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Immune Training Unlocks Innate Potential

Wilbur M. Song[1] and Marco Colonna[1,*]
[1]Department of Pathology and Immunology, Washington University School of Medicine, Saint Louis, MO, USA
*Correspondence: mcolonna@wustl.edu
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Trained immunity is a form of innate immune memory with distinct features from classical adaptive immune memory. In the current issues of *Cell* and *Cell Host & Microbe*, five studies from the International Trained Immunity Consortium shed light on mechanisms and functional consequences of this phenomenon on cellular and whole-organism levels.

Immunological memory is a powerful tool for host defense against pathogens, as evidenced by the success of childhood vaccination. In adaptive immune memory, T and B cells develop a highly diverse repertoire of antigen-specific receptors by somatic gene rearrangement. These receptors are clonally distributed. When a clone expressing one of these unique receptors recognizes a foreign antigen with high affinity, it expands and acquires the capacity to clear the insult and then leaves behind memory cells that rapidly respond if the same foreign antigen reappears (Laidlaw et al., 2016; Weisel and Shlomchik, 2017). An innate analog of this phenomenon is the selective expansion of murine natural killer cells carrying the germline-encoded Ly49H receptor upon encountering a specific protein expressed on cells infected with murine cytomegalovirus (Geary and Sun, 2017). This skewing of the natural killer cell population persists over time and leads to a more robust response to reinfection with the same virus. Whether it originates from somatic rearrangement or is germline encoded, classical immunological memory is highly specific and potent. More recently, a completely different form of immunological memory termed "trained immunity" has been described whereby certain stimuli durably prime innate immune cells, especially of the myeloid lineage, to respond more strongly to future stimuli with broad specificity (Netea et al., 2016). Five papers from the International Trained Immunity Consortium in the current issues of *Cell* and *Cell Host & Microbe* have now provided insights on how trained immunity may operate on the cellular and whole-organism levels to boost beneficial immune responses.

Bekkering et al. utilize a typical *in vitro* model of trained immunity in which differentiated myeloid cells are stimulated (trained) for 24 hr, rested for 5 days, and then stimulated again to measure the secondary cytokine response (Bekkering et al., 2018). Interestingly, they find that fluvastatin, but not a downstream inhibitor of cholesterol synthesis, blocks training by oxidized low-density lipoprotein, Bacillus Calmette-Guérin (BCG) vaccine (against tuberculosis), or β-glucan. From these results, they zero in on the cholesterol synthesis intermediate mevalonate and show that mevalonate itself could promote training (Figure 1), suggesting that mevalonate enhances IGF-1 receptor (IGF-1R) signaling through mTOR activation and glycolysis, which are required for training. Although effect sizes of both exogenous mevalonate treatment and IGF-1R inhibition on trained immunity were modest, peripheral blood monocytes from individuals with hyperimmunoglobulin D (hyper-IgD) syndrome, an autoinflammatory disease associated with elevated mevalonate, produced more proinflammatory cytokines than did control monocytes. This novel mechanism raises the tantalizing possibility that statins, a widely used drug class, may block trained immunity and thus ameliorate human inflammatory disease. Further studies are needed to prove that this promising *in vitro* pathway is meaningful for trained immunity *in vivo* and in human diseases.

The other four papers apply the concepts of trained immunity *in vivo* and hint that on the whole-organism level, trained immunity might operate in additional, fundamentally distinct ways. Mitroulis et al., Christ et al., and Kaufmann et al. all find that transient inflammatory conditions expand myeloid-biased or myeloid-committed hematopoietic progenitors, enhancing future myeloid cell responses on a population basis (Mitroulis et al., 2018; Christ et al., 2018; Kaufmann et al., 2018) (Figure 1). Mitroulis et al. investigate metabolomic and transcriptomic effects of systemic β-glucan injection on hematopoietic stem cells. In the short term, β-glucan upregulated various pathways associated with cell proliferation, including cell cycle genes, cholesterol biosynthesis, and glycolysis. Interestingly, these changes accompany expansion of myeloid-biased, but not lymphoid-biased, hematopoietic progenitors based on lineage surface markers and transcription factors. This myeloid skewing persisted for up to a month, and transplantation of hematopoietic stem cells from β-glucan-trained mice yielded myeloid-skewed immune populations in untrained recipients. Blockade of either glycolysis or IL-1β signaling prevents preferential myeloid expansion. Moreover, training increases STAT5 phosphorylation, which is downstream of GM-CSF. They conclude that IL-1β and GM-CSF are required for this myeloid-skewing effect. Importantly, β-glucan training affects functional phenotypes. Upon lipopolysaccharide challenge, β-glucan-trained mice make more myeloid progenitors and accumulate less DNA damage (Figure 1). Chemotherapy-induced mortality and DNA damage are also reduced in β-glucan-trained mice. Kaufmann et al. performed a very similar study using a different microbial stimulus, the BCG vaccine, also in mouse models. Like Mitroulis et al., the authors find myeloid-biased proliferation of hematopoietic progenitors in response to BCG. When T cell-depleted BCG-trained bone marrow is

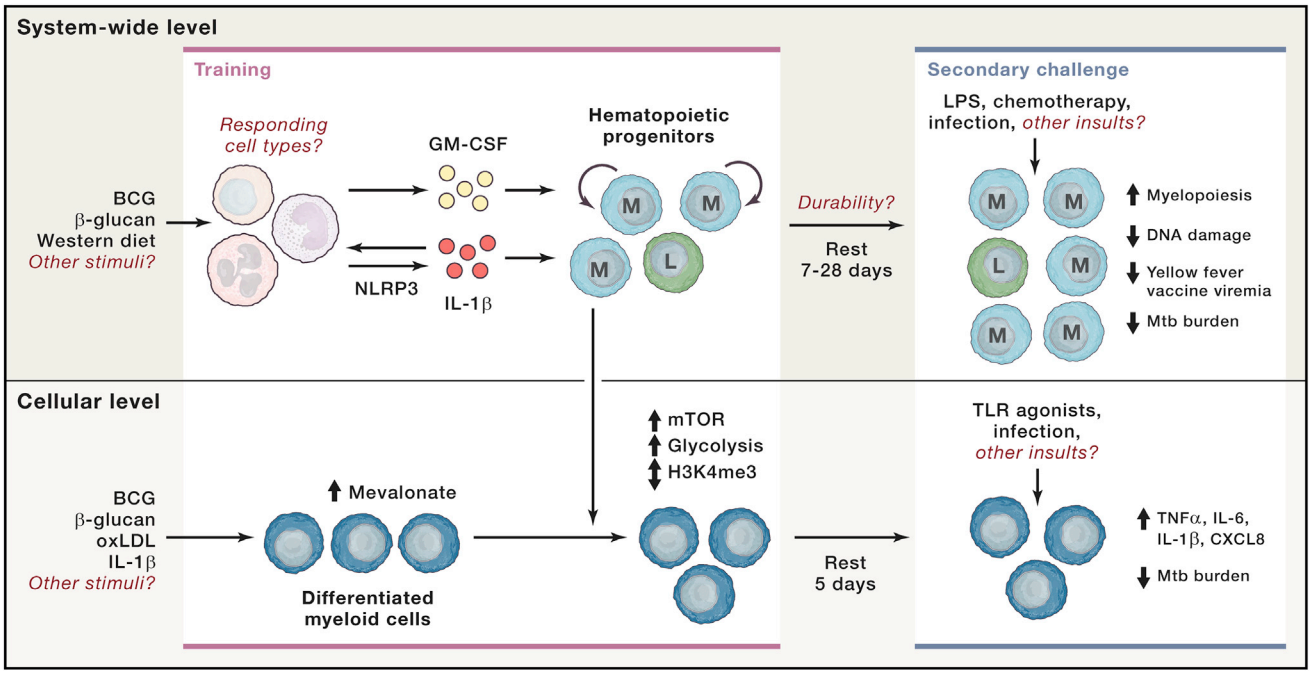

Figure 1. Trained Immunity Can Act at the System-wide Level on Myeloid Lineage Progenitors or at the Cellular Level on Differentiated Myeloid Cells

At the system-wide level (top), inflammation is induced by a variety of insults, including the BCG vaccine, β-glucan, or a Western diet combined with *Ldlr* deficiency. Systemic inflammation leads to NLRP3 inflammasome-dependent production of IL-1β, as well as production of GM-CSF. These cytokines signal myeloid lineage progenitors to proliferate and undergo epigenetic changes, leading to preferential expansion of the myeloid lineage (M) over the lymphoid lineage (L) within the hematopoietic stem cell compartment. Upon secondary challenge, "trained" individuals have a boosted immune response compared to untrained individuals, leading to better functional outcomes. At the cellular level (bottom), various stimuli can induce epigenetic changes that impact microbial clearance and secretion of inflammatory cytokines in response to TLR agonists *in vitro*. This enhancement depends on glycolysis, mevalonate synthesis, and mTOR activation. Several unanswered questions remain, as represented by red italicized phrases, including the specificity of the stimuli that can be used for training and for challenge, the durability of the trained state, and the identity of cells that respond to training stimuli *in vivo*.

transplanted into irradiated recipients, mice exhibit better responses against *Mycobacterium tuberculosis*. Moreover, bone marrow-derived macrophages from trained bone marrow have better responses against *M. tuberculosis in vitro*. Interestingly, these phenotypes all depend on IFNγ signaling.

Christ et al. approach the same question using a conceptually distinct model: rather than injecting the microbial products β-glucan or BCG, they model metabolic syndrome-induced autoinflammation by feeding *Ldlr*-deficient mice a high-fat Western diet (WD). As expected, after 4 weeks of WD, mice had higher circulating inflammatory cytokines, increased proliferation and higher proportions of myeloid lineage precursors and differentiated cells, and more activated monocytes than mice kept on a conventional diet (CD); lipopolysaccharide injection synergizes with WD in upregulating inflammatory genes. The most intriguing findings from this study come from observing WD-fed mice that were switched back to conventional diet for 4 weeks (WD→CD), at which point systemic inflammatory markers and metabolic parameters had already returned to normal. WD→CD myeloid progenitors exhibit a transcriptional profile more similar to that of WD than that of CD and have a distinct pattern of chromatin accessibility, and WD→CD monocytes remain activated. *Ex vivo*, monocytes and bone marrow cells from WD→CD, like WD, secrete higher levels of inflammatory cytokines in response to Toll-like receptor (TLR) agonists than CD, similar to *in vitro* training. Mechanistically, Nlrp3-dependent inflammasome activation and IL-1β production are required for the inflammatory effects of WD. Although the authors did not investigate *in vivo* secondary responses, they offer important evidence that both myeloid progenitors and differentiated cells can exhibit trained immunity phenotypes in response to an autoinflammatory state.

Finally, Arts et al. investigate trained immunity *in vivo* in a controlled human study (Arts et al., 2018). The authors recruited volunteers to undergo a trained immunity regime in which they were given the BCG vaccine or placebo as training stimulus and challenged 4 weeks later with the live attenuated yellow fever vaccine (YFV), which causes detectable viremia and induces inflammatory responses (Figure 1). Their major finding is that BCG-vaccination subjects have lower peak viremia at day 5 after receiving YFV, although viremia at days 3 and 7 are unchanged. As expected, peripheral blood mononuclear cells isolated from BCG-trained individuals produce more proinflammatory cytokines in response to a variety of stimuli. While many differences are subtle and/or variable, the authors bolster their claim by correlating viremia with *ex vivo* results from the

same subjects. In support of a role for trained immunity in viremia, the magnitude of the *ex vivo* IL-1β training response inversely correlates with peak viremia. Subjects with lower peak viremia also exhibit distinguishing epigenetic markers, including a specific H3K27ac peak near the *NOD2* gene, which had been previously implicated in BCG-induced trained immunity. Because of the link to IL-1β, the authors attempt to train monocytes *ex vivo* with IL-1β itself and find that IL-1β can elicit many characteristics of trained immunity.

Overall, these studies establish that trained immunity can operate by both myeloid skewing of the hematopoietic compartment and cell-intrinsic changes and provide proof of principle that training can influence subsequent immune responses *in vivo*. At the same time, their mechanistic insights raise important questions moving forward. Key features of immunological memory include durability and specificity (Figure 1). Several experiments in these papers use a relatively late 4-week time point; furthermore, all studies find persistent epigenetic changes, which are encouraging as a sign of stable changes, after training. On the other hand, these papers point toward a striking lack of specificity. A consistent finding is that IL-1β, which is produced in diverse inflammatory conditions, is necessary and perhaps sufficient to induce training. Kaufmann et al. report strong dependence of their phenotypes on IFNγ but do not exclude a role for IL-1β. Mitroulis et al. suggest that IGF-1 can directly induce training via mTOR activation and glycolysis. Thus, a vast array of mTOR-activating and/or IL-1β-inducing stimuli could also potentially induce a trained state. Moreover, the epigenetic changes induced by different training stimuli only partially overlap, despite all correlating with enhanced inflammatory cytokine production. Thus, the precise mechanism of trained immunity may not be well defined, generalizable, or predictable *in vivo* (Figure 1). As an example, WD strongly suppressed cholesterol synthesis in myeloid progenitors, which might theoretically inhibit mevalonate-dependent training. The specificity for secondary challenge is also an important issue (Figure 1). If trained immunity is entirely non-specific, then promoting training may predispose to autoimmunity, and blocking training may lead to susceptibility to tumors and infections. These studies link trained immunity to functional outcomes, an important step forward; now, more work needs to be done to demonstrate that these effects can be durable, specific, predictable, and clinically significant.

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