71.
 15. Winglee K, Eloë-Fadrosch E, Gupta S, Guo H, Fraser C, Bishai W. Aerosol *Mycobacterium tuberculosis* infection causes rapid loss of diversity in gut microbiota. *PLoS One* 2014;9:e97048.
 16. Millington KA, Gooding S, Hinks TS, Reynolds DJ, Lalvani A. *Mycobacterium tuberculosis*-specific cellular immune profiles suggest bacillary persistence decades after spontaneous cure in untreated tuberculosis. *J Infect Dis* 2010;202:1685–1689.
 17. Lewinsohn DA, Gold MC, Lewinsohn DM. Views of immunology: effector T cells. *Immunol Rev* 2011;240:25–39.
 18. Jasenosky LD, Scriba TJ, Hanekom WA, Goldfeld AE. T cells and adaptive immunity to *Mycobacterium tuberculosis* in humans. *Immunol Rev* 2015;264:74–87.
 19. Malherbe ST, Shenai S, Ronacher K, Loxton AG, Dolganov G, Kriel M, Van T, Chen RY, Warwick J, Via LE, *et al.*; Catalysis TB–Biomarker Consortium. Persisting positron emission tomography lesion activity and *Mycobacterium tuberculosis* mRNA after tuberculosis cure. *Nat Med* 2016;22:1094–1100.
 20. Goodridge HS, Ahmed SS, Curtis N, Kollmann TR, Levy O, Netea MG, Pollard AJ, van Crevel R, Wilson CB. Harnessing the beneficial heterologous effects of vaccination. *Nat Rev Immunol* 2016;16:392–400.
 21. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature* 2012;486:207–214.
 22. Macovei L, McCafferty J, Chen T, Teles F, Hasturk H, Paster BJ, Campos-Neto A. The hidden ‘mycobacteriome’ of the human healthy oral cavity and upper respiratory tract. *J Oral Microbiol* 2015;7:26094.

Copyright © 2017 by the American Thoracic Society