ANNUAL REVIEWS

Annual Review of Virology

Shifting the Immune Memory Paradigm: Trained Immunity in Viral Infections

Esther J.M. Taks,[1] Simone J.C.F.M. Moorlag,[1] Mihai G. Netea,[1,2] and Jos W.M. van der Meer[1]

[1]Department of Internal Medicine and Radboud Center for Infectious Diseases, Radboud University Medical Centre, Nijmegen, Netherlands; email: Jos.vanderMeer@radboudumc.nl

[2]Department of Immunology and Metabolism, Life and Medical Sciences Institute, University of Bonn, Bonn, Germany

Annu. Rev. Virol. 2022. 9:469–89

First published as a Review in Advance on June 8, 2022

The *Annual Review of Virology* is online at virology.annualreviews.org

https://doi.org/10.1146/annurev-virology-091919-072546

Keywords

trained immunity, innate immune memory, viral infection, epigenetics, vaccination, functional reprogramming

Abstract

Trained immunity is defined as the de facto memory characteristics induced in innate immune cells after exposure to microbial stimuli after infections or certain types of vaccines. Through epigenetic and metabolic reprogramming of innate immune cells after exposure to these stimuli, trained immunity induces an enhanced nonspecific protection by improving the inflammatory response upon restimulation with the same or different pathogens. Recent studies have increasingly shown that trained immunity can, on the one hand, be induced by exposure to viruses; on the other hand, when induced, it can also provide protection against heterologous viral infections. In this review we explore current knowledge on trained immunity and its relevance for viral infections, as well as its possible future uses.

THE INITIATION OF INNATE HOST DEFENSE AGAINST VIRAL INFECTIONS

Despite the major advancements in science and medicine since the discovery of viruses in the late nineteenth century, viral infections still account for a large burden of morbidity and mortality worldwide. Many questions remain about the antiviral host defense mechanisms and specifically the role of the innate immune system for protection against viral infections. An increased understanding of the innate host defense against viruses can provide exciting new approaches to viral infectious diseases. This review highlights the role of the innate immune system in the antiviral immune response and elaborates on how memory characteristics of innate immune cells, also called trained immunity, can aid an adequate defense against viruses.

Upon exposure to a viral pathogen, the innate immune system is the first line of defense. After successfully passing through the first barriers including the skin and mucous membranes, viruses come into contact with innate immune cells such as myeloid cells and later natural killer (NK) cells. Pathogen-associated molecular patterns (PAMPs), such as viral nucleic acids, are recognized by pattern recognition receptors (PRRs) on these cells. Several families of PRRs are involved in innate antiviral responses including Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), C-type lectin receptors (CLRs), inflammasomes, and DNA sensors (1). Interaction of PAMPs with these PRRs leads to the generation of various antiviral effectors, most importantly the production of type-I interferons (IFNs).

Because viruses are obligate intracellular pathogens, recognition mostly occurs within the cell in endosomes. Specifically, TLR3, TLR7/8, and TLR9 are involved in the recognition of viral nucleic acids in humans. Activation of these receptors results in MyD88-dependent nuclear factor kappa B (NF-κB) and interferon response factor 7 (IRF7) activation and Toll/interleukin (IL)-1 receptor domain-containing adaptor inducing IFN-β (TRIF)-dependent type-I IFN production. However, there is evidence that various viral envelope proteins can also be sensed by surface TLRs such as TLR2/6 and TLR4, while subsequently using signaling molecules such as MyD88 to activate NF-κB (2–4). The production of type-I IFNs is the result of cell activation by the various recognition receptors present on both immune and nonimmune cells (e.g., epithelial cells). The induction of signaling pathways by these cytokines, such as the Janus kinase signal transducer and activator of transcription proteins (JAK-STAT) pathway, results in an antiviral state mediated by interferon-stimulated genes (ISGs).

Recognition of viruses can also occur through activation of the inflammasomes, such as the AIM2 inflammasome and the NLRP3 inflammasome (5, 6). Their key role is the cleavage of procaspase-1 to caspase-1 that subsequently leads to cleavage of inactive precursors into active IL-1β, IL-18, and gasdermin D. IL-1β recruits neutrophils to the infected cell, and both IL-1β and IL-18 activate the adaptive immune response mediated by helper and cytotoxic T cells. Especially the NLRP3 inflammasome plays an important role in the antiviral response, as it recognizes viral components and related damage, and it inhibits viral replication upon activation (7).

NK cells are lymphoid cells of the innate immune system, although they lack specific antigen receptors. They are particularly important in the defense against viruses, as they act much faster than cytotoxic T lymphocytes to kill infected cells. Their activation is dependent on activating receptors known as immunoreceptor tyrosine-based activating motifs (ITAMs) and inhibitory receptors, also known as ITIMs, in which the binding of the inhibitory receptor generally dominates the activating receptor. NK cells have two cytotoxic proteins, perforins and granzymes, that are able to kill specific target cells. Additionally, type-I IFNs mediate the production of interferon gamma (IFN-γ) by NK cells. IFN-γ boosts the adaptive immune response and biases it toward a cytotoxic response, aiding in the destruction of intracellular pathogens.

In summary, the first steps in the host defense against viruses involve the activation of various innate pathways leading to the killing of infected cells, the production of inflammatory cytokines, and the induction of the antiviral state in uninfected cells. Furthermore, the activation of the innate immune system initiates the adaptive immune response.

THE CLASSICAL VIEW OF IMMUNE MEMORY

Because of the memory characteristics of the immune system, the host immune response improves upon secondary infection. Immune memory has previously been defined as the state of the immune system induced by infection or vaccination that confers an improved, faster, more efficient immune response upon secondary exposure. It is generally associated with activation of the adaptive immune system resulting in the formation of specific antibodies and T lymphocytes that are able to react very rapidly and efficiently to neutralize the pathogen. Neutralizing antibodies have been associated with protective immunity post infection or vaccination, and they are often used as correlates of protection (8). A stronger and more rapid production of antibodies has therefore been targeted as the basis for successful immunization and vaccination (9). However, induction of potent memory cellular responses, which are T cell mediated, is gaining increased interest and is a crucial component of protection after vaccination (10).

After encountering an antigen, B cells differentiate into antibody-secreting plasma cells, with the help of T helper cells. T helper cells can recognize antigen on B cells and induce their differentiation. Upon activation, the antibody-secreting cells secrete antibodies, which are the soluble form of the specific B cell membrane receptors that were able to bind to the antigen. The functions of antibodies are the neutralization of viral particles and toxins, the opsonization of viral particles to enhance uptake by phagocytic cells, and the activation of the complement system. Upon secondary encounter with the same antigen, the antibody response is faster and more efficient by producing more antibodies with higher affinity through clonal expansion of the particular B cells. Besides antibodies, the adaptive immune system also has a memory response through memory T cells. After an antigen has been cleared, most T cells that have proliferated during the active phase of the infection will die. However, a remainder of T cells will differentiate into central, peripheral, resident, and effector memory T cells. These cells can be present lifelong independent of antigen exposure. When challenged, memory T cells also undergo clonal expansion, resulting in an improved cellular immune response.

While antigen-specific immune memory based on B and T cells is a crucial component of host defense, increasing evidence during the last decade has demonstrated that certain infections and vaccines can induce heterologous protection toward pathogens different from those causing the initial infection. One explanation for such heterologous responses is cross-reactivity, which is when specific receptors on lymphocytes also recognize antigens that are molecularly similar to the targeted antigen. Heterologous lymphocyte responses upon activation by cytokines released by innate immune cells can also contribute to nonspecific protection against infections (11). However, in this review we focus on the property of innate immune cells to build adaptive characteristics, a de facto innate immune memory termed trained immunity (12) (**Figure 1**), and how these responses can be elicited by viral pathogens or protect against viral infections.

TRAINED IMMUNITY: THE CONCEPT OF INNATE IMMUNE MEMORY

Several lines of evidence suggest that innate immune responses develop memory characteristics after viral infections (13). Here we outline the evolutionary, epidemiological, and experimental arguments.

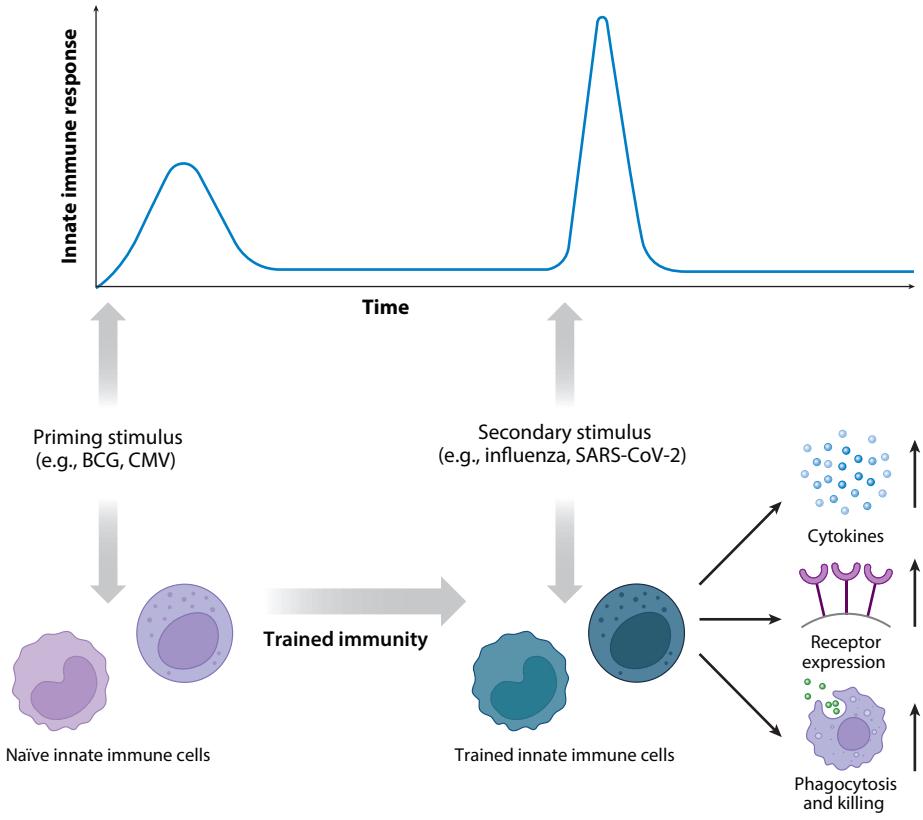

Figure 1

The effect of trained immunity. Trained innate immune cells respond more robustly and faster upon secondary stimulation. Training results in increased cytokine production, increased receptor expression, and enhanced phagocytosis and killing. Abbreviations: BCG, Bacillus Calmette-Guérin; CMV, cytomegalovirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2. Figure adapted from images created with BioRender.com.

Evolutionary Background

Only vertebrate animals have lymphocytes that can exert a classical adaptive immune response. Plants and invertebrates do not possess such an adaptive immune system; however, upon secondary exposure to pathogens, they do show enhanced host defense, a direct demonstration that innate immunity can adapt after an infection. In plants, this memory-like state is referred to as systemic acquired resistance (SAR) and has also been shown to be induced by viral infections (14). Inoculation of leaves of the tobacco plant *Nicotiana tabacum* with diluted tobacco mosaic virus (TMV) leads to a resistance upon secondary challenge with the same pathogen (14). This effect was seen not just in the inoculated leaves but also in other parts of the plant. Such an enhanced response to secondary infection can be explained only by the presence of memory-like characteristics in its innate immune response. It has been shown that other plant infections are accompanied by epigenetic changes such as chromatin remodeling, DNA methylation, and RNA silencing (15, 16). There is even evidence that these alterations can be inherited by their progeny, leading to transgenerational SAR (17).

Similarly, invertebrate animals do not possess lymphocytes and a classical adaptive immune system. However, upon infection, the mealworm beetle *Tenebrio molitor* displays a prolonged antimicrobial response that could provide protection against reinfection (18, 19). In *Aedes aegypti* mosquitoes, priming with inactivated dengue virus during larval stages results in reduced viral load when they are exposed to dengue virus as adults through RNA interference (RNAi) (20). Additionally, after exposure to RNA viruses, these mosquitoes have a memory-like immune response that is carried on to their offspring and is referred to as transgenerational immune priming (TGIP). There is convincing evidence that epigenetic mechanisms and RNAi play a major role in this protection and are nonspecific (21).

The presence of memory characteristics in organisms that have only an innate immune system indicates that the adaptive immune system is not, as generally thought, solely responsible for memory responses in vertebrates.

Epidemiological Background

Already in the early days of vaccination, evidence emerged that some vaccines provide more protection than the protection offered against the specific pathogen for which they were developed. Epidemiological studies have shown that especially live-attenuated vaccines have nonspecific effects, meaning that vaccinated individuals are better protected against infectious diseases other than the one they were vaccinated against. Although initially these nonspecific effects were largely attributed to socioeconomic aspects and increased health-seeking behavior of vaccinees, they were later demonstrated in randomized clinical trials to be true biological effects (22). Subsequently, innate immune memory provides an immunological explanation for this broader protection against infections.

The first evidence of this enhanced protection against heterologous infections by vaccines came from studies with Bacillus Calmette-Guérin (BCG), a vaccine containing an attenuated strain of *Mycobacterium bovis* and originally developed to protect against tuberculosis. In epidemiological studies of its efficacy as early as 1931, it was already seen that there was significantly less mortality among vaccinated children compared to those who were not vaccinated (23, 24). This difference could not be fully explained by the protection against tuberculosis alone and was found to be due to a decrease in death from neonatal sepsis and respiratory tract infections (24).

The Effects of Viral Vaccines

These beneficial effects were not limited to the bacterial vaccine BCG; after routine vaccination with various live-attenuated viral vaccines, reduced overall mortality was also reported. Decades after routine *Vaccinia* vaccination, the vaccine that was used to prevent and ultimately eradicate smallpox, had been implemented in Guinea-Bissau, an observational study showed a reduced mortality ratio of 0.60 (0.41–0.87) in adults with Vaccinia scars (25, 26). Measles virus–containing vaccines, such as the measles-mumps-rubella (MMR) vaccine, were shown to decrease childhood mortality (27). Other observational studies confirmed this effect and reported a reduced mortality rate of 0.51 (0.27–0.98) (28). The most pronounced differences in mortality were seen in death due to pneumonia and malaria. A recent meta-analysis by a World Health Organization Strategic Advisory Group of Experts confirmed the protective effect of MMR and BCG vaccines against mortality in children beyond their target diseases (29).

During a shortage of diphtheria-tetanus-pertussis (DTP) vaccine in Guinea-Bissau, the effect of the simultaneously given oral polio vaccine (OPV) was analyzed by looking at the case-fatality rate in a pediatric ward. It showed that children who had received OPV only, instead of OPV and DTP, had a lower case fatality rate (30). Other epidemiologic studies in Guinea-Bissau have

confirmed this observation when looking at child mortality rates in OPV-vaccinated and/or DTP-vaccinated children before they received the measles vaccine (31). It has been hypothesized that DTP, an inactivated vaccine, may display inhibitory effects on the beneficial nonspecific effects seen after live-attenuated viral vaccines such as OPV, MMR, and *Vaccinia*. Currently we do not know whether live organisms are essential to attain these effects; in fact, we know that inanimate compounds such as beta-glucans (β-glucans), oxidized low-density lipoproteins, and monosodium urate crystals are also able to induce trained immunity (32, 33).

These studies show that individuals vaccinated with certain vaccines have a more general protection against infectious diseases. The specific memory induced by the adaptive immune system cannot explain these findings due to its antigen specificity, and trained immunity that induces a nonspecific memory response upon exposure to pathogens explains these effects.

Experimental Background

Several murine studies on viral infections have explored this broader memory response. Mice latently infected with murine gammaherpesvirus 68 or murine cytomegalovirus (MCMV) were found resistant to *Listeria monocytogenes* and *Yersinia pestis* through elevated production of tumor necrosis factor-α (TNF-α) and IFN-γ by macrophages for at least 3 months (34). Similarly, when NK cells are exposed to MCMV memory, NK cells are generated that are able to exhibit an enhanced inflammatory response 70 days later when they are restimulated with antibodies that bind to the NK1.1 or Ly49H receptor (35). Most of these results seem to be due to the increased proinflammatory cytokine production that is characteristic of trained immunity upon restimulation; an increase in IFNs in vaccinated animals is considered to contribute to these effects.

Similar mechanisms seem to be responsible for protection by trained immunity against other viral infections. Mice treated with N^{α}-acetylmuramyl-l-alanyl-d-isoglutaminyl-N^{ε}-stearoyl-l-lysine [muramyl dipeptide (MDP)-Lys(L18)] appear to be resistant to Sendai virus and show increased IFN production on the first day after this viral infection (36). Macrophages from BCG-vaccinated mice are better able to reduce influenza A viral titers compared to control animals, and this leads to reduced inflammation and less pulmonary injury (37). BCG vaccination also improves resistance to encephalomyocarditis and to infection with herpes simplex virus (HSV) type 1 (38). Likewise, the vaccine improves the survival rate of newborn mice infected with HSV-2 (39). When exposed to ectromelia virus, mice vaccinated with BCG have a lower mortality than control animals, and they produce significantly more IFN in the peritoneum and the spleen (40).

In in vitro studies with human cells, the underlying mechanisms for this nonspecific induction of resistance have been investigated. Monocytes primed with Vaccinia virus and restimulated with bacterial pathogens show increased production of IL-6 and TNF-α. In contrast, cells exposed to MVA85A (a tuberculosis vaccine consisting of a recombinant strain of modified Vaccinia virus expressing *Mycobacterium tuberculosis* antigen 85A) become tolerant, with a decreased production of IL-6 and TNF-α upon restimulation. The immune tolerance induced by MVA85A is also likely to be caused by epigenetic reprogramming, as it could be reversed by the use of a histone methyltransferase inhibitor (41). Immune tolerance is on the opposite side of the spectrum compared to trained immunity, and it can be detrimental in diseases characterized by defective host defense such as sepsis (32, 42).

In vitro studies and the studies in mouse models were recently complemented by a model for experimental human viral infection in which human volunteers recently vaccinated with BCG were subsequently exposed to yellow fever vaccine. This vaccine contains an attenuated version of the yellow fever virus, and it induces a very mild infection with viremia that is detectable by polymerase chain reaction. The BCG-vaccinated individuals that subsequently received the

yellow fever vaccine showed reduced viremia compared to control individuals that received only the yellow fever vaccine, demonstrating the control of a nonmycobacterial infection by BCG (43).

BCG also has effects on HSV-1 and HSV-2 infections in humans. Multiple clinical studies, mainly in the 1970s, were performed using administration of BCG in patients with recurrent herpes genitalis and labialis (44, 45). Similar to the animal studies, these studies showed a beneficial effect, i.e., patients saw clinical improvement and the rate of recurrence reduced. Only one randomized controlled trial (RCT) was done, in which patients received either BCG vaccination or the, unknown at that time, nonspecific immunomodulatory *Candida* sp. skin test antigen, which showed that both could lead to a reduction in recurrence, while no significant difference between the two immunomodulators was seen (46).

Besides these vaccines that are still commonly used, there have been other examples of immunomodulators used to improve nonspecific host defense mechanisms against infections. Nonspecific resistance and macrophage activation through pathogen-derived components such as Broncho-Vaxom and Biostim (both extracts of bacterial cultures) for chronic or recurrent infections were previously used (47). Another example is Polidin, an immunomodulatory vaccine licensed in 1966 containing heat-killed bacteria from 13 different species, which provides nonspecific improved antimicrobial resistance. It was used in Romania in the second half of the twentieth century as a preventive measure against infections during flu season, but it was also given as adjuvant therapy against various infections and in burn patients (48).

Collectively, these arguments support the concept that certain vaccines can induce long-term protection against heterologous infections, including those caused by viral pathogens (**Table 1**). Due to the heterologous (antigen-independent) nature of the protection induced by these vaccines, memory characteristics built within innate immune cells are most likely an important mechanism mediating these effects.

MOLECULAR AND CELLULAR MECHANISMS UNDERLYING TRAINED IMMUNITY

The various mechanisms that are involved in the induction and effects of trained immunity can be identified and described at different levels, and these include cellular, immunological, transcriptional, and metabolic mechanisms.

Cellular Mechanisms

Several innate immune cells are known to acquire a memory-like response. The best-studied cells are monocytes/macrophages and NK cells (49–51).

Monocytes are bone marrow–derived cells that differentiate into macrophages when entering tissues. They are important in the recognition, phagocytosis, and killing of microbial pathogens. Monocytes and macrophages contribute to antiviral host defense in several ways. First, these cells produce defensins, molecules that block viral infection by interacting with the viral envelope, that are also able to trigger cytokine production and macrophage chemotaxis. Second, they produce IFNs and proinflammatory cytokines that subsequently activate other immune cells with direct antiviral effect (such as NK cells and cytotoxic T cells). Third, myeloid cells present antigens to T cells and contribute to induction of adaptive immune responses.

Depending on the training stimuli, different pathways induce trained immunity in monocytes and macrophages. β-glucan induces trained immunity through the Dectin-1/Raf1-dependent pathway, whereas BCG uses nucleotide-binding oligomerization domain 2 (NOD2) signaling. Which pathways mediate the training induced by viruses is currently unknown. Trained immunity improves the response against viruses through monocytes and macrophages by increasing

Table 1 Overview of evidence supporting trained immunity

Argument	Stimulus	Studied in	Effect(s)	Reference(s)
Evolutionary	Tobacco mosaic virus	*Nicotiana tabacum*	Systemic acquired resistance; resistance upon secondary challenge	14
			Epigenetic changes	16, 17
			Transgenerational	15
	Lipopolysaccharide	*Tenebrio molitor*	Long-lasting response against *Metarhizium anisopliae* after priming	19
			Transgenerational immune priming	18
	Dengue	*Aedes aegypti*	Reduction in viral load	20
			Epigenetic modifications and RNA interference in transgenerational immune priming	21
Epidemiological	BCG	Human	Reduced childhood mortality; reduction in neonatal sepsis and respiratory tract infections	23, 24
	Vaccinia	Human	Reduced mortality rate	25, 26
	Measles-mumps-rubella	Human	Reduced childhood mortality	27, 28
	Oral polio vaccine	Human	Lower case fatality rate in pediatric ward	30, 31
Experimental	Murine gammaherpesvirus-68; MCMV	Mice	Resistance to *Listeria monocytogenes* and *Yersinia pestis*	34
	MCMV	Mice	Prolonged inflammatory response in natural killer memory cells	35
	Muramyl dipeptide-Lys syn. L18	Mice	Resistance to Sendai virus; increased interferon production	36
	BCG	Mice	Reduction in influenza A titers; reduced inflammation and associated pulmonary injury	37
			Improved resistance against HSV-1	38
			Improved survival in HSV-2 infection	39
			Reduced mortality rate; enhanced interferon production	40

Abbreviations: BCG, Bacillus Calmette-Guérin; HSV, herpes simplex virus; MCMV, murine cytomegalovirus.

the production of monocyte- and macrophage-derived cytokines, leading to several downstream antiviral effects.

NK cells are considered to be at the interface of the innate and adaptive immune system and share many characteristics with T cells. NK cells survey for missing self and have various important functions within the antiviral immune response. NK cells have several mechanisms by which they provide protection: antibody-dependent cellular cytotoxicity (ADCC), secretion of IFN-γ and TNF-α upon activation, and cytolytic granule-mediated cell apoptosis of virus-infected cells. Besides infection, cytotoxicity can alternatively be achieved through cytokine-driven pathways, particularly type-I IFNs.

NK cells carry both specific and nonspecific memory responses. There are different types of NK cell memory, including virus-induced, hapten-induced, and cytokine-induced memory (52). Nonspecific NK memory responses are mediated through epigenetic imprinting of the *Ifng* locus and result in an enhanced production of proinflammatory cytokines, especially IL-1β, as IFN-γ enhances the release of IL-1β in humans (35, 53). Even though NK cells do not possess

antigen-specific receptors, they do have the ability to respond more rapidly and robustly upon restimulation. This memory has been seen after viral infection with human cytomegalovirus (HCMV), exposure to haptens such as 2,4-dinitro-1-fluorobenzene, and cytokines. These memory NK cells also have an increased life span. When mice are infected with MCMV, the NK cell population expands and contracts, similar to T cells. Upon reinfection, these NK cells were able to produce higher amounts of IFN-γ compared to naïve NK cells. Adoptive transfer of the memory NK cells from mice with previous exposure to MCMV to a naïve host also preserves this enhanced response in the acceptor (35).

Innate lymphoid cells (ILCs) are a population of innate lymphocytes that are closely related to NK cells. Group 1 ILCs (ILC1s) rapidly produce IFN-γ at the infection site to limit replication of viruses before NK cells can do so. Tissue-resident ILC1s possess a persisting memory response after MCMV infection. They undergo expansion and functional changes, including epigenetic reprogramming, that result in an enhanced protection against secondary MCMV challenge (54). Tissue resident ILC1s are found in the salivary gland and the liver. ILC1s also migrate to the salivary gland and expand in response to infection or inflammatory signals.

Not much is known about the role of neutrophils in the antiviral response, although there is evidence that a lack of neutrophils has a significant negative effect on the outcome of viral infections. In knock-out mice depleted of neutrophils, viral titers are significantly higher and there was increased mortality upon exposure to infection with HSV-1, influenza virus, and a neurotropic JHM strain of mouse hepatitis virus (55–58).

Progenitor HSCs as well as neutrophils can be trained (50). When granulocyte-monocyte progenitors are trained, their transcriptome shows preference toward production and maturation of neutrophils (59, 60). Among the changes that occur after restimulation, an upregulation of type-I IFN signaling was noticed (60). The training of these cells has mostly been investigated for its antitumor activity; however, as previously described, type-I IFN is important in viral infections. Even though neutrophil training has not been performed in the context of viral infections, one may speculate that training of neutrophils also contributes to trained immunity responses against viruses. More research is needed to evaluate the role of trained immunity of neutrophils and their progenitor cells in viral infection.

In contrast to the adaptive immune response, which provides lifelong protection against certain viruses, the effects of trained immunity are thought to be shorter lived, based on immunological studies that report waning of trained immunity in the course of one year (61). Other arguments for a longer duration have come from epidemiological studies (26, 62, 63). However, a duration of years raises the question of how this is maintained, as the lifespan of innate immune cells is limited. This means there must also be central induction of trained immunity in HSCs and/or the other progenitors of myeloid cells.

In mouse studies, increased cytokine production after secondary stimulation is seen for at least 3 months after BCG vaccination (64). After administration of β-glucan, there is an expansion of myeloid progenitor cells in the bone marrow. This indicates that not just mature innate immune cells are involved in trained immunity but also their precursors. In this way the reprogramming lasts longer than the life span of the circulating innate immune cells (65). A different study confirmed the involvement and reprogramming of HSCs and their ability to confer this memory to the cells derived from these HSCs, such as bone marrow–derived macrophages (BMDMs) (59).

Similarly, in human volunteers, BCG vaccination induces transcriptomic changes in the HSCs and other progenitor cells. There is upregulation of genes involved in myeloid and granulocytic function and priming; specifically, transcription factor HNF1A/B plays a key role in the reprogramming. Transcriptomic changes were also seen in $CD14^+$ monocytes that derive from these HSCs, while no differences in the number of cells were seen (66).

Immunological Effector Mechanisms

Trained immunity leads to an enhancement of the effector innate immune responses when restimulated. Among these effector mechanisms, the most relevant for antiviral host defense are pathogen recognition, cytokine production, and killing mechanisms (the latter mediated especially by NK cells and other ILCs). Typically, trained cells produce higher amounts of innate proinflammatory and anti-inflammatory cytokines when restimulated.

The increased cytokine production after reinfection is an essential functional consequence of trained immunity. This mechanism is most relevant for monocytes and macrophages, which are the main producers of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α and chemokines such as CXCL9, CXCL10, and CXCL11. Cytokines and chemokines have an important antimicrobial effect by activating macrophages and neutrophils, potentiating phagocytosis, and stimulating the killing of pathogens and infected cells in case of viruses.

IL-1β is a cytokine with a wide range of effects. One of these is costimulation of T cells. IL-1β is linked to increased antibody production through stimulation of IL-6 production. In an in vivo experimental setting of BCG followed by yellow fever vaccination, it was shown that the upregulation of IL-1β after BCG vaccination is strongly correlated to a reduction of viremia due to the yellow fever 17D vaccine strain, independent of IFN-γ. Interestingly, besides the reduction of circulating yellow fever virus, there was a lower concentration of circulating inflammatory cytokines in BCG-vaccinated individuals, while the concentration of neutralizing antibodies was similar (43).

Type-I IFNs are cytokines produced by fibroblasts and monocytes, innate immune cells, and are well known for their antiviral effects. They bind to the IFN-α/β receptor (IFNAR) and inhibit viral replication at various stages as well as promote apoptosis of infected cells through ISGs and p53 activity. Unfortunately, the type-I IFNs have not yet been thoroughly studied in the context of trained immunity. It has been shown that training of bone marrow GMPs with β-glucan leads to increased concentrations of IFN-α in bone marrow plasmacytoid dendritic cells (DCs) and macrophages (60). What impact trained immunity has on the production of type-I IFNs in the context of virus infections remains to be investigated in future studies.

The increase in cytokine production also has several downstream effects, for instance on the functioning of the adaptive immune system. BCG vaccination has been shown to improve the immunogenicity of subsequent influenza and hepatitis B vaccination (67, 68). This could be explained by the enhanced cytokine response, for example IL-6 promoting B and T cell differentiation, antibody synthesis, and memory cell formation. Trained immunity could thus potentially influence antibody responses to other vaccines as well.

The expression of various receptors on immune cells also changes in trained cells. In monocytes from BCG-vaccinated volunteers, there is an increased expression of TLR4 and CD11b compared to prevaccination (64). The enhanced expression of CD11b is interesting for viral infections, as it is a marker for $CD8^+$ cytotoxic T cell activation and memory response (69). β-Glucan-trained monocytes express more TLR2 (70).

Memory NK cells, as an example of trained cells, also show enhanced expression of certain receptors compared to resting naïve NK cells. In the context of MCMV infection, the expression of the Ly49H receptor on NK cells is an indicator for MCMV-specific memory NK cells. They highly express killer cell lectin-like receptor G1 (KLRG1), an inhibitory receptor also present on $CD8^+$ T cells (35). The results of these changes are improved IFN-γ production and more robust degranulation, which lead to enhanced killing capacity by NK cells. The human counterparts of these $Ly49H^+$ NK cells are thought to be CD94-$NKG2C^+$ NK cells, which expand after reinfection with HCMV and remain elevated for a prolonged time (71, 72).

Transcriptional and Epigenetic Mechanisms

One of the most important molecular mechanisms for the induction of trained immunity is the epigenetic reprogramming of innate immune cells, leading to an enhanced capacity to respond transcriptionally. Epigenetic regulation of gene transcription can take place at different levels: chromatin accessibility influenced by histone modifications [e.g., methylation at histone H3 lysine 4 (H3K4) and histone H3 lysine 9 (H3K9), acetylation at histone H3 lysine 27 (H3K27)], DNA methylation, and long noncoding RNA (lncRNA) expression modulation (73, 74). A combination of the genome-wide epigenetic modifications mediated by these diverse mechanisms leads to memory-like responses in various cell types (**Figure 2**).

After stimulation cells reprogram in order to meet the need to respond to the stimulation, and the unpacking of chromatin around gene regions involved in the immune response allows for the transcription of proinflammatory genes. The initial activation of proinflammatory genes goes hand in hand with the accumulation of open chromatin marks such as H3K4me3 on promoters. Immune priming lncRNAs are also upregulated by the initial stimulation and regulate the deposition of epigenetic marks on gene promoters (75, 76). Not only are gene promoter regions affected, but also modulation of H3K4me1 and H3K27Ac on enhancer regions seems to be involved in regulation of trained immunity (77).

IFN-β stimulation leaves chromatin marks on ISGs leading to transcriptional memory. After mouse embryonic fibroblasts are exposed to IFN-β, a secondary stimulation with encephalomyocarditis virus after washout of the initial IFN-β leads to improved antiviral activity. This effect was also seen in BMDMs. This memory response can also be considered a form of trained immunity and is especially interesting in the context of viral infections (78).

It is important to realize that the epigenetic reprogramming of cells can go in two directions: toward trained immunity but also toward immune tolerance. During the latter, the epigenetic changes result in diminished expression of inflammatory genes, and this may ultimately lead to immune paralysis, such as seen in sepsis (79). Recent studies have suggested that coronavirus disease 2019 (COVID-19) can induce immune paralysis in a minority of patients (80), but this needs to be investigated in more detail.

After stimulation, several of these modifications may persist, leaving the cell in a trained state that facilitates a rapid and robust transcription after secondary stimulation. This epigenetic reprogramming is considered a hallmark of trained immunity without which training cannot take place.

Metabolic Modulation of Trained Immunity

The link between intracellular signaling through immune receptors and the long-term epigenetic changes that underlie the change in cell function is provided by a rewiring of the cellular metabolism (**Figure 3**). In turn, epigenetic changes also influence genes involved in metabolic processes, such as those involved in glycolysis. In parallel, upregulation of genes responsible for increased proinflammatory cytokine production occurs (81, 82).

During induction of trained immunity, activation of the Akt/mammalian target of rapamycin (mTOR) pathway induces a metabolic switch also known as the Warburg effect, in which cell metabolism switches from oxidative phosphorylation to aerobic glycolysis. This induces a further stimulation of the cell, as well as provides a faster, yet less efficient, source of energy in the form of ATP for the cell. However, the Krebs cycle is changing from a catabolic to an anabolic state to produce citrate and acetyl-coenzyme A (CoA) that subsequently enters the lipid-synthesis pathway. Additionally, Krebs cycle metabolites are cofactors of epigenetic enzymes and impact

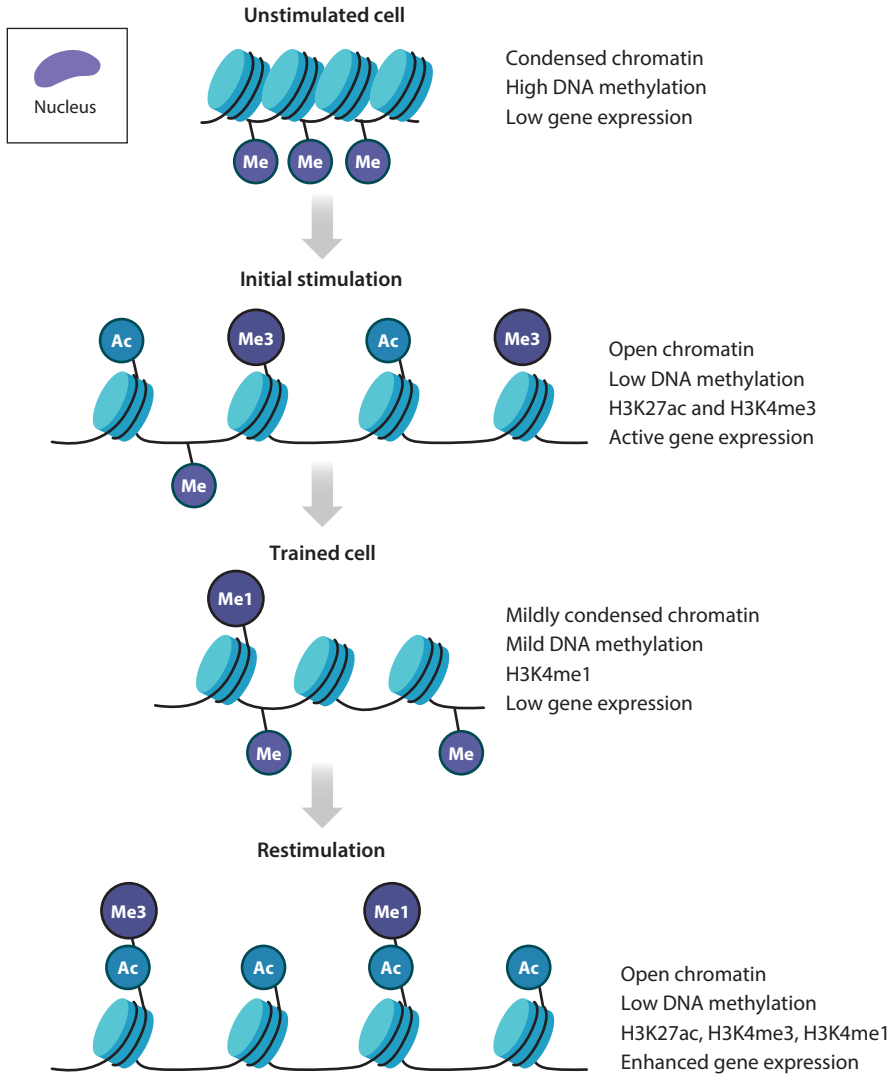

Figure 2

The epigenetic mechanisms that result in the enhanced immune response upon secondary stimulation in trained immunity. Upon initial stimulation chromatin opens, DNA methylation is low and there is active gene expression. A trained cell does not return to an unstimulated state; chromatin only mildly condenses, and there is mild DNA methylation. This allows for enhanced gene expression upon restimulation. Figure adapted from images created with BioRender.com.

the remodeling of chromatin: For example, accumulation of fumarate inhibits KDM5 histone demethylases, thus allowing the persistence of methylation marks on histones (81). In turn, succinate also promotes the activity of TLR3 and TLR7, receptors relevant for viral recognition (83). At the same time, the cholesterol pathway metabolite mevalonate amplifies trained immunity through the insulin-like growth factor 1 receptor (IGF1R) and phosphatidylinositol-3-kinase (PI3K)/mTOR pathway (74).

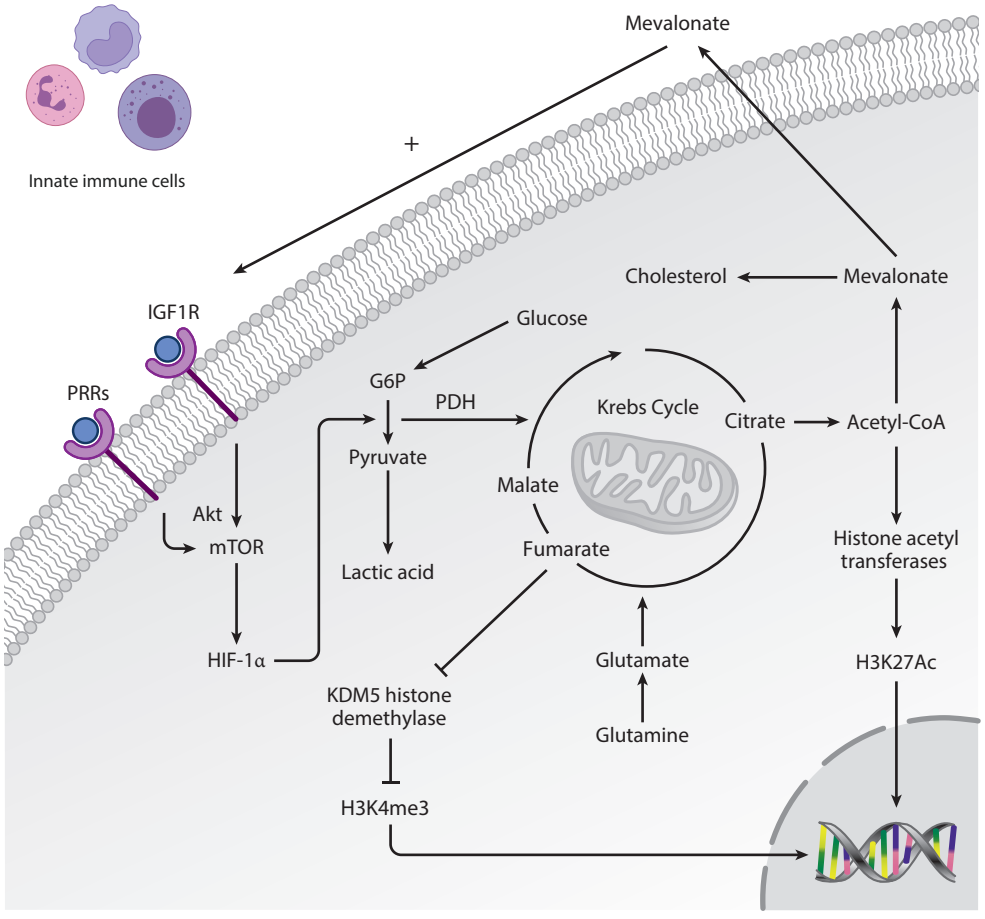

Figure 3

Metabolic pathways involved in trained immunity. Trained immunity results in a metabolic switch in which the cellular metabolism instead of oxidative phosphorylation uses aerobic glycolysis as a faster source of energy. Meanwhile, the Krebs cycle goes from a catabolic to an anabolic state. Metabolites from these pathways are involved in the remodeling of chromatin and amplifying the trained state of the cell. Abbreviations: CoA, coenzyme A; G6P, glucose 6-phosphate; HIF-1α, hypoxia-inducible factor 1-alpha; IGF1R, insulin-like growth factor 1 receptor; mTOR, mammalian target of rapamycin; PDH, pyruvate dehydrogenase; PRR, pattern recognition receptor. Figure adapted from images created with BioRender.com.

COVID-19 AND TRAINED IMMUNITY

While we have focused in this review on the impact of trained immunity on viral infections in general, we should also reflect on its importance in the context of the COVID-19 pandemic. Based on the growing body of evidence that certain vaccines induce trained immunity and heterologous protection against infections, several clinical trials using vaccines that induce trained immunity have been designed to combat COVID-19 (**Table 2**). An improved innate immune response could result in a milder course of the disease and prevent further spreading of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (84). Using trained immunity–inducing vaccines as bridge

Table 2 Nonspecific effects of vaccines in the COVID-19 pandemic

Vaccine	Study design	Effect(s) on COVID-19	Reference
BCG	Cohort	Reduced seroprevalence of anti-SARS-CoV-2 immunoglobulin G; reduced self-reported COVID-19 symptoms	85
	Ecological	Negative association between BCG vaccine coverage and COVID-19 mortality	86
	Cohort	Reduced incidence of self-reported sickness and extreme fatigue	89
	RCT	Reduced incidence of COVID-19 diagnosis	90
		No effect on COVID-19 incidence	91
		Increased neutralization by antibodies after COVID-19 vaccination after recent BCG vaccination	93
	Mice	Sterilizing immunity using BCG-adjuvanted COVID-19 vaccine	92
Influenza	Cohort	49% risk reduction for SARS-CoV-2 positivity	94
		24% reduced odds of SARS-CoV-2 positivity; 42% reduced odds of needing hospitalization; 55% reduced odds of needing ventilation when infected; shorter COVID-19-related hospital stay	96
		29% reduced odds of symptomatic disease	97
Pneumococcal	Cohort	52% reduced odds of symptomatic disease	97
Measles-mumps-rubella	RCT	48% risk reduction for symptomatic COVID-19; 76% risk reduction in COVID-19 treatment	99
	Cohort	38% reduction of COVID-19-related hospitalization; 32% reduction of COVID-19 intensive care unit admission or death	100
Recombinant zoster	Cohort	16% risk reduction for COVID-19; 32% risk reduction for associated hospitalization	101
Oral polio vaccine	Cohort	Fewer symptomatic infections	102

Abbreviations: BCG, Bacillus Calmette-Guérin; COVID-19, coronavirus disease 2019; RCT, randomized controlled trial; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

vaccinations until a specific vaccine (or a vaccine adapted to a new viral mutant) is developed could limit morbidity and mortality in the population and mitigate the effects of the pandemic.

Epidemiological and ecological studies have found a correlation between a history of BCG vaccination and reduced seroprevalence of SARS-CoV-2 immunoglobulin G (IgG) and self-reported health complaints (85), as well as reduced mortality in countries that have an ongoing BCG national vaccianation program (86, 87). However, these studies are not definitive proof of a direct negative relation between BCG vaccination and SARS-CoV-2, as they are prone to bias. Several randomized placebo-controlled trials are currently underway in many different countries to analyze whether BCG vaccination could provide protection against COVID-19 in health care workers (NCT04328441, NCT04327206, NCT04461379, NCT04648800), and there are also studies in various countries where this effect is analyzed in the elderly (88) (NCT04417335, NCT04414267, NCT04537663, NCT04475302).

Surrounding these trials, there were some concerns that the immune boost by BCG could amplify hyperinflammation when individuals become infected with COVID-19. One study has shown that recent BCG vaccination was safe and might be associated with a lower incidence of self-reported sickness and of extreme fatigue during the first wave of COVID-19 in the Netherlands. It must be mentioned that there was limited access to SARS-CoV-2 testing during the reported period, and this retrospective study had only small numbers of participants (89). However, the hypothesis that recent BCG vaccination does not cause more severe disease was supported by

the data of the ACTIVATE-2 clinical trial, in which elderly individuals revaccinated with BCG did not display more symptoms. Moreover, this study identified fewer diagnoses of COVID-19 in BCG-vaccinated individuals than in the control group (90). However, the first results of the BCG-CORONA-ELDERLY trial in the Netherlands did not show significant effects of BCG vaccination on the incidence of total COVID-19 diagnoses. No conclusions could be drawn regarding the severity of infection due to the limited number of cases of severe COVID-19 requiring hospitalization. Interestingly, recently BCG-vaccinated individuals exhibited an increase in IL-6 after in vitro stimulation with SARS-CoV-2, as well as an improved serological response after COVID-19 infection. In addition, BCG-vaccinated individuals that were subsequently infected with SARS-CoV-2 had a higher antibody response (91). All in all, it is too early to tell whether BCG vaccination has beneficial effects on COVID-19, and the results of additional studies are eagerly awaited to provide definitive answers regarding the effects on incidence and severity of the disease.

With regard to SARS-CoV-2 vaccination, there is evidence of a similar synergistic effect of BCG (re)vaccination on the specific antibody response. In mice, a single dose of BCG-adjuvanted COVID-19 vaccine results in sterilizing immunity against SARS-CoV-2 infection (92). In humans, recent revaccination of health care workers with BCG before SARS-CoV-2 vaccination showed increased titers of neutralizing antibodies compared to placebo (93).

Other vaccines have also been investigated for protective effects against COVID-19. An interesting example of this is vaccination with quadrivalent inactivated influenza vaccine (QIV), a vaccine that is offered yearly to health care personnel and the elderly. One study found that the incidence of COVID-19 in health care personnel vaccinated in flu season 2019/2020 was lower than that in unvaccinated personnel. This is also in line with experiments in vitro, in which restimulation with heat-killed SARS-CoV-2 after training with QIV results in greater production of IL-1RA (94). IL-1RA is anti-inflammatory that can reduce the severe inflammation seen in COVID-19-related respiratory problems and has even been used as treatment for hospitalized patients (95). Additionally, several clinical studies have shown that there are fewer COVID-19 diagnoses and a reduced risk of COVID-19 hospitalization and mortality in influenza-vaccinated individuals (96–98).

Studies on the MMR vaccine showed that it does not prevent SARS-CoV-2 infection, but it reduces the risk of symptomatic COVID-19 by 48% after only one dose of MMR; even better results were obtained with two doses (99). Although trained immunity after MMR vaccine has not yet been demonstrated, it is thought to provide protection against SARS-CoV-2 through cross-reactive memory T cells reactivated by SARS-CoV-2 (100).

A large cohort study on recombinant $AS01_B$-adjuvanted herpes zoster vaccination and COVID-19 found a risk reduction of 16% for COVID-19 diagnosis and of 32% for associated hospitalization (101). The AS01 adjuvant is thought to induce heterologous protection, possibly due to the induction of trained immunity.

Finally, OPV has also been suggested as a potential strategy against COVID-19. In one study, Iranian women who were exposed to children who had received OPV were reported to have fewer symptomatic SARS-CoV-2 infections than women without such exposure. The excreted attenuated polio virus would have led to exposure of the former group of women (102).

FUTURE PERSPECTIVES

In summary, the innate immune response is important as a first line of defense against viruses. The nonspecific innate immune memory, known as trained immunity, contributes to an enhanced immune response against viral infectious diseases. This memory response is mediated by

functional reprogramming of innate immune cells by certain vaccines and involves epigenetic, metabolic, cellular, and immunological changes that are not limited to one generation of cells. There is, however, heterogeneity of trained immunity, which can be partially explained by host factors such as gender, age, genetics, and diet, but also seasonality and kind of stimulus (103). Most studies have focused on the induction of training with bacterial and fungal products, but the role of viruses and their ligands in training is largely unexplored.

Currently the major knowledge gap in trained immunity that requires further investigation most urgently is the duration of effective protection. Also, the relevance of timing of initial stimulation in relation to other vaccinations and natural exposure to disease has not been researched. Throughout life, natural exposure, environmental determinants, and physiological changes could greatly affect trained immunity. It is still unknown how the antiviral immune response benefits from these.

Considering the protective effects induced by trained immunity, one could consider its use for clinical applications. Trained immunity can provide additional beneficial nonspecific effects after vaccination, which gives a new approach to the development of new vaccines. Knowing how long trained immunity persists can provide arguments to adapt vaccination programs to include trained immunity–inducing vaccines or adjuvants to further reduce the impact infectious diseases have on morbidity and mortality. Its use can also lead to vaccination in a broader public health approach, aiming to reduce pathology of more than just a single pathogen.

This is promising not only for vulnerable populations, in which vaccines do not always work well, but also for diseases for which vaccines are not very successful. The largest vulnerable population that can benefit from improved vaccines is the elderly population. Many vaccines lack efficacy in elderly people due to aging of the immune system, of which the adaptive immune system and vaccination responses are most affected. Trained immunity may improve their vaccine responses, as it makes use of the innate immune system, which remains largely intact in old age. In addition, it might be beneficial to use trained immunity–inducing stimuli as adjuvants to existing vaccines. In that way, vaccines may be more effective and also provide heterologous protection and thereby prevent other (viral) infectious diseases.

Unfortunately, currently most vaccines in use that induce trained immunity are live-attenuated vaccines. Live-attenuated vaccines cannot always be administered safely to immunocompromised individuals, such as people infected with human immunodeficiency virus or using immunosuppressive medication. More research into noninfectious agents that can provide similar nonspecific protection and are safe for use could be a breakthrough in the protection of vulnerable populations. Another application that can be envisaged is the use of viral ligands as inducers of trained immunity. These might be used in clinical conditions of immunosuppression, such as sepsis or cancer. Indeed, the use of bacterial or fungal structures as immunomodulators in cancer has been recently proposed, and initial data are already promising (60, 104). However, little is currently known of viral compounds with potential trained immunity–inducing properties, and future studies are warranted.

In conclusion, trained immunity definitely plays a role in the antiviral immune response. Some viruses are able to induce trained immunity by themselves, but the host can also be better protected against viral infections by vaccines and other compounds that induce trained immunity. More research is necessary to explore the full beneficial potential of trained immunity in human viral infections.

DISCLOSURE STATEMENT

M.G.N. is scientific founder of Trained Therapeutix Discovery and is owner of two patents on modulation of trained immunity.

LITERATURE CITED

1. Lester SN, Li K. 2014. Toll-like receptors in antiviral innate immunity. *J. Mol. Biol.* 426:1246–64
2. Barbalat R, Lau L, Locksley RM, Barton GM. 2009. Toll-like receptor 2 on inflammatory monocytes induces type I interferon in response to viral but not bacterial ligands. *Nat. Immunol.* 10:1200–7
3. Murawski MR, Bowen GN, Cerny AM, Anderson LJ, Haynes LM, et al. 2009. Respiratory syncytial virus activates innate immunity through Toll-like receptor 2. *J. Virol.* 83:1492–500
4. Kurt-Jones EA, Popova L, Kwinn L, Haynes LM, Jones LP, et al. 2000. Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. *Nat. Immunol.* 1:398–401
5. Ekchariyawat P, Hamel R, Bernard E, Wichit S, Surasombatpattana P, et al. 2015. Inflammasome signaling pathways exert antiviral effect against Chikungunya virus in human dermal fibroblasts. *Infect. Genet. Evol.* 32:401–8
6. Hamel R, Dejarnac O, Wichit S, Ekchariyawat P, Neyret A, et al. 2015. Biology of Zika virus infection in human skin cells. *J. Virol.* 89:8880–96
7. Allen IC, Scull MA, Moore CB, Holl EK, McElvania-TeKippe E, et al. 2009. The NLRP3 inflammasome mediates in vivo innate immunity to influenza A virus through recognition of viral RNA. *Immunity* 30:556–65
8. Plotkin SA, Plotkin SA. 2008. Correlates of vaccine-induced immunity. *Clin. Infect. Dis.* 47:401–9
9. Mason RA, Tauraso NM, Spertzel RO, Ginn RK. 1973. Yellow fever vaccine: direct challenge of monkeys given graded doses of 17D vaccine. *Appl. Microbiol.* 25:539–44
10. McElhaney JE, Xie D, Hager WD, Barry MB, Wang Y, et al. 2006. T cell responses are better correlates of vaccine protection in the elderly. *J. Immunol.* 176:6333–39
11. Goodridge HS, Ahmed SS, Curtis N, Kollmann TR, Levy O, et al. 2016. Harnessing the beneficial heterologous effects of vaccination. *Nat. Rev. Immunol.* 16:392–400
12. Netea MG, Quintin J, van der Meer JW. 2011. Trained immunity: a memory for innate host defense. *Cell Host Microbe* 9:355–61
13. Quintin J, Cheng SC, van der Meer JW, Netea MG. 2014. Innate immune memory: towards a better understanding of host defense mechanisms. *Curr. Opin. Immunol.* 29:1–7
14. Ross AF. 1961. Systemic acquired resistance induced by localized virus infections in plants. *Virology* 14:340–58
15. Luna E, Bruce TJ, Roberts MR, Flors V, Ton J. 2012. Next-generation systemic acquired resistance. *Plant Physiol.* 158:844–53
16. Diezma-Navas L, Pérez-González A, Artaza H, Alonso L, Caro E, et al. 2019. Crosstalk between epigenetic silencing and infection by tobacco rattle virus in *Arabidopsis*. *Mol. Plant Pathol.* 20:1439–52
17. Luna E, Ton J. 2012. The epigenetic machinery controlling transgenerational systemic acquired resistance. *Plant Signal. Behav.* 7:615–18
18. Moret Y. 2006. "Trans-generational immune priming": specific enhancement of the antimicrobial immune response in the mealworm beetle, *Tenebrio molitor*. *Proc. R. Soc. B* 273:1399–405
19. Moret Y, Siva-Jothy MT. 2003. Adaptive innate immunity? Responsive-mode prophylaxis in the mealworm beetle, *Tenebrio molitor*. *Proc. R. Soc. B* 270:2475–80
20. Vargas V, Cime-Castillo J, Lanz-Mendoza H. 2020. Immune priming with inactive dengue virus during the larval stage of *Aedes aegypti* protects against the infection in adult mosquitoes. *Sci. Rep.* 10:6723
21. Mondotte JA, Gausson V, Frangeul L, Suzuki Y, Vazeille M, et al. 2020. Evidence for long-lasting transgenerational antiviral immunity in insects. *Cell Rep.* 33:108506
22. Jensen KJ, Larsen N, Biering-Sørensen S, Andersen A, Eriksen HB, et al. 2015. Heterologous immunological effects of early BCG vaccination in low-birth-weight infants in Guinea-Bissau: a randomized-controlled trial. *J. Infect. Dis.* 211:956–67
23. Näslund C. 1932. Resultats des experiences de vaccination par le BCG poursuivies dans le Norrbotten (Suède) (Septembre 1927–Décembre 1931). In *Vaccination Preventative de Tuberculose, Rapports et Documents*. Paris: Institut Pasteur
24. Aaby P, Roth A, Ravn H, Napirna BM, Rodrigues A, et al. 2011. Randomized trial of BCG vaccination at birth to low-birth-weight children: beneficial nonspecific effects in the neonatal period? *J. Infect. Dis.* 204:245–52

25. Aaby P, Gustafson P, Roth A, Rodrigues A, Fernandes M, et al. 2006. Vaccinia scars associated with better survival for adults. An observational study from Guinea-Bissau. *Vaccine* 24:5718–25
26. Jensen ML, Dave S, Schim van der Loeff M, da Costa C, Vincent T, et al. 2006. Vaccinia scars associated with improved survival among adults in rural Guinea-Bissau. *PLOS ONE* 1:e101
27. Aaby P, Martins CL, Garly ML, Balé C, Andersen A, et al. 2010. Non-specific effects of standard measles vaccine at 4.5 and 9 months of age on childhood mortality: randomised controlled trial. *BMJ* 341:c6495
28. Veirum JE, Sodemann M, Biai S, Jakobsen M, Garly ML, et al. 2005. Routine vaccinations associated with divergent effects on female and male mortality at the paediatric ward in Bissau, Guinea-Bissau. *Vaccine* 23:1197–204
29. Higgins JP, Soares-Weiser K, López-López JA, Kakourou A, Chaplin K, et al. 2016. Association of BCG, DTP, and measles containing vaccines with childhood mortality: systematic review. *BMJ* 355:i5170
30. Aaby P, Rodrigues A, Biai S, Martins C, Veirum JE, et al. 2004. Oral polio vaccination and low case fatality at the paediatric ward in Bissau, Guinea-Bissau. *Vaccine* 22:3014–17
31. Øland CB, Mogensen SW, Rodrigues A, Benn CS, Aaby P. 2020. Reduced mortality after oral polio vaccination and increased mortality after diphtheria-tetanus-pertussis vaccination in children in a low-income setting. *Clin. Ther.* 43(1):172–84
32. Ifrim DC, Quintin J, Joosten LA, Jacobs C, Jansen T, et al. 2014. Trained immunity or tolerance: opposing functional programs induced in human monocytes after engagement of various pattern recognition receptors. *Clin. Vaccine Immunol.* 21:534–45
33. Crişan TO, Cleophas MC, Oosting M, Lemmers H, Toenhake-Dijkstra H, et al. 2016. Soluble uric acid primes TLR-induced proinflammatory cytokine production by human primary cells via inhibition of IL-1Ra. *Ann. Rheum. Dis.* 75:755–62
34. Barton ES, White DW, Cathelyn JS, Brett-McClellan KA, Engle M, et al. 2007. Herpesvirus latency confers symbiotic protection from bacterial infection. *Nature* 447:326–29
35. Sun JC, Beilke JN, Lanier LL. 2009. Adaptive immune features of natural killer cells. *Nature* 457:557–61
36. Ishihara C, Mizukoshi N, Iida J, Kato K, Yamamoto K, Azuma I. 1987. Suppression of Sendai virus growth by treatment with N^{α}-acetylmuramyl-l-alanyl-d-isoglutaminyl-N^{ε}-stearoyl-l-lysine in mice. *Vaccine* 5:295–301
37. Spencer JC, Ganguly R, Waldman RH. 1977. Nonspecific protection of mice against influenza virus infection by local or systemic immunization with Bacille Calmette-Guérin. *J. Infect. Dis.* 136:171–75
38. Floc'h F, Werner GH. 1976. Increased resistance to virus infections of mice inoculated with BCG (Bacillus calmette-guérin). *Ann. Immunol.* 127:173–86
39. Starr SE, Visintine AM, Tomeh MO, Nahmias AJ. 1976. Effects of immunostimulants on resistance of newborn mice to herpes simplex type 2 infection. *Proc. Soc. Exp. Biol. Med.* 152:57–60
40. Suenaga T, Okuyama T, Yoshida I, Azuma M. 1978. Effect of *Mycobacterium tuberculosis* BCG infection on the resistance of mice to ectromelia virus infection: participation of interferon in enhanced resistance. *Infect. Immun.* 20:312–14
41. Blok BA, Jensen KJ, Aaby P, Fomsgaard A, van Crevel R, et al. 2019. Opposite effects of *Vaccinia* and modified *Vaccinia* Ankara on trained immunity. *Eur. J. Clin. Microbiol. Infect. Dis.* 38:449–56
42. Saeed S, Quintin J, Kerstens HH, Rao NA, Aghajanirefah A, et al. 2014. Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. *Science* 345:1251086
43. Arts RJW, Moorlag SJCFM, Novakovic B, Li Y, Wang SY, et al. 2018. BCG vaccination protects against experimental viral infection in humans through the induction of cytokines associated with trained immunity. *Cell Host Microbe* 23:89–100.e5
44. Bierman SM. 1976. BCG immunoprophylaxis of recurrent herpes progenitalis. *Arch. Dermatol.* 112:1410–15
45. Hippmann G, Wekkeli M, Rosenkranz AR, Jarisch R, Götz M. 1992. [Nonspecific immune stimulation with BCG in Herpes simplex recidivans. Follow-up 5 to 10 years after BCG vaccination]. *Wien. Klin. Wochenschr.* 104:200–4 (In German)
46. Douglas JM, Vontver LA, Stamm WE, Reeves WC, Critchlow C, et al. 1985. Ineffectiveness and toxicity of BCG vaccine for the prevention of recurrent genital herpes. *Antimicrob. Agents Chemother.* 27:203–6
47. Hadden JW. 1993. Immunostimulants. *Trends Pharmacol. Sci.* 14:169–74

48. POLIDIN [drug sheet]. Bucuresti, Romania: Institutul Cantacuzino; 2012
49. Bekkering S, Blok BA, Joosten LA, Riksen NP, van Crevel R, Netea MG. 2016. *In vitro* experimental model of trained innate immunity in human primary monocytes. *Clin. Vaccine Immunol.* 23:926–33
50. Moorlag SJCFM, Rodriguez-Rosales YA, Gillard J, Fanucchi S, Theunissen K, et al. 2020. BCG vaccination induces long-term functional reprogramming of human neutrophils. *Cell Rep.* 33:108387
51. Hammer Q, Romagnani C. 2017. About training and memory: NK-cell adaptation to viral infections. *Adv. Immunol.* 133:171–207
52. O'Leary JG, Goodarzi M, Drayton DL, von Andrian UH. 2006. T cell– and B cell–independent adaptive immunity mediated by natural killer cells. *Nat. Immunol.* 7:507–16
53. Kleinnijenhuis J, Quintin J, Preijers F, Joosten LA, Jacobs C, et al. 2014. BCG-induced trained immunity in NK cells: role for non-specific protection to infection. *Clin. Immunol.* 155:213–19
54. Weizman O-E, Song E, Adams NM, Hildreth AD, Riggan L, et al. 2019. Mouse cytomegalovirus-experienced ILC1s acquire a memory response dependent on the viral glycoprotein m12. *Nat. Immunol.* 20:1004–11
55. Tumpey TM, Chen SH, Oakes JE, Lausch RN. 1996. Neutrophil-mediated suppression of virus replication after herpes simplex virus type 1 infection of the murine cornea. *J. Virol.* 70:898–904
56. Zhou J, Stohlman SA, Hinton DR, Marten NW. 2003. Neutrophils promote mononuclear cell infiltration during viral-induced encephalitis. *J. Immunol.* 170:3331–36
57. Tate MD, Deng YM, Jones JE, Anderson GP, Brooks AG, Reading PC. 2009. Neutrophils ameliorate lung injury and the development of severe disease during influenza infection. *J. Immunol.* 183:7441–50
58. Tate MD, Brooks AG, Reading PC. 2008. The role of neutrophils in the upper and lower respiratory tract during influenza virus infection of mice. *Respir. Res.* 9:57
59. Kaufmann E, Sanz J, Dunn JL, Khan N, Mendonça LE, et al. 2018. BCG educates hematopoietic stem cells to generate protective innate immunity against tuberculosis. *Cell* 172:176–90.e19
60. Kalafati L, Kourtzelis I, Schulte-Schrepping J, Li X, Hatzioannou A, et al. 2020. Innate immune training of granulopoiesis promotes anti-tumor activity. *Cell* 183:771–85.e12
61. Kleinnijenhuis J, Quintin J, Preijers F, Benn CS, Joosten LA, et al. 2014. Long-lasting effects of BCG vaccination on both heterologous Th1/Th17 responses and innate trained immunity. *J. Innate Immun.* 6:152–58
62. de Castro MJ, Pardo-Seco J, Martinón-Torres F. 2015. Nonspecific (heterologous) protection of neonatal BCG vaccination against hospitalization due to respiratory infection and sepsis. *Clin. Infect. Dis.* 60:1611–19
63. Rieckmann A, Villumsen M, Sørup S, Haugaard LK, Ravn H, et al. 2017. Vaccinations against smallpox and tuberculosis are associated with better long-term survival: a Danish case-cohort study 1971–2010. *Int. J. Epidemiol.* 46:695–705
64. Kleinnijenhuis J, Quintin J, Preijers F, Joosten LA, Ifrim DC, et al. 2012. Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. *PNAS* 109:17537–42
65. Mitroulis I, Ruppova K, Wang B, Chen L-S, Grzybek M, et al. 2018. Modulation of myelopoiesis progenitors is an integral component of trained immunity. *Cell* 172:147–61.e12
66. Cirovic B, de Bree LCJ, Groh L, Blok BA, Chan J, et al. 2020. BCG vaccination in humans elicits trained immunity via the hematopoietic progenitor compartment. *Cell Host Microbe* 28:322–34.e5
67. Leentjens J, Kox M, Stokman R, Gerretsen J, Diavatopoulos DA, et al. 2015. BCG vaccination enhances the immunogenicity of subsequent influenza vaccination in healthy volunteers: a randomized, placebo-controlled pilot study. *J. Infect. Dis.* 212:1930–38
68. Scheid A, Borriello F, Pietrasanta C, Christou H, Diray-Arce J, et al. 2018. Adjuvant effect of Bacille Calmette-Guérin on hepatitis B vaccine immunogenicity in the preterm and term newborn. *Front. Immunol.* 9:29
69. McFarland HI, Nahill SR, Maciaszek JW, Welsh RM. 1992. CD11b (Mac-1): a marker for CD8+ cytotoxic T cell activation and memory in virus infection. *J. Immunol.* 149:1326–33
70. Quintin J, Saeed S, Martens JHA, Giamarellos-Bourboulis EJ, Ifrim DC, et al. 2012. *Candida albicans* infection affords protection against reinfection via functional reprogramming of monocytes. *Cell Host Microbe* 12:223–32

71. Gumá M, Angulo A, Vilches C, Gómez-Lozano N, Malats N, López-Botet M. 2004. Imprint of human cytomegalovirus infection on the NK cell receptor repertoire. *Blood* 104:3664–71
72. Petersen L, Roug AS, Skovbo A, Thysen AH, Eskelund CW, Hokland ME. 2009. The CD94/NKG2C-expressing NK cell subset is augmented in chronic lymphocytic leukemia patients with positive human cytomegalovirus serostatus. *Viral Immunol.* 22:333–37
73. Arts RJW, Carvalho A, La Rocca C, Palma C, Rodrigues F, et al. 2016. Immunometabolic pathways in BCG-induced trained immunity. *Cell Rep*. 17:2562–71
74. Bekkering S, Arts RJW, Novakovic B, Kourtzelis I, van der Heijden C, et al. 2018. Metabolic induction of trained immunity through the mevalonate pathway. *Cell* 172:135–46.e9
75. Fanucchi S, Fok ET, Dalla E, Shibayama Y, Börner K, et al. 2019. Immune genes are primed for robust transcription by proximal long noncoding RNAs located in nuclear compartments. *Nat. Genet.* 51:138–50
76. Wang KC, Yang YW, Liu B, Sanyal A, Corces-Zimmerman R, et al. 2011. A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. *Nature* 472:120–24
77. Novakovic B, Habibi E, Wang S-Y, Arts RJW, Davar R, et al. 2016. β-Glucan reverses the epigenetic state of LPS-induced immunological tolerance. *Cell* 167:1354–68.e14
78. Kamada R, Yang W, Zhang Y, Patel MC, Yang Y, et al. 2018. Interferon stimulation creates chromatin marks and establishes transcriptional memory. *PNAS* 115:E9162–71
79. Domínguez-Andrés J, Novakovic B, Li Y, Scicluna BP, Gresnigt MS, et al. 2019. The itaconate pathway is a central regulatory node linking innate immune tolerance and trained immunity. *Cell Metab*. 29:211–20.e5
80. Giamarellos-Bourboulis EJ, Netea MG, Rovina N, Akinosoglou K, Antoniadou A, et al. 2020. Complex immune dysregulation in COVID-19 patients with severe respiratory failure. *Cell Host Microbe* 27:992–1000.e3
81. Arts RJ, Novakovic B, Ter Horst R, Carvalho A, Bekkering S, et al. 2016. Glutaminolysis and fumarate accumulation integrate immunometabolic and epigenetic programs in trained immunity. *Cell Metab*. 24:807–19
82. Cheng SC, Quintin J, Cramer RA, Shepardson KM, Saeed S, et al. 2014. mTOR- and HIF-1α-mediated aerobic glycolysis as metabolic basis for trained immunity. *Science* 345:1250684
83. Arts RJ, Joosten LA, Netea MG. 2016. Immunometabolic circuits in trained immunity. *Semin. Immunol.* 28:425–30
84. Netea MG, Giamarellos-Bourboulis EJ, Domínguez-Andrés J, Curtis N, van Crevel R, et al. 2020. Trained immunity: a tool for reducing susceptibility to and the severity of SARS-CoV-2 infection. *Cell* 181:969–77
85. Rivas MN, Ebinger JE, Wu M, Sun N, Braun J, et al. 2021. BCG vaccination history associates with decreased SARS-CoV-2 seroprevalence across a diverse cohort of health care workers. *J. Clin. Invest.* 131(2):e145157
86. Urashima M, Otani K, Hasegawa Y, Akutsu T. 2020. BCG vaccination and mortality of COVID-19 across 173 countries: an ecological study. *Int. J. Environ. Res. Public Health* 17(15):5589
87. Green CM, Fanucchi S, Dominguez-Andres J, Fok ET, Moorlag SJCFM, et al. 2020. COVID-19: A model correlating BCG vaccination to protection from mortality implicates trained immunity. medRxiv 2020.04.10.20060905. **https://doi.org/10.1101/2020.04.10.20060905**
88. Ten Doesschate T, Moorlag SJCFM, van der Vaart TW, Taks E, Debisarun P, et al. 2020. Two randomized controlled trials of Bacillus Calmette-Guérin vaccination to reduce absenteeism among health care workers and hospital admission by elderly persons during the COVID-19 pandemic: a structured summary of the study protocols for two randomised controlled trials. *Trials* 21:481
89. Moorlag SJCFM, van Deuren RC, van Werkhoven CH, Jaeger M, Debisarun P, et al. 2020. Safety and COVID-19 symptoms in individuals recently vaccinated with BCG: a retrospective cohort study. *Cell Rep. Med.* 1:100073
90. Tsilika M, Taks E, Dolianitis K, Kotsaki A, Leventogiannis K, et al. 2021. ACTIVATE-2: a double-blind randomized trial of BCG vaccination against COVID19 in individuals at risk. medRxiv 2021.05.20.21257520. **https://doi.org/10.1101/2021.05.20.21257520**

91. Moorlag SJCFM, Taks E, Ten Doesschate T, van der Vaart TW, Janssen AB, et al. 2022. Efficacy of Bacillus Calmette-Guérin vaccination against respiratory tract infections in the elderly during the COVID-19 pandemic. *Clin. Infect. Dis.* 2022:ciac182
92. Counoupas C, Johansen MD, Stella AO, Nguyen DH, Ferguson AL, et al. 2021. A single dose, BCG-adjuvanted COVID-19 vaccine provides sterilising immunity against SARS-CoV-2 infection. *NPJ Vaccines* 6:143
93. Ramos-Martinez E, Falfán-Valencia R, Pérez-Rubio G, Andrade WA, Rojas-Serrano J, et al. 2021. Effect of BCG revaccination on occupationally exposed medical personnel vaccinated against SARS-CoV-2. *Cells* 10:3179
94. Debisarun PA, Gössling KL, Bulut O, Kilic G, Zoodsma M, et al. 2021. Induction of trained immunity by influenza vaccination—impact on COVID-19. *PLOS Pathog.* 17(10):e1009928
95. Kyriazopoulou E, Huet T, Cavalli G, Gori A, Kyprianou M, et al. 2021. Effect of anakinra on mortality in patients with COVID-19: a systematic review and patient-level meta-analysis. *Lancet Rheumatol.* 3:e690–97
96. Conlon A, Ashur C, Washer L, Eagle KA, Hofmann Bowman MA. 2021. Impact of the influenza vaccine on COVID-19 infection rates and severity. *Am. J. Infect. Control* 49:694–700
97. Patwardhan A, Ohler A. 2021. The flu vaccination may have a protective effect on the course of COVID-19 in the pediatric population: When does severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) meet influenza? *Cureus* 13:e12533
98. Fink G, Orlova-Fink N, Schindler T, Grisi S, Ferrer APS, et al. 2021. Inactivated trivalent influenza vaccination is associated with lower mortality among patients with COVID-19 in Brazil. *BMJ Evid. Based Med.* 26:192–93
99. Fedrizzi EN, Girondi JBR, Sakae TM, Steffens SM, de Souza Silvestrin AN, et al. 2021. Efficacy of the measles-mumps-rubella (MMR) vaccine in the reducing the severity of COVID-19: an interim analysis of a randomised controlled clinical trial. medRxiv 2021.09.14.21263598. **https://doi.org/10.1101/2021.09.14.21263598**
100. Mysore V, Cullere X, Settles ML, Ji X, Kattan MW, et al. 2021. Protective heterologous T cell immunity in COVID-19 induced by the trivalent MMR and Tdap vaccine antigens. *Med* 2:1050–71.e7
101. Bruxvoort KJ, Ackerson B, Sy LS, Bhavsar A, Tseng HF, et al. 2021. Recombinant adjuvanted zoster vaccine and reduced risk of COVID-19 diagnosis and hospitalization in older adults. *J. Infect. Dis.* 2021:jiab633
102. Habibzadeh F, Sajadi MM, Chumakov K, Yadollahie M, Kottilil S, et al. 2021. COVID-19 infection among women in Iran exposed versus unexposed to children who received attenuated poliovirus used in oral polio vaccine. *JAMA Netw. Open* 4:e2135044
103. Moorlag SJCFM, Matzaraki V, van Puffelen JH, van der Heijden C, Keating S, et al. 2021. An integrative genomics approach identifies KDM4 as a modulator of trained immunity. *Eur. J. Immunol.* 52(3):431–46
104. Priem B, van Leent MMT, Teunissen AJP, Sofias AM, Mourits VP, et al. 2020. Trained immunity-promoting nanobiologic therapy suppresses tumor growth and potentiates checkpoint inhibition. *Cell* 183:786–801.e19