CLINICAL IMPLICATIONS OF BASIC RESEARCH

Elizabeth G. Phimister, Ph.D., *Editor*

Trained Innate Immunity, Epigenetics, and Covid-19

Alberto Mantovani, M.D., and Mihai G. Netea, M.D.

Innate immunity is mediated by different cell types and cell-associated or fluid-phase pattern-recognition molecules and plays a key role in tissue repair and resistance against pathogens.[1] Exposure to selected vaccines, such as bacille Calmette–Guérin (BCG) or microbial components, can increase the baseline tone of innate immunity and trigger pathogen-agnostic antimicrobial resistance (known as trained innate immunity). Such training is directly relevant to resistance against infectious diseases, including Covid-19. A recent study by de Laval et al.[2] pinpoints a driver of durable innate immune memory conferred by myeloid cells (monocytes, macrophages, and neutrophils).

Myeloid cells are central players in innate immunity: they produce effector molecules and contribute to the activation, orientation, and regulation of adaptive immune responses. Diversity and plasticity are fundamental properties of myeloid cells, particularly macrophages. To some extent, these properties are imprinted through ontogenetic origin (embryonal vs. adult bone marrow), but they are also influenced by environmental cues in the tissue. Moreover, in response to microbial molecules, metabolic products, or cytokines, macrophages increase effector function ("activation"), are primed for short-term responses ("priming"), or become unresponsive ("tolerance"). Microbial components can also cause long-term imprinting ("training") of innate immunity and myeloid-cell function (Fig. 1).[3] (This type of imprinting is distinct from genomic imprinting whereby methyl groups are added to DNA in or near specific genes.)

The cellular basis of trained immunity and heterologous protection against secondary infections resides in the functional reprogramming of innate immune cells, which were first observed in invertebrates.[4] Selected live attenuated vaccines (such as BCG) or fungal structures (such as beta-glucans and lipopolysaccharide) can durably boost antimicrobial function in myeloid cells; this function is associated with changes in chromatin conformation, which in turn alter accessibility to the regulatory elements in DNA that govern gene transcription, in these cells. Evidence supports the convergence of multiple regulatory layers, including changes in chromatin organization at the level of the topologically associated domains of DNA (genomic regions where interactions among specific DNA sequences occur preferentially, as compared with sequences located outside the domain), in transcription of long noncoding RNAs, in DNA methylation, and in reprogramming of cellular metabolism. Stimulation of innate immune cells can leave an "epigenetic scar" — a pattern of exposed enhancers and promoters of host-defense genes. Each element of the scar has, by virtue of its exposure, heightened responsiveness in its ability to influence gene expression.

The experimental observations of durable effects induced at the level of myeloid cells by microbial products (such as lipopolysaccharide or beta-glucans) or vaccines (such as BCG) present a conundrum, because mature myeloid cells such as monocytes and dendritic cells have a relatively short half-life, at least in the circulatory system. How trained immunity is maintained in myeloid cells over the course of months or even years has been unknown. The work by de Laval et al. provides some insight. Using mouse models, they showed that the long-time induction of innate immune memory is generated through transcriptomic, epigenetic, and functional reprogramming of myeloid progenitors (hematopoietic stem cells) in the

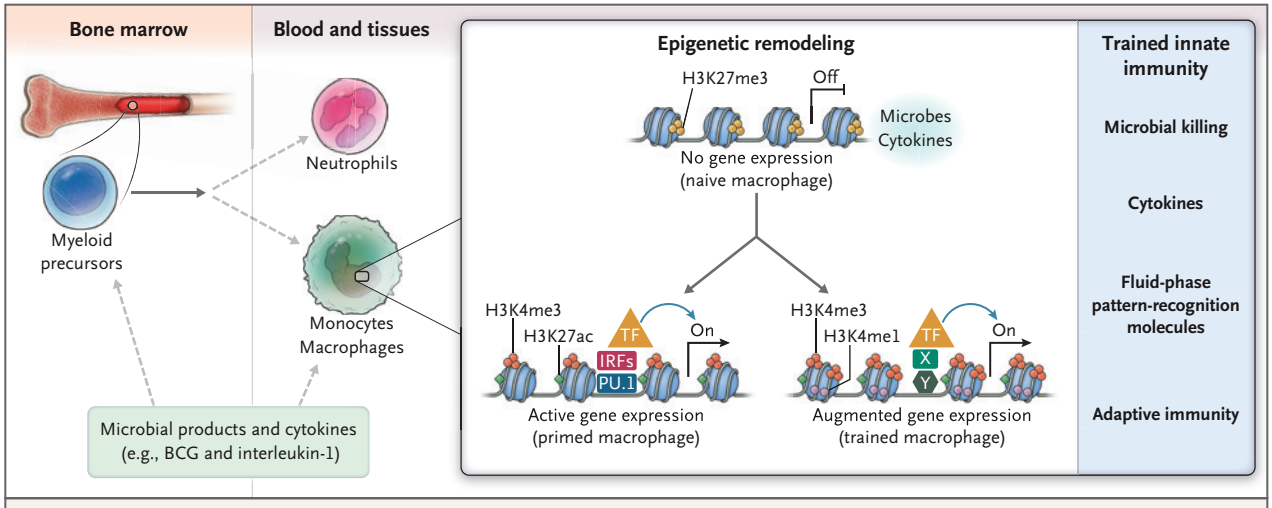

Figure 1. Cellular and Molecular Mechanisms Underlying Trained Innate Immunity.

Exposure to microbial signals, particularly from bacille Calmette–Guérin (BCG), and to cytokines trains myelomonocytic cells with enhanced effector function against microbial agents. Training can occur at the level of bone marrow hematopoietic stem cells or of mature macrophages. Training-mediated augmentation of myelomonocytic-cell function depends on reshaping of the epigenetic landscape driven at the level of stem cells by the pioneering transcription factor (TF) CCAAT/enhancer-binding protein β (C/EBPβ),[2] transcription of long noncoding RNA, and metabolic rewiring. Trained myeloid cells show enhanced killing capacity and increased production of cytokines, chemokines, and fluid-phase pattern-recognition molecules. Moreover, they are better suited to triggering adaptive immune responses. Training is likely to underlie the off-target pathogen-agnostic function of BCG and possibly other vaccines. Interferon regulatory factors (IRFs) and PU.1 are TFs. X and Y indicate TFs that are involved in the regulation of specific genes in trained macrophages.

bone marrow. They discovered that this reprogramming (or imprinting) is dependent on the transcription factor CCAAT/enhancer-binding protein β (C/EBPβ) and can be induced by exposure to lipopolysaccharide, a component of the cell wall of *Pseudomonas aeruginosa* and other gram-negative bacteria. The imprinting led to a transient myeloid bias (i.e., a transient orientation of hematopoiesis toward increased production of myeloid cells), which was associated with protection against later infection by *P. aeruginosa*. Their report thus provides a potential mechanistic blueprint for the long-term effect of vaccines with live attenuated microorganisms, such as BCG or measles, on cells conferring innate immunity (Fig. 1). The finding that epigenetic memory undergirds innate immunity at the level of bone marrow progenitors is consistent with a myeloid bias in bone marrow hematopoietic stem cells of healthy volunteers 3 months after BCG vaccination.[5]

The relative importance of imprinting of myeloid-cell function in hematopoietic stem cells, as described by de Laval et al., as compared with that of mature macrophages,[1] remains to be elucidated. No less important is defining the time frame of training and the extent to which it informs off-target pathogen-agnostic protection. Children undergo an intense vaccination program involving adjuvants, which is perhaps relevant to their relative resistance to Covid-19.[6] In the same vein, it would be important to ascertain whether the receipt of influenza vaccine with or without an adjuvant by elderly persons has resulted in differences in the function of heterologous mechanisms of protection and subsequent differences in susceptibility to Covid-19.

Some epidemiologic studies suggest that BCG has a pleiotropic effect that decreases the incidence of other infections.[7] Several trials to determine whether BCG can help prevent Covid-19 are under way; findings from these trials could reinforce a role of trained immunity in illness prevention or amelioration (the second author of this article is leading two of them). The use of BCG in the prophylaxis or treatment of Covid-19 outside of controlled clinical trials is, of course, not recommended.

Disclosure forms provided by the authors are available at NEJM.org.

From the Humanitas Clinical and Research Center IRCCS and Humanitas University, Milan (A.M.); the William Harvey Research Institute, Queen Mary University of London, London (A.M.); the Department of Internal Medicine and Radboud Center for Infectious Diseases, Radboud University, Nijmegen, the Netherlands (M.G.N.); and Immunology and Metabolism, Life, and Medical Sciences Institute, University of Bonn, Bonn, Germany (M.G.N.).

1. Locati M, Curtale G, Mantovani A. Diversity, mechanisms, and significance of macrophage plasticity. Annu Rev Pathol 2020;15:123-47.
2. de Laval B, Maurizio J, Kandalla PK, et al. C/EBPβ-dependent epigenetic memory induces trained immunity in hematopoietic stem cells. Cell Stem Cell 2020;26:793.
3. Netea MG, Domínguez-Andrés J, Barreiro LB, et al. Defining trained immunity and its role in health and disease. Nat Rev Immunol 2020;20:375-88.
4. Kurtz J, Franz K. Innate defence: evidence for memory in invertebrate immunity. Nature 2003;425:37-8.
5. Cirovic B, de Bree LCJ, Groh L, et al. BCG vaccination in humans elicits trained immunity via the hematopoietic progenitor compartment. Cell Host Microbe 2020;28(2):322-334.e5.
6. Castagnoli R, Votto M, Licari A, et al. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in children and adolescents: a systematic review. JAMA Pediatr 2020 April 22 (Epub ahead of print).
7. Escobar LE, Molina-Cruz A, Barillas-Mury C. BCG vaccine protection from severe coronavirus disease 2019 (COVID-19). Proc Natl Acad Sci U S A 2020;117:17720-6.

DOI: 10.1056/NEJMcibr2011679