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Current Opinion in Pharmacology

# Trained immunity as a novel therapeutic strategy

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Recent studies have shown that upon certain vaccinations or infections human innate immune cells can undergo extensive metabolic and epigenetic reprogramming, which results in enhanced immune responses upon heterologous re-infection, a process termed *trained immunity*. Trained immunity has also been shown to be inappropriately activated in inflammatory diseases. This provides the potential for identifying novel therapeutic targets: potentiation of trained immunity could protect from secondary infections and reverse immunotolerant states, while inhibition of trained immunity might reduce excessive immune activation in chronic inflammatory conditions. By targeting specific mechanisms of trained immunity on either immunologic, metabolic or epigenetic level, novel therapeutic approaches could be developed.

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**Current Opinion in Pharmacology** 2018, **41**:52–58

This review comes from a themed issue on **Immunomodulation**

Edited by **Ed Lavalle** and **James McLachlan**

For a complete overview see the Issue and the Editorial

Available online 24th April 2018

**https://doi.org/10.1016/j.coph.2018.04.007**



## Introduction

The ability to induce memory is an important feature of the adaptive immune system, yet an increasing body of evidence shows that the innate immune system is also able to mount a memory response. It is known that plants and insects, which lack adaptive immunity, show enhanced nonspecific protection to reinfection [1–3]. Moreover, in *Candida albicans* vaccinated mice it has been shown that a partial protection to reinfection can be induced through T-cell and B-cell independent mechanisms [4]. Recently, it has been shown that infections and vaccinations are able to induce an enhanced immune response in monocytes upon nonspecific restimulation, a process termed trained immunity. β-Glucan, a major cell wall component of *C. albicans*, as well as the Bacillus Calmette-Guérin (BCG) vaccine are able to induce trained immunity, whereas lipopolysaccharide (LPS) stimulation in monocytes results in tolerance [5–7]. β-Glucan and BCG induce trained immunity in monocytes via pattern recognition receptors (PRRs) dectin-1 and NOD2 respectively, leading to enhanced signaling of the Akt (protein kinase B)–mTOR (mammalian target of rapamycin)–HIF-1α (hypoxia-inducible factor-1α) pathway, modifications in metabolic pathways, and epigenetic rewiring [5,6] (Figure 1). Unfortunately, not only microbial ligands can induce trained immunity after infection or vaccination, but also endogenous ligands can inappropriately activate trained immunity, thereby contributing to chronic inflammation, as has been shown in atherosclerosis [8] or gout [9].

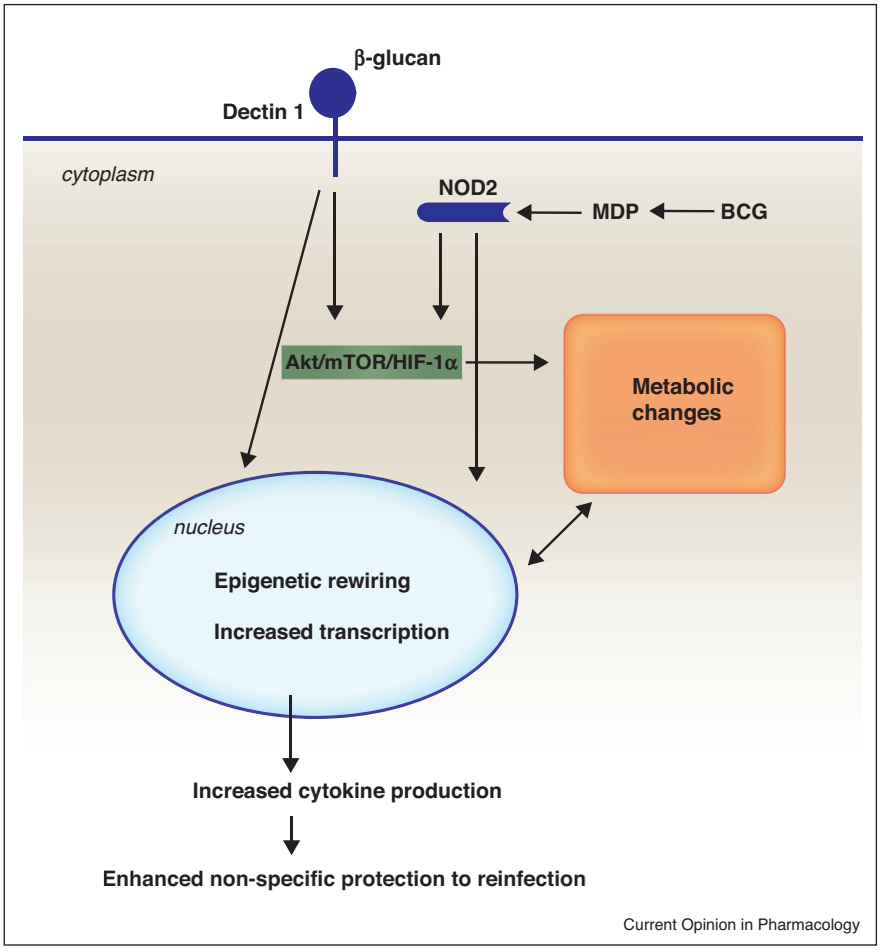
The recent insights in trained immunity could have important implications for the development of novel therapeutic targets for immune-mediated diseases. Interestingly, BCG vaccinated children in West Africa show a lower overall mortality by decreased morbidity due to infections [10••], and healthy volunteers vaccinated with BCG show an enhanced pro-inflammatory cytokine profile upon nonspecific restimulation in *ex vivo* monocytes, persisting for at least three months [5]. Currently, inducers of trained immunity are already used to treat diseases, such as muramyl tripeptide (MTP) for osteosarcoma [11] and BCG in bladder cancer [12] through mechanisms that likely involve induction of autophagy [13]. In addition to improvement of vaccines, it would be very interesting to modulate trained immunity in humans with maladaptive immune responses to restore a balanced immune function.

In this review, we will provide a short overview on the role of trained immunity in the pathophysiology of diseases, and discuss potential therapeutic targeting strategies in the context of trained immunity on the immunological, metabolic and epigenetic level.

## Trained immunity in the pathophysiology of diseases

Enhanced immune responses induced by trained immunity, as reflected by an increased interleukin (IL)-6, IL-1β, and tumor necrosis factor α (TNFα) production, might lead in the long term to development and/or persistence of chronic inflammatory conditions such as arthritis or atherosclerosis. It is already known that a high glucose environment drives a pro-inflammatory profile

**Figure 1**

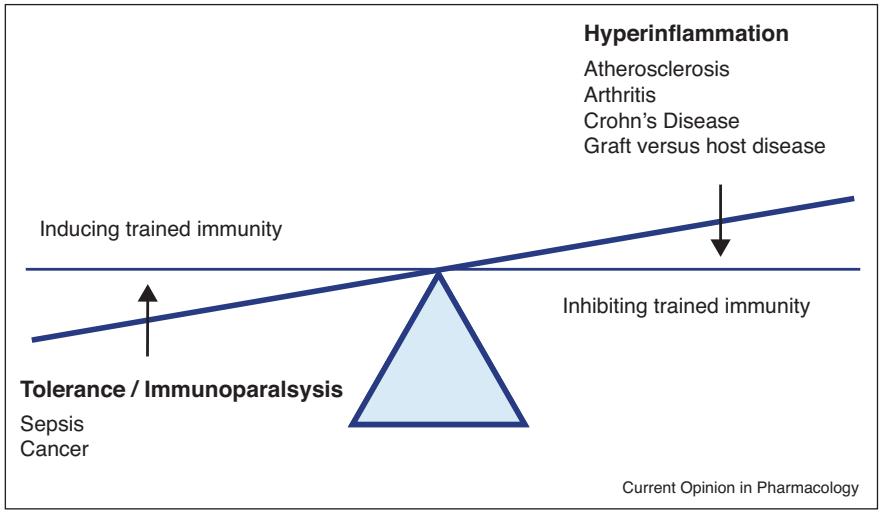



The concept of trained immunity. β-Glucan and BCG induce trained immunity in monocytes via pattern recognition receptors dectin-1 and NOD2 respectively, leading to enhanced signaling of the Akt–mTOR–HIF-1α pathway, modifications in metabolic pathways, and epigenetic rewiring. This results in increased pro-inflammatory cytokine production and enhanced non-specific protection to reinfection.

which remains even after introduction to a normal glucose environment, called hyperglycemic memory [14]. Furthermore, metformin, an mTOR inhibitor leading to inhibition of trained immunity [15••,16••], is associated with anti-inflammatory effects in atherosclerosis and is administered to type 2 diabetes patients [17,18]. Recently, it has been shown that endogenous danger ligands such as oxidized low density lipoprotein (oxLDL) or lipoprotein A, both involved in the pathogenesis of atherosclerosis, are able to induce training in monocytes *in vitro*. By training monocytes with oxLDL, genes involved in metabolic processes important for atherogenesis are induced, and histone methylation plays a crucial role in the long-term persistence of these effects. The trained monocytes have a propensity to differentiate into foam cells and show enhanced expression of scavenger receptors [8]. Moreover, a western type diet induces trained immunity in a mice model for atherosclerosis, which was dependent on the NLRP3 inflammasome. This occurs already at the level of granulocyte-monocyte precursors and persisted for several months [19•].

By contrast, enhanced immune responses induced by trained immunity might be beneficial in immunotolerant states occurring in sepsis or cancer. In sepsis, an imbalanced interaction between epigenetic and metabolic pathways in immune cells is observed [20], and *in vitro* it has been shown that tolerized and trained macrophages have a different epigenetic and metabolic state [15••,21]. Importantly, the training stimulus β-glucan is able to reverse LPS-induced tolerance in monocytes *ex vivo* from volunteers with experimental endotoxemia, by recovering the induction of ~60% of tolerized genes and reversing histone modifications [22•]. Similarly, tumor-associated macrophages have a long-term phenotype of anti-inflammatory M2 macrophages, and exert inhibitory effects on $CD8^+$ cytotoxic lymphocytes [23]. Therefore, inducers of trained immunity are of great

**Figure 2**




Trained immunity in the pathophysiology of immune-mediated diseases. Tolerant states in for example sepsis and cancer could possibly be reversed by inducing trained immunity. By contrast, inhibiting trained immunity could dampen hyperinflammatory conditions in for example atherosclerosis.

interest as treatment to reverse immunotolerant states in diseases such as sepsis or cancer (Figure 2).

## Therapeutic strategies to target trained immunity

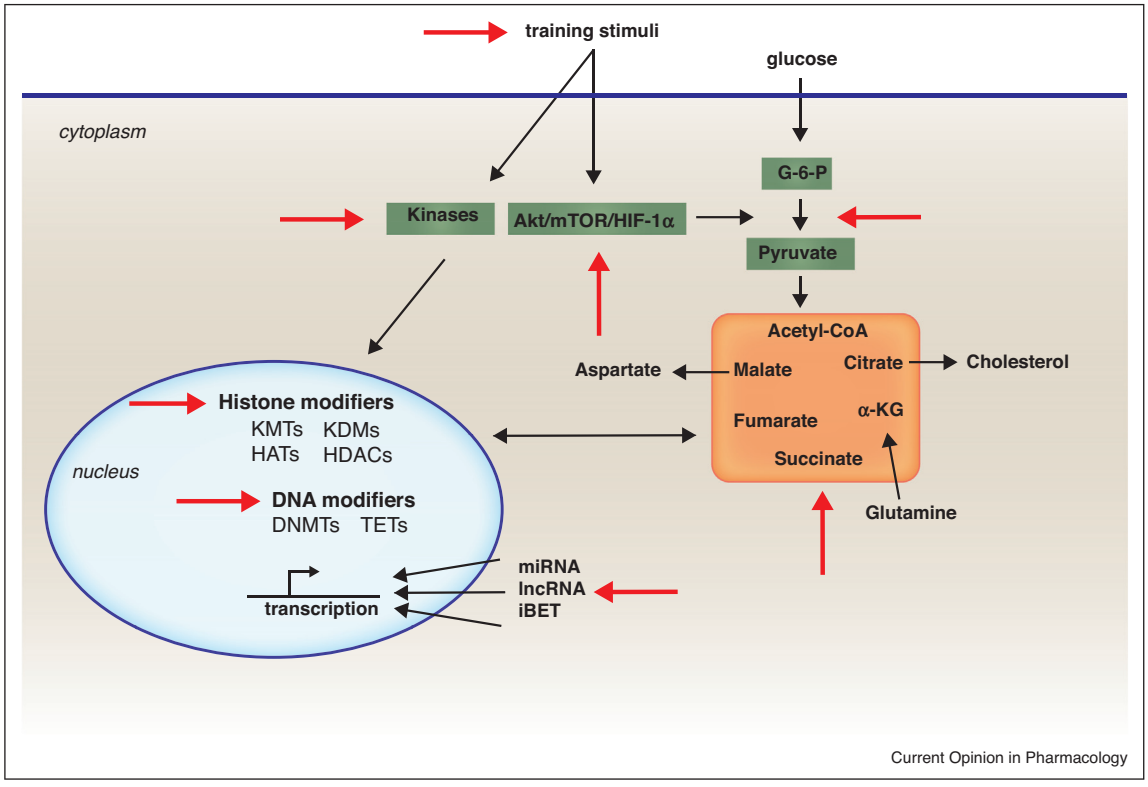
Induction of trained immunity is achieved by interaction of immunologic signaling, metabolic changes and epigenetic modifications. Targeting these components for therapeutic purposes represents an interesting novel approach (Figure 3).

### Immunological targets

Stimuli which are able to induce training could act as a protective mechanism for subsequent infections by the means of vaccinations, or as a therapeutic approach in maladaptive immune states. β-Glucan as an inducer of trained immunity for therapeutic purposes has already entered clinical trials. Earlier studies suggest anticarcinogenic effects [24] and increased resistance to infections after β-glucan administration [25]. Although oral β-glucan administration to healthy volunteers did not induce trained immunity in monocytes *ex vivo* [26], intravenous administration of clinical grade β-glucan has passed regulatory hurdles and is now in clinical trials for treatment of cancer in combination with checkpoint inhibitors (Clinical Trials database; Imprime PGG, U.S. National Library of Medicine). In addition, BCG strongly induces trained immunity in vaccinated healthy volunteers, as shown by enhanced pro-inflammatory cytokine production in *ex vivo* monocytes upon nonspecific reinfection [27]. As mentioned before, BCG is currently administered to patients with bladder cancer after transurethral resection of the tumor [12]. Although the underlying mechanism is unclear, activation of innate immune cells and production of pro-inflammatory cytokines suggest a protective role of trained immunity. This hypothesis is supported by the fact that the synthetic mycobacterial structure MTP-phosphatidylethanolamine (mifamurtide) has beneficial effects and is approved for the treatment of osteosarcoma to stimulate anticarcinogenic activity by macrophages [11]. Additionally, the muramyl dipeptide (MDP) derivate murabutide promotes non-specific protection to bacterial and viral infections and decreases lethality of mice injected with LPS [28], and paclitaxel conjugated to MDP shows immunoenhancing capacity [29]. Liposome-encapsulated MDP (or derivates) is able to migrate to an inflammatory environment, and is therefore a promising therapeutic strategy to target trained immunity responses.

In addition, another approach aims to inhibit intracellular immunological pathways essential for training in monocytes and macrophages, such as Akt–mTOR–HIF-1α [15••,16••]. Everolimus, an mTOR activator rapamycin analog, is currently used to treat various types of cancer [30], and metformin is currently administered to type 2 diabetes patients [17]. Their beneficial effects likely also involves modulation of trained immunity. Various Akt and HIF-1α inhibitors are currently being studied, however, no inhibitor is yet approved for administration in humans, partially due to their complex regulatory network [31]. Inhibition of kinases such as Raf-1 and Rip2, or PI3K and ERK are of interest since they are activated after triggering of dectin-1 and NOD2 receptors respectively, or by oxLDL. Importantly, it is known that crosstalk and compensation between different pathways occurs, therefore a combinatorial approach

**Figure 3**




Therapeutic strategies to target trained immunity. Trained immunity could be targeted in different ways, by targeting (I) inducers of trained immunity, (II) signaling molecules, (III) metabolic patways, (IV) epigenetic enzymes, or (V) non-coding RNAs (indicated by red arrows). *Abbreviations*: G-6-P, glucose-6-phosphate; KTMs, lysine methyl transferases; HATs, histone acetyl transferases; DNMTs, DNA methyltransferases.

is recommended when inhibiting signaling molecules. Furthermore, inhibiting these proteins will have significant consequences for other cellular processes due to their complex interacting network.

## Epigenetic targets

The development of epigenetic modulators is of great interest due to accumulating evidence of the importance of epigenetic regulation in various diseases, including inflammatory conditions [32]. Targeting epigenetic modifiers is interesting since epigenetic changes can be stably maintained but are also able to adapt to changing environments. Moreover, epigenetic reprogramming is at the basis of the long-term induced memory of innate immune cells.

In β-glucan-induced trained monocytes, modifications in histone 3 lysine 27 (H3K27) acetylation as well as H3K4 mono-methylation and trimethylation were observed in gene promoters involved in trained immunity, resulting in transcriptionally active chromatin [15••,21,22•]. Since inhibiting histone methyltransferases abrogates β-glucan-induced and BCG-induced training [5,6] this could be an interesting therapeutic target. Although lysine-specific demethylase 1 inhibition by pargyline did not influence β-glucan-induced trained immunity [6], this could be due to the poor inhibitory activity of pargyline at micromolar concentrations [33]. On the other hand, the broad jumonji histone demethylase inhibitor JIB-04 decreased trained immunity responses through modulation of the inhibitory histone modification H3K9 (Moorlag SJCFM, *et al.* submitted), supporting the value of this therapeutic strategy. Furthermore, the small molecule inhibitor of the BET family of bromodomains I-BET151 (GSK 1210151A) is able to block LPS-induced tolerance when co-administered in human monocytes *in vitro*, however it could not reverse tolerance in a therapeutic manner if tolerance was already induced by LPS and is therefore probably not an effective treatment in monocytes that already experienced an inflammatory response [22•]. During β-glucan-induced trained immunity, lower expression of sirtuins is also observed, which deacetylate histones as well as non-histone targets [15••]. Interestingly, an increased $NAD^+$/NADH ratio and lactate levels are observed during training in monocytes, which are able to respectively activate or inhibit histone deacetylases (HDACs) [34,35]. Induction of sirtuin-1 results in a more anti-inflammatory state with increased fatty acid

oxidation [35]. This suggests that sirtuin-1 might be an interesting target in trained or tolerized monocytes. Currently, the class I/II HDAC inhibitor vorinostat (suberoylanilide hydroxamic acid) is approved for cancer treatment [36], and multiple HDAC inhibitors, including ITF-2357 and SAHA, have been shown to reduce inflammation in inflammatory diseases [37]. Of note, certain epigenetic enzyme inhibitors exhibit broad substrate specificity, and enzymes might alter their function in different cell types and environments, which may lead to unwanted effects.

BCG is also able to induce trained immunity in NK cells [38], which is accompanied with specific DNA methylation patterns as well as modulated cytokine responses in CMV-infected individuals [39,40]. Although metabolites such as fumarate, α-ketoglutarate (α-KG) and succinate, which are able to affect the Ten-eleven translocation (TET) family of DNA demethylases [41•,42], accumulate during training, no role for DNA methylation was observed in β-glucan trained monocytes [22•].

Micro (mi)RNAs have recently emerged as critical regulators of gene expression in immune cells. miRNAs have a relatively long half-life and could therefore be interesting in the context of trained immunity by their persistence after removal of the primary stimulus, especially since myeloid cells have limited proliferative capacity. miR-155 expression is increased in response to inflammatory signals and promotes M1 polarization and inflammation [43], and miR-155 knock-out mice exhibit early resistance to mycobacteria due to reduced growth of bacteria in infected monocytes/macrophages [44]. miR-146a, expression of which is increased following LPS stimulation, is suggested to play a key role in endotoxin tolerance by abrogating hyperinflammation [43]. Furthermore, various studies have described a role for long non-coding (lnc)RNAs in innate immunity. For example, lncRNA ANRIL correlates with severity in atherosclerosis [45], and long non-intergenic ncRNA Cox2 and THRIL are respectively essential for TLR-induced expression of IL-6 and regulation of TNFα in monocytes [46,47]. Further understanding of the role of non-coding RNAs is required to assess their function in the context of trained immunity.

## Metabolic targets

Modulation of energy metabolism is essential for functioning of cells, and it is becoming increasingly clear that during innate immune responses specific metabolic signatures occur [48]. Both β-glucan and BCG-induced training results in upregulation of aerobic glycolysis via epigenetic modifications at essential glycolytic genes, leading to an increased lactate production and increased $NAD^+$/NADH ratio [15••,16••,41•]. Induction of glutaminolysis, aspartate and cholesterol synthesis pathways are involved in β-glucan-induced trained immunity, whereas oxidative phosphorylation (OXPHOS) plays a minor role [15••,41•,49]. The metabolic switch from OXPHOS towards aerobic glycolysis, called the Warburg effect, is essential since inhibiting glycolysis inhibits β-glucan-induced trained immunity *in vitro* [15••]. Although OXPHOS is downregulated in β-glucan-induced trained immunity, succinate, fumarate and malate accumulate in monocytes [41•]. Interestingly, during BCG-induced training the metabolic changes are dependent on epigenetic modifications at metabolic genes, yet epigenetic changes are also dependent on the induction of glycolysis and glutaminolysis [16••], thereby linking metabolic and epigenetic processes. Several metabolites and metabolic enzymes can act as substrates or cofactors for epigenetic-modifying enzymes (Table 1) [42,50,51]. Of note, fumarate itself is able to induce training in monocytes *in vitro* by increasing H3K4me3 and H3K27ac and inhibiting degradation of HIF-1α [41•]. Moreover, the decreased expression of the lysine demethylase (KDM) 5 family of HDACs, which are responsible for H3K4 demethylation, is inhibited by fumarate thereby maintaining accessible chromatin. Interestingly, during tolerance no KDM5 activity is observed, and this could be restored by α-KG, a cofactor for KDMs [41•]. Recently, it has been shown that β-glucan-induced trained immunity depends on mevalonate, a metabolite of the cholesterol synthesis pathway. Statins are able to block mevalonate and can thereby prevent trained immunity. This could be very relevant for Hyper-IgD syndrome (HIDS) patients which accumulate mevalonate and have monocytes with a trained phenotype [52•].

**Table 1**

**Metabolites/enzymes affect epigenetic enzymes.**

| Metabolite/enzyme | Function |
|---|---|
| $NAD^+$ | Stimulate sirtuins (HDACs) |
| Acetyl-CoA | Stimulate HATs |
| α-KG | Stimulate TET family (DNMTs)<br>Inhibits KDMs |
| Succinate and fumarate | Inhibits TET family (DNMTs)<br>Stimulate KDMs |

Altered metabolism in immune cells is observed in many diseases, including cancer, for which currently multiple agents targeting metabolism are in trials or approved for cancer treatment [53]. Some of these drugs have also been studied in the context of inflammation, such as drugs which block the glycolysis pathway, 2-deoxy-D-glucose and dichloroacetic acid [54], which are both able to abrogate trained immunity *in vitro* [16••]. Additionally, administration of etomoxir, which blocks lipid metabolism, as well as 6-diazo-5-oxo-L-norleucine (DON), which inhibits glutamine uptake and metabolism, have been shown to be beneficial in respectively graft versus host disease and after transplantation to prevent rejection

[54]. Toxicity is a main challenge in the development of metabolic inhibitors. If this can be overcome, it would be a very promising target in the context of trained immunity.

## Conclusions and future prospective

Therapeutic targeting of trained immunity on the immunologic, metabolic or epigenetic level is a promising strategy to treat maladaptive immune responses in humans. Especially the development of epigenetic modulators is of great interest since epigenetic modifications are dynamic and reversible, and are at the basis of long-term trained immunity. In the future, knowledge needs to be gained to better understand the mechanisms mediating trained immunity in specific diseases to develop novel effective targeting strategies.

## Funding and declaration of interest

MGN was supported by an ERC Consolidation Grant (#310372) and a Spinoza grant from the Netherlands Organization for Scientific Research (NWO). MGN, LABJ and JCHMW received funding from Europees Fonds voor Regionale Ontwikkeling (EFRO) (PROJ-00587).

## Conflict of interest statement

Nothing declared.

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