Vaccination for *Mycobacterium tuberculosis* infection: reprogramming CD4 T-cell homing into the lung

DL Barber[1]

Development of effective tuberculosis vaccines is hampered by insufficient understanding of protective immunity. Here, Woodworth *et al.*[1] show secondary effector CD4 T cells generated after Mtb challenge of H56/CAF01 vaccinated mice display superior lung homing compared with primary effectors. Vaccination generates large populations of parenchymal lung effector cells by inducing CXCR3$^+$KLRG1$^-$ cells that continuously migrate from lymph nodes to lung, and limiting the generation of non-protective CX3CR1$^+$KLRG1$^+$ intravascular effectors, providing insight vaccine-mediated protection against tuberculosis.

Mycobacterium tuberculosis is the leading cause of global mortality due to infectious disease, despite the availability of curative chemotherapies and the mostly widely used vaccine in the world, bacille Calmette–Guérin (BCG) (WHO 2015). BCG immunization partially protects against the most severe forms of childhood tuberculosis, but it has little or no ability to prevent adolescent and adult tuberculosis, where the majority of transmission occurs. The development of an effective vaccine against Mtb infection would have a dramatic impact on global public health and is urgently needed. Progress in the development of novel TB vaccination strategies, however, has been hindered by the inadequate understanding of the precise mechanisms of protective immunity. Here I briefly trace how some recent advances in the understanding of CD4 T-cell differentiation and lung-homing during Mtb infection are now converging with observations on T cell correlates of vaccine-mediated protection to give important new insights into vaccination against tuberculosis.

DICHOTOMY OF PD-1$^+$ AND KLRG1$^+$ MTB-SPECIFIC TH1 CELLS AS CORRELATE OF VACCINE MEDIATED PROTECTION

One of the first studies to identify functionally distinct subsets of pulmonary Mtb-specific CD4 T cells found that two inhibitory receptors, PD-1 and KLRG1, are differentially expressed and can be used to discriminate CD4 T cells that are either highly proliferative or produce relatively elevated amounts of IFNγ, respectively.[2] Moreover, it was shown that PD-1$^+$ CD4 T cells convert into KLRG1$^+$ CD4 T cells as they proliferate while KLRG1$^+$ T cells proliferate poorly and do not change phenotype, indicating that PD-1$^+$ CD4 T cells are less differentiated compared with KLRG1$^+$ cells. This dichotomy has since been further explored in several studies investigating correlates of protection following vaccination as well as the basic T-cell biology of these two subsets.

On the basis of this paradigm, studies in the mouse model have found that vaccine-induced protection against aerosol Mtb infection is associated with a predominance of KLRG1$^-$ CD4 T cells. Upon Mtb challenge, vaccine-specific effector CD4 T cells in the lungs of mice that are protected by immunization with BCG or protein/adjuvant vaccines produce more IL-2 and express much less KLRG1 in comparison with primary Mtb-specific effector T cells generated after infection of naïve mice.[3] It was later shown that many of the KLRG1$^-$ vaccine-specific CD4 T cells that are present in the lungs of vaccinated mice following Mtb challenge also express the inhibitory receptor PD-1 but display little evidence of functional exhaustion.[4]

[1]T lymphocyte Biology Unit, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA. Correspondence: D Barber, (barberd@niaid.nih.gov)

Published online 14 December 2016. doi:10.1038/mi.2016.110

Collectively, these data have led to the hypothesis that protective vaccination for Mtb infection elicits less-differentiated CD4 T cells, as indicated by the lack of KLRG1 expression and increased PD-1 and IL-2 production, that resist differentiation into KLRG1^{+} CD4 T cells.

MIGRATORY CAPACITY OF MTB-SPECIFIC CD4 T-CELL SUBSETS

In parallel studies investigating T cell migration during Mtb infection, it was found that PD-1^{+} and KLRG1^{+} Mtb-specific CD4 T cells differ in their ability to migrate into the lungs and control Mtb infection[5] Using the intravascular staining approach that allows for the discrimination between cells in the lung vasculature and lung parenchyma, it was demonstrated that CXCR3^{+}PD-1^{+} CD4 T cells localize in the lung tissue parenchyma, while CX3CR1^{+}KLRG1^{+} CD4 T cells reside in the lung-associated blood vasculature. Moreover, CD4 T cells purified from the lung parenchyma migrate back into the lung parenchyma and suppress growth of Mtb, while CD4 T cells purified from the lung vasculature fail to migrate into the lungs and poorly protect against Mtb infection. These data showing that CD4 T cells must migrate into the lung to control Mtb are consistent with the observation that direct recognition of MHCII on Mtb-infected macrophages by CD4 T cells is required for suppression of bacterial replication.[6] It was later demonstrated that BCL6 is required to generate lung parenchymal CXCR3^{+}PD-1^{+} CD4 T-cell responses while ICOS and CXCR5 are required to sustain them, showing that the host-protective Mtb-specific effector CD4 T cells utilize pathways similar to those used by conventional memory CD4 T cells generated after complete clearance of an acute infection.[7] Collectively, these data indicate that to control Mtb infection Ag-specific CD4 T cells must extravasate out of the blood, migrate into granulomas and directly interact with Mtb infected macrophages, but only the more memory-like CXCR3^{+}PD-1^{+} subset of T cells is able to do so. Once CD4 T cells differentiate so far as the CX3CR1^{+}KLRG1^{+} stage, they lose the ability to enter the lung and fail to protect against pulmonary Mtb infection.

VACCINATION ENHANCES PULMONARY RESPONSES BY IMPACTING CD4 T-CELL DIFFERENTIATION

The promising candidate Mtb vaccine H56/CAF01 is a fusion of three Mtb antigens in a liposome-based adjuvant. H56 immunization offers robust, long-lived protection in mice and greatly boosts BCG-mediated protection in cynomolgus macaques.[8] Moreover, H56/CAF01 is currently under clinical development, making it a key example for investigating the correlates of vaccine-mediated protection. Here, Woodworth *et al*[1] evaluate the lung homing capacity and recirculation properties of effector CD4 T cells that expand following Mtb challenge of either unimmunized or H56/CAF01-vaccinated mice. Using the intravascular staining approach, the authors found that H56/CAF01 immunization leads to an early and large increase in the number of lung-parenchymal CD4 T cells compared with responses in unimmunized animals, and alterations to the phenotype of T cells in the lung blood vasculature. Unlike the primary CD4 T cell response to Mtb infection where most of the CD4 T cells in the vasculature are CX3CR1^{+}KLRG1^{+} non-protective cells, most of the CD4 T cells in the vasculature of immunized mice are CXCR3 + KLRG1^{-}, consistent with the lung-homing phenotype. Accordingly, it was found that adoptively transferred secondary effector CD4 T cells FACS purified from the lung vasculature after challenge of immunized mice migrate into the lung parenchyma much more efficiently compared with primary effector CD4 T cells taken from the lung vasculature of unimmunized mice. Furthermore, Woodworth *et al* also show that treatment of mice 7 weeks post infection with the S1P1 agonist FTY720 (which traps T cells in lymph nodes by preventing egress) causes a rapid depletion of the CXCR3^{+}KLRG1^{-} CD4 T cells from the vasculature. These data collectively indicate that immunized mice largely contain CXCR3^{+}KLRG1^{-} CD4 T cells in both the vasculature and parenchymal of the lung, in contrast to the prominent accumulation of non-protective CX3CR1^{+}KLRG1^{+} CD4 T cells observed in unimmunized mice. Moreover, H56/CAF01 immunization induces sustained migration of CXCR3^{+}KLRG1^{-} CD4 T cells from lymph nodes into the lung parenchyma, thereby maintaining the elevated numbers of parenchymal cells at the site of infection even at late time points (**Figure 1**).

Collectively these data argue that in addition to the enhanced precursor frequency of Mtb-specific CD4 T cells present at the time of challenge, the differentiation of CD4 T cells upon infection is also improved by vaccination. That is, H56/CAF01 immunization generates a population of lung homing CXCR3^{+}KLRG1^{-} memory CD4 T cells that maintains lung homing capacity and largely resists differentiating into non-protective CX3CR1^{+}KLRG1^{+} intravascular secondary effector cells upon recall after Mtb challenge. Indeed, in immunized mice Mtb-specific CD4 T cells that recognize antigens not included in the vaccine underwent extensive differentiation into CX3CR1^{+}KLRG1^{+} non-protective phenotype CD4 T cells, showing the ability to resist differentiation into this more terminal effector like state was only found in the recalled vaccine-specific memory CD4 T cells. Importantly, this provides a proof-of-principle that large numbers of lung parenchymal CD4 T cells can be generated and sustained with vaccination regimens that impact the differentiation state and therefore lung migrating capacity of the Ag-specific T cells that are recalled after infection challenge.

IMPLICATIONS FOR TB VACCINE DEVELOPMENT

Previous work in the study of vaccine elicited CD8 T-cell responses in mice have found that repeated rounds of boosting results in ever increasing amounts of KLRG1 expression and decreasing ability to proliferate and produce IL-2.[9] In other words, repeated boosting has been associated with the development of traits that are associated with a nonprotective phenotype in the

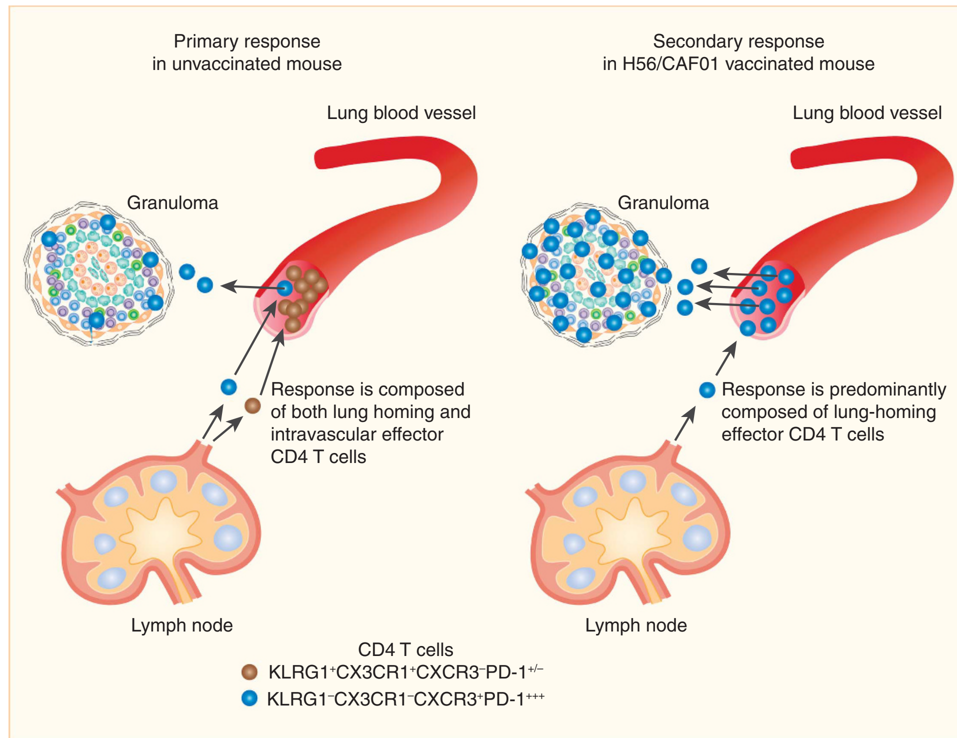

Figure 1 H56/CAF01 vaccination for Mtb infection generates large lung parenchymal effector populations upon recall by selectively inducing secondary CD4 T cells with enhanced lung-homing capacity. See text for details.

setting of pulmonary Mtb infection. In the present report by Woodworth *et al*, however, H56/CAF01 immunization, which included an initial subcutaneous inoculation and two subsequent booster injections 2 weeks apart, had the opposite effect on the CD4 T-cell responses and actually imparted the vaccine-specific T cells the ability to resist the accumulation of KLRG1^{+} terminal effector-like cells. Therefore, an important question in the future will be to better understand the mechanisms through which H56/CAF01 immunization protects CD4 T cells from terminal differentiation. In the setting of CD8 T-cell responses specific for systemic, acute pathogens like LCMV and *Listeria monocytogenes* it has been shown that memory cells are predisposed to adopting a terminal effector phenotype upon recall due to heighted amounts of IL-12R, but that limiting the amount of IL-12 can prevent Tbet driven terminal differentiation of recalled memory cells.[10] Therefore, although it is somewhat counter-intuitive, it is possible that vaccination regimens that induce limited amounts of IL-12 driven T-cell polarization and differentiation could result in superior protection against Mtb. In support of this hypothesis, it was recently shown that Tbet haploinsufficient mice have reduced levels of KLRG1^{+} ESAT-6-specific CD4 T cells and mediate enhanced control of Mtb infection.[11] The precise roles of IL-12 and Tbet in CD4 T-cell differentiation during Mtb infection, however, have not been well examined. Moreover, TB vaccine development has been largely empirical, and the direct impact of various vaccine strategies on T cell quality is not routinely, systematically evaluated.

The T cell markers being used in the mouse models of Mtb infection described herein will hopefully prove useful for the evaluation of the quality of CD4 T-cell responses in humans. Currently it is not clear if Mtb-specific CX3CR1^{+}KLRG1^{+} terminal effectors are even generated in human tuberculosis. Furthermore, it is not known what types of vaccination regimens induce Mtb-specific terminal effectors versus the more desirable less-differentiated memory cells that can resist terminal differentiation upon recall and thereby sustain high numbers of parenchymal T cells. In all, this report by Woodworth *et al* shows that the ability to induce CD4 T cells that generate secondary effectors with an efficient lung-homing phenotype upon challenge may be a useful criteria with which to evaluate new candidate tuberculosis vaccines.

ACKNOWLEDGMENTS

D.L.B. is supported by the Intramural Research Program of the NIAID/NIH.

DISCLOSURE

The author declares no conflict of interest.

© 2017 Society for Mucosal Immunology

REFERENCES

1. Woodworth, J.S. *et al.* Subunit vaccine H56/CAF01 induces a population of circulating CD4 T cells that traffic into the Mycobacterium tuberculosis-infected lung. *Mucosal Immunol.;* e-pub ahead of print 24 August 2016 (this issue).
2. Reiley, W.W. *et al.* Distinct functions of antigen-specific CD4 T cells during murine Mycobacterium tuberculosis infection*Proc. Natl. Acad. Sci.* **107**, 19408–19413 (2010).

3. Lindenstrøm, T., Knudsen, N.P.H., Agger, E.M. & Andersen, P. Control of chronic mycobacterium tuberculosis infection by CD4 KLRG1- IL-2-secreting central memory cells. *J. Immunol.* **190**, 6311–6319 (2013).
4. Woodworth, J.S. *et al.* Protective CD4 T cells targeting cryptic epitopes of Mycobacterium tuberculosis resist infection-driven terminal differentiation. *J. Immunol.* **192**, 3247–3258 (2014).
5. Sakai, S. *et al.* Cutting edge: control of Mycobacterium tuberculosis Infection by a Subset of Lung Parenchyma-Homing CD4 T Cells. *J. Immunol.* **192**, 2965–2969 (2014).
6. Srivastava, S. & Ernst, J.D. Cutting edge: Direct recognition of infected cells by CD4 T cells is required for control of intracellular Mycobacterium tuberculosis in vivo. *J. Immunol.* **191**, 1016–1020 (2013).
7. Moguche, A.O. *et al.* ICOS and Bcl6-dependent pathways maintain a CD4 T cell population with memory-like properties during tuberculosis. *J. Exp. Med.* **212**, 715–728 (2015).
8. Lin, P.L. *et al.* The multistage vaccine H56 boosts the effects of BCG to protect cynomolgus macaques against active tuberculosis and reactivation of latent Mycobacterium tuberculosis infection. *J. Clin. Invest.* **122**, 303–314 (2012).
9. Masopust, D., Ha, S-J., Vezys, V. & Ahmed, R. Stimulation history dictates memory CD8 T cell phenotype: implications for prime-boost vaccination. *J. Immunol.* **177**, 831–839 (2006).
10. Joshi, N.S. *et al.* Increased numbers of preexisting memory CD8 T cells and decreased T-bet expression can restrain terminal differentiation of secondary effector and memory CD8 T cells. *J. Immunol.* **187**, 4068–4076 (2011).
11. Torrado, E. *et al.* Interleukin 27R regulates CD4+ T cell phenotype and impacts protective immunity during Mycobacterium tuberculosis infection. *J. Exp. Med.* **212**, 1449–1463 (2015).