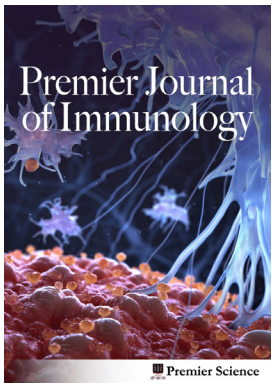

Expanding Horizons of Trained Immunity: Implications in Cancer and Pathogen Resistance

Jainu Ajit

ABSTRACT

Immunological memory has traditionally been attributed to the adaptive immune system. However, recent research shows that innate immune cells can also "remember" past infections and respond more effectively to subsequent exposures. This phenomenon, known as trained immunity or innate immune memory, has significant implications for various health applications. Depending on the type of training stimuli, the enhanced immune responses can last anywhere from a few days to several months. Emerging evidence suggests that these mechanisms can be harnessed to develop innovative anti-cancer therapeutics. This review examines the current landscape of trained immunity molecules, their applications in pre-clinical and clinical cancer models, and the challenges and future directions in this promising field.

Keywords: Trained immunity, Innate immune memory, Anti-cancer therapeutics, Epigenetic remodeling, Metabolic reprogramming

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Basics of Trained Immunity

The immune system comprises two main components: the innate and adaptive arms. The innate immune system serves as the body's first line of defense against invading pathogens. After encountering infectious agents, innate immune cells, such as macrophages, neutrophils, mast cells, etc., secrete cytokines and chemokines to control it. While this response is rapid and non-specific, the adaptive immune response develops later but generates prolonged, antigen-specific effects. T and B-cells are components of the adaptive immune system that recognize and respond to antigens presented by antigen-presenting cells (APCs).[1]

Cells of the innate immune system express pattern recognition receptors (PRRs) that recognize various molecular patterns common to infectious agents and damaged cells called pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs). Activation by PAMPs and DAMPs on innate immune cells generates a rapid response. The adaptive immune response is slow to develop yet retains immunological memory of previous insults, thereby protecting the host from subsequent infections. However, recent research has identified that cells of the innate immune system also retain a memory of past inflammatory insults, and this feature is called Trained Immunity or Innate Immune Memory (Figure 1).[2]

The stimulation of certain PRRs induces trained immunity.[3] After the first stimulation, innate immune cells undergo epigenetic remodeling, which results in an open chromatin architecture enabling faster transcription of pro-inflammatory genes when challenged with an infectious agent. This epigenetic remodeling results in the trained cell continuing in a heightened state of responsiveness, protecting the host from infections from the same or unrelated pathogen. While only specific PAMPs and DAMPs are known to induce training, there is less specificity regarding which pathogens against which trained immune cells might respond better. Hence, the nature of heterologous protection varies between different training stimuli. Additionally, although innate immune cells like monocytes are short-lived, trained immunity responses can be extended by targeting progenitor cells in the bone marrow and spleen. As a result, the length of the trained response can vary from a few days to several months, also depending on the nature of the training stimuli.[4]

The most widely studied inducers of trained immunity are the Bacillus Calmette-Guérin (BCG) vaccine and the fungal cell wall component called β-glucan. BCG is recognized by multiple PRRs, including toll-like receptors (TLRs): TLR2, TLR4, and NOD2.[5] Additionally, trained immune responses from BCG last for 3 months to a year.[6] β-glucan recognition by Dectin-1 induces training responses in several innate immune cells and lasts up to 28 days.[7,8] Various other molecules, vaccines, and infectious agents have also been reported to induce trained immunity and will be explored in other sections of this review. Trained immunity produces protection against unrelated infectious agents; however, this review will primarily focus on the anti-cancer properties of training.

Mechanisms of Trained Immunity

Trained immunity is mediated by epigenetic and metabolic reprogramming of innate immune cells. These mechanisms are interdependent, and metabolites from various cellular pathways influence histone remodeling in distinct ways specific to the training agent. Ultimately, training results in chromatin remodeling, allowing faster transcription of inflammatory cytokines to control subsequent pathogenic challenges (Figure 2).

Metabolic Regulation of Trained Immunity

Innate immune cells use oxidative phosphorylation (OXPHOS) to fuel their energy demands. After training, cells require a quicker source of energy to maintain their elevated state of responsiveness. Therefore, β-glucan trained macrophages undergo a shift from OXPHOS to glycolysis, called the Warburg effect, to supplement the increased energy demands and involve the Akt-mTOR-HIF1a signaling pathway.[9] Additionally, β-glucan trained macrophages also undergo an increase in cholesterol metabolism to secrete mevalonate, a metabolite that activates the IGF1-R and mTOR pathway.[10] BCG training induces increased

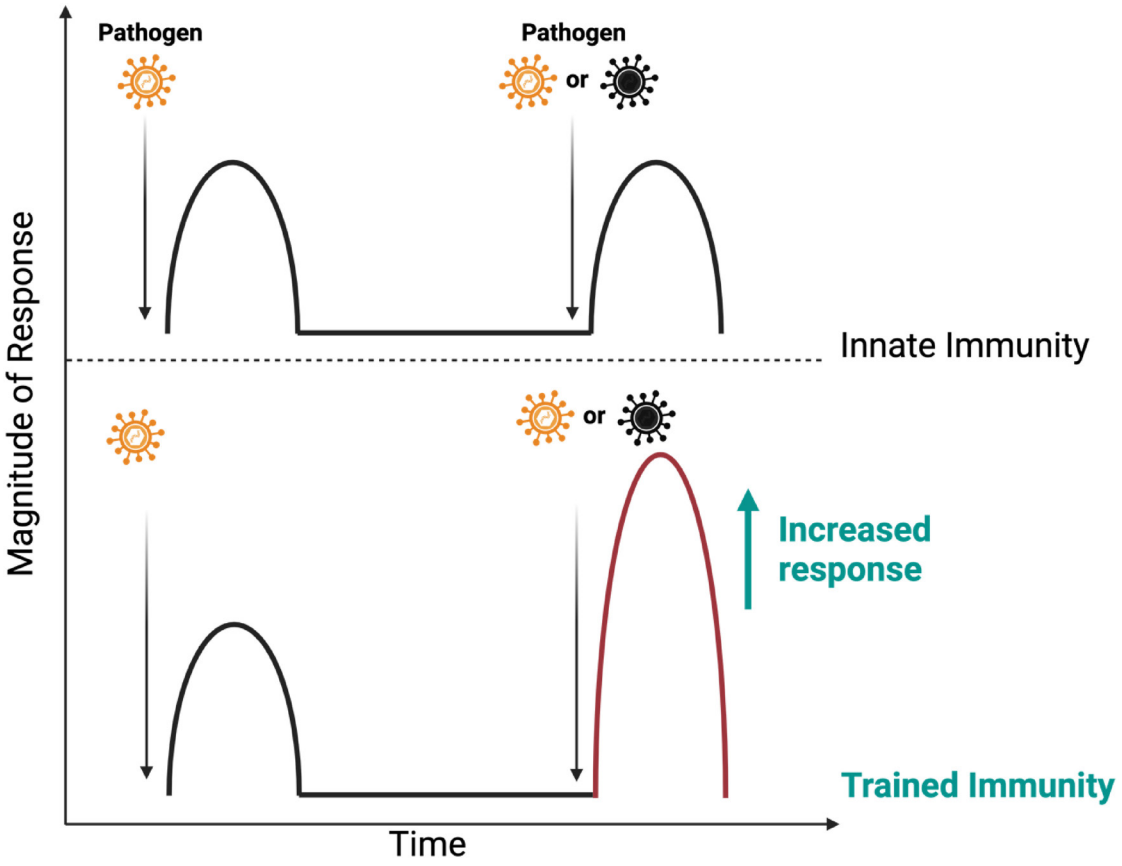

Fig 1 | Schematic representation of trained immunity
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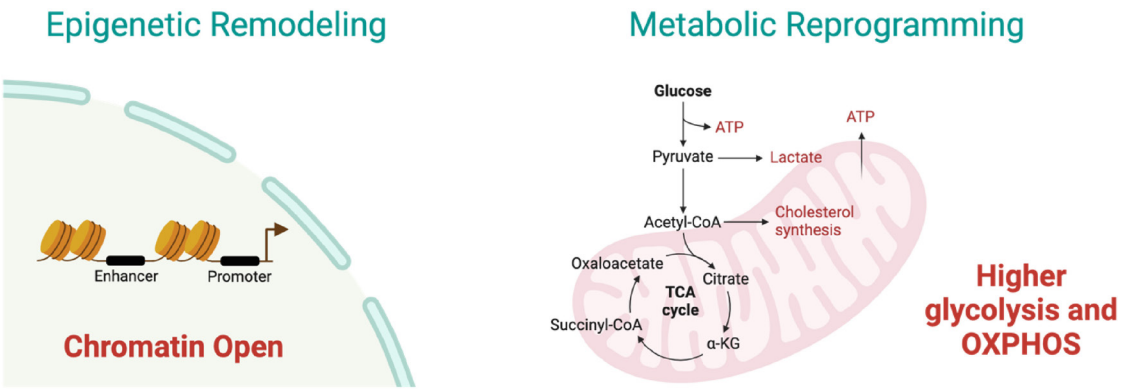

Fig 2 | Epigenetic and metabolic regulation of trained immunity
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glycolysis, glutamine synthesis, and OXPHOS to promote training phenotype.[11]

Epigenetic Regulation of Trained Immunity

Pro-inflammatory genes in myeloid cells are in a repressed configuration. Training with specific stimuli induces epigenetic changes by opening chromatin by histone modifications. This results in the enhanced recruitment of transcription factors, facilitating quicker synthesis of pro-inflammatory cytokines. Two main epigenetic changes are associated with training: histone 3 lysine 27 acetylation at distal enhancers and histone 3 lysine 4 trimethylation at promoters.[4] Epigenetic changes can persist even after the immunologic insult, thereby providing long-lasting training effects.[12]

Correlations Between Metabolic and Epigenetic Pathways of Training

Metabolites from cellular metabolism pathways regulate chromatin remodeling. For example, fumarate accumulation after β-glucan training inhibits histone demethylase, thereby increasing histone methylations. Similarly, lactates inhibit histone deacetylases,

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increasing chromatin opening and transcription.[12] Mevalonate, a metabolite derived from cholesterol synthesis, also influences histone modifications.[10]

Central and Peripheral Trained Immunity

Trained immunity effects can either be systemic or tissue-specific, called central training or peripheral training, respectively. Understanding the breadth of training effects makes it easier to harness trained immunity in developing novel anti-cancer therapies.

Central Trained Immunity

Central trained immunity alters hematopoietic stem and progenitor cells (HSPCs), primarily in the bone marrow, leading to prolonged and durable training responses. For example, BCG-induced training results in enhanced myelopoiesis at the expense of lymphopoiesis. Kaufmann et al. reported that BCG-trained hematopoietic stem cells (HSCs) generated macrophages that enhanced resistance against mycobacterial infection.[13] Other studies validated this observation by reporting alterations to the HSPC compartment in the bone marrow, spleen, and draining lymph node.[14] BCG-induced training on dendritic cells (DCs), increasing their antigen-presentation, and improved subsequent development of cytotoxic T-lymphocytes.[15]

Besides BCG, β-glucan, and lipopolysaccharides (LPS) have also been demonstrated to induce central training effects in the bone marrow. Mitroulis et al. demonstrated that β-glucan-induced myelopoiesis altered glucose metabolism and cholesterol biosynthesis pathways. Trained myeloid cells exhibited enhanced IL-1β and granulocyte-macrophage colony-stimulating factor secretion and protected against chemotherapy-induced myelosuppression in mice models.[16] Similarly, de Laval et al. noted increased myelopoiesis following acute stimulation with LPS, which induced C/EBPβ-dependent chromatin accessibility-mediated training effects.[17]

Tissue-Resident or Peripheral Trained Immunity

Multiple studies demonstrate enhanced training effects on lung-resident macrophages following exposure to LPS, protecting against subsequent pneumococcal infections. Kang et al. reported enhanced monocyte recruitment to the lung following LPS training.[18] Zahalka et al. linked this effect to type-1 interferon signaling, fatty acid oxidation, and glutaminolysis.[19] Other studies identified lung-resident alveolar macrophages (AMs) as key to enhancing bacterial resistance while maintaining tissue homeostasis.[20,21]

Further studies by Mai et al. emphasized the necessity of an optimal lung environment to generate protective training responses and trained AMs following bacterial exposure.[22] While O'Hara et al. demonstrated trained innate immunity mediated by γδT and NKT cells in the lungs following BCG vaccination, Wang et al. found that trained AMs were able to induce efficient memory and cytotoxic T cells when BCG vaccination was followed by RSV vaccination.[23,24]

Additionally, β-glucan training increased goblet cells and mucus production in the intestine, leading to an upregulation of type-2 responses characterized by elevated secretion of IL-4, IL-5, and IL-13.[25] In a zebrafish model, β-glucan was found to elevate trimethyl-histone H3 lysine 4 (H3K4me3) modifications on mitophagy-related genes, thereby alleviating septic liver injury.[26]

Combined Effects

Many studies report that both central and peripheral training effects can be induced by targeting the bone marrow and specific tissues. For instance, Xu et al. reported that the virulence protein PepO from *Streptococcus pneumoniae* modulates hematopoiesis, promoting the formation of myeloid cells. Additionally, PepO also stimulates training in peritoneal macrophages, enhancing their bactericidal capacity.[27] Similarly, the subcutaneous administration of the BCG vaccine affects multiple sites, including bone marrow, gut microbiota, and lungs. Changes in the intestinal microbiome lead to alterations in circulating metabolites, which induce memory macrophages in the lung.[28] Additionally, Kang et al. found that neutrophil-mediated protection against *S. pneumoniae* infections arises from training effects in the lung following BCG vaccination.[29] Furthermore, multiple doses of LPS have been shown to influence brain-resident macrophages, with the training effects lasting over 6 months.[30]

Trained Immunity and Anti-cancer Effects

Trained immunity acts on peripheral immune cells and bone marrow progenitors. Training increases the secretion of pro-inflammatory cytokines, phagocytosis, and ROS production on subsequent challenges, including cancers. Moreover, training acts on APCs like DCs, increasing their maturation, migration to the lymph node, and subsequent presentation to T cells, eliciting tumor-specific responses. Within the tumor microenvironment (TME), macrophages undergo reprogramming to an inflammatory M1 phenotype on training. M1 macrophages exhibit increased phagocytosis and respiratory burst activity with increased anti-tumor properties and autophagy.[31]

Myeloid-derived suppressor cells (MDSCs) inhibit the activity of T cells and promote the immune escape of tumor cells. Trained immunity decreases the number of MDSCs in the tumor and spleen, thereby converting the TME into an immune-responsive state. Additionally, the recruitment of pro-inflammatory macrophages further restricts the MDSC effect, improving the impact of immunotherapy. Multiple studies report the successful combination of training with existing checkpoint blockade therapy to enhance anti-tumor effects further.[32]

This review highlights existing training stimuli and related mechanisms of protection against infectious agents and tumor growth (Figure 3).

BCG-Induced Trained Immunity

Epigenetic reprogramming is key to training effects induced by the BCG vaccine. BCG binds to the NOD-2 receptor, leading to epigenetic remodeling, inducing increased histone 3 lysine 4 trimethylation at promoter

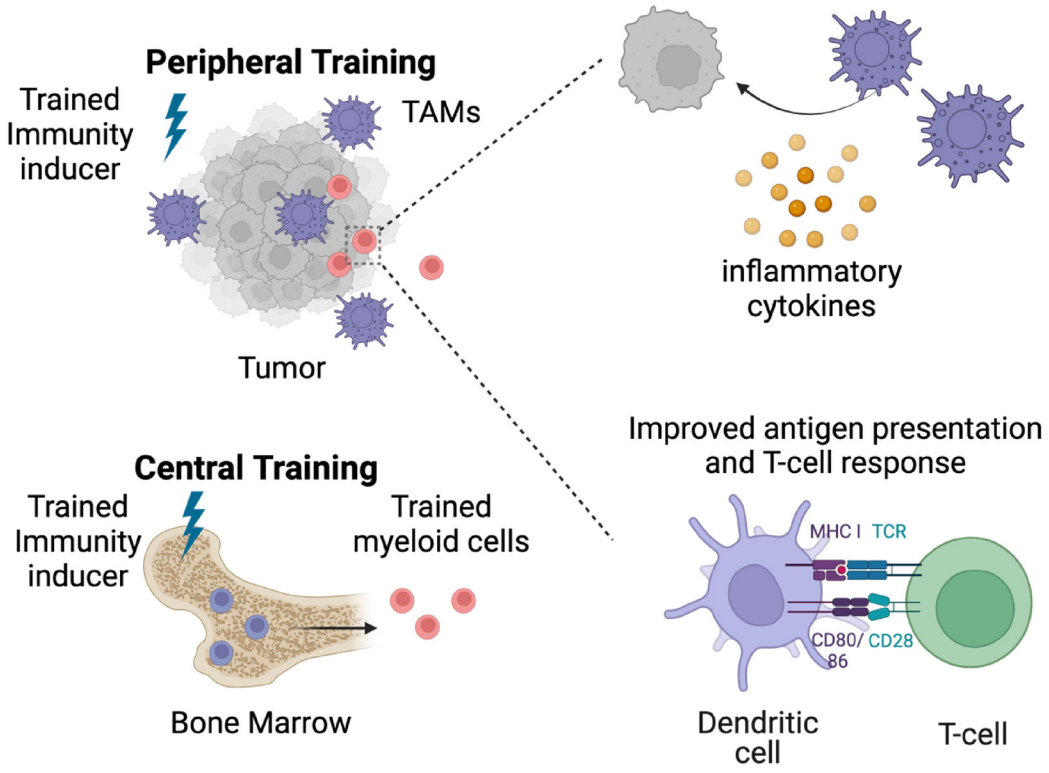

Fig 3 | Schematic representation of trained immunity effects against cancer
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sites for monocyte inflammatory cytokines like TNF-α and IL1β. Following BCG vaccination, there is an up-regulation of TLR4 and CD11b, a sevenfold increase in IFN-γ production, and improved resistance to unrelated pathogens for over 3 months.[33] Apart from epigenetic modifications, trained immunity is associated with metabolic changes, including an increase in glycolysis and glutaminolysis.[11]

Röring et al. observed an inhibitory role of IL-10 on BCG-induced training in human monocytes.[34] IL-10 prevented ROS production, and circulating levels of IL-10 negatively correlated to training responses in BCG-vaccinated adults. On the other hand, resveratrol potentiated BCG-induced training in human monocytes by enhancing histone acetylation on the promoter regions of IL-6 and TNF-a genes without changing metabolic cycles.[35]

BCG vaccination also induces training in other immune cells, including natural killer (NK) cells and neutrophils, with long-lasting heterologous protection.[6,8] It induces transcriptional changes in γδ-T cells, enhancing protection against bacterial and fungal infection.[36] Notably, heterologous TH1 and TH17 responses remained elevated for up to a year after BCG vaccination.[37] Besides immune cells, BCG also targets cells in the bone marrow, leading to central training effects that induce long-lasting effects. Kaufmann et al. demonstrated that BCG accesses the bone marrow and induces myelopoiesis, generating macrophages with enhanced protection against M. tuberculosis.[13] Cirovic et al. confirmed these findings and highlighted the key role of the hepatic nuclear factor family of proteins in regulating transcriptional changes associated with these training effects.[14]

BCG Training in Anti-tumor Effects

BCG vaccination during childhood is linked to a reduced risk of lung cancer, leukemia, and lymphomas.[38–40] Monocytes isolated from BCG-vaccinated patients with non-muscle-invasive bladder cancer (NMIBC) produced increased IL-12 when challenged with LPS. Moreover, BCG-vaccinated patients had significantly longer disease-free survival rates of 84% compared to only 22% in the unvaccinated cohort, and they also experienced a longer time to recurrence.[41] Even with the same vaccine, it is essential to distinguish systemic vs tissue-specific delivery methods. For instance, Atallah and colleagues showed that intravenous BCG vaccination promoted anti-tumor effects by modifying the TME, whereas intravesical delivery promoted tumor growth. This suggests that the systemic or central training effects of BCG are key to modifying the tumor microenvironment, increasing cytotoxic T cells, and decreasing MDSCs. Additionally, systemic BCG training improved DC function, increasing antigen uptake and presentation, thereby promoting T cell proliferation.[15]

Zhang et al. reported a novel approach to enhance targeting of the TME by using macrophage membrane to camouflage BCG. The authors noted that this new delivery technique synergized with checkpoint blockade without causing toxicity.[42] Apart from TME remodeling, BCG promotes anti-tumor effects by increasing autophagy. Moreover, certain single nucleotide polymorphisms in an autophagy gene ATG2B correlated with the progression and recurrence of bladder cancer

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in BCG-vaccinated patients.[43] Singh et al. developed an innovative strategy to improve anti-tumor effects by engineering BCG to express high levels of another PAMP called cyclic di-AMP that activates the stimulator of interferon genes (STING) receptor. This modified construct elicited higher levels of pro-inflammatory cytokines and trained myeloid cells, providing better protection against bladder cancer.[44]

β-glucan-Induced Trained Immunity

Quintin et al. observed functional reprogramming and enhanced cytokine production by monocytes exposed to *Candida albicans* upon reinfection with the same antigen mediated by Dectin-1.[45] Subsequent Raf-1 signaling pathway led to stable epigenetic modifications like histone methylations at promoter regions of inflammatory cytokines. Further studies revealed increased glucose metabolism, lactate production, and glycolysis in β-glucan-trained monocytes via the mTOR-HIF-1α pathway.[9] SHIP-1, a phosphatase, is a negative regulator of trained immunity by inhibiting PI3K signaling downstream of Dectin-1. Saz-Leal and colleagues deduced the role of SHIP-1 or SH2-containing inositol 50-phosphatase 1 in β-glucan-induced training.[46] The authors reported that the training effects of β-glucan are enhanced by myeloid-specific deletion of SHIP-1 and better protection against Candida infection. In addition to SHIP-1, recent studies have highlighted key modulators of β-glucan-induced training, including miR-9-5p-mediated metabolic rewiring, GSH-dependent antioxidation, and C5a receptor (C5aR)-driven mTOR activation.[47,48] Shim et al. reported that a Complement C5aR activating Co1 peptide amplified β-glucan training in peritoneal macrophages.[49]

Mitroulis et al. observed that β-glucan induced bone marrow myelopoiesis, elevated response to LPS challenge, and protection from side effects after chemotherapy.[16] Bono and colleagues deduced the mechanistic links and reported that HSPCs were activated following Dectin-1 binding, differentiating into macrophages, contributing to emergency myelopoiesis, and improving disease resistance.[50] HSPC reprogramming was further confirmed by Moorlag et al., who also reported an increase in myelopoiesis and IL-1 signaling following β-glucan administration.[8] HSPC reprogramming leads to transient mobilization at the spleen, which is then reprogrammed for both myelopoiesis and enhanced pro-inflammatory cytokine production against an infectious challenge.[51]

HSPC reprogramming by β-glucan points to the induction of long-lasting training effects. However, considerable variability in the duration of training was observed with different sources and solubility profiles of β-glucan. Moreover, some soluble glucans were also found to induce tolerance effects.[52] However, Garcia-Valtanen and colleagues observed that β-glucan-induced training effects only lasted 7 days.[53] Additionally, delivery vehicles play an important role in determining the outcome of β-glucan. For example, Ardali et al. noted tolerance induced by β-glucan delivered in an oil-in-water adjuvant in a porcine model.[54]

β-glucan Training in Anti-cancer Effects

β-glucan induces central and peripheral training. For example, Kalafati and colleagues demonstrated that mice pre-treated with β-glucan elicited epigenetic rewiring of granulopoiesis and neutrophil reprogramming, generating an anti-tumor phenotype. The authors identified the role of type 1 interferon signaling in inducing this trained phenotype and reported that the adoptive transfer of neutrophils could inhibit tumor growth.[55] In another study, β-glucan was trafficked to the pancreas, resulting in peripheral training characterized by an influx of innate immune cells, like monocytes and macrophages, with enhanced anti-tumor cytotoxicity. This contributed to reduced tumor growth and prolonged survival, an effect enhanced in combination with immunotherapy.[56] Additionally, β-glucan training in lung interstitial macrophages was found to be mediated by the sphingosine-1-phosphate metabolite, resulting in mitochondrial fission. Trained cells inhibited tumor metastasis and prolonged survival in multiple mouse melanoma models.[57]

Apart from whole glucan preparations, other delivery vehicles and combinations have also been explored to induce potent anti-tumor responses. For example, Vuscan and colleagues developed a blend of two β-glucan formulations from Saccharomyces cerevisiae that targets multiple PRRs like Dectin-1, CR3, and TLR4. This blend induced potent anti-tumor responses in mouse melanoma and bladder cancer models.[58] Similarly, Woeste et al. found that β-glucan improves the effect of irreversible electroporation, a non-thermal tumor ablation method. Combining β-glucan increased innate immune cell infiltration to the pancreatic TME.[59]

β-glucan is also used to develop personalized cancer vaccines. For example, Chen and colleagues engineered an inactivated probiotic *Escherichia coli* Nissle 1917 encapsulated with β-glucan and tumor antigens. The new construct effectively trained macrophages, differentiating them into an M1 phenotype, facilitating DC activation and T cell function, and being capable of inducing prophylactic and therapeutic anti-tumor response.[60] Recent work utilized polymeric platforms to achieve kinetic control over training responses. Ajit and colleagues demonstrated that the molecular weight of poly(lactic-co-glycolic acid) (PLGA) nanoparticles can be tailored to control the release of encapsulating β-glucan, capable of achieving controlled training and potent anti-tumor responses.[61]

Infection-Induced Trained Immunity

Sepsis is a life-threatening condition that results in organ dysfunction due to a systemic release of inflammatory molecules. Multiple studies have shown that sepsis leads to trained immunity. For example, Bomans and colleagues found that bone marrow monocytes exhibit enhanced glycolysis and training responses after sepsis.[62] Another study reported that sepsis reprograms granulocytes, increasing inflammatory cytokine secretion, respiratory bursts, and phagocytosis by upregulating glycolysis and fatty acid synthesis.[63]

Crabtree et al. studied the effects of *Plasmodium falciparum* on inducing training effects and observed that it was mediated by soluble signals secreted from lymphocytes. This crosstalk increased the number of cytokine-producing monocytes and DCs.[64] Similarly, viral infections also lead to training effects. For instance, human monocytes cultured with extracellular vesicles (EVs) containing the HIV-1 protein Nef changed their epigenetic architecture and cholesterol metabolism.[65] Additionally, hepatitis-B-infected mothers induce in-utero training in the fetal immune system characterized by high levels of IFN-α2 and IL-12p40 cytokines and lesser IL-10. This enhances the anti-bacterial resistance of neonatal immune cells *in vitro*.[66]

Bacterial infections trigger a training effect by binding to various PRRs like TLRs and nucleotide-binding and oligomerization domain-like receptors (NLRs). Research has shown that intranasally administered heat-killed mycobacteria induced robust training responses through a Syk/HIF-1a-dependent mechanism.[67] Kain and colleagues discovered that *Mycobacterium avium* induces HSPC reprogramming, and trained cells respond to unrelated pathogens by upregulating IFN-g genes.[68] Frauenlob et al. demonstrated the training effects of Helicobacter pylori on monocytes by the accumulation of NF-kB proteins.[69] Additionally, the *S. pneumoniae* virulence protein PepO enhances macrophage function and increases protection against various pathogens. The activation mechanism involves epigenetic reprogramming of macrophages and the release of complement C3-mediated activation of B-cells in the peripheral cavity, resulting in peripheral and central training.[27] Lasaviciute and colleagues explored the training effect of the secretome from a probiotic bacteria called *Limosilactobacillus reuteri*, which induced a mixed training phenotype in human monocytes associated with histone modifications.[70] Secondary challenges induced higher levels of IL-6 and IL-1b but low TNF-a, IL-23, and IL-27, which were crucial for T helper cell function. Furthermore, Q-fever-causing *Coxiella burnetii* also induces training in CD14+ monocytes, resulting in elevated levels of IL-1b-, IL-6 and IL-8.[71]

Vaccine-Induced Trained Immunity

Apart from the BCG vaccine, other vaccinations have also been shown to induce innate immune training. For example, Cervarix and Gardasil, which are human papillomavirus vaccines, increase the expression of TLRs and inflammatory cytokines in macrophages.[72] Gu et al. demonstrated training of AMs by a single intranasal immunization of *Acinetobacter baumannii*, protecting against bacterial pneumonia.[73] The immune-stimulating components of vaccines play a crucial role in determining their training effects. For example, while vaccinia-induced robust training via epigenetic reprogramming in human primary monocytes, a recombinant strain of modified vaccinia Ankara MVA85A did not have the same effect.[74]

Small-Molecule-Induced Trained Immunity

Besides pathogens, simplified molecular structures called PAMPs also induce training. LPS are components of gram-negative bacteria cell walls that induce training in innate immune cells depending on the secondary challenge and local environment. Mast cells exposed to LPS underwent training in response to a candida infection but demonstrated tolerance when challenged with LPS.[75] In another study, Zahalka and colleagues found that intranasal exposure to LPS elicited training in AMs. This resulted in enhanced protection against a pneumococcal challenge linked to better type 1 IFN signaling. However, the adoptive transfer of trained AMs worsened symptoms and damaged tissue during a subsequent challenge, highlighting the impact of the local environment in dictating the magnitude and type of response elicited.[19]

Infectious-Agents-Induced Trained Immunity in Anti-Cancer Responses

Cholera B subunit induced training in DCs, increasing recruitment at the skin and lymph nodes. These cells infiltrated tumors and activated exhausted CD8+ T cells, protecting against melanoma challenge.[76] Antigens derived from *Leishmania braziliensis* activated multiple TLRs, altered metabolism, and exerted potent anti-tumor effects in mice with non-Hodgkin lymphoma.[77]

Similarly, bacteria-derived outer membrane vesicles activated inflammasome signaling and IL-1β secretion, increasing the number of APCs and generating potent anti-tumor responses in multiple cancer models *in vivo*.[78] In another study, an outer membrane vesicle-based nanohybrid was developed that effectively trained bone marrow progenitors and monocytes, reprograming TAMs and eliciting tumor suppression in MC38 and B16F10 models.[79]

Viral infection-induced training also promotes anti-tumor responses. For example, Wang et al. found that influenza-trained respiratory mucosal-resident AMs infiltrate tumors. The training was associated with epigenetic and metabolic reprogramming. Additionally, they reported the crucial role of IFN-γ and NK cells in mediating the anti-tumor response.[80]

Other TI Inducers in Anti-Tumor Responses

Muramyl dipeptides (MDP) are derived from the cell walls of mycobacteria and activate NOD2 receptors, inducing trained immunity.[33] Li and colleagues designed a biphasic delivery system composed of PLGA nanoparticles that encapsulated MDP and a tumor antigen combined with nanoparticles loading β-glucan embedded in a sodium alginate hydrogel. Immunization with this vaccine completely abrogated tumor growth in mice by inducing training phenotype, leading to efficient DC maturation and migration to the lymph node.[81] In another study, Priem et al. developed a nanobiologic platform encapsulating muramyl tripeptide, a synthetic lipophilic analog of MDP, that promoted myelopoiesis and remodeled the TME with potent anti-tumor response in a B16F10 melanoma model.[82]

Targeting epigenetic and metabolic intermediates is another strategy to modulate trained immunity. For example, Mourits et al. showed that an inhibitor

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of histone methyltransferase G9a amplifies trained immunity responses in monocytes of NMIBC patients treated with BCG. This effect is mediated by decreased H3K9me2 at the promoters of inflammatory genes and also increases cellular metabolism.[83] In another study, Yang et al. targeted a metabolic intermediate, metformin, to activate NK cells for enhanced anti-tumor responses. They synthesized a MnO_2 nanoparticle to load Metformin, which activates the cGAS-STING pathway, with improved barrier penetration and radiotherapy effects.[84] A nanoparticle construct composed of curdlan, a type of β-glucan, in combination with chitosan derivative, elicited increased glycolysis and OXPHOS *in vivo*. Enhanced training in macrophages with this construct successfully prevented lung metastasis in a B16F10 model.[85]

Cancer-Treatment-Induced Trained Immunity

Cancer treatment using radiotherapy and ultrasound also induces training effects. For instance, Voshart and colleagues showed that microglia harvested from rats exposed to radiotherapy secreted higher inflammatory cytokines to subsequent inflammatory insults.[86] This finding points to a potential cause for cognitive decline in radiotherapy patients. Meanwhile, Yang et al. showed that low-intensity ultrasound upregulates trained immunity enzymes in cancer cells, promoting anti-tumor response.[87]

Table 1 | Details of Human Clinical Trials Using Trained Immunity-Inducing Molecules for Anti-cancer Effects

Study	Cancer	TI Inducer	Effect	Mechanism
Paré et al.[88]	NMIBC; n = 7	BCG	Recurrence-free survival associated with higher histone methylation	H3K4me3 levels in MAPK pathway genes after 5 weeks of BCG treatment in the recurrence-free group
Alves Costa Silva.[89]	Stage IIIB/C melanoma; n = 148	Gut microbiota composition	*Faecalibacterium prausnitzii* is the main beneficial taxon for no recurrence at 2 years	
Singh et al.[90]	Brain and CNS (BCNS) tumors - observational study	BCG vaccination	Significantly lower BCNS cancer incidence in countries with neonatal BCG vaccine	
van Puffelen et al.[91]	NMIBC -	BCG intravesical	• Increased production of TNF and IL-1β • Protective effects against respiratory infections (37% decreased risk)	Enhanced inflammatory activity
Broquet et al.[92]	Cancer	Sepsis	Sepsis survivors had a lower cumulative incidence of cancers than matched non-severe infection survivors	Mediated by sepsis-trained resident macrophages that trigger tissue residency of T cells via CCR2 and CXCR6
Derré et al.[93]	NMIBC; n = 24	BCG Intravesical combined with cancer vaccine	Increase of vaccine-specific T cells in the bladder upon BCG	
Föhse et al.[94]	Neuroendocrine sarcoma	BCG with Checkpoint therapy	Safe and Effective combination therapy with fewer side effects	
Graham et al.[41]	NMIBC; n = 33	BCG	Higher disease-free survival in the BCG group	

A few of these molecules have been explored in human clinical studies and are summarized in Table 1.[31]

Challenges

Although most of the studies in this review report positive anti-tumor effects of trained immunity, many mechanistic details are still unclear. For example, training induces glycolysis in innate immune cells, which is also the preferred metabolic pathway for tumor cells.[32] Therefore, it is essential to clarify how training specifically induces glycolysis in immune cells and to understand the mechanisms through which an anti-tumor phenotype is established.[4]

The tumor immune microenvironment is influenced by innate immune cells, which can exert both tumor-promoting and tumor-suppressive functions. While trained immunity enhances host defense, persistent epigenetic reprogramming may also result in maladaptive responses that support tumor progression and metastasis. Maladaptive or inappropriately activated trained immunity has been implicated in various chronic inflammatory and autoimmune conditions, where it worsens disease progression. These underlying conditions, combined with environmental factors, stress, and comorbidities, can complicate the use of trained immunity as a reliable anti-cancer strategy. Furthermore, the impact of trained immunity on cancer is expected to differ depending on tumor types and stages. Therefore, it is crucial to carefully assess potential risks and therapeutic outcomes.[95]

Only a few trained immunity-inducing molecules have been rigorously studied to date. While recent research identified novel trained immunity-inducing small molecules, in-depth mechanistic insight is still lacking.[96] Small molecules provide an advantage over BCG vaccine and other polymers for their favorable immunomodulatory profiles. Encapsulation and delivery of such small molecules also make it easier to achieve cell-specific training effects.

A better understanding of the correlation between administration routes and training effects is crucial to harnessing the positive effects of training in cancer therapy. For example, an intravenous BCG administration targeting bone marrow progenitors is advantageous for generating long-lasting central training rather than tissue-specific delivery. Similarly, developing novel delivery vehicles that target the bone marrow offers an innovative solution to induce central training.[82]

Future Perspectives

Role of Circadian Rhythm in Modulating Trained Immunity

Long et al. conducted the first large-scale randomized controlled trial to evaluate the effects of vaccination timing. They found that receiving the influenza vaccine in the morning results in a stronger antibody response.[97] Ince et al. suggested that this enhanced response is related to the rhythmic nature of DC migration to the draining lymph node, which peaks during the day. They proposed that this time-dependent response is an evolutionary adaptation designed to

prime immune responses against pathogens when encounters are most likely, especially during social interactions and search for food.[98]

Interestingly, trained immune responses also display a similar pattern. de Bree et al. reported that monocytes isolated from individuals vaccinated in the morning with the BCG vaccine demonstrated enhanced training effects compared to those vaccinated in the evening.[99] No studies have thus far looked at the effects of timing of training in the induction of anti-tumor responses. Since training influences many cell types with an inherent circadian clock, like neutrophils, macrophages, and DCs, more studies need to be performed to elucidate these mechanisms and potential links to anti-tumor responses.

Sex-Specific Differences in Training Effects

Koeken et al. found that inflammatory markers were higher in males than females before vaccination. However, males exhibited significantly lower systemic inflammation after the BCG vaccine than women.[100] However, de Bree and colleagues reported no effect of estrogen or dihydrotestosterone on BCG-induced training in primary monocytes, although direct stimulation decreased pro-inflammatory cytokine secretion. Sun et al. demonstrated that estradiol promotes β-glucan-induced trained immunity, resulting in greater resistance to sepsis in female mice compared to male mice.[101]

Conversely, Earhart et al. reported a reduction in the heat-killed *Candida albicans* training response in females compared to males, resulting in decreased survival from opportunistic infections such as *Burkholderia gladioli*. They highlighted the significant role of the estrous cycle and circulating serum progesterone levels in mediating reduced training effects in females.[102] These findings highlight the need to understand sex-based differences in training responses for improved patient care and anti-cancer strategies.

Lifestyle Effects Like Diet, Exercise, and Environmental Factors on Trained Immunity Responses

A ketogenic diet rich in saturated fatty acids and a high-salt diet enhance training effects by rewiring HSCs, leading to the downregulation of the NR4a family and decreased mitochondrial OXPHOS.[103,104] Multiple studies have also examined the effects of a Western diet on trained immunity. Studies also indicate that a Western diet can impact trained immunity, as Christ et al. found it boosts NLRP3-mediated reprogramming in myeloid progenitor cells, while Wu et al. noted its protective role against colitis via the mevalonate pathway.[105,106]

Enhanced training effects in adipose macrophages were observed during weight gain and loss cycles, linked to increased glycolysis and OXPHOS, leading to increased IL-6 and TNF-a levels after the LPS challenge.[107] Interestingly, Zhang et al. found that pre-operative exercise induced an anti-inflammatory trained immunity phenotype in Kupffer cells, attenuating liver injury and inflammation during surgery.[108] Similar anti-inflammatory effects were observed in BMDMs from mice that underwent chronic moderate-intensity training, which was associated with improved mitochondrial quality and decreased ROS production.[109] Regular early-life exercise was also found to exert anti-inflammatory beneficial effects that mitigate sepsis.[110]

Environmental factors like bisphenol A, commonly found in plastics, have been shown to trigger training in human monocytes. The authors observed a positive correlation between circulating bisphenol A levels and TNFα concentration.[111] Urban communities in Tanzania exhibit enhanced inflammatory profiles, highlighting the influence of the environment on health.[112] Overall, diet, exercise, and environmental exposure shape trained immune responses, but how these interplay with anti-tumor responses remains unclear, necessitating further research in cancer patients.

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