REVIEW SUMMARY

INNATE IMMUNITY

Trained immunity: A program of innate immune memory in health and disease

Mihai G. Netea,* Leo A. B. Joosten, Eicke Latz, Kingston H. G. Mills, Gioacchino Natoli, Hendrik G. Stunnenberg, Luke A. J. O'Neill, Ramnik J. Xavier

BACKGROUND: Host immune responses are classically divided into innate immune responses, which react rapidly and nonspecifically upon encountering a pathogen, and adaptive immune responses, which are slower to develop but are specific and build up immunological memory. The dogma that only adaptive immunity can build immunological memory has recently been challenged by studies showing that innate immune responses in plants and invertebrates (organisms lacking adaptive immune responses) can mount resistance to reinfection. Furthermore, in certain mammalian models of vaccination, protection from reinfection has been shown to occur independently of T and B lymphocytes. These observations led to the hypothesis that innate immunity can display adaptive characteristics after challenge with pathogens or their products. This de facto immunological memory has been termed "trained immunity" or "innate immune memory."

ADVANCES: In recent years, emerging evidence has shown that after infection or vaccination, prototypical innate immune cells (such as monocytes, macrophages, or natural killer cells) display long-term changes in their functional programs. These changes lead to increased responsiveness upon secondary stimulation by microbial pathogens, increased production of inflammatory mediators, and enhanced capacity to eliminate infection. Mechanistic studies have demonstrated that trained immunity is based on epigenetic reprogramming, which is broadly defined as sustained changes in transcription programs and cell physiology that do not involve permanent genetic changes, such as mutations and recombination. Histone modifications with chromatin reconfiguration have proven to be a central process for trained immunity, but other mechanisms—such as DNA methylation or modulation of microRNA and/or long noncoding RNA expression—are also expected to be involved. This leads to transcriptional programs that rewire the intracellular immune signaling of innate immune cells but also induce a shift of cellular metabolism from oxidative phosphorylation toward aerobic glycolysis, thus increasing the innate immune cells' capacity to respond to stimulation. Trained immunity programs have evolved as adaptive states that enhance fitness of the host (e.g., protective effects after infection or vaccination, or induction of mucosal tolerance toward colonizing microorganisms). Proof-of-principle experimental studies support the hypothesis that trained immunity is one of the main immunological processes that mediate the nonspecific protective effects against infections induced by vaccines, such as bacillus Calmette-Guérin or measles vaccination. However, when inappropriately activated, trained immunity programs can become maladaptive, as in postsepsis immune paralysis or autoinflammatory diseases.

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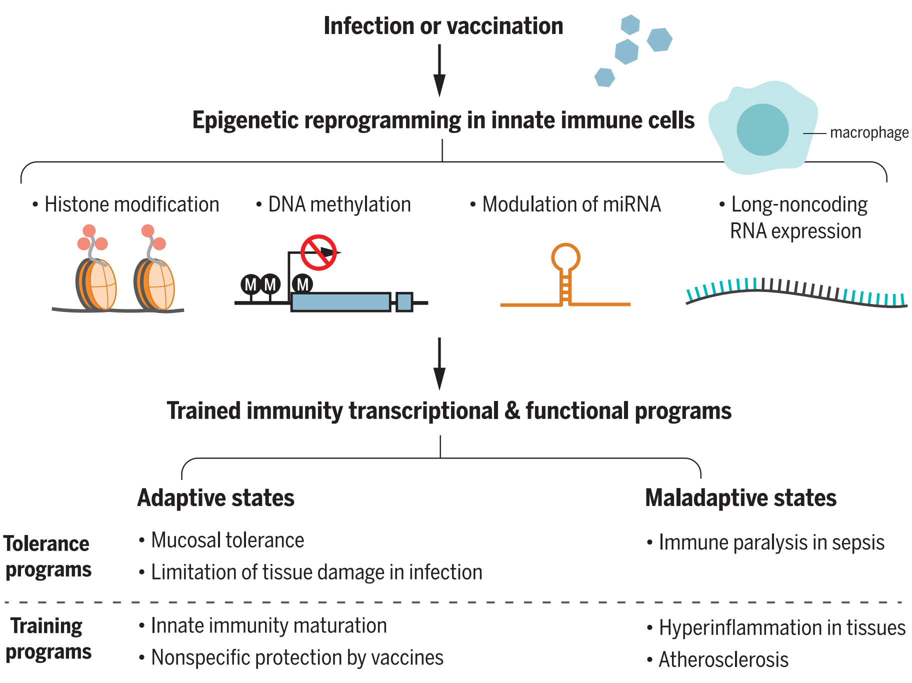
OUTLOOK: The discovery of trained immunity has revealed an important and previously unrecognized property of human immune responses. This advance opens the door for future research to explore trained immunity's effect on disease, for both diseases with impaired host defense, such as postsepsis immune paralysis or cancers, and autoinflammatory diseases, in which there is inappropriate activation of inflammation. These findings have considerable potential for aiding in the design of new therapeutic strategies, such as new generations of vaccines that combine classical immunological memory and trained immunity, the activation of trained immunity for the treatment of postsepsis immune paralysis or other immune deficiency states, and modulation of exaggerated inflammation in autoinflammatory diseases. ■



Innate immune activation by infections or vaccinations leads to histone modifications and functional reprogramming of cells (such as monocytes, macrophages, or NK cells) termed "trained immunity" or "innate immune memory." Trained immunity evolved to lead to adaptive states that protect the host during microbial colonization or after infections. However, in certain situations, trained immunity may result in maladaptive states such as postsepsis immune paralysis or hyperinflammation. miRNA, microRNA.

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REVIEW

INNATE IMMUNITY

Trained immunity: A program of innate immune memory in health and disease

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The general view that only adaptive immunity can build immunological memory has recently been challenged. In organisms lacking adaptive immunity, as well as in mammals, the innate immune system can mount resistance to reinfection, a phenomenon termed "trained immunity" or "innate immune memory." Trained immunity is orchestrated by epigenetic reprogramming, broadly defined as sustained changes in gene expression and cell physiology that do not involve permanent genetic changes such as mutations and recombination, which are essential for adaptive immunity. The discovery of trained immunity may open the door for novel vaccine approaches, new therapeutic strategies for the treatment of immune deficiency states, and modulation of exaggerated inflammation in autoinflammatory diseases.

Host immune responses are classically divided into innate immune responses, which react rapidly and nonspecifically upon encountering a pathogen, and adaptive immune responses, which are slower to develop but are specific (due to antigen receptor gene rearrangements) and result in classical immunological memory. This schematic distinction has been challenged by the discovery of pattern recognition receptors (PRRs) that confer some specificity to the recognition of microorganisms by innate immune cells (*1*), as well as by a growing body of literature showing that the innate immune system can adapt its function after previous insults (*2*, *3*). Protection against reinfection has been reported, not only in plants and invertebrates that do not have adaptive immunity (*4*), but also in mammals, with old and new studies demonstrating cross-protection between infections with different pathogens (*5*). These studies have led to the hypothesis that innate immunity can be influenced by previous encounters with pathogens or their products, and this property has been termed "trained immunity" or "innate immune memory."

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Compared with classical immunological memory, trained immunity has a number of defining characteristics. First, it involves a set of cells [myeloid cells, natural killer (NK) cells, and innate lymphoid cells (ILCs)] and germline-encoded recognition and effector molecules (e.g., PRRs, cytokines) that are different from those involved in classical immunological memory. Second, and in contrast to classical immunological memory that depends on gene rearrangement and proliferation of antigen-specific lymphocyte clones, the increased responsiveness to secondary stimuli during trained immunity is not specific for a particular pathogen and is mediated through signals impinging on transcription factors and epigenetic reprogramming. These are broadly defined as sustained changes in transcription programs through epigenetic rewiring, leading to changes in cell physiology that do not involve permanent genetic changes such as mutations and recombination. Finally, trained immunity relies on an altered functional state of innate immune cells that persists for weeks to months, rather than years, after the elimination of the initial stimulus.

In this context, it important to note that some innate immune cells, such as NK cells, display both trained immunity characteristics, as defined above, and antigen-dependent (or even antigen-specific) immunity related to the classical immunological memory mediated by T and B lymphocytes (see below for a detailed description). In addition, it is important to clearly discriminate between trained immunity and other immunological processes, such as immune cell activation and differentiation. During immune cell activation, transcription of genes takes place at the time of stimulation in response to a ligand directly acting on the cell. In contrast, during trained immunity, innate immune cells display gene- or locus-specific changes in their chromatin profiles, induced by a previous stimulation. However, these changes allow increasing response to restimulation of the cells through both the same and different PRRs. The discrimination between trained immunity and immune cell differentiation is more difficult and, to a certain degree, is even semantic: One could argue that macrophage differentiation could also be considered an example of trained immunity. However, immune cell differentiation can (and does) also occur during homeostatic conditions, whereas trained immunity is defined as a reaction to a foreign insult. In addition, although the term "circulating differentiated monocyte" could also be used instead of "trained monocyte," we believe that this may be confusing, as monocyte differentiation is generally considered equivalent to the process through which blood monocytes differentiate into macrophages in the tissues. Moreover, differentiated cells such as macrophages can be trained as well (e.g., after infection or vaccination), and thus their capacity to display increased function should be defined differently than cell differentiation.

Defining the properties of trained immunity will critically integrate our understanding of host defense. In this Review, we will describe this concept and discuss recent data that support its important role in health and disease. We will not delve into classical immunological memory, as this topic has already been the subject of many thorough reviews.

Immunological memory in plants and invertebrate animals

A first line of evidence that the innate immune system has the capacity to build memory to previous insults comes from a plethora of immunological studies in plants. Collectively, these studies provide compelling evidence of the capacity to respond more efficiently to reinfection, a phenomenon termed "systemic acquired resistance" (SAR) (*6*). The molecular mechanisms and biochemical mediators of SAR are largely known (*6*), with epigenetic-based rewiring of host defense playing a central role (*7*). In addition, there is increasing evidence to suggest that innate immunity displays memory traits, not only in plants but also in invertebrate animals (*4*). For example, the microbiota has been shown to induce innate immune memory to protect mosquitoes against *Plasmodium* (*8*), the social insect *Bombus terrestris* displays innate immune memory against three different pathogens (*9*), and the tapeworm *Schistocephalus solidus* induces memory in the copepod crustacean (*10*). In these models, the organism is protected against reencounter with the pathogen by an improved clearance of the infection. It is therefore reasonable to conclude that immunological memory is found in plants and lower animals (*3*, *4*), as well as in vertebrates.

Table 1. Overview of innate immune memory mechanisms described for various types of innate immune cells.

Innate immune cell type	Primary challenge	Type of memory	Pathway involved	Mechanism	References
Monocytes and macrophages	LPS	Tolerance/trained immunity	TLR4/MAPK-dependent ATF7-dependent	Epigenetic changes: latent enhancers (H3K4me1), other modifications (H3K4me3, H2K27me, H3K9me2)	Foster *et al.* (*50*), Ostuni *et al.* (*75*), Yoshida *et al.* (*80*)
Monocytes and macrophages	β-glucan, *Candida* infection, BCG vaccination	Trained immunity	Dectin-1/Raf1/Akt-dependent STAT1-dependent NOD2-dependent	Epigenetic changes (H3K4me1, H3K4me3, H2K27Ac, H3K9me2), metabolic rewiring	Quintin *et al.* (*26*), Saeed *et al.* (*51*), Cheng *et al.* (*79*), Yoshida *et al.* (*80*)
NK cells	Hapten-induced influenza A, vaccinia virus, HIV-1 infection	Antigen-specific	Not described	CXCR6-dependent, NKG2D-dependent	O'Leary *et al.* (*30*), Paust *et al.* (*57*), Gillard *et al.* (*58*), Reeves *et al.* (*64*)
NK cells	CMV infection	Antigen-dependent	Atg3-mediated mitophagy	BNIP3/BNIP3L-dependent	Sun *et al.* (*31*), O'Sullivan *et al.* (*61*)
NK cells	CMV infection	Trained immunity	Stable down-regulation of adaptors and transcription factors (e.g., Syk, PLZF)	Epigenetic modification of gene promotors DNA methylation	Lee *et al.* (*65*), Schlums *et al.* (*34*)

Several mechanisms have been proposed to account for innate immune memory in invertebrates, including the sustained up-regulation of immune regulatory pathways [such as the Toll and Imd receptors on the haematocytes (*11*)] or of the bacterial peptidoglycan recognition molecules and lectins (*12*), and quantitative and phenotypic changes in immune cell populations (*8*). Alternatively, memory may be due to the presence of diversity-generating mechanisms in insects, such as generation of variation in fibrinogen-related proteins (probably acting as pathogen sensors) with high rates of diversification at the genomic level through point mutations and recombinatorial processes (*13*). The Toll-like receptors (TLRs)—the animal counterpart of Toll in *Drosophila*—also show great diversity in the sea urchin, which has an estimated 222 receptors (*14*).

Innate immune memory in vertebrates

The presence of memory characteristics in innate host defense of different plant and animal lineages suggests that innate immune memory may be present in vertebrates as well (Table 1). Important clues indicating that vertebrate innate immunity also has adaptive characteristics came from experimental studies in mice showing that priming (or training) of mice with microbial ligands of PRRs can protect against a subsequent lethal infection. For example, trained immunity induced by β-glucan (a polysaccharide component of mainly fungal cell walls) induces protection against infection with *Staphylococcus aureus* (*15*, *16*). Similarly, the peptidoglycan component muramyl dipeptide induces protection against *Toxoplasma* (*17*), and prophylactic treatment with TLR9 agonists (such as oligodeoxynucleotides containing unmethylated CpG dinucleotides) 3 days before the infection protects against sepsis and meningitis caused by *Escherichia coli* (*18*). Furthermore, flagellin can induce protection against *S. pneumonia* (*19*) and rotavirus (*20*), the latter being independent of adaptive immunity and induced by dendritic cell–derived interleukin (IL)–18, which in turn drives production of IL-22 by epithelial cells. In addition to microbial ligands, there is evidence that certain proinflammatory cytokines may induce trained immunity: Injection of mice with one dose of recombinant IL-1 3 days before infection with *Pseudomonas aeruginosa* protected the mice against mortality (*21*). The nonspecific character of the trained immunity provides evidence against a classical immunological memory effect and instead suggests the activation of nonspecific innate immune mechanisms.

Compelling evidence that trained immunity is induced in vertebrates and mediates at least some of the protective effects of vaccination came from studies showing that immunization of mice with bacillus Calmette-Guérin [(BCG), the tuberculosis vaccine that is also the most commonly used vaccine worldwide] induces T cell–independent protection against secondary infections with *Candida albicans* or *Schistosoma mansoni* (*22*, *23*). The hypothesis that trained immunity can be elicited in vertebrates is further supported by studies investigating the mechanism of protection against disseminated candidiasis conferred by attenuated strains of *C. albicans*. For example, when an attenuated PCA-2 strain of *C. albicans* that is incapable of germination is injected in mice, protection is induced against the virulent strain CA-6 (*24*). This protection was also induced in athymic mice and *Rag1*-deficient animals (i.e., those that cannot rearrange their antigen receptors), demonstrating a lymphocyte-independent mechanism (*25*, *26*). The protection against reinfection was instead dependent on macrophages (*24*) and proinflammatory cytokine production (*27*), both prototypical innate immune components.

In addition to BCG and *C. albicans*, some viral and parasitic organisms can exert protective effects through mechanisms independent of adaptive immunity. Herpesvirus latency increases resistance to the bacterial pathogens *Listeria monocytogenes* and *Yersinia pestis* (*28*), with protection achieved through enhanced production of the cytokine interferon-γ (IFN-γ) and systemic activation of macrophages. Similarly, infection with the helminth parasite *Nippostrongylus brasiliensis* induces a long-term macrophage phenotype that, on one hand, damages the parasite and, on the other, induces T and B lymphocyte–independent protection from reinfection (*29*).

Other studies have shown that NK cells also display immune memory. This was first demonstrated in mice that showed hapten-induced contact hypersensitivity dependent on NK cells that persisted for at least 4 weeks (*30*). Consistent with this notion, several subsequent studies reported that infection with murine cytomegalovirus (mCMV) induces immunological memory independent of T and B cells (*31*–*34*). The protection in these models is mediated by NK cells, which proliferate and persist in lymphoid and nonlymphoid organs. Upon reinfection, these memory NK cells undergo a secondary expansion, rapidly degranulating and releasing cytokines, thus inducing a protective immune response (*31*). Additionally, NK cells have been shown to prime monocytes in the bone marrow during infection, and this may also induce long-term effects on innate immune responses (*35*).

In addition to experimental studies showing induction of innate immune memory in mice, emerging data suggest that similar trained immunity effects can be generated in humans.

First, a large number of epidemiological studies have shown nonspecific beneficial effects of live vaccines—such as BCG, measles vaccines, and oral polio vaccine—against infections other than the target diseases (*36*). The identification of these nonspecific (or heterologous) effects suggests that these vaccines induce trained immunity that protects against unrelated pathogens. This hypothesis was proposed in proof-of-principle trials with BCG vaccine in healthy adult volunteers (*37*) and was subsequently validated in clinical trials in newborn children vaccinated with BCG (*38*) or exposed in utero to hepatitis B vaccine (*39*). Second, certain infections such as malaria can also induce a state of hyperresponsiveness that is functionally equivalent to the induction of trained immunity (*40*, *41*). Finally, nonspecific protective effects through innate immunity-dependent mechanisms are provided by the use of BCG for treatment of malignancies such as bladder cancer (*42*), melanoma (*43*), leukemia (*44*), and lymphoma (*45*): Although direct inflammatory effects are probably important, long-term innate immune memory persisting between the BCG treatments is also likely to be involved. In this respect, Buffen *et al.* have recently suggested that these anticancer effects of BCG are directly dependent on the capacity to mount trained immunity, as individuals unable to mount trained immunity due to autophagy defects show a diminished recurrence-free survival after BCG treatment in bladder carcinoma (*46*).

Taken together, these complementary murine and human studies suggest that innate immune responses have the capacity to be primed or trained and thereby exert a new type of immunological memory upon reinfection, for which the term "trained immunity" has been proposed (Fig. 1).

Mechanisms responsible for mediating trained immunity

Innate immune cells that build innate immune memory

Innate immune memory properties have been described in several cell populations, including monocytes, macrophages, and NK cells. Preliminary observations suggest that similar characteristics may also be present in other cell types, such as ILCs or polymorphonuclear leukocytes. Unlike lymphocytes, innate immune cells do not express rearranging antigen receptor genes, but they do express PRRs and other receptors that allow them to recognize and respond to pathogen-derived structures [pathogen-associated molecular patterns (PAMPs)] and endogenous danger signals (damage-associated molecular patterns) (*47*, *48*). Although these responses are not specific to the degree conferred by antigen receptors, there is evidence to suggest that expression of distinct members of PRR families (e.g., TLRs, NOD-like receptors, C-type lectin receptors, RIG-I–like receptors, or combinations thereof) on macrophages and dendritic cells triggers different signaling pathways that lead to innate immune responses that are tailored to the particular type of pathogen encountered (*49*).

Among the various cell types implicated in innate immune memory, the major focus has been on monocytes, macrophages, and NK cells. We note that this attention does not necessarily mean that these cells are more amenable to training than other innate immune cells. Instead, this focus may merely reflect historical connection of these cells with lipopolysaccharide (LPS)–induced tolerance (LPS is a component of Gram-negative bacterial cell walls). Some of the earliest evidence that macrophages may exhibit memory-like features came from investigations of LPS-induced tolerance at the molecular level (*50*). In one such study, gene-specific chromatin modifications were associated with silencing of genes coding for inflammatory molecules while priming other genes coding for antimicrobial molecules (*50*). These findings suggested that macrophages could be primed by LPS to become more or less responsive to subsequent activation signals. This observation was expanded by studies demonstrating that exposure of monocytes and macrophages to *C. albicans* or β-glucan enhanced their subsequent response to stimulation with unrelated pathogens or PAMPs, a process termed "trained immunity" (*26*). Training was demonstrated to be accompanied by significant reprogramming of chromatin marks (*26*, *50*, *51*), as detailed further below. Besides bacterial and fungal pathogens, monocytes and macrophages can also mount trained immunity responses after infection with parasitic (*29*) and viral (*28*) pathogens.

Regarding trained immunity in monocytes, it is important to consider the life span of these cells. Monocytes are cells with a short half-life in circulation, with recent studies suggesting it to be up to 1 day (*52*). The observation that trained monocytes have been identified in the circulation of BCG-vaccinated individuals for at least 3 months after vaccination (*37*) suggests that reprogramming must take place at the level of progenitor cells in the bone marrow as well. Indeed, recent evidence has emerged to indicate that innate immune memory can be transferred via hematopoietic stem and progenitor cells. Macrophages derived from hematopoietic stem and progenitor cells rendered tolerant by TLR2 ligand exposure and transferred to irradiated mice retain a tolerant phenotype and produce lower amounts of inflammatory cytokines and reactive oxygen species in response to inflammatory stimulation (*53*). Furthermore, exposure of mouse skin to ultraviolet radiation induces immunosuppression that was originally attributed to defective T cell priming by dendritic cells (*54*) but was subsequently shown to involve epigenetic reprogramming and a long-lasting effect on dendritic cell progenitors in the bone marrow that altered the function of their differentiated progeny (*55*). In addition, recent studies have suggested that microbiota can induce long-term functional reprogramming of bone marrow progenitors—and, subsequently, dendritic cells—to protect against *Entamoeba histolytica* (*56*). Whether vaccines such as BCG automatically stimulate trained immunity and also confer or induce similar effects at the level of progenitor cells remains to be established.

Emerging evidence suggests that NK cells also respond more vigorously after a previous challenge. NK cell memory has been documented after exposure to cytokine combinations (e.g., IL-12, IL-15, and IL-18) (*32*) or hapten sensitization, which induced long-lived NK cells that mediate contact hypersensitivity and long-lived antigen-specific recall responses, independently of B and T cells (*30*). In addition, NK cells undergo expansion during virus infections, such as those with mCMV (*31*), influenza A (*57*), or vaccinia virus (*58*). Studies of CMV infection have shown that NK cell activation may provide T cell–independent protection against reinfection by rapidly degranulating and producing cytokines (*31*). Furthermore, adoptive transfer experiments have demonstrated that activated NK cells can proliferate in vivo and protect naïve recipient mice against virus infection, which suggests that they could confer protective immunological memory. The nonspecific protective effects of BCG infection have also been linked with activation of NK cells. NK cells from BCG-vaccinated individuals have enhanced proinflammatory cytokine production in response to mycobacteria and other unrelated pathogens, and studies in mice have shown that BCG confers nonspecific protection against *C. albicans*, at least partially through NK cells (*59*).

A number of mechanisms have been put forward that may mediate the memory properties of NK cells: some of them are responsible for induction of innate immune memory and others for the survival of the NK memory cells. The former include enhanced responsiveness of the IL-12/IFN-γ axis (*32*) or the activation of the costimulatory molecule DNAM-1 (DNAX accessory molecule-1, also known as CD226) on the membrane of the cells (*60*). However, survival of the memory NK cells during the contraction phase after mCMV infection necessitates mitophagy through an *Atg3*-dependent mechanism (*61*).

The issue of specificity of the NK memory immune responses is complex. Evidence that NK memory is specific was provided by the demonstration that, in the mouse, mCMV-induced NK cells protected against mCMV but not Epstein-Barr virus, another herpesvirus (*62*). Notably, mCMV impaired heterologous immunity against influenza and *L. monocytogenes* (*57*, *63*). Memory responses of NK cells toward other stimuli such as haptens and viruses also induced antigen-specific immune memory (*41*). Another important aspect concerns the mechanisms responsible for the persistence of NK memory cells. NK cell memory of haptens and viruses depends on CXCR6, a chemokine receptor on hepatic NK cells that is required for the persistence of memory NK cells but not for antigen recognition (*41*). In addition, recent studies revealed evidence of NK memory in primates: Splenic and hepatic NK cells from adenovirus 26–vaccinated macaques efficiently lysed antigen-matched but not antigen-mismatched targets 5 years after vaccination. These data demonstrate that robust, durable,

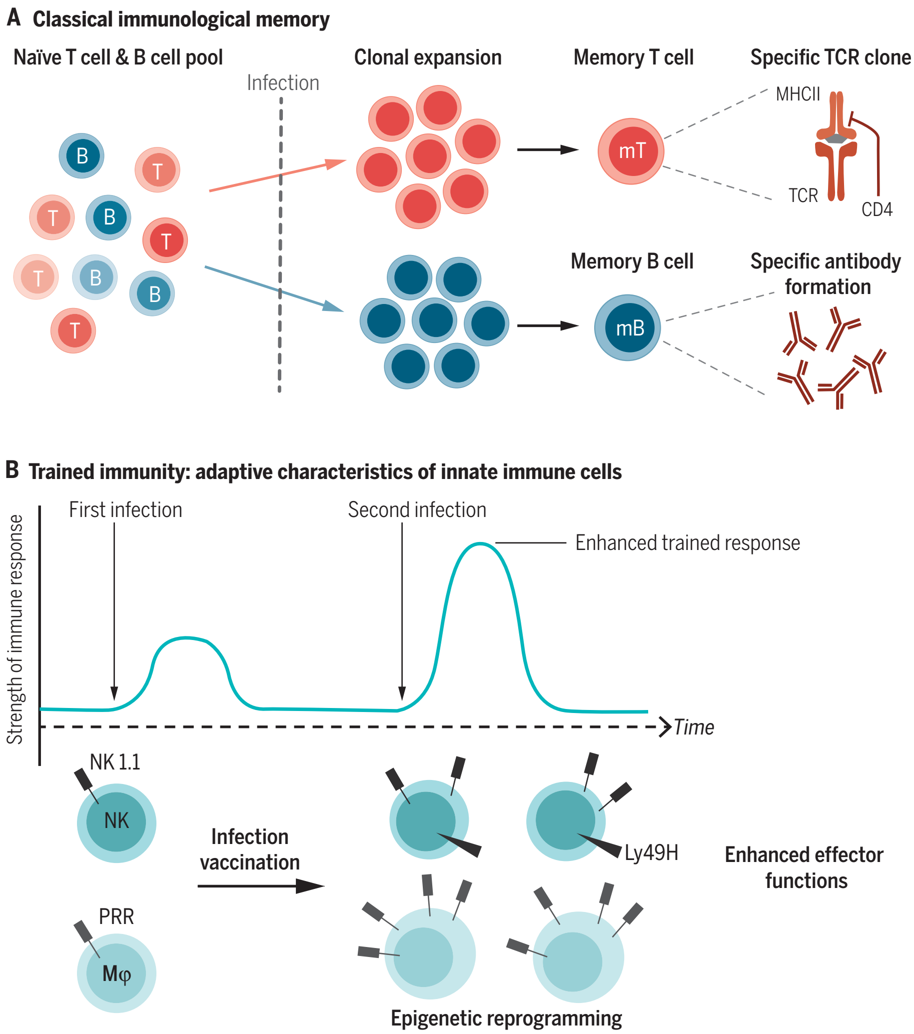

Fig. 1. Classical immunological memory versus trained immunity. (**A**) Classical adaptive immunological memory involves gene recombination in B and T lymphocytes, which confers high specificity and, very often, long-term, pathogen-specific protection (up to decades). (**B**) Trained immunity defines a de facto innate immune memory that induces enhanced inflammatory and antimicrobial properties in innate immune cells, resulting in an increased nonspecific response to subsequent infections and improved survival of the host. Mϕ, macrophage.

antigen-specific NK cell memory can be induced in primates after both infection and vaccination. This finding has important implications for the development of vaccines against HIV-1 and other pathogens (*64*).

In addition to studies showing antigen-specific mechanisms of NK cell immune memory, other recent investigations have suggested that memory in NK cells is also mediated by epigenetic changes. In a study in patients recovering from CMV virus infection, the DNA methylation patterns of NK cells and cytotoxic T cells were similar, and very different from those of canonical NK cells. Subsequently, the capacity of these adaptive NK cells to secrete cytokines was modulated, and this was dependent on the transcription factor promyelocytic leukemia zinc finger (PLZF) (*34*, *65*) Similarly, another study showed that these memory-like NK cells are defective in the Syk-dependent stimulation pathway, which is correlated with epigenetic changes at the level of the gene promoter (*60*).

Taken together, the published data suggest that NK immune memory is complex and may display aspects of both antigen-dependent (and, in certain circumstances, antigen-specific) memory and epigenetic reprogramming as seen in trained immunity.

The molecular basis of trained immunity: Transcriptional and epigenetic reprogramming

A distinguishing feature of the trained innate immune cell is its ability to mount a qualitatively different—and to some extent quantitatively stronger—transcriptional response compared with untrained cells when challenged with pathogen or danger signals. The molecular bases of such enhanced activation of a subset of inflammatory genes are only partially defined, but evidence supports the convergence of multiple regulatory layers, including changes in chromatin organization and the persistence of microRNAs (miRNAs) induced by the primary stimulus.

In myeloid cells, many loci encoding inflammatory genes are in a repressed configuration (*66*–*68*), as inferred by their limited accessibility to nucleases (used as tools to probe chromatin structure), the low acetylation of the nucleosomal histones, and the very low amount of RNA polymerase II loaded onto both the coding body of the genes and the genomic regulatory elements (enhancers and promoters) that control their expression (*69*). Upon primary stimulation, the changes observed at these loci, in terms of gain in chromatin accessibility, increased histone acetylation and RNA polymerase II recruitment, are massive, and are of magnitudes not commonly observed in other responses to microenvironmental changes. These considerable alterations—which, in some cases, result in the activation of gene expression that is hundreds of times higher than baseline levels in a short window of time—are driven by the recruitment of stimulation-responsive transcription factors (e.g., NF-κB, AP-1, and STAT family members) to enhancers and gene promoters, which are usually premarked by lineage-determining transcription factors such as PU.1 (*70*–*73*). In turn, transcription factors control the recruitment of coactivators (including histone acetyltransferases and chromatin remodelers) (*67*, *68*) that locally modify chromatin to make it more accessible to transcriptional machinery.

Maintenance of such enhanced accessibility may underlie the more efficient induction of genes primed by the initial stimulation (*50*). Moreover, because histone modifications are specifically bound by recognition domains contained in various proteins implicated in transcriptional control (as in the case of the bromodomain–acetyl lysine interaction) (*74*), the persistence of histone modifications deposited at promoters or enhancers after the initial stimulus may itself affect the secondary response (*26*). The possible contribution of chromatin modifications to trained immunity must be examined while accounting for the different stability of individual covalent chromatin modifications, with more stable modifications (e.g., histone methylation) being potentially more suitable to perpetuate a functional change than those with a typically short half-life (e.g., histone acetylation). Therefore, the observed long-term persistence of some histone modifications in myeloid cells after removal of the initial activation stimulus may reflect either their stability or, alternatively, the sustained activation of the upstream signaling pathways and transcription factors that control their deposition.

One interesting paradigm is provided by latent or de novo enhancers (*75*, *76*), which are genomic regulatory elements that are epigenetically unmarked or marked at low levels in unstimulated cells but gain histone modifications characteristic of enhancers [such as monomethylation of histone H3 at K4 (H3K4me1)] only in response to specific stimuli. In vitro, upon removal of the stimulus that triggered their functionalization, a fraction of latent enhancers retain their modified histones and can undergo a stronger activation in response to restimulation (Fig. 2). This observation is reminiscent of the fact that in vivo macrophages acquire repertoires of active enhancers that are largely instructed by the microenvironmental signals specific to a given tissue and are thus substantially different, depending on the organ in which a macrophage is located (77, 78). In turn, such signals act by specifically inducing regulation by distinct combinations of transcription factors that are eventually responsible for the activation of different sets of genes mediated by epigenetic modifying enzymes. Transferring macrophages from one tissue to another results in an extensive reprogramming of the enhancer repertoire (78). Therefore, a complex equilibrium exists between mechanisms that promote the persistence of the modified epigenome instructed by the previously encountered stimuli and mechanisms that reprogram it in response to a changing environment. The very same dynamic equilibrium probably underlies the persistence of chromatin states that are relevant to enhanced transcriptional responses in trained immunity.

Recent studies have investigated the changes in epigenomic programs in innate immune cells during the induction of trained immunity. One early study proposed that changes in epigenetic status underlie the repression of inflammatory genes during LPS tolerance. However, genes mainly involved in antimicrobial responses were either normally produced or even displayed an increased production capacity (50). The repression of inflammatory mediator production and the potentiation of antimicrobial proteins synthesis were accompanied by histone-repressive or -activating marks, respectively. Similarly, exposure of monocytes and macrophages to *C. albicans* or β-glucan modulated their subsequent response to stimulation with unrelated pathogens or PAMPs, and the changed functional landscape of the trained monocytes was accompanied by epigenetic reprogramming (26, 51). Pathway analysis identified important immunological (cAMP-PKA activation) and metabolic (aerobic glycolysis) pathways that play crucial roles in the induction of trained immunity (51, 79). Additionally, a recent study showed that both LPS and β-glucan induce trained immunity through a mitogen-activated protein kinase (MAPK)–dependent pathway that phosphorylates the transcription factor ATF7, subsequently reducing the repressive histone mark H3K9me2 (80). Moreover, the immunological networks activated in trained monocytes depend on STAT1 activation (80), and the importance of STAT1 for the induction of trained immunity is supported by the defects in trained immunity reported in patients with chronic mucocutaneous candidiasis due to *STAT1* mutations (81).

BCG vaccination has also been shown to result in an increase in inflammatory mediators produced by monocytes from healthy volunteers, which correlated with parallel changes in a histone modification associated with gene activation (37). Similar to observations for monocytes and macrophages, the induction of CMV-induced NK cell memory at least partially relies on epigenetic reprogramming, which is linked to reduced expression of PLZF (34) and the tyrosine kinase SYK (65). Human CMV also drives epigenetic priming of the *IFNG* locus in NK cells, which "tags" the gene and leads to consistent IFN-γ production in a subset of NK cells, providing a molecular basis for the adaptive feature of these cells (82). The epigenetic machinery of the immune system may also be hijacked by certain bacterial pathogens, such as *L. monocytogenes* (83), and this may represent a more general mechanism of escape from host defense (84, 85).

microRNAs may also contribute to trained immunity (86), mainly because of the reportedly long half-life of these molecules (87) that, combined with the limited proliferative ability of myeloid cells, would result in their persistence after removal of the primary stimulus. Among miRNAs, miR-155 may have particular relevance because its up-regulation in response to inflammatory signals (such as microbial components) is associated with the hyperactivation of myeloid cells, possibly due to the derepression of phosphatases that negatively regulate transducers of several signaling pathways (88). It is reasonable to predict that myeloid cells expressing miR-155 in a sustained manner would remain in a primed, hypersensitive state: Upon exposure to a secondary stimulus of identical strength, these cells could respond in an enhanced manner compared with their response to the primary stimulation.

Although the discussion above addresses the role of epigenetic programing as a mechanism for mediating innate immune memory, one crucial aspect remains unknown: What cellular processes induce and maintain these epigenetic changes? There is increasing evidence to suggest that rewiring of cellular metabolism is involved, with a role for metabolites as cofactors for enzymes involved in epigenetic modulation of gene transcription.

Immunometabolic circuits: The role of cellular metabolites for shaping the epigenetic program of trained innate immune cells

Recent work revealed extensive rewiring of metabolic pathways in different immune cells upon activation (89). The best example concerns macrophages, where the M1 phenotype (i.e., macrophages activated with LPS and IFN-γ, producing mainly inflammatory cytokines) and M2 phenotype (macrophages activated by IL-4–related cytokines and expressing genes involved in tissue repair) use distinct metabolic pathways (90, 91). M1 macrophages are largely glycolytic, with impairment of oxidative phosphorylation and disruption of the Krebs cycle at two steps: after citrate and after succinate. Citrate is withdrawn for fatty acid biosynthesis (which enables the increased production of inflammatory prostaglandins), whereas succinate activates the transcription factor HIF1α, which regulates a wide range of genes, including the one encoding the inflammatory mediator IL-1β (90, 91). In M2 macrophages, the Krebs cycle is intact. A key feature is the synthesis of uridine diphosphate

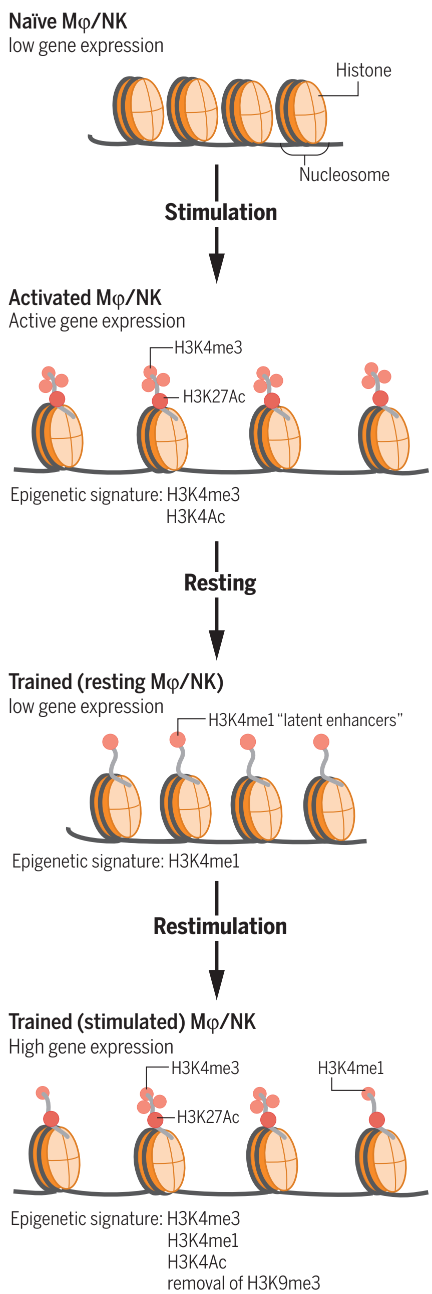

Fig. 2. Epigenetic rewiring underlies the adaptive characteristics of innate immune cells during trained immunity. Initial activation of gene transcription is accompanied by the acquisition of specific chromatin marks, which are only partially lost after elimination of the stimulus. The enhanced epigenetic status of the innate immune cells, illustrated by the persistence of histone marks such as H3K4me1 characterizing "latent enhancers," results in a stronger response to secondary stimuli upon rechallenge.

N-acetylglucosamine from glucose and glutamine, which is needed for the extensive glycosylation occurring in receptors such as mannose-binding lectin, which are hallmarks of the M2 phenotype (*91*).

The importance of cellular metabolism for macrophage programming suggests that similar mechanisms may play a role for the long-term functional changes in monocytes and macrophages during trained immunity. In line with this, an important role for a shift from oxidative phosphorylation toward glycolysis through an Akt/mTOR/HIF-1α–dependent pathway has recently been reported to be essential for trained immunity induced by β-glucan (*51*, *79*). Whether and how this shift influences epigenetic processes in trained immunity is still under investigation, but important clues have been provided by studies linking chromatin regulation to intermediary metabolism (*92*, *93*). In this respect, a critical metabolic intermediate that is increased in trained monocytes (acetyl–coenzyme A) is required for histone acetylation. Additionally, the ratio of the Krebs cycle metabolites α-ketoglutarate and succinate is a critical determinant for the activity of two families of enzymes controlling epigenetic modifications: the JMJ family of lysine demethylases and the TET family of methyl-cytosine hydroxylases (*51*, *94*). These enzymes require α-ketoglutarate as a cofactor, whereas succinate limits their activity (Fig. 3). An additional possibility for innate immune memory may be that stimulation of macrophages causes an elevation in the level of succinate; this would then inhibit JMJD3, leading to enhanced H3K27 trimethylation of particular genes (e.g., those associated with the M2 phenotype), thus suppressing their expression (*95*). This process would maintain a proinflammatory phenotype of trained macrophages upon restimulation. Important links between altered metabolites and epigenetic changes have also been demonstrated in LPS-induced tolerance, in which nicotinamide adenine dinucleotide–dependent activation of class III histone deacetylases (sirtuins) functions with sirtuin-1 and sirtuin-6 in coordinating a switch from glucose to fatty acid oxidation (*96*). The remaining challenges are to explain how these potentially nonspecific functions of metabolites could have locus- and/or gene-specific effects and to provide direct evidence for metabolites altering the activity of enzymes that modify DNA and histones during trained immunity.

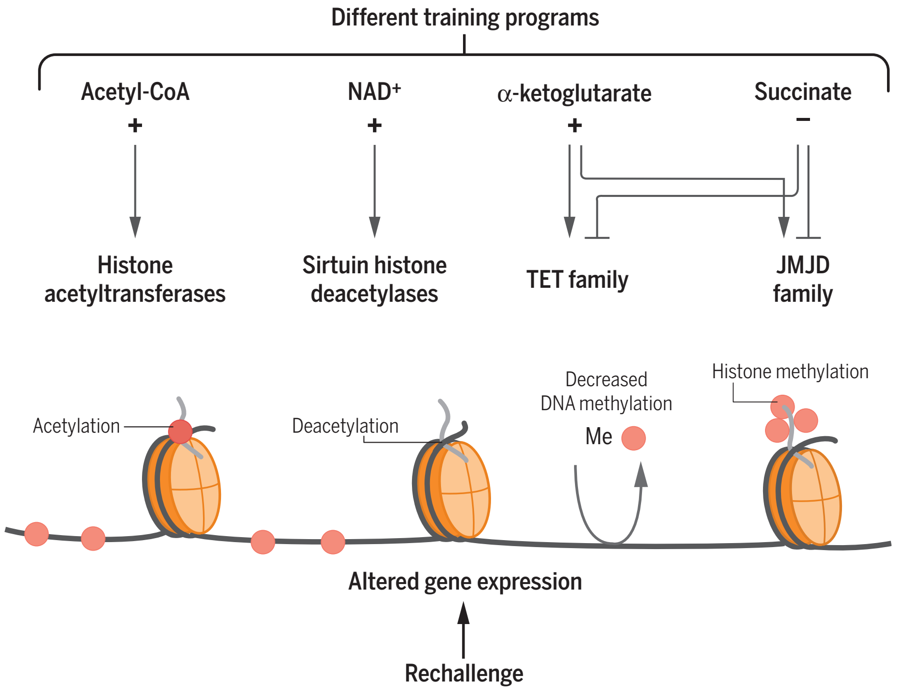

Fig. 3. Stimulation of innate immune cells with training stimuli induces changes in cellular metabolism. Various metabolites function as cofactors for epigenetic enzymes, which in turn induce chromatin and DNA modifications, modulate gene transcription, and result in different trained immunity programs. CoA, coenzyme A; NAD$^+$, nicotinamide adenine dinucleotide; Me, methyl.

Adaptive and maladaptive programs

As described above, trained immunity most likely evolved as a primitive form of immune memory, aimed to provide improved protection of the host against reinfection, with beneficial effects for survival. It is also likely that trained immunity plays an important role in ontogeny, enabling the maturation of the innate immune system of the newborn (*97*), a process in which microbiota plays an important role (*98*). In line with the notion that microbiota might influence the functional program of immune cells, a recent study showed increased H3K4me3 in NK cells from conventionally housed mice compared with germ-free animals (*99*). However, there may also be situations in which reprogramming of innate immunity and increased inflammatory responses to exogenous or endogenous stimuli could have deleterious effects.

Several pathological conditions have been described in which innate immune reprogramming may have adverse effects. During LPS-induced tolerance, reprogramming of innate immune cells probably plays a beneficial role in maintaining a relatively high threshold of cellular activation in organs where LPS naturally occurs at physiological levels, such as in the gastrointestinal tract (*50*). In contrast, in the case of systemic activation of innate immune cells during sepsis, LPS-induced tolerance can contribute to immune paralysis, placing the individual at greater risk for opportunistic infections (*100*). Persistent silencing of important host defense genes, possibly due to epigenetic mechanisms, has been proposed to mediate these effects (*101*, *102*). Hence, maladaptive responses that inappropriately affect cell populations, such as systemic monocytes (as opposed to local tissue-resident macrophages), can have detrimental effects for the host.

Deleterious systemic consequences of trained immunity have also been documented. In general, trained immunity is an adaptation that results in the long-lasting capacity to respond more strongly to stimuli (*36*). Although this type of high-alert immune state has beneficial effects during host defense, it could also trigger enhanced tissue damage during chronic inflammatory conditions in which trained immunity is induced by endogenous ligands of innate receptors. For example, there is strong epidemiological evidence for an increased susceptibility of atherosclerosis in patients with autoimmunity or chronic inflammatory conditions such as rheumatoid arthritis (*103*). It is tempting to speculate that the maladaptive state of innate immune cells triggered by the underlying chronic inflammatory condition would change the local immune responsiveness of immune cells in atherosclerotic lesions and that this could contribute to increased disease risk (*104*). It is also possible that Western-type diets, which are known to trigger systemic inflammatory responses, can precipitate maladaptive trained immune responses. A strong argument for this hypothesis is the recent demonstration of trained immunity induced by oxidized low-density lipoprotein in human monocytes via epigenetic reprogramming (*105*). Furthermore, this type of maladaptation of innate immune cells could be a culprit for other common inflammatory diseases prevalent in Western societies, such as type 2 diabetes or Alzheimer's disease. In diabetes, a bout of hyperglycemia can result in long-term deleterious effects, a process termed "hyperglycemic memory." This condition is accompanied by sustained NF-κB activation by increased H3K4me1 and decreased H3K9me3 at selected genes (*106*).

The data presented above indicate that the adaptive ability of innate immune cells to tune their responses to changing environments appears to be an important feature that evolved to prepare innate immune cells for unpredictable events, such as invading pathogens. However, the epigenetic mechanisms that control the memory of the environmental trigger may also lead to persistence of disease-associated phenotypes. Hence, altering the changed epigenetic landscape by pharmacologic means or behavioral changes could be a promising strategy to restore homeostatic healthy gene expression patterns.

Trained immunity: A modified steady-state of innate immunity after infection

In this Review, we reappraised the various arguments pointing to the presence of innate immune memory in plants, lower animals, and vertebrates. We defined trained immunity as a nonspecific immunological memory resulting from rewiring the epigenetic program and the functional state of the innate immune system, eventually resulting in protection against secondary infections. We also compared data assessing the mechanisms of tolerance and trained immunity. However, one important question remains: Are tolerance and training two fundamentally divergent functional programs, or do they represent different facets of the same phenomenon?

Considering the traditional appraisal of the effects of tolerance as a hypoinflammatory state and trained immunity resulting in an increased production of proinflammatory cytokines, these two programs may seem to be functional opposites. However, one must consider the evidence carefully: Whole-genome transcriptional and epigenetic analyses have clearly demonstrated that while in the process of LPS-induced tolerance, many proinflammatory genes are down-regulated, and others are not modified or even up-regulated (*50*). Similarly, the assessment of the trained immunity program induced by β-glucans also shows that it contains both up- and down-regulated genes (*51*). Thus, both tolerance and training evidently represent manifestations of long-term epigenetic reprogramming of the innate immune system after encountering an infection or a microbial ligand.

A crucial aspect of trained immunity that needs further investigation is its duration. In vitro studies of monocytes and macrophages have demonstrated long-term memory effects lasting days (*26*, *75*), whereas experimental studies have reported effects that extended for weeks (*26*, *107*). Epidemiological studies on the nonspecific effects of vaccines such as BCG or measles have suggested positive effects on susceptibility to infections, lasting for months and even years (*36*), although it is highly unlikely for this protection to be as long-lived as classical immunological memory. These data are supported by proof-of-principle studies demonstrating the presence of trained immunity effects on circulating monocytes of volunteers for 3 months and even 1 year after vaccination with BCG (*108*). This would imply effects of vaccination on bone marrow progenitors as well, as pointed out earlier. More studies are warranted to better describe the duration of trained immunity effects after infection and vaccination.

Conclusions and future directions for research

The arguments presented above suggest that trained immunity is a fundamental property of host defense in the mammalian immune response. Whereas classical immunological memory mediated by T and B lymphocytes is specific and antigen-dependent, with antigen specificity being mediated by gene rearrangement in specific lymphocyte clones that undergo expansion and contraction, trained immunity (innate immune memory) is nonspecific and mediated through epigenetic reprogramming in myeloid cells or NK cells. An important difference between classical immunological memory and trained immunity also concerns the persistence of the effects: Memory within trained immunity has a shorter duration than classical adaptive immune memory.

Much remains to be learned in this exciting new field over the coming years. First, the molecular mechanisms that mediate trained immunity should be elucidated at the level of the cell types involved, and the immunological, metabolic, and epigenetic processes mediating it need to be unraveled further. It will be also important to delineate the duration of innate immune memory and its effect on the innate immune cell precursors in the bone marrow and tissue macrophage populations. Second, the fast progress of cutting-edge technologies such as single-cell transcriptomics and epigenomics—in particular, DNA methylation—will permit the identification of the potential novel subpopulations of cells that are prone to displaying innate immune memory characteristics. This will enhance our understanding of immunological processes and open up possibilities for new therapeutics that target specific cell subpopulations. Third, future research should explore the effect of trained immunity on disease: its role in diseases with impaired host defense, such as postsepsis immune paralysis or cancers, as well as its role in autoinflammatory and autoimmune diseases in which maladaptive programs may be in place.

Finally, the concept of innate immune memory has considerable potential for aiding in the design of novel therapeutic approaches, with at least three potential lines of investigation: (i) the design of new-generation vaccines that combine adaptive and innate immune memory, as recently proposed with a novel *Bordetella pertussis* vaccine (*109*); (ii) the use of inducers of trained immunity for the treatment of immune paralysis, such as the muramyl dipeptide preparation mufamurtide for osteosarcoma (*110*) or β-glucan in various cancer types (*111*); and (iii) the modulation of the potentially deleterious consequences of trained immunity in autoinflammatory diseases (e.g., the potential use of the recently described iBET inhibitors). Only when these investigations are accomplished will the discovery of trained immunity reach its full therapeutic potential.

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Trained immunity: A program of innate immune memory in health and disease

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Training immune cells to remember

Classical immunological memory, carried out by T and B lymphocytes, ensures that we feel the ill effects of many pathogens only once. Netea *et al.* review how cells of the innate immune system, which lack the antigen specificity, clonality, and longevity of T cell and B cells, have some capacity to remember, too. Termed ''trained immunity,'' the property allows macrophages, monocytes, and natural killer cells to show enhanced responsiveness when they reencounter pathogens. Epigenetic changes largely drive trained immunity, which is shorter lived and less specific than classical memory but probably still gives us a leg up during many infections.

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