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Trained Immunity: Reprogramming Innate Immunity in Health and Disease

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Abstract

Traditionally, the innate and adaptive immune systems are differentiated by their specificity and memory capacity. In recent years, however, this paradigm has shifted: Cells of the innate immune system appear to be able to gain memory characteristics after transient stimulation, resulting in an enhanced response upon secondary challenge. This phenomenon has been called trained immunity. Trained immunity is characterized by nonspecific increased responsiveness, mediated via extensive metabolic and epigenetic reprogramming. Trained immunity explains the heterologous effects of vaccines, which result in increased protection against secondary infections. However, in chronic inflammatory conditions, trained immunity can induce maladaptive effects and contribute to hyperinflammation and progression of cardiovascular disease, autoinflammatory syndromes, and neuroinflammation. In this review we summarize the current state of the field of trained immunity, its mechanisms, and its roles in both health and disease.

HISTORICAL PERSPECTIVE

As early as in 1931, in the Swedish province of Norrbotten, Carl Naeslund discovered that after the introduction of bacillus Calmette-Guérin (BCG) vaccination in neonates—who are known to have immature adaptive immune responses—mortality in the region dropped significantly. This drop was more than could be explained by a decrease in tuberculosis alone, and he was "tempted to find an explanation for this much lower mortality among vaccinated children in the idea that BCG provokes a nonspecific immunity. . ." (1, p. 629). It took almost a century to understand that the innate immune system can indeed provide protection against reinfection by building a heterologous immunological memory in innate immune cells, a process that ten years ago was termed trained immunity (2).

In plants and invertebrate animals, which both lack an adaptive immune system, the innate immune system is long known to present memory characteristics. Plants that survive an infection obtain long-term protection against reinfection, a phenomenon called systemic acquired resistance (SAR) (3). SAR spreads from the site of infection throughout the entire plant through signaling molecules, subsequently inducing an enhanced expression of pattern-recognition receptors (PRRs) and the secretion of antimicrobial proteins (4). This is mediated via epigenetic changes and can even be even transmitted to the progeny through seeds (5). In invertebrates such as *Drosophila melanogaster*, a sublethal dose of *Streptococcus pneumoniae* primes macrophages and protects the animal for life against a secondary, otherwise lethal reinfection with the same pathogen, despite the fact that the organism does not have an adaptive immune system (6). A similar phenomenon was observed in beetles and was shown to go beyond even the first generation (7, 8). Even though the definition and mechanisms of innate immune memory in vertebrates were only unraveled in the last decade, older studies showed evidence of BCG protecting athymic nude mice against a secondary lethal infection with *Candida albicans* (9), through a process driven by macrophages (10). In humans, multiple epidemiological studies have shown that live attenuated vaccines can protect against heterologous microorganisms different from the target pathogen (11, 12). In conclusion, plants, invertebrates, and vertebrates are all capable of developing adaptive characteristics in innate immune cells, a de facto memory, which results in a nonspecific increased effector function of the innate immune cell itself. The mechanisms and characteristics of this innate immune memory, or trained immunity, substantially differ from those of the specific memory induced in cells of the adaptive immune system by gene recombination.

TRAINED IMMUNITY VERSUS TOLERANCE

The innate immune system is thus capable of remembering first encounters, resulting in a functional change upon secondary stimulation. This can either enhance or repress immune cell function. The first stimulation will lead to reprogramming of innate immune cells, which can result in an increased effector function upon secondary stimulation, a process now known as trained immunity (2), or a repressed effector function upon secondary stimulation, a process called tolerance. Tolerance and trained immunity are both innate immune memory phenotypes and are mediated largely through similar molecular mechanisms (metabolic and epigenetic reprogramming), although with opposite functional results depending on the initial stimulus and the specific pathways that are induced (**Figure 1**). The immunological phenotype of trained immunity in humans lasts from months (13) to at least one year (14), but earlier studies described nonspecific effects of vaccination in neonates that can have a duration of up to five years (15). Finally, there are recent studies that even indicate transgenerational effects of trained immunity, similar to what has been described for innate immune memory in invertebrates. In newborns vaccinated with BCG, nonspecific beneficial effects and survival were increased when their mothers had prior BCG

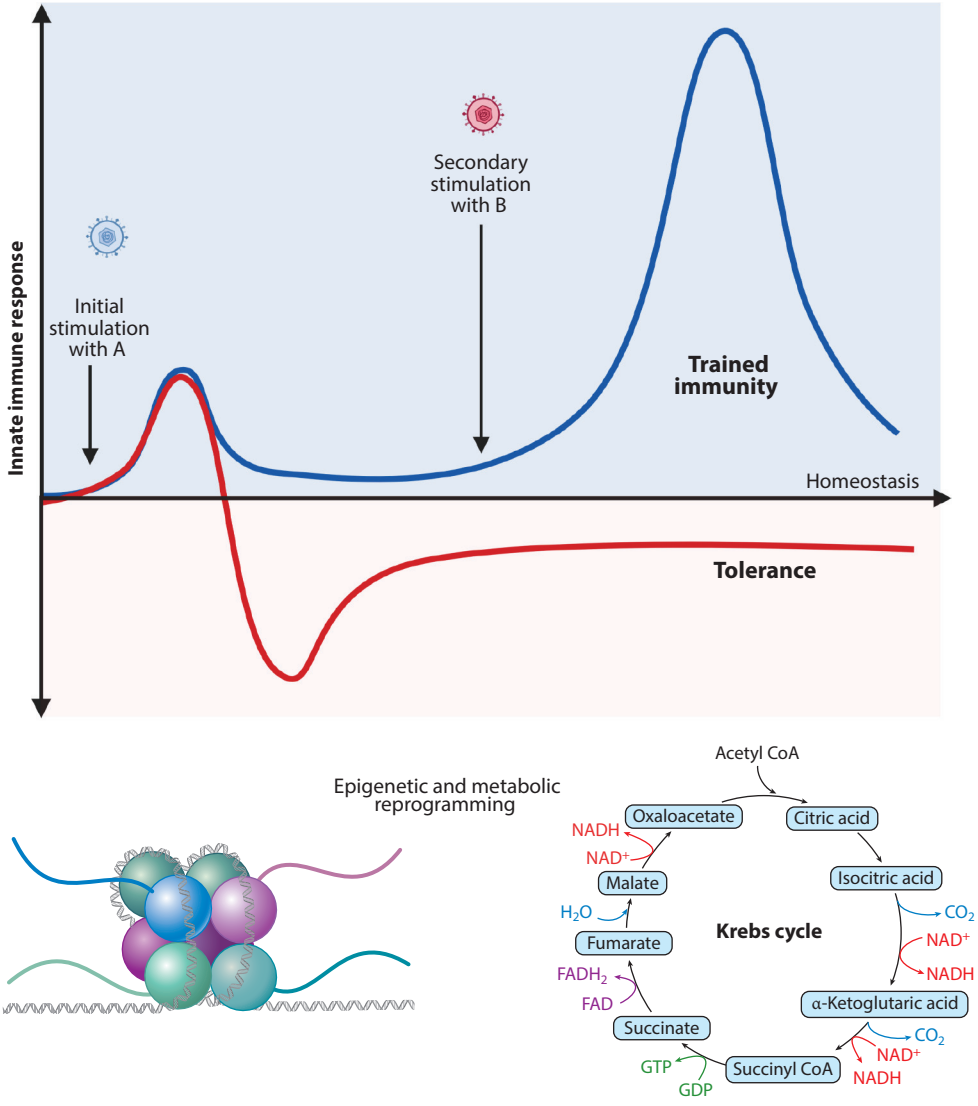

Figure 1

Trained immunity versus tolerance. Microbial or endogenous stimuli (stimulus A) can activate innate immune cells and induce a primary response. Innate immune cells will produce cytokines or activate other effector functions. Depending on the dose and stimulus, subsequent anti-inflammatory mechanisms can be induced, and a secondary stimulation (stimulus B) will not lead to another proinflammatory response, so as to limit tissue damage (tolerance). However, when trained immunity is induced, this leads to enhanced innate immune effector function upon secondary stimulation (compared to the initial response). Both training and tolerance are mediated via metabolic and epigenetic changes, although each phenotype has its own signature: Different genes and pathways will be activated or repressed by different epigenetic and metabolic changes. Figure adapted from image created with BioRender.com.

vaccination (and scarring) as well, indicating a synergistic transgenerational effect of the BCG vaccine (16).

Inducers of Trained Immunity

Induction of trained immunity has been studied for microbial but more recently also for nonmicrobial stimuli. These studies have focused on myeloid cells such as monocytes and macrophages, but trained immunity can also be induced in innate lymphoid populations such as natural killer (NK) cells and innate lymphoid cells (ILCs). Examples of microbial stimuli known to induce trained immunity in monocytes are live-attenuated vaccines such as the BCG vaccine (13), the newer live attenuated tuberculosis vaccine MTBVAC (17), the oral polio vaccine (18), the smallpox vaccine (19), and the measles vaccine (20). The fungal pathogen *Candida albicans* and its cell wall component β-glucan (21) were among the first described inducers of trained immunity in vitro as well as in vivo and are the most used in experimental studies on trained immunity. More recently the malaria pathogen *Plasmodium falciparum* (22) and hepatitis B virus (11) were added to the growing list of microbial inducers of trained immunity. Immune tolerance, on the other hand, can be induced by lipopolysaccharide (LPS) endotoxins from *Escherichia coli* (23) and other microbial ligands (24), although at lower concentration LPS is also able to induce trained immunity as well (24). This dichotomy of dose-dependent training or tolerance is described for more danger-associated molecular patterns and pathogen-associated molecular patterns (24). Nonmicrobial endogenous inducers of trained immunity include lipoproteins such as oxidized low-density lipoprotein (oxLDL) (25), lipoprotein(a) [Lp(a)] (26), and activators of the liver X receptor pathways (27, 28); uric acid (29, 30); catecholamines (31); aldosterone (32); S100-alarmin (33); and interferons (34). Importantly, all inducers of trained immunity affect the inflammatory functions of myeloid cells, although with different potential. In general, microbial inducers of trained immunity such as β-glucan and BCG have a greater potential to induce the inflammatory effector function upon secondary stimulation than endogenous stimuli such as lipids. Importantly, besides its capacity to induce trained immunity, BCG is also a strong inducer of T helper type 1 (Th1) responses and has been employed as a therapeutic alternative in pathologies characterized by exacerbated Th2 responses, such as allergies and asthma (35, 36). Furthermore, due to its strong immunogenicity, BCG has also been used as an adjuvant to enhance the protective capacity of different types of vaccines, such as veterinary vaccines (37) and cancer vaccines (38).

MECHANISMS OF TRAINED IMMUNITY

Whereas in adaptive immune memory gene recombination leads to a long-lived specific memory phenotype resulting in the generation of memory cells, trained immunity develops through different intracellular mechanisms: Upon first encounter of a stimulus, myeloid cells adapt through epigenetic and metabolic reprogramming (**Figure 2**), resulting in hyperresponsiveness upon secondary stimulation. The metabolic and epigenetic changes that underlie trained immunity are intertwined, and our knowledge of which pathways are involved and interact is still increasing (39). Importantly, although some metabolic and epigenetic changes appear to be common denominators in trained immunity, such as the Akt/PI3K/mTOR pathway and changes in histone methylation and acetylation in promoters and enhancers of proinflammatory genes, different stimuli can activate different trained immunity programs.

Metabolic Changes Underlying Trained Immunity

Several metabolic pathways are involved in training innate immune cells (**Figure 2**), such as glycolysis, oxidative phosphorylation (OXPHOS), the TCA cycle, and lipid metabolism. Most of the

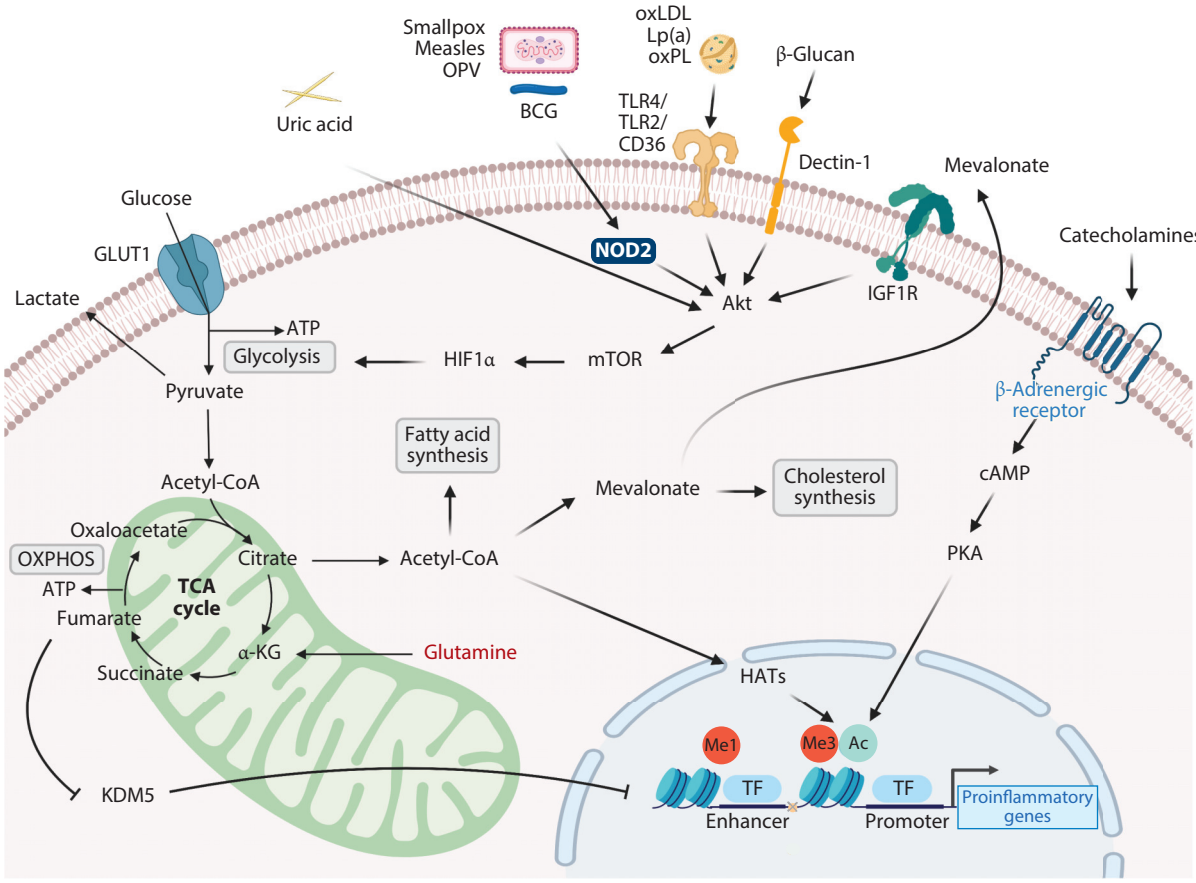

Figure 2

Molecular mechanisms of trained immunity. Inducers of trained immunity, such as β-glucan, BCG, uric acid, and several (oxidized) lipids, activate intracellular metabolic pathways, each via different receptors. The most common pathway is the Akt-HIF1α-mTOR pathway, which ultimately leads to the upregulation of glycolysis, the TCA cycle, and also the cholesterol synthesis pathway. Metabolites from these pathways can in turn regulate epigenetic remodeling of histones; for example, acetyl-CoA serves as an acetyl donor for histone acetyltransferases. Fumarate, on the other hand, a TCA cycle metabolite, inhibits lysine-demethylase KDM5, thereby increasing histone methylation, another epigenetic mark associated with trained immunity. Catecholamines, via activation of the beta-adrenergic receptor, are inducing epigenetic and metabolic changes via the cAMP-PKA pathway. Abbreviations: Ac, acetylation; ATP, adenosine triphosphate; α-KG, α-ketoglutarate; BCG, bacillus Calmette-Guérin; cAMP, cyclic adenosine monophosphate; GLUT1, glucose transporter 1; HAT, histone acetyl transferase; HIF1α, hypoxia-inducible factor 1 alpha; IGF1R, insulin-like growth factor 1 receptor; KDM5, lysin-specific demethylase 5; Lp(a), lipoprotein(a); Me1, monomethylation; Me3, trimethylation; mTOR, mammalian target of rapamycin; NOD2, nucleotide-binding oligomerization domain–containing protein 2; OPV, oral polio vaccine; oxLDL, oxidized low-density lipoprotein; OXPHOS, oxidative phosphorylation; oxPL, oxidized phospholipid; PKA, protein kinase A; TCA, tricarboxylic acid; TF, transcription factor; TLR, Toll-like receptor. Figure adapted from image created with BioRender.com.

evidence listed here comes from studies using an in vitro protocol for inducing trained immunity (40), although the majority of findings are confirmed in in vivo animal models or human studies.

Glycolysis plays a central role in cellular metabolism, and its upregulation is essential during the activation of macrophages (41). Upregulation of glycolysis results in an increased uptake of glucose, which is subsequently converted into pyruvate and transformed into lactate, which is then

released from the cell. Although glycolysis is much less efficient than OXPHOS for the generation of ATP, glycolytic enzymes can be rapidly induced. Cheng et al. (42) showed that in β-glucan-trained monocytes, glucose consumption is increased, indicating upregulation of the glycolysis pathway. Arts et al. (43) confirmed that glucose was converted into lactate via an increased activity of the glycolysis pathway. Several metabolites from this pathway can act as cofactors for DNA and histone methyltransferases (44), indicating a connection between metabolic pathways and epigenetic reprogramming. In β-glucan-trained monocytes, genes involved in glycolysis such as hexokinase and pyruvate kinase are epigenetically modified with activating histone marks (23). Just as in β-glucan-induced training, BCG- and oxLDL-trained monocytes are also characterized by an upregulation of glycolysis (45, 46).

The TCA cycle uses the energy from acetyl-CoA and transfers it to OXPHOS, which results in ATP production. In the first studies with β-glucan-trained monocytes, it was shown that β-glucan induces a shift from OXPHOS to glycolysis, also known as the Warburg effect (42). Instead of providing energy by ATP production, there is an anabolic repurposing of TCA metabolites (43). Glutamine replenishment of the TCA cycle leads to the accumulation of fumarate, which can induce trained immunity by inhibiting the activity of histone demethylase KDM5. This is followed by persistence of histone methylation and opening of the chromatin that is needed for gene transcription and the increased production of proinflammatory cytokines upon restimulation. Subsequent studies showed that the balance between OXPHOS and glycolysis is dependent on the specific concentration of β-glucan used in the studies: A lower dose of β-glucan that was also associated with a hyperresponsive trained phenotype induced an upregulation of both OXPHOS and glycolysis in trained monocytes (47). This activation of OXPHOS is due to enrichment of the activating histone modification H3K4me1 on the enhancers of specific metabolic enzymes by the histone methyltransferase Set7 (47). In BCG- and oxLDL-trained monocytes, a similar upregulation of both OXPHOS and glycolysis is also observed (45, 48). Other intermediates from the TCA cycle such as α-ketoglutarate, a cofactor for JmjC demethylases (49); succinate; and acetyl-CoA play important roles in the regulation of inflammation as well. Succinate can stabilize HIF1α, thereby promoting IL-1β transcription (50). Acetyl-CoA serves as an acetyl donor for histone acetyl transferases (51), which are upregulated in the context of trained immunity. Examples of acetylated genes in trained immunity are genes for hexokinase and lactate dehydrogenase, which in turn promote glycolysis (52). Finally, itaconate, another TCA cycle metabolite, is an important node between trained immunity and tolerance in that it inhibits inflammation and trained immunity. In this sense, itaconate is able to mimic the tolerizing effects of LPS and inhibit the induction of trained immunity by β-glucan through the inhibition of mitochondrial metabolism, leading to decreased responsiveness after secondary stimulation (53).

During the induction of trained immunity by β-glucan, in addition to upregulation of glycolysis and OXPHOS and repurposing of the TCA cycle, an upregulation of cholesterol synthesis pathway genes was observed (23). Inhibition of the cholesterol synthesis pathway by statins prevented the induction of training for β-glucan, BCG, and oxidized LDL, indicating that the cholesterol synthesis pathway is essential for trained immunity (54). Further characterization of the pathway revealed that accumulation of mevalonate is responsible for the induction and further amplification of trained immunity through the IGF1R-Akt-mTOR pathway. Further evidence for a role for mevalonate in trained immunity comes from the characterization of monocytes from patients with deleterious mutations in mevalonate kinase who subsequently accumulate high levels of mevalonate. These patients with the hyperimmunoglobulin D syndrome (HIDS) are characterized by recurrent attacks of sterile inflammation and fever, and their monocyte phenotype resembles an endogenous trained immune phenotype, with increased cytokine production, upregulation of glycolysis, and epigenetic changes (54).

Fatty acid synthesis contributes to inflammation by inducing intracellular stress and activation of the NLRP3 inflammasome (55), whereas fatty acid oxidation is generally believed to be anti-inflammatory. A role for the fatty acid synthesis pathway in trained immunity was demonstrated by Mitroulis et al. (56), who showed that β-glucan training induces a reduction in fatty acid metabolites in hematopoietic progenitors. As a result, intracellular lipids in β-glucan-trained cells appeared shorter and more saturated. Inhibition of the fatty acid synthesis pathway during the first 24-h stimulation period, however, does not prevent training (43). On the other hand, inhibition of fatty acid synthesis during restimulation blunts the trained immunity phenotype (32). Trained immunity induced by aldosterone induces fatty acid synthesis via activation of the mineralocorticoid receptor, ultimately leading to increased trimethylation of lysine 4 on histone 3 (H3K4me3) on genes involved in the fatty acid synthesis pathway as well as an increased production of IL-6 and TNF-α. Preincubation of aldosterone-trained cells with a fatty acid synthesis inhibitor before restimulation prevented augmented cytokine production, showing a role for fatty acid synthesis in the secondary effector function. Monocyte-derived macrophages from patients with hyperaldosteronism, who also have increased arterial wall inflammation, showed a similar increase in TNF-α production upon stimulation compared to trained cells in vitro (57). In contrast, monocytes from patients with sepsis, characterized by immune tolerance, showed lower proinflammatory cytokine production accompanied by impaired beta-oxidation.

Trained immunity is dependent on distinct epigenetic programs. Due to a first encounter with a stimulus, monocytes are rapidly activated and upregulate gene transcription, which is associated with a rapid acquisition of activating histone modifications. After removal of the stimulus, however, some of these epigenetic marks persist, which leads to faster and stronger activation of gene transcription upon secondary stimulation (58). Important epigenetic marks involved in trained immunity are H3K4me3, which marks active promotors; H3K4me1, which marks distal enhancers; and H3K27 acetylation, which marks both active enhancers and promotor regions (23, 59) (**Figure 1**). Repressive histone marks important for trained immunity are H3K9me3 and H3K27me3, which are reduced during training in some cases (45, 60, 61), although these did not play a role in the early priming phase of innate immune memory induced by β-glucan in vitro (59), indicating stimulus-specific epigenetic changes.

In addition to histone modifications, long noncoding RNAs (lncRNAs) were described to link epigenetic and metabolic changes in trained immunity (62, 63) through immune gene–priming lncRNAs that function as transporters for methyl transferases within a topologically associated domain of the chromatin that contains trainable genes. However, this mechanism is only studied in β-glucan-trained cells and warrants further investigation in other models of trained immunity. Finally, the contribution of DNA (de)methylation to the development of trained immunity needs to be investigated in more detail. Preliminary studies show that the induction of LPS tolerance in vitro leads to stable and long-term specific DNA methylation changes, but less is known about these signatures in trained immunity (59). In a recent study with BCG-vaccinated human adults in vivo, DNA methylation appeared useful to discriminate responders (human volunteers that undergo trained immunity upon BCG vaccination) from nonresponders. Enhanced containment of *Mycobacterium tuberculosis* replication after BCG vaccination in responders was accompanied by a wide loss of DNA methylation on promoters of inflammatory genes compared to nonresponders (64, 65). Further studies of the role of DNA methylation in trained immunity are warranted.

REPROGRAMMING OF HEMATOPOIETIC PROGENITOR CELLS

Trained immunity was first described for mature myeloid cells such as monocytes and NK cells (13, 21, 66). The average half-life of myeloid cells in the circulation is only a few days, yet circulating

cells with a trained immunity phenotype have been described to exist months or even a year following in vivo induction of trained immunity with BCG. This strongly suggests that progenitor cells might also be reprogrammed in the context of trained immunity. Indeed, recent work showed that trained immunity can occur in bone marrow progenitor cells in addition to training occurring in mature myeloid cells. Mitroulis et al. (56) showed that modulation of bone marrow progenitors is an integral component of β-glucan training in mice in vivo. β-Glucan training induced reprogramming of hematopoietic stem cell progenitors via IL-1β, and this was also associated with an upregulation of glycolysis and the cholesterol synthesis pathway in progenitor cells, similar to the case of trained monocytes. This resulted in the expansion of myeloid-biased hematopoietic stem and progenitor cells (HSPCs), which was essential for increased protection against reinfection. BCG was also shown to train and expand hematopoietic stem cells in a mouse model in vivo, a process that was dependent on IFN-γ signaling (67). Bone marrow–derived macrophages of BCG-trained mice subsequently acquired protection against infection with *M. tuberculosis*, which was accompanied by epigenetic changes such as H3K4me3 and H3K27ac. Transplantation of the bone marrow of BCG-trained mice into naive mice then conferred protection against reinfection, confirming long-term reprogramming of the bone marrow. These observations were confirmed in a study with human volunteers in which vaccination with BCG led to transcriptional and epigenetic reprogramming of the bone marrow hematopoietic stem cell progenitors up to 90 days after initial vaccination, resulting in a myeloid bias and increased protection against reinfection (68, 69). Combined transcriptional and genetic analysis demonstrated an important role of transcription factors such as hepatic nuclear factors 1a and 1b in this process (69). In $Ldlr^{-/-}$ mice, a four-week Western diet followed by a four-week chow diet induced similar reprogramming of the bone marrow via transcriptomic and epigenetic changes (70). This was mediated via the NLRP3 inflammasome and IL-1β and resulted in increased proliferation and enhanced innate immune responses in the mice. The role of bone marrow reprogramming in other settings of trained immunity is under investigation.

CENTRAL VERSUS PERIPHERAL INDUCTION OF TRAINED IMMUNITY

Monocytes and their bone marrow progenitors are not the only myeloid cells to undergo trained immunity. Similar properties have been described for various populations of tissue macrophages and dendritic cells (DCs). Indeed, one can distinguish activation of trained immunity at the level of bone marrow progenitors and monocytes (central activation) from activation of myeloid cells such as macrophages and DCs in the peripheral tissues (peripheral activation) (**Figure 3**). In the lung, macrophages are capable of obtaining a memory phenotype upon a viral challenge. Lungs of mice that had previously been exposed to gammaherpesvirus showed decreased dust mite–induced asthma complications. This phenotype was dependent on long-term memory in monocyte-derived alveolar macrophages conferring protection against allergic responses (71). Adenovirus infection induced remodeling of resident macrophages, resulting in more pronounced antibacterial immunity upon reinfection (72). This was dependent on $CD8^+$ T lymphocytes, thereby showing close collaboration between trained immunity and adaptive immunity. Finally, Hoyer et al. (73) showed that local injury such as sepsis, myocardial infarction (MI), and stroke could lead to macrophage memory in remote organs, leaving them susceptible for increased activation. In the lung specifically, this was dependent on local IFN-γ priming. Alveolar macrophages are also sensitive to inflammation-induced epigenetic changes leading to long-term immunoparalysis and tolerance (74).

Microglia, resident macrophages of the central nervous system, play a vital role in normal brain functions but are also sensitive to innate immune memory under certain conditions (75). In mouse

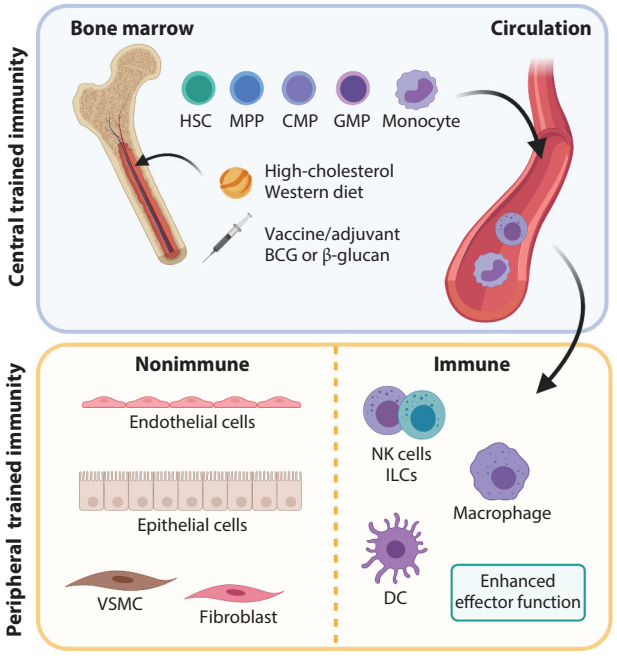

Figure 3

Central versus peripheral trained immunity. Although trained immunity was first discovered in circulating cells such as monocytes and NK cells, these cells have a short life span; it is shorter than the duration of trained immunity in humans in vivo. We now know that progenitor cells of the bone marrow can also obtain a memory phenotype, for example through vaccination or a Western diet, that is then transmitted to their progeny, the cells in circulation. Eventually, these circulating cells migrate to tissues, where they have enhanced effector functions as tissue macrophages and NK cells. Additionally, other immune cells in the tissues, such as DCs and ILCs, are capable of obtaining a trained immunity phenotype as well. Finally, nonimmune cells such as endothelial cells, epithelial cells, fibroblasts, and VSMCs are described to have memory capacities as well (78, 79, 94–96). Abbreviations: BCG, bacillus Calmette-Guérin; CMP, common myeloid progenitor; DC, dendritic cell; GMP, granulocyte-macrophage progenitor; HSC, hematopoietic stem cell; ILC, innate lymphoid cell; MPP, myeloid progenitor population; NK, natural killer; VSMC, vascular smooth muscle cell. Figure adapted from image created with BioRender.com.

models of Alzheimer disease and stroke, both innate immune training and tolerance were shown in brain-resident microglia via epigenetic reprogramming. This memory lasted for at least six months (76) but is also thought to be transgenerational (77). The important role played by chronic inflammation in neurodegenerative processes makes trained immunity a potential important therapeutic target in Alzheimer and Parkinson diseases.

The field of DC memory is relatively new, although there are indications that DCs can obtain a memory phenotype as well. In the lung, local modulation of DCs after the resolution of pneumonia resulted in long-term susceptibility to secondary infections, indicating DC tolerance (78). This was further studied by Hole et al., who showed that DCs can be trained to exhibit enhanced transcriptional activation upon secondary stimulation, a process driven by epigenetic modifications (79). The induction of DC memory might be useful in optimizing vaccination strategies for better clearance of infections. Indeed, one can argue that the adjuvanticity effect on antigen-presenting cells is a functional program similar to induction of trained immunity.

TRAINED IMMUNITY IN LYMPHOID IMMUNE CELLS

In addition to myeloid cells, trained immunity has been described for several populations of cells of the lymphoid lineage, such as NK cells and ILCs (80, 81). NK cells can obtain a memory phenotype and enhanced effector function when exposed to BCG in humans in vivo. In healthy human volunteers, BCG vaccination leads to enhanced production of cytokines by NK cells three months later (66). Similarly, the influenza vaccine is capable of inducing NK cell memory, resulting in enhanced NK cell activation upon secondary stimulation (82). However, previous cytomegalovirus (CMV) infection can impair the induction of NK cell memory to influenza (83). In a human experimental malaria model, NK cell memory lasted at least for four months, but here the induction of NK cell memory required the help of T cells (84). Other microbial stimuli described to induce NK cell memory are haptens (85) and CMV (86). But not only microbial stimuli can induce NK cell training. A combination of IL-12, IL-15, and IL-18 was shown to induce memory-like NK cells, resulting in effector cells with superior control of leukemic cells (87) and enhanced IFN-γ production (88). This affects tumor immune surveillance of NK cells and might be very significant for clinical immunotherapy. In the uterus, training of NK cells and subsequent epigenetic modifications are essential for their function in successful placentation (89). Mechanistically, both metabolic and epigenetic reprogramming are shown to underlie NK cell memory, similar to trained immunity in monocytes (90–93).

ILC memory research is still in its infancy, but recent studies show that liver-resident group 1 ILCs (ILC1s) can be primed in a non-antigen-specific fashion, inducing stable transcriptional, epigenetic, and phenotypic changes up to one month after CMV infection (94). This is driven by cytokines. ILC2s can also obtain immune memory characteristics upon stimulation with allergens (95), similar to hapten-induced NK cell memory, as described above. Further research into the roles of ILC memory in health and disease is warranted.

TRAINED IMMUNITY IN NONIMMUNE CELLS

In addition to immune cells, nonimmune cells can also play a role in the response to pathogens and can produce cytokines and antimicrobial factors. Recently, it has been suggested that they could also be capable of developing memory characteristics, in a concept called expanded trained immunity (96). Indeed, in mesenchymal stem cells, trained immunity can be induced via microRNA expression and DNA methylation changes upon LPS exposure. Proinflammatory cytokine expression was maintained even in the absence of the stimulus (97). In the skin, epithelial stem cells are capable of immune memory after chemical, mechanical, and microbial stimulation, resulting in enhanced healing capacity. This was mediated via epigenetic changes similar to monocyte training and has been termed inflammatory memory (98). Furthermore, epithelial progenitor cells in the respiratory tract can acquire memory during allergic inflammatory disease, which is mediated via long-term epigenetic changes (99). A similar observation was made for mature bronchial epithelial cells that can remember infection through epigenetic regulation. *Pseudomonas aeruginosa* flagellin induces trained immunity in epithelial cells and increased proinflammatory cytokine production upon unrelated secondary stimulation, mediated via histone acetylation and H3K9 methylation (100).

Endothelial cells can produce cytokines and chemokines, and they play a major role in the development of atherosclerotic plaques. El-Osta et al. (101) showed that exposure of cultured endothelial cells in vitro to transient high glucose concentrations caused persistent hyperinflammation through epigenetic changes and altered gene expression during a subsequent period of normoglycemia, a phenomenon known as hyperglycemic memory. Furthermore, oxidized

phospholipids present on Lp(a) were recently shown to induce metabolic reprogramming of endothelial cells, resulting in a proinflammatory and atherogenic phenotype (102), similar to monocyte training by Lp(a) (26). Whether these changes are long-lasting is a topic for future studies. Human coronary smooth muscle cells are also amenable to the induction of trained immunity. oxLDL or BCG priming of smooth muscle cells in vitro led to increased proinflammatory cytokine production upon restimulation seven days later, which was mediated via mTOR-HIF1α signaling and changes in glycolysis and the cholesterol synthesis pathway as well as epigenetic changes (103).

Finally, synovial fibroblasts can become persistently activated via long-term epigenetic changes resulting in enhanced inflammation upon stimulation in rheumatoid arthritis (104). In periodontal disease, human gingival fibroblasts can also sustain an inflammatory phenotype (105), a process that involves epigenetic changes (106, 107). Interestingly, not all fibroblasts can obtain a memory phenotype, as dermal fibroblasts, for example, were not able to undergo trained immunity (108). Further research is warranted to assess the role of trained immunity in (non)immune cells in vivo.

TRAINED IMMUNITY IN HEALTH AND DISEASE

From an evolutionary perspective, trained immunity most likely evolved to protect the host against reinfection, in species that lack adaptive immunity or in newborns that do not yet have functional adaptive immunity. A maladaptive effect of trained immunity, however, can potentially contribute to several disease states in which hyperinflammation or immunosuppression is detrimental for disease pathology. The induction of trained immunity or lack thereof can thereby contribute to both health and disease (**Figure 4**).

Protection Against Reinfection

Neonates are fully dependent on their innate immune system, in addition to antibody supplementation in breast milk. It was therefore initially surprising that vaccination with BCG led to protection against unrelated secondary infections, both viral and bacterial (12, 109, 110). It is now known that several live attenuated vaccines have this beneficial, nonspecific protective effect leading to decreased childhood mortality. Inactivated vaccines such as the diphtheria-tetanus-pertussis (DTP) vaccine have been suggested to induce an opposing program and can diminish the induction of trained immunity and possibly even lead to increased childhood mortality, especially among vaccinated girls (20, 111). This is important for future vaccination strategies and also for the order in which vaccination programs are designed. These epidemiological studies in neonates have been complemented by investigations showing nonspecific protective effects of BCG in adults. In adolescents already vaccinated with BCG at birth, revaccination with either BCG or the new H4IC31 vaccine leads to a 70% reduction of respiratory tract infections compared to the placebo controls (112). In adult human volunteers, BCG vaccination leads to decreased experimental viral infection via the yellow fever vaccine one month after vaccination, a process mediated by IL-1β (113). Another explorative randomized trial studied the effects of BCG on Vi polysaccharide typhoid fever vaccine, an inactive vaccine that induces tolerance (114). Prior BCG vaccination prevented the tolerizing effects of typhoid vaccine, similar to the effects observed for BCG prior to DTP vaccination (115). In individuals aged more than 65 years, BCG can protect against respiratory tract infections, indicating potential for boosting immune function in the elderly (116, 117). Finally, BCG vaccination was shown to alter the clinical and immunological response to malaria in a human trial, indicating a potential for malaria vaccine strategies (118).

A number of animal and human studies have now demonstrated that trained immunity induced by several different stimuli can give rise to increased protection against reinfection in the

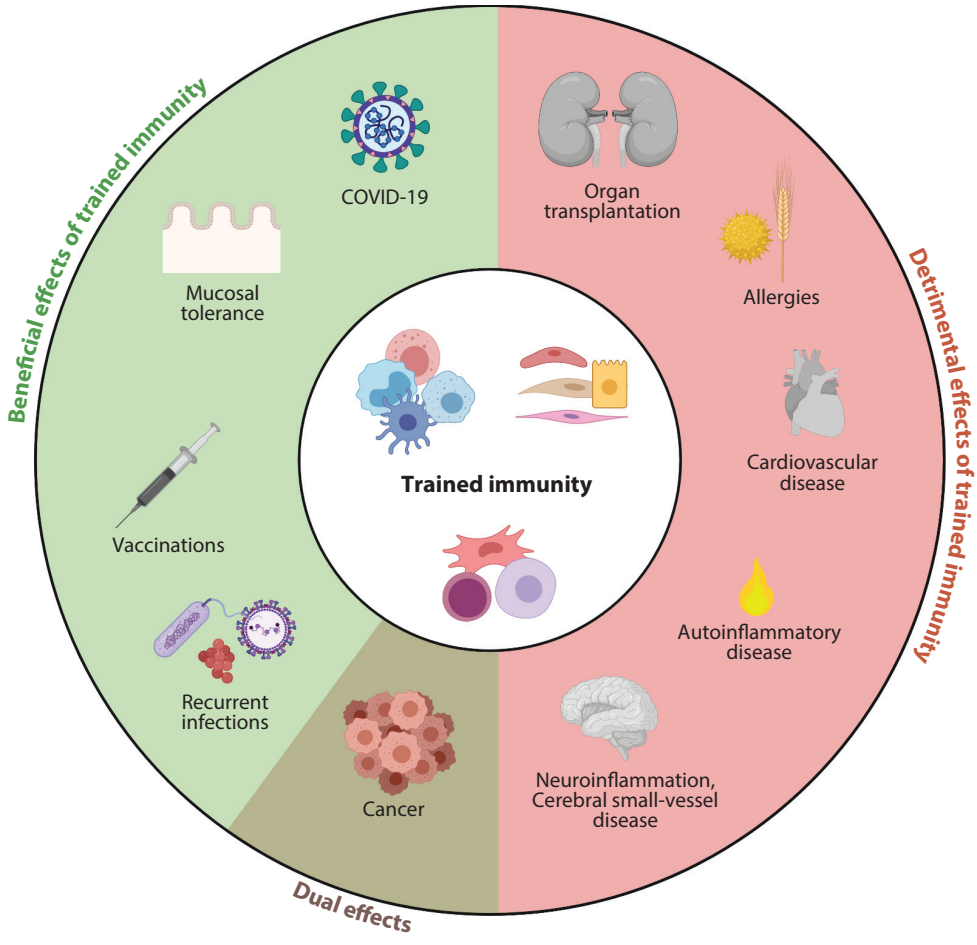

Figure 4

Trained immunity in health and disease. Trained immunity is associated with both health and disease states, and therefore there is therapeutic potential in either inducing it or inhibiting it. Trained immunity can induce increased protection against reinfection, which can be used to optimize vaccination strategies or for protection against, for example, COVID-19. It is important in the induction of mucosal tolerance, and it is already being used to treat bladder cancer. On the other hand, several chronic inflammatory diseases are characterized by an inappropriate trained immunity phenotype, such as cardiovascular diseases, allergies, transplantation rejection, and autoinflammatory diseases. Here, it will be more beneficial to inhibit trained immunity. Abbreviation: COVID-19, coronavirus disease 2019. Figure adapted from image created with BioRender.com.

host. In a recent mouse model, β-glucan training led to protection against *Leishmania* infection via upregulated expression of IL-1 and IL-32 (68). A similar IL-1β-dependent protection against *M. tuberculosis* infection was shown upon β-glucan training in mice, a process that was also mediated via reprogramming of the bone marrow. Mice latently infected with gammaherpesvirus or CMV show antigen-aspecific resistance to infection with *Listeria monocytogenes* and *Yersinia pestis*, a process that was mediated by increased innate immune activation (119). Intraperitoneal stimulation with CpG dinucleotides protected neutropenic mice against intracerebral *Escherichia coli*

infection, indicating that the protection against reinfection can also be induced in remote organs and this protection can cross the blood-brain barrier (120). Locally in the lungs, respiratory infection with attenuated *Bordetella pertussis* protected against the highly pathogenic influenza A virus by dampening cytokine production, indicating protection by the induction of immune tolerance (121). S100-alarmin-induced training, on the other hand, protected newborns from neonatal sepsis and immune tolerance (33). This is important, as sepsis (and the accompanying immune tolerance) generally leads to increased susceptibility to secondary infections (122). Not only can trained immunity prevent sepsis, but immunoparalysis in sepsis patients can even be reversed by the induction of trained immunity (123), a process that was also unraveled mechanistically in vitro and involves reversal of epigenetic changes induced by immune tolerance (59). In conclusion, optimal induction of trained immunity can lead to enhanced protection against infections and reverse immune-tolerized states.

Trained Immunity Against SARS-CoV-2 Infection?

Based on the evidence that BCG induces rapid viral clearance in several human studies (113) and can lead to protection against reinfection, trained immunity was hypothesized to be a tool for reducing susceptibility to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (124). SARS-CoV-2 infection is mild in most cases but can rapidly progress to severe pneumonia and hyperinflammatory phenotypes in a proportion of patients. Elderly individuals and those with comorbidities often leading to a compromised response to viral infections are at increased risk of developing severe disease. It is now proposed that boosting of the innate immune response in these individuals by inducing trained immunity might protect against severe SARS-CoV-2 infection at least for a limited amount of time and until a specific vaccine is developed (124). Although the first epidemiological studies seemed to indicate lower infection rates in countries with active BCG vaccination policies (125), causality cannot be established and several biases cannot be excluded, such as demographics, genetics, and infectious burden (126). Also, reporting and diagnosing of coronavirus disease 2019 (COVID-19) cases differ greatly among countries, making these epidemiological studies hard to interpret. Furthermore, it is not known whether a trained monocyte phenotype persists for more than one year after vaccination (14). Therefore, several randomized controlled trials have now started to (re)induce heterologous protection against SARS-CoV-2 using BCG (127, 128), and the oral polio vaccine and measles vaccine are under consideration for additional randomized controlled trials (129).

Trained Immunity in Cancer

In cancer, the activation of the immune system is a double-edged sword. Efficient activation of the immune system can induce antitumor effects and elimination of cancer, but excessive or chronic inflammation can also promote tumor progression. BCG vaccination is shown to have antitumor effects and is already in use for the treatment of bladder cancer, melanoma, leukemia, and lymphoma (130–132). The antitumor capacity of BCG is regulated via the induction of trained immunity, a process in which autophagy plays an important role (133). Furthermore, the induction of trained immunity by β-glucan was shown to protect against chemotherapy-induced myelosuppression and inhibit the growth of Lewis lung carcinoma (56, 134). Indeed, β-glucan has long been used as an immunostimulatory agent in cancer in countries in East Asia, and in the United States it is currently in clinical trials in combination with checkpoint inhibitors (**https://www.clinicaltrials.gov**). Further studies are warranted to fully understand the role of trained immunity in cancer and to harness its therapeutic potential (135).

Trained Immunity in Intestinal Homeostasis

In the gut, innate immune memory likely can have both a beneficial role and a deleterious effect in inflammatory bowel disease. Cells of the gastrointestinal tract are continuously exposed to relatively high concentrations of LPS through the microbiome and diet. In this sense, intestinal stromal cells are able to provide long-lasting immune responses against diverse pathogens and trigger the quick recruitment of immune cells to the site of infection (136). Cellular tolerance and an increased threshold of cellular activation are continuously induced in the healthy gut mucosa and are likely beneficial for preventing hyperinflammation (137). The gut microbiota is hypothesized to regulate the induction of innate immune memory (138), but external stimuli such as β-glucans can also affect intestinal inflammation and epithelial barrier function (139) in both beneficial and deleterious ways. Using a model of dextran sodium sulfate (DSS)-induced colitis, Heinsbroek et al. (140) showed that orally delivered β-glucans can aggravate intestinal inflammation. On the other hand, mice lacking dectin-1, the receptor for β-glucans, also exhibit increased susceptibility to DSS-induced colitis; this also occurs in humans with specific polymorphisms in dectin-1 (141). Furthermore, prolonged oral treatment of mice with antifungal drugs increases disease severity in models of chronic colitis as well as chronic allergic airways disease, indicating the importance of a healthy fungal community in the gut but also highlighting the influence of gut microbiota on peripheral immune responses and allergic diseases (142). Further research is warranted to better understand the roles of trained immunity in the gut in both health and disease.

Cardiometabolic Disease

Monocytes and macrophages play a pivotal role in the disease progression of cardiometabolic disorders, such as atherosclerosis, diabetes, and obesity. Several lines of evidence now indicate that in these chronic inflammatory cardiometabolic diseases, trained immunity can play a detrimental role (143–145). First of all, trained immunity may explain the known epidemiological association between the infectious burden and increased cardiovascular disease risk, as discussed in Reference 146. Secondly, in addition to microbial stimuli, several endogenous atherogenic stimuli are able to induce trained innate immunity in monocytes in vitro, such as oxLDL (25), Lp(a) (26), catecholamines (31), and aldosterone (32).

Furthermore, hyperglycemia can induce innate immune memory via long-term epigenetic changes in both monocytes and endothelial cells (101, 147, 148). In vitro, training of monocytes with oxLDL in high-glucose conditions boosted cytokine production capacity compared to oxLDL training in normoglycemic conditions (46). Recently, it was shown that diabetes also induces enhanced proliferation of HSPCs in the bone marrow and a myeloid bias, leading to augmented circulating myeloid cell numbers and enhanced atherosclerosis (149, 150). This was regulated via the interaction between endothelial cells and bone marrow progenitor cells. Furthermore, in an atherosclerosis-prone *Ldlr*$^{-/-}$ mouse model, a four-week Western diet induced long-term reprogramming of cells of the myeloid lineage, a process that originated in changes in bone marrow progenitor cells (70). These inflammatory changes persisted for at least four weeks after reversal of the diet to normal chow.

In human observational studies, monocytes from patients with symptomatic atherosclerosis exhibited a trained immunity phenotype with increased proinflammatory cytokine production, accompanied by metabolic and epigenetic changes compared to healthy controls (61). This was confirmed in another study by Shirai et al. (151), who showed that monocytes from patients with coronary atherosclerosis have a proinflammatory phenotype and enhanced glycolytic activity, even after differentiation into macrophages in vitro. In treatment-naive patients with familial hypercholesterolemia, monocytes showed a trained immunity phenotype, in contrast to healthy

controls, characterized by an increased proinflammatory phenotype, that persisted up to three months after lipid-lowering therapy with statins, indicating a memory capacity and an involvement of the bone marrow (60). A similar hyperinflammatory monocyte phenotype was observed in patients with elevated levels of Lp(a) (26), who also had increased vascular wall inflammation as measured with FDG-PET/CT (fluorodeoxyglucose positron emission tomography/computed tomography). This proinflammatory phenotype was not reversed upon Lp(a) lowering with PCSK9 for 90 days (152). Lp(a) was recently shown to induce hematopoietic reprogramming as well (153).

Catecholamines induce trained immunity in monocytes in vitro. In patients with pheochromocytoma, who have chronic increased levels of catecholamines, increased systemic inflammation and an elevated cytokine production capacity of myeloid cells were observed. These did not decline after surgical removal of the tumor, which indicates a memory phenotype in the circulating cells. And indeed, trained immunity was confirmed by epigenetic analysis showing long-term enriched H3K4me3 in promoter regions of proinflammatory genes (31).

In a small proof-of-principle study, it was shown that lifestyle intervention might be an interesting therapeutic target for decreasing inflammation in patients at risk for cardiovascular diseases. In a group of obese and hypertensive patients, sedentary behavior was reduced over a 16-week period, which induced a reduction in cytokine production capacity upon secondary stimulation. This was accompanied by anti-inflammatory changes in intracellular metabolism, such as a decrease in glycolysis and OXPHOS. It would be interesting to study whether these observed changes were also accompanied by long-term epigenetic changes. Lifestyle changes are increasingly seen as a potential therapy for cardiovascular disease and the underlying innate immune memory, and it will be interesting to study the effects on trained immunity (154).

Neurodegenerative Disease

Microglia are the resident macrophages of the central nervous system and play a vital role in normal brain function and pathologies. Microglial immune memory has now been suggested as an underlying cause of neuroinflammation (75). As mentioned above, microglia can be trained, so as to result in metabolic and epigenetic changes. This was first shown by Wendeln et al. (76), who showed that both immune training and tolerance can be induced in brain-resident macrophages via changes in H3K4me1 and H3K27ac, which lasted for at least six months. The imprinted memory, in the case of trained immunity induction, resulted in exacerbated cerebral inflammation and beta-amyloidosis in a mouse model of Alzheimer disease. When tolerance was induced, on the other hand, disease pathology was alleviated. Similar context-dependent observations in the microglia were made by Datta et al. (155), who showed that this process was dependent on histone acetyltransferases HDAC1/2. We now know that microglial immune memory can be induced by both microbial (156) and endogenous stimuli such as stress (157), and it is thought to be transgenerational (77) or at least long-term, with an important role for IL-1β in early life (158–160).

In the brain, vascular diseases such as arteriolosclerosis underlie cognitive decline and dementia. Here, trained immunity might also contribute to the pathology of disease. In an elderly cohort of individuals with cerebral small-vessel disease, monocytes showed a proinflammatory phenotype and disease progression associated with increased cytokine production capacity (161).

Autoinflammatory and Allergic Diseases

Besides cardiovascular and neuroinflammatory diseases, patients with several other autoinflammatory diseases or allergies have been described to suffer from the detrimental effects of hyperinflammation in the context of trained immunity (162). Patients with HIDS suffer from recurrent febrile episodes and hyperinflammation and are characterized by mevalonate kinase deficiency

(163). Monocytes isolated from these patients exhibit a trained phenotype, as shown by increased production of proinflammatory cytokines as well as metabolic and epigenetic rewiring. It is now known that the accumulation of mevalonate is responsible for these effects, through a positive loop activated by IGF1R (54). A similar trained immunity phenotype is likely responsible for the hyperinflammatory patterns in other autoinflammatory syndromes such as familial Mediterranean fever, Behcet disease, and Schnitzler syndrome. Future studies are warranted to establish the involvement of trained immunity in these diseases.

In patients with systemic lupus erythematosus, hematopoietic stem and progenitor cells show transcriptomic reprogramming and myeloid skewing similar to what has been described in trained immunity, resulting in increased circulating neutrophil numbers, increased inflammation, and disease progression (164). In gouty arthritis, monocytes become hyperinflammatory due to priming with soluble uric acid, leading to increased proinflammatory cytokine production upon restimulation, which is mediated via epigenetic and metabolic reprogramming (29, 30). In murine autoimmune arthritis, myeloid skewing occurs in HSPCs, resulting in increased inflammation. Whether this is mediated via metabolic and epigenetic changes, however, requires further investigation (165). The therapeutic potential of inducing trained immunity or tolerance in autoinflammatory diseases was elegantly studied by Jeljeli et al. (166). They showed that the induction of LPS tolerance alleviated fibrosis in a mouse model of systemic sclerosis, whereas induction of training by BCG exacerbated disease progression. The induction of two opposing immune programs resulted in opposing disease states, collectively mediated via innate immune activation (166).

Recently, a potential role for trained immunity and epigenetic changes was hypothesized to underlie food allergy (167). Allergic children have an increased innate immune response compared to nonallergic children (168) that is already present in early life (169). Monocytes from children with food allergy show changes in innate immune function resembling a trained phenotype (170), although the underlying epigenetic changes and memory upon removal of the stimulus are still under investigation. The development of food allergy later in life, however, can be predicted from monocyte immune responses at birth, indicating some sort of programmed phenotype (171). However, the induction of a balanced state of trained immunity has also been suggested to prevent (food) allergy. Several studies have shown the beneficial effects of vaccines on the development of (food) allergies (172). Prior BCG vaccination was shown to suppress allergic sensitization in an animal model of allergic airway disease (173). This was confirmed in another study in which gammaherpesvirus infection provided protection against allergic asthma via activation of the innate immune system (71). In a similar fashion, whole-cell pertussis vaccination decreased the risk of IgE-mediated food allergy (174). Large randomized clinical trials are being conducted to study whether BCG vaccination at birth prevents the development of food allergy in countries with low infectious burden (175). To improve prevention or treatment strategies, more research is needed to understand the difference between the potential of trained immunity to prevent food allergy and the detrimental effects of trained immunity once the allergy is established. On the other hand, these data could also mirror different programs of trained immunity. Indeed, trained immunity is a means by which innate immune cells gain a different function through long-term epigenetic changes, rather than a specific transcriptional program. Some of these changes can be protective in certain circumstances (a protective trained immunity program), while other changes can represent the molecular substrate of disease (a deleterious trained immunity program).

Organ Transplantation

The role of innate immune cells in organ transplantation is poorly studied, and the focus has long been on the role of the adaptive immune system. However, recent advances have shown

that the innate immune system not only plays a role in the initial immune response against allografts but also partially mediates long-term chronic rejection (176). Braza et al. (177) showed that monocytes in the allograft obtain a trained immunity phenotype that is induced by vimentin and HMGB1, and this results in allograft rejection. Inhibition of trained immunity by mTOR high-density lipoprotein (HDL) nanoparticles, on the other hand, leads to successful organ transplantation, indicating a potential for transplantation therapeutics (177).

CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVES

With our recent increasing understanding of the role of trained immunity in health and disease, we are also observing a growing knowledge gap. Trained immunity is defined as a long-term enhanced secondary effector function upon a first stimulation, but we are increasingly realizing that the underlying mechanisms and subsequent secondary phenotype can differ for each stimulus or in different health or disease states. This might also impact the way we need to boost trained immunity to achieve health, such as by using nonspecific effects of vaccines, or inhibit it in chronic inflammatory diseases. We have only begun to unravel the role of trained immunity in (inflamm)aging. Further research is therefore warranted to study the diversity of stimuli and disease pathologies, the accompanying intracellular metabolic and epigenetic changes that are induced, and the subsequent increased inflammatory response upon secondary stimulation, in order to optimize the therapeutic potential that trained immunity encompasses (178).

Ideal targets for designing novel approaches for therapeutics are the central mechanisms underlying trained immunity, such as metabolic and epigenetic changes. Both these processes are amenable for therapeutic targeting, as has been suggested in several reviews (178–180). Epigenetic enzymes such as histone demethylase KDM5 and histone methyltransferase Set7 are known to regulate trained immunity (43, 47), and Set7 is even implicated in bone marrow reprogramming by β-glucan. These two enzymes are therefore interesting targets for preventing trained immunity. Another group of potential epigenetic modifiers is bromodomain and extraterminal domain inhibitors (BETis). BETis are increasingly recognized for their therapeutic potential in inflammation (181), as they interfere with the recognition of acetylated histone marks, areas that could well be primed by trained immunity for increased transcription upon secondary stimulation. An example of a BETi that is under investigation is iBET151. iBET151 was shown to suppress the immune response during a fungal infection and prevent the induction of trained immunity (182). iBET151 was also shown to reduce chronic inflammation in rheumatoid arthritis and diabetes (183, 184). Several other BETis are now studied for their anti-inflammatory potential. Next to dampening the effects of trained immunity and the written epigenetic marks, it would be interesting to reverse trained immunity and remove the accompanying epigenetic marks. Would simply removing the stimulus for long enough be sufficient to reverse trained immunity and the accompanying epigenetic changes? Further research is much warranted to study reversal of a trained phenotype and the enzymes that remove histone marks.

In addition to epigenetic enzymes, metabolic changes are the second interesting therapeutic target in trained immunity. Even though different metabolic pathways have been described for different trained immunity stimuli, most stimuli share the activation of the PI3K/Akt/mTOR pathway and subsequent induction of glycolysis (**Figure 2**), which would therefore be an interesting first target. Several small molecules have been shown to reduce glycolysis in vivo, for example 3-PO, a partial glycolysis inhibitor that can significantly reduce atherosclerosis development in an atherosclerotic mouse model (185). Interestingly, glycolysis is not completely blocked by 3-PO, thereby guaranteeing normal immune function of the innate immune cell when needed for fighting infections. Secondly, rapamycin-loaded HDL-nanoparticles have been shown to specifically

target the Akt-mTOR pathway in cells of the innate immune system. With the use of rapamycin-loaded HDL nanoparticles, trained immunity induced by allograft transplantation could be inhibited and allograft rejection was prevented (177). A similar nanoparticle approach was used to target macrophages in an atherosclerotic mouse model. Duivenvoorden et al. (186) showed that statin-loaded HDL nanoparticles were able to prevent plaque formation and inflammation by specifically targeting macrophages. This corresponds with our finding that statins are able to prevent trained immunity in vitro (54). Unfortunately, statins were unable to reverse already established trained immunity phenotypes in patients with hypercholesterolemia (60), indicating that reversal and prevention might need two different therapeutic approaches.

In conclusion, trained immunity is a recently described property of myeloid cells that allows them to undergo long-term functional reprogramming upon a short interaction with a stimulatory agent. This interaction can either induce long-term improvement of host defense and protection against heterologous infections when induced adaptively by infections or vaccinations or lead to an inflammatory scar when augmented inappropriately by endogenous ligands, leading to inflammation-mediated diseases. Understanding the pathways and mechanisms that mediate trained immunity will have the potential to improve the efficacy of vaccination on the one hand and to provide new therapeutic targets in inflammatory and autoimmune diseases on the other hand.

DISCLOSURE STATEMENT

M.G.N. and L.A.B.J. are scientific founders of TTxD and are owners of two patents on modulation of trained immunity. L.A.B.J. is an SAB member of Olatec Pharmaceuticals.

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LITERATURE CITED

1. Naeslund C. 1931. Résultats des expériences de vaccination par le BCG poursuivies dans le Norrbotten (Suède) (Septembre 1927–Décembre 1931). *Rev. Tuberc.* 12:617–36
2. Netea M, Quintin J, van der Meer J. 2011. Trained immunity: a memory for innate host defense. *Cell Host Microbe* 9(5):355–61
3. Durrant WE, Dong X. 2004. Systemic acquired resistance. *Annu. Rev. Phytopathol.* 42(1):185–209
4. Fu ZQ, Dong X. 2013. Systemic acquired resistance: turning local infection into global defense. *Annu. Rev. Plant Biol.* 64(1):839–63
5. Reimer-Michalski EM, Conrath U. 2016. Innate immune memory in plants. *Semin. Immunol.* 28(4):319–27
6. Pham LN, Dionne MS, Shirasu-Hiza M, Schneider DS. 2007. A specific primed immune response in *Drosophila* is dependent on phagocytes. *PLOS Pathog.* 3(3):e26
7. Tate AT, Andolfatto P, Demuth JP, Graham AL. 2017. The within-host dynamics of infection in trans-generationally primed flour beetles. *Mol. Ecol.* 26(14):3794–807

8. Moret Y, Siva-Jothy MT. 2003. Adaptive innate immunity? Responsive-mode prophylaxis in the mealworm beetle, *Tenebrio molitor*. *Proc. R. Soc. B* 270(1532):2475–80
9. Tribouley J, Tribouley-Duret J, Appriou M. 1978. Influence du bacille de Calmette et Guérin (BCG) sur la réceptivité de la souris nude vis-à-vis de *Schistosoma mansoni* [Effect of Bacillus Calmette-Guérin (BCG) on the receptivity of nude mice to *Schistosoma mansoni*]. *C. R. Seances Soc. Biol. Fil.* 172(5):902–4
10. van't Wout JW, Poell R, van Furth R. 1992. The role of BCG/PPD-activated macrophages in resistance against systemic candidiasis in mice. *Scand. J. Immunol.* 36(5):713–20
11. Hong M, Sandalova E, Low D, Gehring AJ, Fieni S, et al. 2015. Trained immunity in newborn infants of HBV-infected mothers. *Nat. Commun.* 6:6588
12. Jensen KJ, Larsen N, Sorensen SB, 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(6):956–67
13. Kleinnijenhuis J, Quintin J, Preijers F, Joosten LAB, Ifrim DC, et al. 2012. Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. *PNAS* 109(43):17537–42
14. Kleinnijenhuis J, Quintin J, Preijers F, Benn CS, Joosten LAB, et al. 2014. Long-lasting effects of BCG vaccination on both heterologous Th1/Th17 responses and innate trained immunity. *J. Innate Immun.* 6(2):152–58
15. Nankabirwa V, Tumwine JK, Mugaba PM, Tylleskär T, Sommerfelt H, et al. 2015. Child survival and BCG vaccination: a community based prospective cohort study in Uganda. *BMC Public Health* 15(1):175
16. Berendsen M, Øland C, Bles P, Jensen A, Kofoed P, et al. 2020. Maternal priming: Bacillus Calmette-Guérin (BCG) vaccine scarring in mothers enhances the survival of their child with a BCG vaccine scar. *J. Pediatr. Infect. Dis. Soc.* 9(2):166–72
17. Tarancón R, Domínguez-Andrés J, Uranga S, Ferreira AV, Groh LA, et al. 2020. New live attenuated tuberculosis vaccine MTBVAC induces trained immunity and confers protection against experimental lethal pneumonia. *PLOS Pathog.* 16(4):e1008404
18. Lund N, Andersen A, Hansen ASK, Jepsen FS, Barbosa A, et al. 2015. The effect of oral polio vaccine at birth on infant mortality: a randomized trial. *Clin. Infect. Dis.* 61(10):1504–11
19. Rieckmann A, Villumsen M, Sørup S, Klingen Haugaard L, 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(2):695–705
20. Higgins JPT, 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
21. 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(2):223–32
22. Schrum JE, Crabtree JN, Dobbs KR, Kiritsy MC, Reed GW, et al. 2018. Cutting edge: *Plasmodium falciparum* induces trained innate immunity. *J. Immunol.* 200(4):1243–48
23. Saeed S, Quintin J, Kerstens HHD, Rao NA, Aghajanirefah A, et al. 2014. Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. *Science* 345(6204):1251086
24. Ifrim DC, Quintin J, Joosten LAB, 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(4):534–45
25. Bekkering S, Quintin J, Joosten LAB, van der Meer JWM, Netea MG, Riksen NP. 2014. Oxidized low-density lipoprotein induces long-term proinflammatory cytokine production and foam cell formation via epigenetic reprogramming of monocytes. *Arterioscler. Thromb. Vasc. Biol.* 34(8):1731–38
26. van der Valk FM, Bekkering S, Kroon J, Yeang C, Van den Bossche J, et al. 2016. Oxidized phospholipids on lipoprotein(a) elicit arterial wall inflammation and an inflammatory monocyte response in humans. *Circulation* 134(8):611–24
27. Sohrabi Y, Sonntag GVH, Braun LC, Lagache SMM, Liebmann M, et al. 2020. LXR activation induces a proinflammatory trained innate immunity-phenotype in human monocytes. *Front. Immunol.* 11:353

28. Fontaine C, Rigamonti E, Nohara A, Gervois P, Teissier E, et al. 2007. Liver X receptor activation potentiates the lipopolysaccharide response in human macrophages. *Circ. Res.* 101(1):40–49
29. Crisan TO, Cleophas MCP, 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(4):755–62
30. Crişan TO, Cleophas MCP, Novakovic B, Erler K, van de Veerdonk FL, et al. 2017. Uric acid priming in human monocytes is driven by the AKT-PRAS40 autophagy pathway. *PNAS* 114(21):5485–90
31. van der Heijden CDCC, Groh L, Keating ST, Kaffa C, Noz MP, et al. 2020. Catecholamines induce trained immunity in monocytes in vitro and in vivo. *Circ. Res.* 127(2):269–83
32. van der Heijden CDCC, Keating ST, Groh L, Joosten LAB, Netea MG, Riksen NP. 2020. Aldosterone induces trained immunity: the role of fatty acid synthesis. *Cardiovasc. Res.* 116(2):317–28
33. Ulas T, Pirr S, Fehlhaber B, Bickes MS, Loof TG, et al. 2017. S100-alarmin-induced innate immune programming protects newborn infants from sepsis. *Nat. Immunol.* 18(6):622–32
34. Kamada R, Yang W, Zhang Y, Patel MC, Yang Y, et al. 2018. Interferon stimulation creates chromatin marks and establishes transcriptional memory. *PNAS* 115(39):E9162–71
35. Tarancón R, Uranga S, Martín C, Aguiló N. 2019. *Mycobacterium tuberculosis* infection prevents asthma and abrogates eosinophilopoiesis in an experimental model. *Allergy* 74(12):2512–14
36. Cavallo GP, Elia M, Giordano D, Baldi C, Cammarota R. 2002. Decrease of specific and total IgE levels in allergic patients after BCG vaccination: preliminary report. *Arch. Otolaryngol. Head Neck Surg.* 128(9):1058–60
37. Guo Y-J, Wu D, Wang K-Y, Sun S-H. 2007. Adjuvant effects of bacillus Calmette-Guerin DNA or CpG-oligonucleotide in the immune response to *Taenia solium* cysticercosis vaccine in porcine. *Scand. J. Immunol.* 66(6):619–27
38. Nishida S, Tsuboi A, Tanemura A, Ito T, Nakajima H, et al. 2019. Immune adjuvant therapy using Bacillus Calmette-Guérin cell wall skeleton (BCG-CWS) in advanced malignancies: a phase 1 study of safety and immunogenicity assessments. *Medicine* 98(33):e16771
39. Domínguez-Andrés J, Joosten LA, Netea MG. 2019. Induction of innate immune memory: the role of cellular metabolism. *Curr. Opin. Immunol.* 56:10–16
40. Bekkering S, Blok BA, Joosten LAB, Riksen NP, van Crevel R, Netea MG. 2016. In vitro experimental model of trained innate immunity in human primary monocytes. *Clin. Vaccine Immunol.* 23(12):926–33. Erratum. 2017. *Clin. Vaccine Immunol.* 24(5):e00096-17
41. Wang T, Liu H, Lian G, Zhang S-Y, Wang X, Jiang H. 2017. HIF1α-induced glycolysis metabolism is essential to the activation of inflammatory macrophages. *Mediators Inflamm.* 2017:9029327
42. Cheng S-C, Quintin J, Cramer RA, Shepardson KM, Saeed S, et al. 2014. mTOR- and HIF-1-mediated aerobic glycolysis as metabolic basis for trained immunity. *Science* 345(6204):1250684
43. Arts RJW, 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(6):807–19
44. Etchegaray JP, Mostoslavsky R. 2016. Interplay between metabolism and epigenetics: a nuclear adaptation to environmental changes. *Mol. Cell* 62(5):695–711
45. Arts RJW, Carvalho A, La Rocca C, Palma C, Rodrigues F, et al. 2016. Immunometabolic pathways in BCG-induced trained immunity. *Cell Rep.* 17(10):2562–71
46. Keating ST, Groh L, Thiem K, Bekkering S, Li Y, et al. 2020. Rewiring of glucose metabolism defines trained immunity induced by oxidized low-density lipoprotein. *J. Mol. Med.* 98(6):819–31
47. Keating ST, Groh L, van der Heijden CDCC, Rodriguez H, dos Santos JC, et al. 2020. The Set7 lysine methyltransferase regulates plasticity in oxidative phosphorylation necessary for trained immunity induced by β-glucan. *Cell Rep.* 31(3):107548
48. Sohrabi Y, Lagache SMM, Schnack L, Godfrey R, Kahles F, et al. 2019. mTOR-dependent oxidative stress regulates oxLDL-induced trained innate immunity in human monocytes. *Front. Immunol.* 9:3155
49. Xiao M, Yang H, Xu W, Ma S, Lin H, et al. 2012. Inhibition of α-KG-dependent histone and DNA demethylases by fumarate and succinate that are accumulated in mutations of FH and SDH tumor suppressors. *Genes Dev.* 26(12):1326–38

50. Tannahill GM, Curtis AM, Adamik J, Palsson-Mcdermott EM, McGettrick AF, et al. 2013. Succinate is an inflammatory signal that induces IL-1β through HIF-1α. *Nature* 496(7444):238–42
51. Friedmann DR, Marmorstein R. 2013. Structure and mechanism of non-histone protein acetyltransferase enzymes. *FEBS J.* 280(22):5570–81
52. Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR, Thompson CB. 2009. ATP-citrate lyase links cellular metabolism to histone acetylation. *Science* 324(5930):1076–80
53. 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(1):211–20.e5
54. Bekkering S, Arts RJW, Novakovic B, Kourtzelis I, van der Heijden CDCC, et al. 2018. Metabolic induction of trained immunity through the mevalonate pathway. *Cell* 172(1–2):135–46.e9
55. Sheedy FJ, Grebe A, Rayner KJ, Kalantari P, Ramkhelawon B, et al. 2013. CD36 coordinates NLRP3 inflammasome activation by facilitating intracellular nucleation of soluble ligands into particulate ligands in sterile inflammation. *Nat. Immunol.* 14(8):812–20
56. 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(1–2):147–61.e12
57. van der Heijden CD, Smeets EM, Aarntzen EH, Noz MP, Monajemi H, et al. 2020. Arterial wall inflammation and increased hematopoietic activity in patients with primary aldosteronism. *J. Clin. Endocrinol. Metab.* 105(5):e1967–80
58. Netea MG, Domínguez-Andrés J, Barreiro LB, Chavakis T, Divangahi M, et al. 2020. Defining trained immunity and its role in health and disease. *Nat. Rev. Immunol.* 20(6):375–88
59. Novakovic B, Habibi E, Wang SY, Arts RJW, Davar R, et al. 2016. β-Glucan reverses the epigenetic state of LPS-induced immunological tolerance. *Cell* 167(5):1354–68.e14
60. Bekkering S, Stiekema LCA, Bernelot Moens S, Verweij SL, Novakovic B, et al. 2019. Treatment with statins does not revert trained immunity in patients with familial hypercholesterolemia. *Cell Metab.* 30(1):1–2
61. Bekkering S, van den Munckhof I, Nielen T, Lamfers E, Dinarello C, et al. 2016. Innate immune cell activation and epigenetic remodeling in symptomatic and asymptomatic atherosclerosis in humans in vivo. *Atherosclerosis* 254:228–36
62. Fok ET, Davignon L, Fanucchi S, Mhlanga MM. 2019. The lncRNA connection between cellular metabolism and epigenetics in trained immunity. *Front. Immunol.* 29(9):3184
63. Fanucchi S, Mhlanga MM. 2019. Lnc-ing trained immunity to chromatin architecture. *Front. Cell Dev. Biol.* 7:2
64. Verma D, Parasa VR, Raffetseder J, Martis M, Mehta RB, et al. 2017. Anti-mycobacterial activity correlates with altered DNA methylation pattern in immune cells from BCG-vaccinated subjects. *Sci. Rep.* 7(1):12305
65. Das J, Verma D, Gustafsson M, Lerm M. 2019. Identification of DNA methylation patterns predisposing for an efficient response to BCG vaccination in healthy BCG-naïve subjects. *Epigenetics* 14(6):589–601
66. Kleinnijenhuis J, Quintin J, Preijers F, Joosten LAB, Jacobs C, et al. 2014. BCG-induced trained immunity in NK cells: role for non-specific protection to infection. *Clin. Immunol.* 155(2):213–19
67. 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(1–2):176–90.e19
68. dos Santos JC, Barroso de Figueiredo AM, Teodoro Silva MV, Cirovic B, de Bree LCJ, et al. 2019. β-Glucan-induced trained immunity protects against *Leishmania braziliensis* infection: a crucial role for IL-32. *Cell Rep.* 28(10):2659–72.e6
69. 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(2):322–34.e5
70. Christ A, Günther P, Lauterbach MAR, Duewell P, Biswas D, et al. 2018. Western diet triggers NLRP3-dependent innate immune reprogramming. *Cell* 172(1–2):162–75.e14
71. Machiels B, Dourcy M, Xiao X, Javaux J, Mesnil C, et al. 2017. A gammaherpesvirus provides protection against allergic asthma by inducing the replacement of resident alveolar macrophages with regulatory monocytes. *Nat. Immunol.* 18(12):1310–20

72. Yao Y, Jeyanathan M, Haddadi S, Barra NG, Vaseghi-Shanjani M, et al. 2018. Induction of autonomous memory alveolar macrophages requires T cell help and is critical to trained immunity. *Cell* 175(6):1634–50.e17
73. Hoyer FF, Naxerova K, Schloss MJ, Hulsmans M, Nair AV, et al. 2019. Tissue-specific macrophage responses to remote injury impact the outcome of subsequent local immune challenge. *Immunity* 51(5):899–914.e7
74. Roquilly A, Jacqueline C, Davieau M, Mollé A, Sadek A, et al. 2020. Alveolar macrophages are epigenetically altered after inflammation, leading to long-term lung immunoparalysis. *Nat. Immunol.* 21(6):636–48
75. Haley MJ, Brough D, Quintin J, Allan SM. 2019. Microglial priming as trained immunity in the brain. *Neuroscience* 405:47–54
76. Wendeln AC, Degenhardt K, Kaurani L, Gertig M, Ulas T, et al. 2018. Innate immune memory in the brain shapes neurological disease hallmarks. *Nature* 556(7701):332–38
77. Perry VH, Holmes C. 2014. Microglial priming in neurodegenerative disease. *Nat. Rev. Neurol.* 10(4):217–24
78. Roquilly A, McWilliam HEG, Jacqueline C, Tian Z, Cinotti R, et al. 2017. Local modulation of antigen-presenting cell development after resolution of pneumonia induces long-term susceptibility to secondary infections. *Immunity* 47(1):135–47.e5
79. Hole CR, Wager CML, Castro-Lopez N, Campuzano A, Cai H, et al. 2019. Induction of memory-like dendritic cell responses in vivo. *Nat. Commun.* 10(1):2955
80. Placek K, Schultze JL, Netea MG. 2019. Immune memory characteristics of innate lymphoid cells. *Curr. Opin. Infect. Dis.* 32(3):196–203
81. O'Sullivan TE, Sun JC, Lanier LL. 2015. Natural killer cell memory. *Immunity* 43(4):634–45
82. Dou Y, Fu B, Sun R, Li W, Hu W, et al. 2015. Influenza vaccine induces intracellular immune memory of human NK cells. *PLOS ONE* 10(3):e0121258
83. Nielsen CM, White MJ, Bottomley C, Lusa C, Rodríguez-Galán A, et al. 2015. Impaired NK cell responses to pertussis and H1N1 influenza vaccine antigens in human cytomegalovirus-infected individuals. *J. Immunol.* 194(10):4657–67
84. McCall MBB, Roestenberg M, Ploemen I, Teirlinck A, Hopman J, et al. 2010. Memory-like IFN-γ response by NK cells following malaria infection reveals the crucial role of T cells in NK cell activation by *P. falciparum*. *Eur. J. Immunol.* 40(12):3472–77
85. 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(5):507–16
86. Sun JC, Ma A, Lanier LL. 2009. Cutting edge: IL-15-independent NK cell response to mouse cytomegalovirus infection. *J. Immunol.* 183(5):2911–14
87. Romee R, Maximillian R, Berrien-Elliott MM, Wagner JA, Jewell BA, et al. 2015. Human cytokine-induced memory-like NK cells exhibit in vivo anti-leukemia activity in xenografted NSG mice and in patients with acute myeloid leukemia (AML). *Blood* 126(23):101
88. Cooper MA, Elliott JM, Keyel PA, Yang L, Carrero JA, Yokoyama WM. 2009. Cytokine-induced memory-like natural killer cells. *PNAS* 106(6):1915–19
89. Gamliel M, Goldman-Wohl D, Isaacson B, Gur C, Stein N, et al. 2018. Trained memory of human uterine NK cells enhances their function in subsequent pregnancies. *Immunity* 48(5):951–62.e5
90. Donnelly RP, Loftus RM, Keating SE, Liou KT, Biron CA, et al. 2014. mTORC1-dependent metabolic reprogramming is a prerequisite for NK cell effector function. *J. Immunol.* 193(9):4477–84
91. Schlums H, Cichocki F, Tesi B, Theorell J, Beziat V, et al. 2015. Cytomegalovirus infection drives adaptive epigenetic diversification of NK cells with altered signaling and effector function. *Immunity* 42(3):443–56
92. Cichocki F, Wu CY, Zhang B, Felices M, Tesi B, et al. 2018. ARID5B regulates metabolic programming in human adaptive NK cells. *J. Exp. Med.* 215(9):2379–95
93. Rasid O, Chevalier C, Camarasa TMN, Fitting C, Cavaillon JM, Hamon MA. 2019. H3K4me1 supports memory-like NK cells induced by systemic inflammation. *Cell Rep.* 29(12):3933–45.e3
94. Weizman O, 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(8):1004–11

95. Martinez-Gonzalez I, Mathä L, Steer CA, Ghaedi M, Poon GFT, Takei F. 2016. Allergen-experienced group 2 innate lymphoid cells acquire memory-like properties and enhance allergic lung inflammation. *Immunity* 45(1):198–208
96. Cassone A. 2018. The case for an expanded concept of trained immunity. *mBio* 9(3):e00570-18
97. Liu GY, Liu Y, Lu Y, Qin YR, Di GH, et al. 2016. Short-term memory of danger signals or environmental stimuli in mesenchymal stem cells: implications for therapeutic potential. *Cell. Mol. Immunol.* 13(3):369–78
98. Naik S, Larsen SB, Gomez NC, Alaverdyan K, Sendoel A, et al. 2017. Inflammatory memory sensitizes skin epithelial stem cells to tissue damage. *Nature* 550(7677):475–80
99. Ordovas-Montanes J, Dwyer DF, Nyquist SK, Buchheit KM, Vukovic M, et al. 2018. Allergic inflammatory memory in human respiratory epithelial progenitor cells. *Nature* 560(7720):649–54
100. Bigot J, Guillot L, Guitard J, Ruffin M, Corvol H, et al. 2020. Respiratory epithelial cells can remember infection: a proof-of-concept study. *J. Infect. Dis.* 221(6):1000–5
101. El-Osta A, Brasacchio D, Yao D, Pocai A, Jones PL, et al. 2008. Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. *J. Exp. Med.* 205(10):2409–17
102. Schnitzler JG, Hoogeveen RM, Ali L, Prange KHM, Waissi F, et al. 2020. Atherogenic lipoprotein(a) increases vascular glycolysis, thereby facilitating inflammation and leukocyte extravasation. *Circ. Res.* 126(10):1346–59
103. Schnack L, Sohrabi Y, Lagache SMM, Kahles F, Bruemmer D, et al. 2019. Mechanisms of trained innate immunity in oxLDL primed human coronary smooth muscle cells. *Front. Immunol.* 10:13
104. Ospelt C, Reedquist KA, Gay S, Tak PP. 2011. Inflammatory memories: Is epigenetics the missing link to persistent stromal cell activation in rheumatoid arthritis? *Autoimmun. Rev.* 10(9):519–24
105. Ara T, Kurata K, Hirai K, Uchihashi T, Uematsu T, et al. 2009. Human gingival fibroblasts are critical in sustaining inflammation in periodontal disease. *J. Periodontal Res.* 44(1):21–27
106. Sohn C, Lee A, Qiao Y, Loupasakis K, Ivashkiv LB, Kalliolias GD. 2015. Prolonged tumor necrosis factor α primes fibroblast-like synoviocytes in a gene-specific manner by altering chromatin. *Arthritis Rheumatol.* 67(1):86–95
107. Klein K, Frank-Bertoncelj M, Karouzakis E, Gay RE, Kolling C, et al. 2017. The epigenetic architecture at gene promoters determines cell type-specific LPS tolerance. *J. Autoimmun.* 83:122–33
108. Crowley T, O'Neil JD, Adams H, Thomas AM, Filer A, et al. 2017. Priming in response to pro-inflammatory cytokines is a feature of adult synovial but not dermal fibroblasts. *Arthritis Res. Ther.* 19(1):35
109. Stensballe LG, Nante E, Jensen IP, Kofoed PE, Poulsen A, et al. 2005. Acute lower respiratory tract infections and respiratory syncytial virus in infants in Guinea-Bissau: a beneficial effect of BCG vaccination for girls; community based case-control study. *Vaccine* 23(10):1251–57
110. 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(2):245–52
111. Aaby P, Mogensen SW, Rodrigues A, Benn CS. 2018. Evidence of increase in mortality after the introduction of diphtheria-tetanus-pertussis vaccine to children aged 6–35 months in Guinea-Bissau: a time for reflection? *Front. Public Health* 6:79
112. Nemes E, Geldenhuys H, Rozot V, Rutkowski KT, Ratangee F, et al. 2018. Prevention of *M. tuberculosis* infection with H4:IC31 vaccine or BCG revaccination. *N. Engl. J. Med.* 379(2):138–49
113. 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(1):89–100.e5
114. Blok BA, Arts RJW, van Crevel R, Aaby P, Joosten LAB, et al. 2020. Differential effects of BCG vaccine on immune responses induced by vi polysaccharide typhoid fever vaccination: an explorative randomized trial. *Eur. J. Clin. Microbiol. Infect. Dis.* 39(6):1177–84
115. Blok BA, de Bree LCJ, Diavatopoulis DA, Langereis JD, Joosten LA, et al. 2020. Interacting, nonspecific, immunological effects of Bacille Calmette-Guérin and tetanus-diphtheria-pertussis inactivated polio vaccinations: an explorative, randomized trial. *Clin. Infect. Dis.* 70(3):455–63

116. 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(1):481. Erratum. 2020. *Trials* 21(1):555
117. Giamarellos-Bourboulis EJ, Tsilika M, Moorlag S, Antonakos N, Kotsaki A, et al. 2020. Activate: randomized clinical trial of BCG vaccination against infection in the elderly. *Cell* 183(2):315–23.e9
118. Walk J, de Bree LCJ, Graumans W, Stoter R, van Gemert GJ, et al. 2019. Outcomes of controlled human malaria infection after BCG vaccination. *Nat. Commun.* 10(1):874
119. Barton ES, White DW, Cathelyn JS, Brett-McClellan KA, Engle M, et al. 2007. Herpesvirus latency confers symbiotic protection from bacterial infection. *Nature* 447(7142):326–29
120. Ribes S, Meister T, Ott M, Redlich S, Janova H, et al. 2014. Intraperitoneal prophylaxis with CpG oligodeoxynucleotides protects neutropenic mice against intracerebral *Escherichia coli* K1 infection. *J. Neuroinflamm.* 11:14
121. Li R, Lim A, Phoon MC, Narasaraju T, Ng JKW, et al. 2010. Attenuated *Bordetella pertussis* protects against highly pathogenic influenza A viruses by dampening the cytokine storm. *J. Virol.* 84(14):7105–13
122. van der Poll T, Opal SM. 2008. Host-pathogen interactions in sepsis. *Lancet Infect. Dis.* 8(1):32–43
123. Cheng SC, Scicluna BP, Arts RJW, Gresnigt MS, Lachmandas E, et al. 2016. Broad defects in the energy metabolism of leukocytes underlie immunoparalysis in sepsis. *Nat. Immunol.* 17(4):406–13
124. Netea MG, Giamarellos-Bourboulis EJ, Domínguez-Andrés J, Curtis N, van Crevel R, et al. 2020. Trained immunity: a tool for reducing susceptibility and severity of SARS-CoV-2 infection. *Cell* 181(5):969–77
125. Gursel M, Gursel I. 2020. Is global BCG vaccination-induced trained immunity relevant to the progression of SARS-CoV-2 pandemic? *Allergy* 75(7):1815–19
126. Faust L, Huddart S, MacLean E, Svadzian A. 2020. Universal BCG vaccination and protection against COVID-19: critique of an ecological study. *Nature Research Microbiology Community*. **https://naturemicrobiologycommunity.nature.com/users/36050-emily-maclean/posts/64892-universal-bcg-vaccination-and-protection-against-covid-19-critique-of-an-ecological-study**
127. O'Neill LAJ, Netea MG. 2020. BCG-induced trained immunity: Can it offer protection against COVID-19? *Nat. Rev. Immunol.* 20(6):335–37
128. Curtis N, Sparrow A, Ghebreyesus TA, Netea MG. 2020. Considering BCG vaccination to reduce the impact of COVID-19. *Lancet* 395(10236):1545–46
129. Aaby P, Benn CS, Flanagan KL, Klein SL, Kollmann TR, et al. 2020. The non-specific and sex-differential effects of vaccines. *Nat. Rev. Immunol.* 20(8):464–70
130. Sylvester RJ, van der Meijden APM, Lamm DL. 2002. Intravesical bacillus Calmette-Guerin reduces the risk of progression in patients with superficial bladder cancer: a meta-analysis of the published results of randomized clinical trials. *J. Urol.* 168(5):1964–70
131. Morton DL, Eilber FR, Holmes EC, Hunt JS, Ketcham AS, et al. 1974. BCG immunotherapy of malignant melanoma: summary of a seven year experience. *Ann. Surg.* 180(4):635–43
132. Sokal JE, Aungst CW, Snyderman M, Sokal PJ. 1974. Delay in progression of malignant lymphoma after BCG vaccination. *N. Engl. J. Med.* 291(23):1226–30
133. Buffen K, Oosting M, Quintin J, Ng A, Kleinnijenhuis J, et al. 2014. Autophagy controls BCG-induced trained immunity and the response to intravesical BCG therapy for bladder cancer. *PLOS Pathog.* 10(10):e1004485
134. Kar UK, Joosten LAB. 2020. Training the trainable cells of the immune system and beyond. *Nat. Immunol.* 21(2):115–19
135. Netea MG, Joosten LAB, van der Meer JWM. 2017. Hypothesis: stimulation of trained immunity as adjunctive immunotherapy in cancer. *J. Leukoc. Biol.* 102(6):1323–32
136. Owens BMJ. 2015. Inflammation, innate immunity, and the intestinal stromal cell niche: opportunities and challenges. *Front. Immunol.* 6:319
137. Foster SL, Hargreaves DC, Medzhitov R. 2007. Gene-specific control of inflammation by TLR-induced chromatin modifications. *Nature* 447(7147):972–78

138. Negi S, Das DK, Pahari S, Nadeem S, Agrewala JN. 2019. Potential role of gut microbiota in induction and regulation of innate immune memory. *Front. Immunol.* 10:2441
139. Han F, Fan H, Yao M, Yang S, Han J. 2017. Oral administration of yeast β-glucan ameliorates inflammation and intestinal barrier in dextran sodium sulfate-induced acute colitis. *J. Funct. Foods.* 35:115–26
140. Heinsbroek SEM, Williams DL, Welting O, Meijer SL, Gordon S, de Jonge WJ. 2015. Orally delivered β-glucans aggravate dextran sulfate sodium (DSS)-induced intestinal inflammation. *Nutr. Res.* 35(12):1106–12
141. Iliev ID, Funari VA, Taylor KD, Nguyen Q, Reyes CN, et al. 2012. Interactions between commensal fungi and the C-type lectin receptor dectin-1 influence colitis. *Science* 336(6086):1314–17
142. Wheeler ML, Limon JJ, Bar AS, Leal CA, Gargus M, et al. 2016. Immunological consequences of intestinal fungal dysbiosis. *Cell Host Microbe* 19(6):865–73
143. Bekkering S, Joosten LAB, van der Meer JWM, Netea MG, Riksen NP. 2013. Trained innate immunity and atherosclerosis. *Curr. Opin. Lipidol.* 24:487–92
144. Bekkering S, Saner C, Riksen NP, Netea MG, Sabin MA, et al. 2020. Trained immunity: linking obesity and cardiovascular disease across the life-course? *Trends Endocrinol. Metab.* 31(5):378–89
145. Thiem K, Stienstra R, Riksen NP, Keating ST. 2019. Trained immunity and diabetic vascular disease. *Clin. Sci.* 133(2):195–203
146. Leentjens J, Bekkering S, Joosten LAB, Netea MG, Burgner DP, Riksen NP. 2018. Trained innate immunity as a novel mechanism linking infection and the development of atherosclerosis. *Circ. Res.* 122(5):664–69
147. Brasacchio D, Okabe J, Tikellis C, Balcerczyk A, George P, et al. 2009. Hyperglycemia induces a dynamic cooperativity of histone methylase and demethylase enzymes associated with gene-activating epigenetic marks that coexist on the lysine tail. *Diabetes* 58(5):1229–36
148. Yun JM, Jialal I, Devaraj S. 2011. Epigenetic regulation of high glucose-induced proinflammatory cytokine production in monocytes by curcumin. *J. Nutr. Biochem.* 22(5):450–58
149. Hoyer FF, Zhang X, Coppin E, Vasamsetti SB, Modugu G, et al. 2020. Bone marrow endothelial cells regulate myelopoiesis in diabetes mellitus. *Circulation* 142(3):244–58
150. Barman PK, Urao N, Koh TJ. 2019. Diabetes induces myeloid bias in bone marrow progenitors associated with enhanced wound macrophage accumulation and impaired healing. *J. Pathol.* 249(4):435–46
151. Shirai T, Nazarewicz RR, Wallis BB, Yanes RE, Watanabe R, et al. 2016. The glycolytic enzyme PKM2 bridges metabolic and inflammatory dysfunction in coronary artery disease. *J. Exp. Med.* 213(3):337–54
152. Viney NJ, van Capelleveen JC, Geary RS, Xia S, Tami JA, et al. 2016. Antisense oligonucleotides targeting apolipoprotein(a) in people with raised lipoprotein(a): two randomised, double-blind, placebo-controlled, dose-ranging trials. *Lancet* 388(10057):2239–53
153. Schnitzler JG, Poels K, Stiekema LCA, Yeang C, Tsimikas S, et al. 2020. Short-term regulation of hematopoiesis by lipoprotein(a) results in the production of pro-inflammatory monocytes. *Int. J. Cardiol.* 315:81–85
154. Schloss MJ, Swirski FK, Nahrendorf M. 2020. Modifiable cardiovascular risk, hematopoiesis, and innate immunity. *Circ. Res.* 126(9):1242–59
155. Datta M, Staszewski O, Raschi E, Frosch M, Hagemeyer N, et al. 2018. Histone deacetylases 1 and 2 regulate microglia function during development, homeostasis, and neurodegeneration in a context-dependent manner. *Immunity* 48(3):514–29.e6
156. Püntener U, Booth SG, Perry VH, Teeling JL. 2012. Long-term impact of systemic bacterial infection on the cerebral vasculature and microglia. *J. Neuroinflamm.* 9:146
157. Ramirez K, Shea DT, McKim DB, Reader BF, Sheridan JF. 2015. Imipramine attenuates neuroinflammatory signaling and reverses stress-induced social avoidance. *Brain Behav. Immun.* 46:212–20
158. Knuesel I, Chicha L, Britschgi M, Schobel SA, Bodmer M, et al. 2014. Maternal immune activation and abnormal brain development across CNS disorders. *Nat. Rev. Neurol.* 10(11):643–60
159. Bilbo SD, Biedenkapp JC, Der-Avakian A, Watkins LR, Rudy JW, Maier SF. 2005. Neonatal infection-induced memory impairment after lipopolysaccharide in adulthood is prevented via caspase-1 inhibition. *J. Neurosci.* 25(35):8000–9
160. Williamson LL, Sholar PW, Mistry RS, Smith SH, Bilbo SD. 2011. Microglia and memory: modulation by early-life infection. *J. Neurosci.* 31(43):15511–21

161. Noz MP, ter Telgte A, Wiegertjes K, Joosten LAB, Netea MG, et al. 2018. Trained immunity characteristics are associated with progressive cerebral small vessel disease. *Stroke* 49(12):2910–17
162. Arts RJW, Joosten LAB, Netea MG. 2018. The potential role of trained immunity in autoimmune and autoinflammatory disorders. *Front. Immunol.* 9:298
163. Van Der Meer JWM, Radl J, Meyer CJLM, Vossen JM, Van Nieuwkoop JA, et al. 1984. Hyperimmunoglobulinaemia D and periodic fever: a new syndrome. *Lancet* 323(8386):1087–90
164. Grigoriou M, Banos A, Filia A, Pavlidis P, Giannouli S, et al. 2019. Transcriptome reprogramming and myeloid skewing in haematopoietic stem and progenitor cells in systemic lupus erythematosus. *Ann. Rheum. Dis.* 79(2):242–53
165. Oduro KA, Liu F, Tan Q, Kim CK, Lubman O, et al. 2012. Myeloid skewing in murine autoimmune arthritis occurs in hematopoietic stem and primitive progenitor cells. *Blood* 120(11):2203–13
166. Jeljeli M, Riccio LGC, Doridot L, Chêne C, Nicco C, et al. 2019. Trained immunity modulates inflammation-induced fibrosis. *Nat. Commun.* 10(1):5670
167. Imran S, Neeland MR, Shepherd R, Messina N, Perrett KP, et al. 2020. A potential role for epigenetically mediated trained immunity in food allergy. *iScience* 23(6):101171
168. Tulic MK, Hodder M, Forsberg A, McCarthy S, Richman T, et al. 2011. Differences in innate immune function between allergic and nonallergic children: new insights into immune ontogeny. *J. Allergy Clin. Immunol.* 127(2):470–78.e1
169. Neeland MR, Koplin JJ, Dang TD, Dharmage SC, Tang ML, et al. 2018. Early life innate immune signatures of persistent food allergy. *J. Allergy Clin. Immunol.* 142(3):857–64.e3
170. Neeland MR, Andorf S, Manohar M, Dunham D, Lyu SC, et al. 2020. Mass cytometry reveals cellular fingerprint associated with IgE^+ peanut tolerance and allergy in early life. *Nat. Commun.* 11(1):1091
171. Zhang Y, Collier F, Naselli G, Saffery R, Tang ML, et al. 2016. Cord blood monocyte-derived inflammatory cytokines suppress IL-2 and induce nonclassic "T_H2-type" immunity associated with development of food allergy. *Sci. Transl. Med.* 8(321):321ra8
172. Arnoldussen DL, Linehan M, Sheikh A. 2011. BCG vaccination and allergy: a systematic review and meta-analysis. *J. Allergy Clin. Immunol.* 127(1):246–53
173. Herz U, Gerhold K, Grüber C, Braun A, Wahn U, et al. 1998. BCG infection suppresses allergic sensitization and development of increased airway reactivity in an animal model. *J. Allergy Clin. Immunol.* 102(5):867–74
174. Estcourt MJ, Campbell DE, Gold MS, Richmond P, Allen KJ, et al. 2019. Whole-cell pertussis vaccination and decreased risk of IgE-mediated food allergy: a nested case-control study. *J. Allergy Clin. Immunol. Pract.* 8(6):2004–14
175. Messina NL, Gardiner K, Donath S, Flanagan K, Ponsonby AL, et al. 2019. Study protocol for the Melbourne Infant Study: BCG for Allergy and Infection Reduction (MIS BAIR), a randomised controlled trial to determine the non-specific effects of neonatal BCG vaccination in a low-mortality setting. *BMJ Open* 9(12):e032844
176. Ochando J, Fayad ZA, Madsen JC, Netea MG, Mulder WJM. 2020. Trained immunity in organ transplantation. *Am. J. Transplant.* 20(1):10–18
177. Braza MS, van Leent MMT, Lameijer M, Sanchez-Gaytan BL, Arts RJW, et al. 2018. Inhibiting inflammation with myeloid cell-specific nanobiologics promotes organ transplant acceptance. *Immunity* 49(5):819–28.e6
178. Mulder WJM, Ochando J, Joosten LAB, Fayad ZA, Netea MG. 2019. Therapeutic targeting of trained immunity. *Nat. Rev. Drug Discov.* 18(7):553–66
179. van der Heijden CDCC, Noz MP, Joosten LAB, Netea MG, Riksen NP, Keating ST. 2018. Epigenetics and trained immunity. *Antioxid. Redox Signal.* 29(11):1023–40
180. Rodriguez RM, Suarez-Alvarez B, Lopez-Larrea C. 2019. Therapeutic epigenetic reprogramming of trained immunity in myeloid cells. *Trends Immunol.* 40(1):66–80
181. Nicodeme E, Jeffrey KL, Schaefer U, Beinke S, Dewell S, et al. 2010. Suppression of inflammation by a synthetic histone mimic. *Nature* 468(7327):1119–23
182. Domínguez-Andrés J, Ferreira AV, Jansen T, Smithers N, Prinjha RK, et al. 2019. Bromodomain inhibitor I-BET151 suppresses immune responses during fungal-immune interaction. *Eur. J. Immunol.* 49(11):2044–50

183. Klein K, Kabala PA, Grabiec AM, Gay RE, Kolling C, et al. 2016. The bromodomain protein inhibitor I-BET151 suppresses expression of inflammatory genes and matrix degrading enzymes in rheumatoid arthritis synovial fibroblasts. *Ann. Rheum. Dis.* 75(2):422–29
184. Fu W, Farache J, Clardy SM, Hattori K, Mander P, et al. 2014. Epigenetic modulation of type-1 diabetes via a dual effect on pancreatic macrophages and β cells. *eLife* 3:e04631
185. Perrotta P, Van Der Veken B, Van Der Veken P, Pintelon I, Roosens L, et al. 2020. Partial inhibition of glycolysis reduces atherogenesis independent of intraplaque neovascularization in mice. *Arterioscler. Thromb. Vasc. Biol.* 40(5):1168–81
186. Duivenvoorden R, Tang J, Cormode DP, Mieszawska AJ, Izquierdo-Garcia D, et al. 2014. A statin-loaded reconstituted high-density lipoprotein nanoparticle inhibits atherosclerotic plaque inflammation. *Nat. Commun.* 5:3065