

Review

# The Role of Innate Priming in Modifying Tumor-associated Macrophage Phenotype

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## Abstract

Tumor-associated macrophages (TAMs) are innate immune cells that exert far reaching influence over the tumor microenvironment (TME). Depending on cues within the local environment, TAMs may promote tumor angiogenesis, cancer cell invasion and immunosuppression, or, alternatively, inhibit tumor progression via neoantigen presentation, tumoricidal reactive oxygen species generation and pro-inflammatory cytokine secretion. Therefore, TAMs have a pivotal role in determining tumor progression and response to therapy. TAM phenotypes are driven by cytokines and physical cues produced by tumor cells, adipocytes, fibroblasts, pericytes, immune cells, and other cells within the TME. Research has shown that TAMs can be primed by environmental stimuli, adding another layer of complexity to the environmental context that determines TAM phenotype. Innate priming is a functional consequence of metabolic and epigenetic reprogramming of innate cells by a primary stimulant, resulting in altered cellular response to future secondary stimulation. Innate priming offers a novel target for development of cancer immunotherapy and improved prognosis of disease, but also raises the risk of exacerbating existing inflammatory pathologies. This review will discuss the mechanisms underlying innate priming including metabolic and epigenetic modification, its relevance to TAMs and tumor progression, and possible clinical implications for cancer treatment.

**Keywords:** tumor-associated macrophage (TAM); innate priming; epigenetic modification; metabolic modification; cancer

## 1. Introduction

The immune system has classically been divided into two arms—the innate and adaptive systems. This division is based primarily on the type of receptor used to distinguish non-self. Cells of the innate system (namely natural killer cells, granulocytes and monocytes/macrophages) recognise foreign material through expression of multiple pattern recognition receptors (PRRs), whereas cells within the adaptive immune system specifically recognise a single antigen. Although originally thought to operate independently, it has become clear that these two arms of immunity act in concert to provide a powerful and effective system of immunosurveillance [1].

Innate immune cells provide the first line of defense against developing tumors through their capacity to not only rapidly detect and eliminate cancer cells, but also stimulate the adaptive immune response [2,3]. However, immunogenic heterogeneity in cancer cells can emerge over clonal generations, allowing for the evasion of immunosurveillance [4]. Mechanisms by which the tumor microenvironment (TME) can potentially influence both the development and function of immune cells include expression of immune checkpoint inhibitors, generation of immune regulatory cells and selection of immune resistant cancer cells [4,5]. Further spatial and temporal heterogeneity within the

TME generates a complex network of interacting immune, non-immune (e.g., fibroblasts, adipocytes and mesenchymal cells) and malignant populations that exert further influence on the immune system's ability to eliminate the tumor [4,6–8].

Macrophages are members of the innate immune system that are proficient phagocytes and serve as core orchestrators of tumor responses [9]. Driven by immunological cues, recruited monocytes and resident macrophages within the TME can be differentiated into what are termed tumor-associated macrophages (TAMs) [10–13]. TAMs represent a major component of the infiltrating immune cells within the TME and have become the focus of increasing research due to their crucial roles in tumor growth, angiogenesis, immune regulation, metastasis and chemoresistance [11,12]. However, the impact of TAM on tumor progression is not fixed as there is considerable functional and phenotypic plasticity in response to the TME derived signals they encounter. TAMs have been broadly grouped as pro-tumor or anti-tumor based on their phenotype and functional capabilities, and changes in the relative number or distribution of these TAM sub-types have been associated with prognosis in numerous cancers [11,14–16]. The functional plasticity of TAMs reflects the metabolic and epigenetic reprogramming that occurs in response to multiple stimulants [17,18].



However, this reprogramming may not solely reflect exposures to stimulants within the TME.

Recent research has suggested that macrophages can be primed by stimulation prior to their arrival in the local TME, which may further impact their phenotype and functional response, leading to potential clinical consequence [19–22]. This adds a further layer of complexity to the understanding of immunotherapy response in patients. This review will discuss the relevance of this innate priming to TAMs, describe the underlying mechanisms driving innate priming and outline the implications for cancer treatment.

## 2. Innate Training versus Innate Tolerance

The first epidemiological data that provided some evidence for the presence of an innate priming process was provided by studies on recipients of live-attenuated *Bacillus Calmette-Guérin* (BCG) vaccine to prevent tuberculosis [23,24]. These studies suggested BCG vaccine induced a broader protection against diseases other than tuberculosis [23,24]. In an international survey consisting of >150,000 children, a 17–37% risk reduction for acute lower respiratory infection was observed in children previously vaccinated against BCG [24]. Murine models have also found that BCG vaccination can induce wider protection against pathogens such as malaria, influenza virus, *Salmonella*, and respiratory syncytial virus [23]. Current evidence suggests that although the adaptive immune system can provide a level of cross-protection, vaccination also induces long-lasting changes in the innate immune system. Although such changes in the innate system are consistent with the process of innate training, the exact mechanisms underlying these observations are currently unknown [23]. Recently, stimulants such as the Influenza vaccine, SARS-CoV-2, Helminth products, butyrate and  $\beta$ -glucan, have also been observed to induce lasting changes in the innate immune system, as reflected in altered phenotype and functionality [20,25–29].

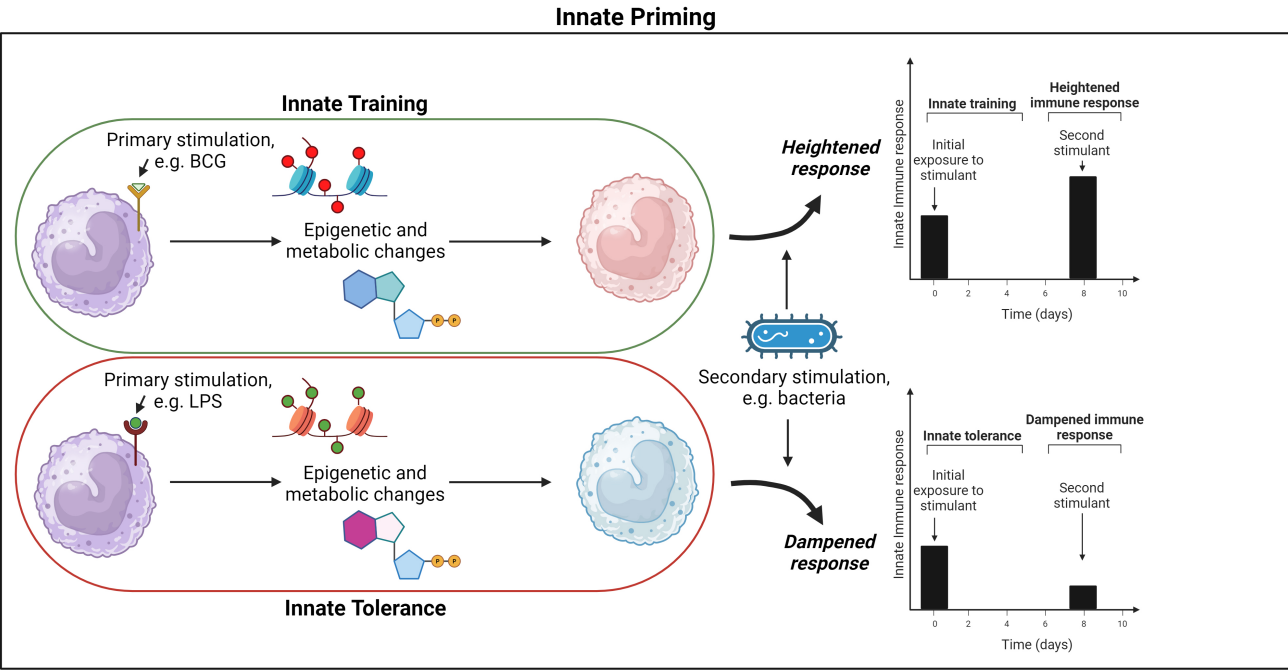
Innate “memory” is often used within literature to discuss lasting changes to innate cell phenotype induced by primary stimulants, but the biological mechanisms underlying this observation differs from those mechanisms involved in adaptive memory. In particular, innate memory does not lead to expansion of a subset of cells that are antigen specific [20,30]. Instead the term innate memory describes a *de facto* memory of a primary stimulant. The “memory” is provided by the induced metabolic and epigenetic reprogramming of differentiated and progenitor innate cells, which result in long lasting changes in innate cell phenotype. These reprogrammed cells subsequently have altered responses to a range of secondary stimuli as compared to the original innate cells [19–21]. In this review, this *de facto* memory will be referred to as “innate priming”. The functional consequence of the epigenetic and metabolic changes that characterise innate priming can result in innate cells exhibiting heightened or dampened responses to sec-

ondary stimulants. Innate priming that results in heightened response to secondary stimulus will be referred to as “innate training” and priming that results in reduced response to secondary stimuli will be referred to as “innate tolerance” (Fig. 1) [31].

A range of exogenous and endogenous compounds have been shown to be capable of inducing innate training (Fig. 1) [20,32,33]. *Ex-vivo*  $\beta$ -glucan, BCG vaccine (exogenous) and oxidized low-density lipoprotein (oxLDL) (endogenous) are commonly used to induce training in macrophages, as they have been shown to alter cytokine responses, receptor expression and reactive oxygen species generation upon restimulation with lipopolysaccharide, while shifting metabolism to a glycolytic system [20,32,34,35]. *In-vivo* murine macrophages have been shown to maintain heightened pro-inflammatory cytokine response to secondary *Cryptococcus neoformans* after initial exposure to an exogenous primary stimulant such as interferon- $\gamma$  (IFN $\gamma$ )-secreting *C. neoformans* strains [36]. In agreement, murine myeloid cells demonstrated heightened response to lipopolysaccharide (LPS) stimulation after the mice experienced a priming event of being fed a western diet [37]. These papers indicate that both exogenous and endogenous stimulants are capable of training the innate immune system and thereby leading to heightened response to non-specific stimulants.

During development of innate tolerance, once primary stimulation concludes, effector genes are silenced instead of being activated prior to secondary stimulation (Fig. 1). This functional reprogramming results in a dampened innate immune response [31]. For example, LPS can induce a strong inflammatory response in macrophages through interaction with receptors including toll-like receptor 4 (TLR4), however, upon re-stimulation, the cells were tolerized and exhibited reduced production of pro-inflammatory mediators and attenuated TLR activation to secondary stimulants [26,38–40]. Interestingly, if LPS was presented to innate cells at low concentrations (<100 pg/mL), priming was induced instead of tolerance [39]. In addition, both duration of resting period and time between stimulation also appear to influence the type of response. An *ex-vivo* study showed that if monocytes were primed with BCG or  $\beta$ -glucan and allowed to rest for 3–6 days that training was induced, but if resting was only permitted for 24 hours the monocytes displayed immune tolerance [20]. These variables further complicate the concept of innate priming and should be addressed in studies that are investigating this area.

The overall status of the current literature is that there are numerous studies describing the occurrence of innate priming with the resultant outcome being either enhanced (termed innate training) or suppressed (termed tolerance) responses to secondary stimulants. The references associated with these particular areas are outlined in Table 1 (Ref. [20,25–29,31,32,34–63]).



**Fig. 1. Priming of macrophages and functional consequence.** Macrophages are activated by a primary stimulant that results in long-lasting epigenetic and metabolic reprogramming, changing the cells phenotypic and functional response to secondary stimulation for a prolonged period; this is termed “innate priming”. If primary stimulation heightens secondary response it is termed ‘innate training’, but if the secondary response is dampened it is termed “innate tolerance”. BCG, Bacillus Calmette-Guérin; LPS lipopolysaccharide. Figure created on BioRender.com (<https://www.biorender.com/>).

**Table 1. Key studies and relevance to topics discussing innate priming.**

|                                                                                               | Study reference                                            |
|-----------------------------------------------------------------------------------------------|------------------------------------------------------------|
| Characterising innate training                                                                | [20], [26], [32], [34], [35], [36], [37], [38], [41]       |
| Characterising innate tolerance                                                               | [20], [26], [31], [38], [39], [40]                         |
| Induction of innate priming with exogenous compounds                                          | [20], [25], [26], [28], [34], [36], [41]                   |
| Induction of innate priming with endogenous compounds                                         | [20], [27], [28], [32], [37], [47], [48], [49]             |
| Effect of priming with a combination of stimulants                                            | [50], [51], [58]                                           |
| Innate priming increasing TAM tumoricidal activity                                            | [44], [45], [46]                                           |
| Epigenetic regulation of innate priming                                                       | [31], [52], [53], [54]                                     |
| Metabolic regulation of innate priming                                                        | [20], [31], [55], [56], [57]                               |
| Clinical implications of innate priming in disease                                            | [20], [25], [28], [29], [41], [49], [59], [60], [61], [62] |
| Cancer Therapies utilising and/or trialling strategies based on or relevant to innate priming | [42], [43], [63]                                           |

TAM, tumor-associated macrophage.

### 3. Innate Priming and Tumor-Associated Macrophages

TAMs are innate cells that have functional plasticity and therefore cannot be considered as strictly pro- or anti-tumorigenic. Depending on the immunological context, TAMs can support anti-tumor immune responses through neo-antigen presentation to the adaptive immune system, tumoricidal reactive oxygen species generation, pro-inflammatory cytokine secretion and induction of cytotoxicity and apoptosis of transformed cells [14–16]. Alternatively, they can promote tumor progression through inducing angiogenesis, cell invasion and immunosuppression [11].

In the literature, TAMs are usually reported to have tumor promoting roles [11,12,64]. However, exceptions can be found and TAMs have been reported to exhibit a more protective role in melanoma and colorectal cancer [15,65]. While previous research has clearly demonstrated the contrasting roles TAM can play in the TME, further work is required to fully understand the stimuli that drive the metabolic and epigenetic development of TAMs in a manner that results in either a tumor promoting or suppressing phenotype.

Macrophages have been well characterized as receptive to innate priming and are the main focus of the literature

exploring this biological mechanism. In most studies investigating innate priming in macrophages, the priming stimulants have been exogenous, including the BCG vaccine and  $\beta$ -glucan [20,41]. Priming macrophages with these exogenous stimulants enhance subsequent production of both pro- and anti-inflammatory cytokines including tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), interleukin 6 (IL6), IL1R $\alpha$  and IL10, and induces the Warburg effect. Although BCG was also reported to upregulate the subsequent production of reactive oxygen species (ROS) in response to zymosan stimulation,  $\beta$ -glucan has the opposite effect [20]. In line with *ex-vivo* work, monocytes isolated from volunteers administered with the BCG vaccine were found to have a lasting profile of upregulated cytokine production including IL1- $\beta$ , TNF and IFN $\gamma$ , and increased expression of activation markers such as cluster of differentiation 11b (CD11b), CD14, and TLR. This heightened state remained for upwards of 3 months after BCG vaccine administration and was demonstrated to provide protection against disseminated candidiasis within severe combined immunodeficiency (SCID) murine models that were B cell and T cell immunodeficient [41]. The effectiveness of the BCG vaccine as an adjuvant therapy for superficial bladder cancer and inoperable stage III in-transit melanoma provides some indirect evidence of TAMs modification by priming, as studies have demonstrated that vaccination promotes proinflammatory TAM phenotypes, with increased tumoricidal activity. [20,42,43,66–68]. Interestingly, studies in the 1980s and 1990s provided evidence that primed TAMs can display enhanced tumor cytotoxic capability, but research in this area then waned [44–46]. Since 2011, research into innate priming has resurfaced and has begun exploring the consequences of innate priming in different diseases [28,69]. Together, these studies provide rationale to explore whether exogenous stimulants can be used as possible adjuvants to cancer treatment, including immunotherapy, as priming monocytes prior to differentiation towards TAMs may influence shifts towards pro- or anti-tumor phenotypes.

Although the literature has predominantly focused on exogenous stimuli in the context of innate priming, recent studies have also pointed to the role of endogenous stimuli. oxLDL has been the most studied endogenous stimulant to induce macrophage priming, but this has now been expanded to include endogenous compounds such as aldosterone, uric acid and butyrate [20,27,47,48]. In monocytes, *ex-vivo* studies have shown that endogenous compounds including oxLDL induce priming effects similar to those induced by exogenous stimulants [20,48]. oxLDL primed macrophages exhibit increased ROS and cytokine production, glycolytic metabolic modification, and enhanced presentation of scavenger receptors including CD36 [20,32]. Moreover, oxLDL stimulation enhances expression of cytokines such as IL-8, TNF- $\alpha$ , IL-6, matrix metalloproteinase 2 (MMP2), monocyte chemoattractant protein-1 (MCP1), and IL18, and increases the rate of foam cell for-

mation [32]. *In-vivo*, feeding Low Density Lipoprotein Receptor (LDLR)<sup>-/-</sup> mice with a high-fat western diet induces innate priming, resulting in bone marrow derived cells exhibiting enhanced TNF and chemokine (C-X-C motif) ligand 1 (CXCL1), and decreased IL6 secretion upon restimulation with TLR ligands. Moreover, these bone marrow derived cells are skewed towards monocytic lineage with upregulation of genes involved in cell proliferation. Circulating myeloid cells also show increased activation status after stimulation with LPS, as evidence by upregulation of CD86 and increased expression of type I interferon genes including *CCL5*, *IRF1*, *IFI203*, *GBP4* and *CXCL10* [37]. With the identification of endogenous compounds capable of inducing innate cell priming, the extent to which innate priming can impact underlying health and disease is a question that needs to be further addressed. Multiple connections have been made between obesity and cancer risk and overall survival [70]. A recent meta-analysis (n = 6,320,365) reported that obesity was associated with improved overall survival in males receiving immunotherapy for metastatic melanoma (hazard ratio (HR), 0.74; 95% CI, 0.57–0.96), lung cancer (HR, 0.86; 95% CI, 0.76–0.98) and renal cell carcinoma (HR, 0.74; 95% CI, 0.53–0.89), compared to patients who were not obese with the same cancer type [71]. Obesity is associated with elevated levels of some plasma metabolites and a number of these, including oxLDL and butyrate, have been shown to prime macrophages [20,27]. Interestingly, a recent murine study of diet induced obesity revealed that leptin, palmitate, insulin, TNF and IFN $\gamma$  are capable of inducing programmed cell death protein 1 (PD-1) expression on TAMs. The PD-1 then provided negative feedback to TAMs suppressing their glycolysis and T cell stimulatory capacity. However, these effects were reversed by immunotherapy induced-PD-1 blockade, resulting in improved anti-tumor immunity [49]. This impact of high-fat western diets on innate priming observed *in-vivo* raises the possibility that obesity-related endogenous compounds may prime innate cells, including monocytes, and this may explain the improved immunotherapy response reported in obese individuals [20,27,37,49,71].

The effects of combinations of stimuli is another crucial factor to consider when discussing innate priming, as *in-vivo* models do not expose the immune system to singular stimulants. Research has shown that although butyrate and oxLDL can individually prime innate cells, when used in combination these priming effects are reduced [20,27,50]. This relationship was also observed when training macrophages with metformin and oxLDL, as metformin inhibits glycolytic pathways [51]. The combined impact of stimulants on the immune system is often overlooked and should be an ongoing consideration within studies investigating innate priming. This may be particularly relevant for TAMs due to the complexity of the TME and systemic stresses individuals receiving treatment for cancer ex-



perience [4,6–8,72,73]. Upregulation of endogenous compounds induced by stress, the presence of neoantigens and systemic inflammation that an individual experience from treatment, and the administration of other medications may all influence priming and how TAMs respond within the TME [73–75]. This demonstrates the necessity of *in-vivo* and clinical work to explore the impact of innate priming on TAMs and therapeutic outcome.

Studies published to date have shown clear evidence that TAMs can undergo innate priming in response to both exogenous and endogenous stimuli and this may result in enhancement or suppression of anti-tumor and/or therapeutic responses. The references associated with these particular areas are outlined in Table 1.

#### 4. Innate Priming Mechanisms

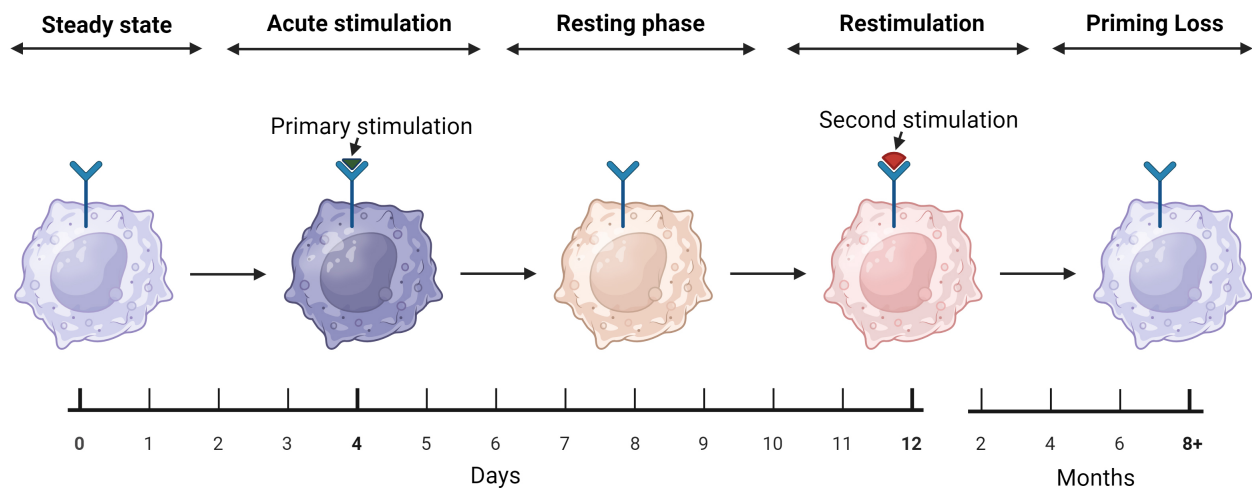
Innate priming can be grouped into four phases—steady state, acute stimulation, resting phase and restimulation (Fig. 2) [31]. Steady state describes unstimulated cells that possess low activity. During acute stimulation, exogenous/endogenous stimulants bind to cytoplasmic and membrane-bound PRRs, which are activated through exogenous pathogen-associated molecular patterns and/or endogenous damage-derived molecular patterns [31,76]. These binding events result in alterations to the epigenome, allowing for transcription of effector genes that orchestrate cellular response to the stimulants [76]. With the cessation of stimulation, the cell enters a resting phase, as the effector factors are no longer required. However, the promoter regions of metabolic and effector genes remain open or become silenced [31]. With rechallenge, the primed (trained or tolerized) cell enters the restimulation phase, which is facilitated by altered metabolic activity and enhanced or suppressed effector response, respectively, depending on the previously modified epigenetic landscape [26,31]. A final phase remains, which is the loss of innate cell priming. Innate priming is not permanent, with heightened responses only lasting for a suggested period of 3–12 months (Fig. 2) [21]. This timeframe is based on epidemiological evidence and needs to be further verified [77]. Furthermore, the way(s) in which innate priming changes during this period also remain to be characterized.

Epigenetic modification is the first pillar of innate priming that can affect TAMs phenotype. The epigenetic landscape drives innate priming, with changes in chromatin architecture facilitating the promotion and silencing of effector genes. In the context of innate priming, epigenetic modification exists at several levels, including methylation, acetylation, enhancer modulation and transcription of non-coding RNAs. For example, following pattern recognition receptors (PRR) activation transcription factors nuclear factor of activated T cells (NFAT), signal transducer and activator of transcription 1 (STAT1) and/or nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) induce the expression of immune-gene prim-

ing long non-coding RNA (IPLs). These IPLs guide histone methyltransferases to the promoter regions of genes including IFN $\gamma$ , making the promoter accessible to RNA polymerases for enhanced transcription. Additionally, through histone modifications, e.g., histone H3 lysine 4 trimethylation (H3K4me3), stochastic noise can be further reduced by maintaining RNA polymerases on promoters in a paused state, increasing expression response [31]. Histone acetylation is another pivotal player in opening chromatin structure, with over 5000 enhancers identified to obtain H3 lysine 27 acetylation (H3K27Ac) after LPS stimulation in bone marrow derived macrophages, a global acetylation event also observed with BCG and  $\beta$ -glucan exposure [52]. Beyond histone modification, DNA methylation has been proposed to regulate innate priming, with research reporting altered methylation profiles in peripheral blood-derived mononuclear cells (PBMCs) up to 8 months after volunteers were administered with the BCG vaccine [53]. However, limited research exists detailing DNA methylations direct role in innate priming [54]. These epigenetic pathways allow for innate priming, as changes in chromatin architecture, DNA structure and upregulation of epigenetic modifiers such as IPLs facilitate the training and/or silencing of genes that direct an immune cell's response to stimuli (Fig. 3) [31].

Within the TME, macrophages are differentiated into TAMs that are typically anti-inflammatory through epigenetic modification [78]. Previous evidence has shown that tumor-derived exosomes can induce TAM expression of jumonji domain-containing protein 3 (JMJD3), a H3K27 demethylase that is associated with anti-inflammatory polarization [79]. However, JMJD3 has also been associated with inducing macrophage expression of pro-inflammatory cytokines in response to inflammatory mediators including LPS [80,81]. This suggests that JMJD3 may be activated prior to reaching the TME by alternative stimuli, including exogenous and endogenous compounds or neoantigens, inducing a pro-inflammatory phenotype as opposed to an immunosuppressive phenotype. However, innate priming may also induce tumor-promoting epigenetic modification within TAMs. With LPS stimulation, H3K4 histone methyltransferase SETD4 has been shown to induce expression of pro-inflammatory cytokine TNF $\alpha$  and IL6 [82]. LPS has also reportedly been shown to induce H3K4 methyltransferase lysine-specific methyltransferase 2H (Ash1L), that suppresses IL-6 and TNF $\alpha$  production through inducing *Tnfrsf3* expression, suggesting inflammatory regulatory pathways within macrophages [83]. These regulatory pathways may promote pro-tumor TAM phenotypes, depending on whether the cells were stimulated and potentially primed prior to arrival at the TME.

Tumors have been reported to manipulate histone acetylation within TAMs, with a recent study reporting that colorectal cancer cells through the secretion of exosomes can downregulate histone deacetylase (HDAC)11 activity,



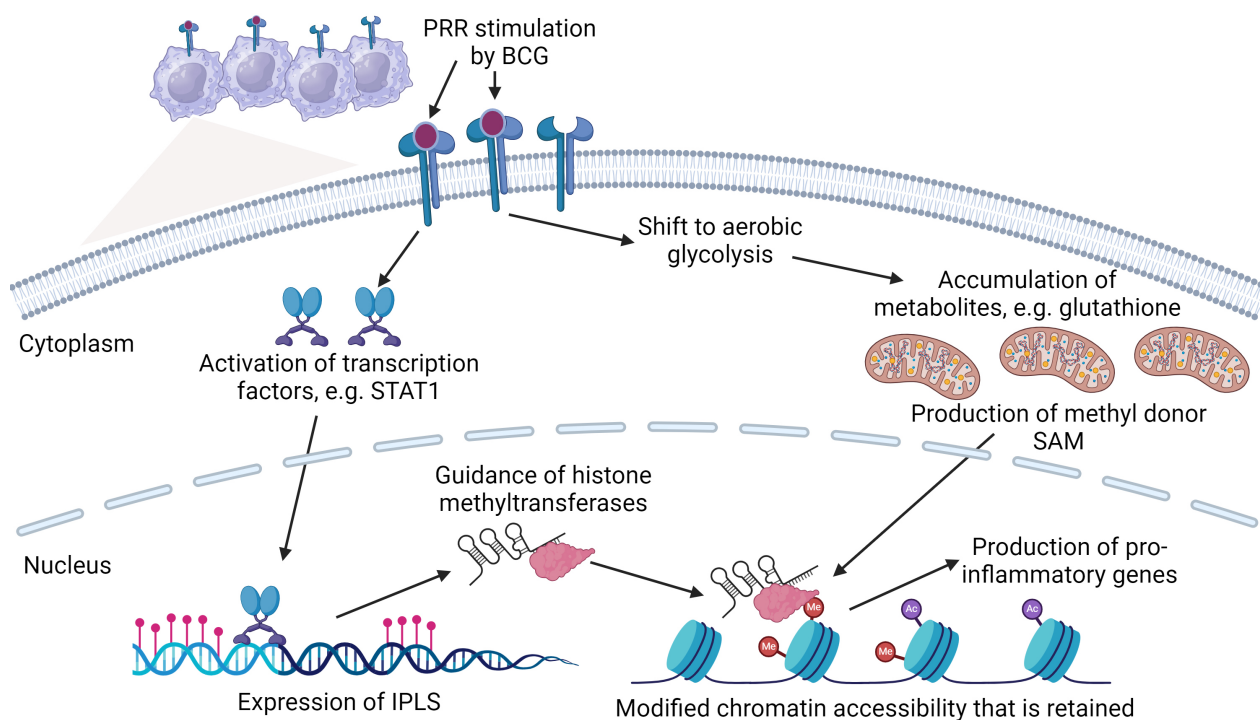
**Fig. 2. The five stages of macrophage priming.** Steady state describes unstimulated macrophages and remains until the innate cell is activated by a stimulant, inducing an immune response that is termed acute stimulation. The primary stimulation induces epigenetic and metabolic reprogramming, changing macrophages phenotype into a primed state. Acute stimulation is followed by the resting phase, where the cell retains its reprogramming but is no longer engaged in an active immune response. The resting phase continues until the macrophage encounters a secondary stimulant, inducing an enhanced (trained) or dampened (tolerized) immune response. The final stage of innate priming is priming loss, as the reprogramming that enhances or dampens secondary response is lost over 3–12 months, returning macrophages back to steady state. Figure created on BioRender.com (<https://www.biorender.com/>).

promoting anti-inflammatory phenotypes [84]. Prior stimulation may also influence anti-inflammatory TAM phenotypes through acetylation mechanisms. Previously, interaction through decoy receptor 3 (DcR3) has been reported to induce histone deacetylation of the class II major histocompatibility complex transactivator (CIITA) promoter, reducing expression of major histocompatibility II (MHC-II) [85]. However, acetylation manipulation can contribute in generating pro-inflammatory phenotypes. Through tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) stimulation, macrophages were reported to produce pro-inflammatory cytokines as HDAC1 was suppressing negative regulation by miR-146a [86]. Moreover, with TL4 activation, increased activity of the tricarboxylic acid (TCA) cycle and glycolysis results in accumulation of acetyl-coenzyme A, facilitating polarization towards pro-inflammatory phenotypes through histone acetylation [87]. Depending on mode of mechanism manipulating histone acetylation profiles within TAMs, this may promote pro- or anti-tumor phenotypes as the cells arrives within the TME, but further evidence is required before conclusive statements can be made.

Previous evidence has shown that tumors can manipulate DNA methylation within TAMs through activating ten-eleven translocation 2 by inducing the interleukin-1 receptor/myeloid differentiation primary response protein 88 (IL1R/MyD88) pathway, resulting in the suppression of pro-inflammatory cytokine production [88]. However, stimulation of macrophages prior to arrival at the TME may also manipulate TAM methylation profiles. Macrophages stimulated with LPS have been reported to upregulate

pro-inflammatory cytokine production through hypermethylation of suppressor of cytokine signaling 1 (SOCS1) from DNA-methyltransferase I (DNMT1) [89]. In agreement, a study characterising the methylation pathway of macrophages exposed to LPS and IFN $\gamma$  reported that hypermethylation of krüppel-like factor 4 (KLF4) allowed for pro-inflammatory polarization [90]. Interestingly, research has shown that estradiol induced DNMT1 activity can downregulate P53 resulting in macrophage polarisation towards anti-inflammatory phenotypes, suggesting downstream consequence of DNMT1 activation and how this may shape TAM response is subjective by the stimulant [91]. These studies provide insight into how TAMs epigenetics may be shaped by prior stimulation and how this may influence the cells phenotype.

Immunometabolism is the second pillar of innate priming and intertwines with the epigenetic landscape. Typically, primed innate cells utilise aerobic glycolysis instead of oxidative phosphorylation (Warburg effect), with maintenance of this metabolic pathway by epigenetic mechanisms fulfilling cellular energetic requirements [55]. However, immunometabolism extends beyond energy fulfilment, with metabolic products having active roles in regulating epigenetic enzymes (Fig. 3) [31]. For example, the accumulation of  $\alpha$ -ketoglutarate is required for the activation of the mTOR-Akt-HIF1 pathway, which is crucial for the initiation of monocyte priming [31,55]. In addition, glutathione accumulation drives methyl donor S-adenosyl methionine production that is necessary in histone methylation, promoting epigenetic modifications that are the basis of innate priming [56,92]. Immunometabolism within



**Fig. 3. The relationship between primed immunity, epigenetics and metabolism.** Bacillus Calmette-Guérin (BCG) interacts with pattern recognition receptors (PRR) to induce transcription factor(s) such as signal transduced and activator of transcription 1 (STAT1) that result in the activation of Immune-gene Priming Long non-coding RNAs (IPLs). IPLs direct epigenetic modifications by guiding proteins including histone methyltransferases to downstream effector genes including pro-inflammatory cytokines. Simultaneously, macrophages shift metabolism to aerobic glycolysis, resulting in the accumulation of metabolites including glutathione. Glutathione promotes the production of s-adenosyl methionine (SAM) that donates methyl groups during epigenetic modification, promoting chromatin restructuring to induce gene expression. Consequently, downstream epigenetic and metabolic pathways are activated and retained, priming macrophages for future encounters with non-specific stimulants. Figure adapted from Fanucchi *et al.* [31], using BioRender.com (<https://www.biorender.com/>).

innate priming will also regulate inflammatory response. The accumulation of lactate from pyruvate within primed cells can in some cases upregulate and downregulate anti-inflammatory and pro-inflammatory gene expression, respectively, through the induction of histone lactatyltation, limiting prolonged inflammation [31,57,93].

Immunometabolism has a pivotal role in modulating the function of TAMs, as the cell responds to different cues within the TME [94]. Acidic pH, lactic acid and IL4 within the TME have all been found to upregulate *Arg1* expression within TAMs, promoting L-arginine degradation and resulting in reduced cytotoxic T cell capacity and promotion of tumor growth [94,95]. Compared to macrophages, TAMs have been shown to possess significantly higher volumes of intracellular lipids [96]. This is not surprising, as pro-tumor TAM phenotypes favor fatty acid oxidation, which leads to induction of genes including *Arg1* within TAMs and reduces T cell proliferation through the degradation of arginine [94,96,97]. In addition, many TMEs possess high concentrations of arachidonic acid, that produces metabolic by-products including prostaglandin E<sub>2</sub>, which has been shown to generate an immunosuppressive pheno-

type within TAMs through the upregulation of programmed death ligand 1 (PD-L1), thus reducing T cell activation [58,98]. Innate priming may influence TAM metabolism within the TME. Treg cells help orchestrate an immunosuppressive environment by suppressing the production of IFN $\gamma$  by T cells and thus enabling the activation of sterol regulatory element-binding transcription factor 1 (SREBP1) in macrophages, which acts to maintain anti-inflammatory TAM metabolomics [99]. Hypothetically, if macrophages were primed by a primary stimulant prior to arrival at the TME and SREBP1 was suppressed by IFN $\gamma$  exposure, this may help mitigate the production of pro-tumor TAM phenotypes. At the same time, it has been reported that oxLDL priming can upregulate surface proteins including CD36, a lipid scavenging receptor, possibly promoting the generation of pro-tumor TAMs [32,96]. A recent *in-vivo* study reported that high-fat diet mice with tumor burden exhibited increased TAM PD-1<sup>+</sup> phenotypes that are dependent on glycolysis and mammalian target of rapamycin complex 1 (TORC1) pathways, resulting in improved anti-tumor immunity with immunotherapy [49]. Glycolytic metabolism is a hallmark of innate priming and with literature demon-

strating that high fat diets can prime macrophages within a murine model, this supports the hypothesis that endogenous compounds are capable of inducing TAM priming that supports enhanced immunotherapy response [31,37,49]. These studies demonstrate how metabolites can potentially manipulate TAMs phenotype to exert greater anti-tumor function, but further evidence is warranted.

Priming of macrophages prior to arrival at the TME may also contribute towards the generation of anti-inflammatory phenotypes. Butyrate has been reported to suppress innate training, inhibiting HDACs from promoting pro-inflammatory cytokine production [50,100]. Itaconate, a derivative from the TCA cycle, has been reported to inhibit succinate dehydrogenase, inducing anti-inflammatory response [101]. However, an itaconate catabolism intermediate, itaconyl-CoA was shown to clear vitamin B12, limiting conversion of S-adenosylhomocysteine (SAH) to methionine, a metabolite reported to reduce production of pro-inflammatory cytokines when allowed to accumulate [31,59]. Moreover, TLR4 activation can induce NAD<sup>+</sup> dependent SIRT1 binding to the promoters of IL1 $\beta$  and TNF $\alpha$  and with RelB recruitment, the resultant complex induces tolerance [60]. LPS stimulation can also inhibit glycolysis, and in turn inflammatory response, by increasing the activity of SIRT6 through upregulating nicotinamide phosphoribosyltransferase expression, augmenting production of NAD<sup>+</sup> [102]. Additionally, prolonged exposure to lactate in environments including adipose tissue can downregulate pro-inflammatory gene expression [57]. These studies provide possible mechanisms on how metabolites may influence TAMs polarisation towards pro- and anti-tumor phenotypes, but direct evidence of metabolites impact on TAMs outside the TME and how it alters functionality to the tumor remains to be revealed. The importance of immunometabolism to both TAMs energetic needs and epigenetic mechanisms may provide opportunities for manipulation of TAMs phenotype and function via priming that may lead to therapeutic benefit. However, immunometabolism may also exacerbate anti-inflammatory phenotypes, warranting further investigating into the role of metabolites on TAM phenotype.

Epigenetic and metabolic reprogramming are the cornerstones of innate priming, enabling innate immune cells to maintain a trained or tolerized response to secondary stimuli. The mechanisms that govern these modifications may be induced prior to reaching the TME, influencing whether TAMs exhibit pro- or anti-tumor phenotypes in response to the tumor challenge. Summary of papers detailing innate priming epigenetic and metabolic reprogramming can be found in Table 1.

## 5. Clinical Implications of Innate Priming

The application of innate priming could be of therapeutic benefit in a range of diseases. Support for the potential of this approach is provided by a study that utilised

transcriptional signatures of monocytes that had undergone *in-vitro* priming to identify primed cells in different diseases. The transcriptional signature of primed monocytes was found within individuals with immune-mediated diseases such as COVID-19, ulcerative colitis and sepsis. This suggests that these primed cells may have a role in these diseases [28].

In the area of immunotherapy, the PD-1/PD-L1 axis has been the focus of considerable clinical interest as blockade of this pathway can overcome suppression of anti-tumor responses and results in long term survival of a proportion of patients [61,103]. However, as progression-free survival is only 20–30% after 5 years, it is clear that enhancement of this approach would be required to extend its life extending benefits to a wider group of patients [103]. Interestingly, transcription signatures associated with myeloid cell activation *in-vitro* were found at higher levels in melanoma patients who responded well to anti-PD1 immunotherapy and exhibited longer overall survival [104]. Additionally, in a urethane-induced inflammation-driven lung adenocarcinoma murine model, TNF $\alpha$  was shown to induce PD-L1 presentation on monocyte-derived macrophages [105]. This suggests that innate priming may contribute towards PDL-1 expression, as upregulation of pro-inflammatory cytokines, including TNF $\alpha$ , are commonly associated with innate training [20]. A number of studies have reported that tumor cells and/or associated exosomes can modulate TAM expression of PD-L1 [106,107]. Furthermore, increased TAM infiltration has been associated with both higher levels of tumor PD-L1 and poor survival [108]. Although, enhanced PD-L1 presentation on TAMs may contribute to tumorigenesis, conversely this expression may also make the patients more responsive to PD-L1 directed immunotherapy [105,109]. Additionally, PD-L1 in some context may promote immune stimulation, as PD-L1 dimerizes with CD80, preventing interaction with cytotoxic T-lymphocyte associated protein 4 (CTLA4) and PD-1 and preserving co-stimulation with CD28 [110]. PD-L1<sup>+</sup> TAMs in breast cancer have been shown to correlate with improved clinical response, as the TAMs induce greater T cell activation and tumor cytotoxicity [111]. In mice, obesity-related compounds including leptin are capable of inducing expression of PD-1 in TAMs, and this TAM sub-type showed increased response to PD-1 directed immunotherapy. While this study did not directly address innate priming, these results indicate that macrophages may be primed by obesity-related compounds prior to arrival at the tumor site, influencing TAM phenotype and response to immunotherapy [49]. These studies provide rationale for further investigation into how innate priming may impact cancer progression, and whether this form of priming may positively, or negatively, influence treatment regimens including immunotherapy.

The relationship between innate priming and cancer therapy may be bidirectional. Prior research has demon-



strated that the release of immunogenic agents from apoptotic tumor cells exposed to oncolytic adenoviruses were capable of polarizing TAMs towards pro-inflammatory phenotypes, promoting extended survival of mice with peritoneal metastasis when receiving anti-PD1 therapy [112]. Release of immunogenic agents during immunotherapy may prime innate cells resulting in the generation of anti-tumor phenotypes that enhance favorable therapy outcomes [37]. Influencing TAMs phenotype heavily shapes the immune landscape of tumors, particularly as TAMs have been identified to be one of the most abundant immune cells within the TME and have been correlated with therapy suppression [113,114]. Through promoting TAM anti-tumor phenotypes with innate priming, this would induce an active tumor immune response, as TAMs are generating a pro-inflammatory environment, recruiting and activating T cells, and engaging in tumoricidal activity [115]. However, it is important to understand how innate priming exacerbates inflammation, as this may also promote development of immune-related adverse events in different circumstances [116,117]. Taken together, these bodies of work suggest that manipulation of innate priming could be of clinical significance to immunotherapy and should become an active area of research.

BCG is already widely used in the oncology clinic as a standard adjuvant therapy for superficial bladder cancer, and for intralesional treatment of inoperable stage III in-transit melanoma [42,43]. Patients with carcinoma *in-situ* and high-risk papillary bladder tumors were reported to have experienced an initial complete response rate of 70–75% and 55–65%, respectively, after administration of the BCG vaccine. However, 40% of individuals relapsed during treatment. Examination of the bladder wall revealed that the vaccine induced gigantocellular and epithelioid granuloma formation, which comprised of fibroblasts, lymphocytes, neutrophils, dendritic cells and macrophages. Moreover, the vaccine upregulated chemokine and cytokine production, induced a Th1 favored response and increased infiltration of inflammatory and cytotoxic cells into the bladder, including macrophages [43]. Additional *in-vivo* and *in-vitro* studies have also shown that BCG vaccination is associated with promoting recruitment of CD8<sup>+</sup> T cells, increased production of cytokines in macrophages including TNF, IL6, IL1 $\beta$  and IFN $\gamma$ , upregulated ROS production, a shift towards glycolysis and overall modulation of inflammatory response [20,29,41,62]. Direct evidence has shown that BCG can prime TAMs to exhibit more favorable anti-tumor phenotypes with enhanced cytotoxic potential, and use of the BCG vaccine as an adjuvant therapy for bladder cancer has demonstrated the therapeutic potential of immune stimulants, including vaccines, that are not derived from the tumor [43–46]. Additionally, a clinical trial reported that administration with imprime  $\beta(1,6)$ -[poly-(1,3)-D-glucopyranosyl]-poly- $\beta(1,3)$ -D-glucopyranose (PGG), a soluble form of  $\beta$ -glucan, and

cetuximab showed modest clinical response in colorectal cancer [63]. While this study did not directly report on response mechanism,  $\beta$ -glucan has been extensively studied and shown to induce innate priming, providing a possible mode of action by imprime PGG [20,63]. This trial provides further insight into the clinical opportunity of harnessing priming-capable immunogenic compounds that are not related to cancer [63]. Together, this provides reason to continue research in this area, particularly as evidence is mounting for the priming capacity of exogenous stimulants such as  $\beta$ -glucan and BCG vaccine for macrophages, providing further insight into the therapeutic potential of innate priming [20,41].

Endogenous compounds may also influence immunotherapy outcome in patient cohorts, with a recent meta-analysis reporting that obese patients with advanced lung cancer, melanoma and renal cell carcinoma had better survival outcomes compared to non-obese patients [71]. In agreement, a recent retrospective analysis of six independent patient cohorts consisting of 2046 patients with metastatic melanoma assigned to various treatment strategies reported obesity was associated with improved survival outcomes in patients with metastatic melanoma receiving anti-PD1 immunotherapy, and that this association was particularly strong in males [118]. Compounds that increase with obesity including acetate, methylglyoxal, estradiol, butyrate and leptin have been found to modulate innate immune cell function, including enhancing pro-inflammatory cytokine production and cytotoxic capacity of macrophages [119–131]. Furthermore, drugs commonly used for co-morbidities associated with obesity, such as metformin, have been reported to downregulate CD206, upregulate the presentation of MHC-II, and increase production of pro-inflammatory cytokines TNF $\alpha$  and IL12 within TAMs [132]. Endogenous compounds including oxLDL have also been shown to induce macrophage priming *in-vitro*, while *in-vivo* studies have shown that high-fat diets can induce myeloid priming [20,37]. These studies provide insight into a recent murine study, which reported that obesity-related compounds induced PD1 presentation on TAMs and this correlated with enhanced glycolysis and T cell activation upon immunotherapy administration. Innate priming provides a potential mechanism that may contribute towards the enhancement in both tumor growth and subsequent immunotherapy response observed in mice fed a high-fat western diet [49].

There have been numerous studies whose results suggest potential links between innate priming, the PD1/PD-L1 axis and immunotherapy responses as summarised in Table 1. Whether the heightened immune response and improved response to immunotherapy described by a number of these *in-vivo* and retrospective patient studies reflect TAM priming by endogenous or exogenous compounds prior to arrival at the tumor site remains to be fully revealed [49,71,118]. However, together these studies provide the

rationale for further investigation into the role of priming and its influence on TAM phenotype and function, and the consequent effects on tumor immune response.

## 6. Limitations of Current Methodologies

While many studies have provided extensive insight into the capacity of innate priming and discussed how this immunological trait may be relevant to disease, common limitations are found within the literature. Many studies have a narrowed characterisation of innate priming through focusing on cytokine output and functional assays including reactive oxygen species generation. While informative of whether primary stimulants elicit alterations in response, detailed information on phenotypical changes remain elusive particularly as the pleiotropic nature of many cytokines are becoming apparent [133]. Moreover, while reactive oxygen species assays do provide information on functional changes, it does not provide information at the cellular level. This is limiting interpretation of the literature characterising innate priming as all ROS molecules are not the same and provide different effects and are involved in different molecular pathways [134]. Additionally, many of the models used within the literature are simplistic, comprising of *in-vitro* and *ex-vivo* culture within a 2D model consisting of single immune cell populations. This model does provide a repeatable environment to trial potential priming compounds, reducing potential result confounders. However, it does not replicate environmental complexity found within *in-vivo* models, as innate cells are exposed to numerous stimulants and communicate with one another to orchestrate an immune response [135]. This is particularly highlighted by studies that reported on attenuated innate priming when cells were exposed to several primary stimulants, representing more physiologically relevant exposure events [50,51]. Furthermore, *in-vivo* models have utilised broad acting primary stimulants and focused on correlating effects with priming events. While providing information on potential consequence of priming in a complex environment, correlating outcomes does not direct to specific biological mechanisms. Research into innate priming is still in its infancy, but the necessary framework to investigate priming and its relevance in disease has been established. However, methodology limitations need to be addressed to further characterise the implications of innate priming.

## 7. Conclusions

In conclusion, exogenous and endogenous compounds have been shown to prime macrophages, resulting in epigenetic modification and metabolic changes that alter phenotype and function, and determine macrophage response to secondary stimulation. Investigations have demonstrated different primary inducers of innate priming and the implications of innate priming in various diseases, many of which are applicable to the TME and may offer potential therapeutic opportunity. In particular, administration of the

BCG vaccine as therapy for bladder cancer and imprime PGG in conjunction with cetuximab to colorectal cancer patients has demonstrated the potential therapeutic benefit of priming-capable immunogenic agents. Together, these studies provide evidence that priming the innate immune system could be a potential strategy to improve the efficacy of cancer therapy [43,63]. To explore the potential therapeutic value of innate priming, future work should further focus on characterizing primed macrophages response to secondary tumor stimulants at an *in-vivo* and *ex-vivo* level. With greater foundational work, this would provide justification to combine innate priming stimulants with existing immunotherapy regimes. Additionally, exploring patients' immune profiles for signatures of innate priming and correlating with treatment outcome would provide confidence that priming could be an effective therapeutic strategy. In summary, innate priming adds to the complexity of the mechanisms that determine whether TAMs exhibit a pro- or anti-tumor phenotype and extends influence on TAMs beyond the immediate TME to potentially include systemic endogenous and/or exogenous factors. Investigating the influence of innate priming on TAM phenotype should become an active area of study, as it offers the potential to discover mechanisms to therapeutically manipulate TAM phenotype to improve current immunotherapy outcomes and create novel treatment regimes against cancer.

## Abbreviations

Ash1L, Lysine-specific Methyltransferase 2H; BCG, Bacillus Calmette-Guérin; CD11b, Cluster of Differentiation 11b; CIITA, Class II Major Histocompatibility Complex Transactivator; CoA, Coenzyme A; CXCL1, Chemokine (C-X-C motif) Ligand 1; DNMT1, DNA-Methyltransferase I; H3K27Ac, H3 Lysine 27 Acetylation; H3K4me3, Histone H3 Lysine 4 Trimethylation; HDACII, Histone Deacetylase II; HR, Hazard Ratio; IFN $\gamma$ , Interferon- $\gamma$ ; IL6, Interleukin 6; IL-1R/MyD88, Interleukin-1 Receptor/Myeloid Differentiation Primary Response Protein 88; IPL, Immune-gene Priming Long non-coding RNA; JMJD3, Jumonji Domain-Containing Protein 3; KLF4, Krüppel-Like Factor 4; LDLR, Low Density Lipoprotein Receptor; LPS, Lipopolysaccharide; MCP1, Monocyte Chemoattractant Protein-1; MHC-II, Major Histocompatibility II; MMP2, Matrix Metalloproteinase 2; mTORC1, Mammalian Target of Rapamycin Complex 1; mTOR-Akt-HIF1, Mammalian Target of Rapamycin – Protein Kinase B – Hypoxia-Inducible Factor 1; NFAT, Nuclear Factor of Activated T Cells; NF- $\kappa$ B, Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells; oxLDL, oxidized low-density lipoprotein; P53, Tumor Protein P53; PBMC, Peripheral Blood-Derived Mononuclear Cells; PD-1, programmed cell death protein 1; PGG,  $\beta$ (1,6)-[poly-(1,3)-D-glucopyranosyl]-poly- $\beta$ (1,3)-D-glucopyranose; PRR, Pattern Recognition Receptor; ROS, Reactive Oxygen Species; SCID, Severe

Combined Immunodeficiency; SETD3, SET Domain Containing 3, Actin N3 (Tan)-Histidine Methyltransferase; STAT1, Signal Transducer and Activator of Transcription 1; SOCS, Suppressor of Cytokine Signaling 1; SREBP1, Sterol Regulatory Element-Binding Transcription Factor 1; TAM, Tumor-Associated Macrophage; TME, Tumor Microenvironment; TLR4, Toll-Like Receptor 4; TNF $\alpha$ , Tumor Necrosis Factor- $\alpha$ .

## Author Contributions

BT, BH and MC involved in conceptualization, literature collection, and figure creation. GW and EP involved in conceptualization and literature collection. BT, BH and MC involved in drafting and reviewing work. GW and EP involved in reviewing work. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

Not applicable.

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## Conflict of Interest

The authors declare no conflict of interest.

## References

- [1] Marshall JS, Warrington R, Watson W, Kim HL. An introduction to immunology and immunopathology. *Allergy, Asthma, and Clinical Immunology: Official Journal of the Canadian Society of Allergy and Clinical Immunology*. 2018; 14: 49.
- [2] Chaplin DD. Overview of the immune response. *The Journal of Allergy and Clinical Immunology*. 2010; 125: S3–S23.
- [3] Iannello A, Thompson TW, Ardolino M, Marcus A, Raulat DH. Immunosurveillance and immunotherapy of tumors by innate immune cells. *Current Opinion in Immunology*. 2016; 38: 52–58.
- [4] Vinay DS, Ryan EP, Pawelec G, Talib WH, Stagg J, Elkord E, *et al.* Immune evasion in cancer: Mechanistic basis and therapeutic strategies. *Seminars in Cancer Biology*. 2015; 35: S185–S198.
- [5] Davis LE, Shalin SC, Tackett AJ. Current state of melanoma diagnosis and treatment. *Cancer Biology & Therapy*. 2019; 20: 1366–1379.
- [6] Chew V, Toh HC, Abastado JP. Immune microenvironment in tumor progression: characteristics and challenges for therapy. *Journal of Oncology*. 2012; 2012: 608406.
- [7] Dagogo-Jack I, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. *Nature Reviews. Clinical Oncology*. 2018; 15: 81–94.
- [8] Tabassum DP, Polyak K. Tumorigenesis: it takes a village. *Nature Reviews. Cancer*. 2015; 15: 473–483.
- [9] Mantovani A, Allavena P, Marchesi F, Garlanda C. Macrophages as tools and targets in cancer therapy. *Nature Reviews. Drug Discovery*. 2022; 21: 799–820.
- [10] Ang AD, Vissers MCM, Burgess ER, Currie MJ, Dachs GU. Gene and Protein Expression Is Altered by Ascorbate Availability in Murine Macrophages Cultured under Tumour-Like Conditions. *Antioxidants (Basel, Switzerland)*. 2021; 10: 430.
- [11] Nielsen SR, Schmid MC. Macrophages as Key Drivers of Cancer Progression and Metastasis. *Mediators of Inflammation*. 2017; 2017: 9624760.
- [12] Loyher PL, Hamon P, Laviron M, Meghraoui-Kheddar A, Goncalves E, Deng Z, *et al.* Macrophages of distinct origins contribute to tumor development in the lung. *The Journal of Experimental Medicine*. 2018; 215: 2536–2553.
- [13] Zhu Y, Herndon JM, Sojka DK, Kim KW, Knolhoff BL, Zuo C, *et al.* Tissue-Resident Macrophages in Pancreatic Ductal Adenocarcinoma Originate from Embryonic Hematopoiesis and Promote Tumor Progression. *Immunity*. 2017; 47: 323–338.e6.
- [14] Wang H, Zhang L, Yang L, Liu C, Zhang Q, Zhang L. Targeting macrophage anti-tumor activity to suppress melanoma progression. *Oncotarget*. 2017; 8: 18486–18496.
- [15] Hussein MR. Tumour-associated macrophages and melanoma tumorigenesis: integrating the complexity. *International Journal of Experimental Pathology*. 2006; 87: 163–176.
- [16] Cendrowicz E, Sas Z, Bremer E, Rygiel TP. The Role of Macrophages in Cancer Development and Therapy. *Cancers*. 2021; 13: 1946.
- [17] Hirahara K, Poholek A, Vahedi G, Laurence A, Kanno Y, Milner JD, *et al.* Mechanisms underlying helper T-cell plasticity: implications for immune-mediated disease. *The Journal of Allergy and Clinical Immunology*. 2013; 131: 1276–1287.
- [18] Lavin Y, Mortha A, Rahman A, Merad M. Regulation of macrophage development and function in peripheral tissues. *Nature Reviews. Immunology*. 2015; 15: 731–744.
- [19] Mulder WJM, Ochando J, Joosten LAB, Fayad ZA, Netea MG. Therapeutic targeting of trained immunity. *Nature Reviews. Drug Discovery*. 2019; 18: 553–566.
- [20] Bekkering S, Blok BA, Joosten LAB, Riksen NP, van Crevel R, Netea MG. In Vitro Experimental Model of Trained Innate Immunity in Human Primary Monocytes. *Clinical and Vaccine Immunology: CVI*. 2016; 23: 926–933.
- [21] Netea MG, Domínguez-Andrés J, Barreiro LB, Chavakis T, Divangahi M, Fuchs E, *et al.* Defining trained immunity and its role in health and disease. *Nature Reviews. Immunology*. 2020; 20: 375–388.
- [22] Zindel J, Kubes P. DAMPs, PAMPs, and LAMPs in Immunity and Sterile Inflammation. *Annual Review of Pathology*. 2020; 15: 493–518.
- [23] Netea MG, van Crevel R. BCG-induced protection: effects on innate immune memory. *Seminars in Immunology*. 2014; 26: 512–517.
- [24] Holm-Delgado MG, Stuart EA, Black RE. Acute lower respiratory infection among Bacille Calmette-Guérin (BCG)-vaccinated children. *Pediatrics*. 2014; 133: e73–e81.
- [25] Debisarun PA, Gössling KL, Bulut O, Kilic G, Zoodsma M, Liu Z, *et al.* Induction of trained immunity by influenza vaccination - impact on COVID-19. *PLoS Pathogens*. 2021; 17: e1009928.
- [26] Quinn SM, Cunningham K, Raverdeau M, Walsh RJ, Curham L, Malara A, *et al.* Anti-inflammatory Trained Immunity Mediated by Helminth Products Attenuates the Induction of T



Cell-Mediated Autoimmune Disease. *Frontiers in Immunology*. 2019; 10: 1109.

- [27] Schulthess J, Pandey S, Capitani M, Rue-Albrecht KC, Arnold I, Franchini F, *et al*. The Short Chain Fatty Acid Butyrate Imprints an Antimicrobial Program in Macrophages. *Immunity*. 2019; 50: 432–445.e7.
- [28] Zhang B, Moorlag SJ, Dominguez-Andres J, Bulut Ö, Kilic G, Liu Z, *et al*. Single-cell RNA sequencing reveals induction of distinct trained-immunity programs in human monocytes. *The Journal of Clinical Investigation*. 2022; 132: e147719.
- [29] Hilligan KL, Namasivayam S, Clancy CS, O'Mard D, Oland SD, Robertson SJ, *et al*. Intravenous administration of BCG protects mice against lethal SARS-CoV-2 challenge. *The Journal of Experimental Medicine*. 2022; 219: e20211862.
- [30] Chi H, Pepper M, Thomas PG. Principles and therapeutic applications of adaptive immunity. *Cell*. 2024; 187: 2052–2078.
- [31] Fanucchi S, Domínguez-Andrés J, Joosten LAB, Netea MG, Mhlanga MM. The Intersection of Epigenetics and Metabolism in Trained Immunity. *Immunity*. 2021; 54: 32–43.
- [32] Bekkering S, Quintin J, Joosten LAB, van der Meer JWM, Netea MG, Riksen NP. Oxidized low-density lipoprotein induces long-term proinflammatory cytokine production and foam cell formation via epigenetic reprogramming of monocytes. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2014; 34: 1731–1738.
- [33] Dore MP, Pes GM. Trained Immunity and Trained Tolerance: The Case of *Helicobacter pylori* Infection. *International Journal of Molecular Sciences*. 2024; 25: 5856.
- [34] Jeljeli M, Riccio LGC, Chouzenoux S, Moresi F, Toullec L, Doridot L, *et al*. Macrophage Immune Memory Controls Endometriosis in Mice and Humans. *Cell Reports*. 2020; 33: 108325.
- [35] Maheshwari A. Innate Immune Memory in Macrophages. *Newborn* (Clarksville, Md.). 2023; 2: 60–79.
- [36] Leopold Wager CM, Hole CR, Campuzano A, Castro-Lopez N, Cai H, Caballero Van Dyke MC, *et al*. IFN- $\gamma$  immune priming of macrophages in vivo induces prolonged STAT1 binding and protection against *Cryptococcus neoformans*. *PLoS Pathogens*. 2018; 14: e1007358.
- [37] Christ A, Günther P, Lauterbach MAR, Duewell P, Biswas D, Pelka K, *et al*. Western Diet Triggers NLRP3-Dependent Innate Immune Reprogramming. *Cell*. 2018; 172: 162–175.e14.
- [38] Saeed S, Quintin J, Kerstens HHD, Rao NA, Aghajani-refah A, Matarese F, *et al*. Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. *Science (New York, N.Y.)*. 2014; 345: 1251086.
- [39] Maitra U, Deng H, Glaros T, Baker B, Capelluto DGS, Li Z, *et al*. Molecular mechanisms responsible for the selective and low-grade induction of proinflammatory mediators in murine macrophages by lipopolysaccharide. *Journal of Immunology (Baltimore, Md.: 1950)*. 2012; 189: 1014–1023.
- [40] Seeley JJ, Ghosh S. Molecular mechanisms of innate memory and tolerance to LPS. *Journal of Leukocyte Biology*. 2017; 101: 107–119.
- [41] Kleinnijenhuis J, Quintin J, Preijers F, Joosten LAB, Ifrim DC, Saeed S, *et al*. Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109: 17537–17542.
- [42] Kremenovic M, Schenk M, Lee DJ. Clinical and molecular insights into BCG immunotherapy for melanoma. *Journal of Internal Medicine*. 2020; 288: 625–640.
- [43] Pettenati C, Ingersoll MA. Mechanisms of BCG immunotherapy and its outlook for bladder cancer. *Nature Reviews. Urology*. 2018; 15: 615–625.
- [44] Seledtsova GV, Seledtsov VI, Kozlov VA. Triggering effects of BCG vaccine on antitumor and interleukin-1 secretory activity of T cell lymphokine-primed macrophages. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*. 1995; 49: 369–374.
- [45] Johnson WJ, Marino PA, Schreiber RD, Adams DO. Sequential activation of murine mononuclear phagocytes for tumor cytotoxicity: differential expression of markers by macrophages in the several stages of development. *Journal of Immunology (Baltimore, Md.: 1950)*. 1983; 131: 1038–1043.
- [46] Leu RW, Herriott MJ. Triggering of interferon gamma-primed macrophages by various known complement activators for non-specific tumor cytotoxicity. *Cellular Immunology*. 1987; 106: 114–121.
- [47] Crişan TO, Cleophas MCP, Novakovic B, Erler K, van de Veer-donk FL, Stunnenberg HG, *et al*. Uric acid priming in human monocytes is driven by the AKT-PRAS40 autophagy pathway. *Proceedings of the National Academy of Sciences of the United States of America*. 2017; 114: 5485–5490.
- [48] van der Heijden CDCC, Keating ST, Groh L, Joosten LAB, Netea MG, Riksen NP. Aldosterone induces trained immunity: the role of fatty acid synthesis. *Cardiovascular Research*. 2020; 116: 317–328.
- [49] Bader JE, Wolf MM, Lupica-Tondo GL, Madden MZ, Reinfield BI, Arner EN, *et al*. Obesity induces PD-1 on macrophages to suppress anti-tumour immunity. *Nature*. 2024; 630: 968–975.
- [50] Cleophas MCP, Ratter JM, Bekkering S, Quintin J, Schraa K, Stroes ES, *et al*. Effects of oral butyrate supplementation on inflammatory potential of circulating peripheral blood mononuclear cells in healthy and obese males. *Scientific Reports*. 2019; 9: 775.
- [51] Riksen NP, Netea MG. Immunometabolic control of trained immunity. *Molecular Aspects of Medicine*. 2021; 77: 100897.
- [52] Sun S, Barreiro LB. The epigenetically-encoded memory of the innate immune system. *Current Opinion in Immunology*. 2020; 65: 7–13.
- [53] Verma D, Parasa VR, Raffetseder J, Martis M, Mehta RB, Netea M, *et al*. Anti-mycobacterial activity correlates with altered DNA methylation pattern in immune cells from BCG-vaccinated subjects. *Scientific Reports*. 2017; 7: 12305.
- [54] Caldwell BA, Li L. Epigenetic regulation of innate immune dynamics during inflammation. *Journal of Leukocyte Biology*. 2024; 115: 589–606.
- [55] Cheng SC, Quintin J, Cramer RA, Shepardson KM, Saeed S, Kumar V, *et al*. mTOR- and HIF-1 $\alpha$ -mediated aerobic glycolysis as metabolic basis for trained immunity. *Science (New York, N.Y.)*. 2014; 345: 1250684.
- [56] Ferreira AV, Koeken VACM, Matzaraki V, Kostidis S, Alarcon-Barrera JC, de Bree LCJ, *et al*. Glutathione Metabolism Contributes to the Induction of Trained Immunity. *Cells*. 2021; 10: 971.
- [57] Ratter JM, Rooijackers HMM, Hooiveld GJ, Hijmans AGM, de Galan BE, Tack CJ, *et al*. *In vitro* and *in vivo* Effects of Lactate on Metabolism and Cytokine Production of Human Primary PBMCs and Monocytes. *Frontiers in Immunology*. 2018; 9: 2564.
- [58] Hao Y, Li D, Xu Y, Ouyang J, Wang Y, Zhang Y, *et al*. Investigation of lipid metabolism dysregulation and the effects on immune microenvironments in pan-cancer using multiple omics data. *BMC Bioinformatics*. 2019; 20: 195.
- [59] Shen H, Campanello GC, Flicker D, Grabarek Z, Hu J, Luo C, *et al*. The Human Knockout Gene CLYBL Connects Itaconate to Vitamin B<sub>12</sub>. *Cell*. 2017; 171: 771–782.e11.
- [60] Liu TF, Yoza BK, El Gazzar M, Vachharajani VT, McCall CE. NAD<sup>+</sup>-dependent SIRT1 deacetylase participates in epigenetic reprogramming during endotoxin tolerance. *The Journal of Biological Chemistry*. 2011; 286: 9856–9864.



- [61] Khoja L, Butler MO, Kang SP, Ebbinghaus S, Joshua AM. Pembrolizumab. *Journal for Immunotherapy of Cancer*. 2015; 3: 36.
- [62] Pasco ST, Anguita J. Lessons from Bacillus Calmette-Guérin: Harnessing Trained Immunity for Vaccine Development. *Cells*. 2020; 9: 2109.
- [63] Segal NH, Gada P, Senzer N, Gargano MA, Patchen ML, Saltz LB. A Phase II Efficacy and Safety, Open-Label, Multicenter Study of Imprime PGG Injection in Combination With Cetuximab in Patients With Stage IV KRAS-Mutant Colorectal Cancer. *Clinical Colorectal Cancer*. 2016; 15: 222–227.
- [64] Riabov V, Gudima A, Wang N, Mickley A, Orekhov A, Kzhyshkowska J. Role of tumor associated macrophages in tumor angiogenesis and lymphangiogenesis. *Frontiers in Physiology*. 2014; 5: 75.
- [65] Larionova I, Tuguzbaeva G, Ponomaryova A, Stakheyeva M, Cherdynseva N, Pavlov V, *et al.* Tumor-Associated Macrophages in Human Breast, Colorectal, Lung, Ovarian and Prostate Cancers. *Frontiers in Oncology*. 2020; 10: 566511.
- [66] Stewart JH, 4th, Levine EA. Role of bacillus Calmette-Guérin in the treatment of advanced melanoma. *Expert Review of Anticancer Therapy*. 2011; 11: 1671–1676.
- [67] Svatek RS, Zhao XR, Morales EE, Jha MK, Tseng TY, Hugen CM, *et al.* Sequential intravesical mitomycin plus Bacillus Calmette-Guérin for non-muscle-invasive urothelial bladder carcinoma: translational and phase I clinical trial. *Clinical Cancer Research: an Official Journal of the American Association for Cancer Research*. 2015; 21: 303–311.
- [68] Liu L, Shi W, Xiao X, Wu X, Hu H, Yuan S, *et al.* BCG immunotherapy inhibits cancer progression by promoting the M1 macrophage differentiation of THP 1 cells via the Rb/E2F1 pathway in cervical carcinoma. *Oncology Reports*. 2021; 46: 245.
- [69] Netea MG, Quintin J, van der Meer JWM. Trained immunity: a memory for innate host defense. *Cell Host & Microbe*. 2011; 9: 355–361.
- [70] Pati S, Irfan W, Jameel A, Ahmed S, Shahid RK. Obesity and Cancer: A Current Overview of Epidemiology, Pathogenesis, Outcomes, and Management. *Cancers*. 2023; 15: 485.
- [71] Petrelli F, Cortellini A, Indini A, Tomasello G, Ghidini M, Nigro O, *et al.* Association of Obesity With Survival Outcomes in Patients With Cancer: A Systematic Review and Meta-analysis. *JAMA Network Open*. 2021; 4: e213520.
- [72] Vignjević Petrinović S, Milošević MS, Marković D, Momčilović S. Interplay between stress and cancer-A focus on inflammation. *Frontiers in Physiology*. 2023; 14: 1119095.
- [73] Greten FR, Grivennikov SI. Inflammation and Cancer: Triggers, Mechanisms, and Consequences. *Immunity*. 2019; 51: 27–41.
- [74] Xie N, Shen G, Gao W, Huang Z, Huang C, Fu L. Neoantigens: promising targets for cancer therapy. *Signal Transduction and Targeted Therapy*. 2023; 8: 9.
- [75] Neophytou CM, Pierides C, Christodoulou MI, Costeas P, Kyriakou TC, Papageorgis P. The Role of Tumor-Associated Myeloid Cells in Modulating Cancer Therapy. *Frontiers in Oncology*. 2020; 10: 899.
- [76] Perkins DJ, Patel MC, Blanco JCG, Vogel SN. Epigenetic Mechanisms Governing Innate Inflammatory Responses. *Journal of Interferon & Cytokine Research: the Official Journal of the International Society for Interferon and Cytokine Research*. 2016; 36: 454–461.
- [77] Nankabirwa V, Tumwine JK, Mugaba PM, Tylleskär T, Sommerfelt H, PROMISE- EBF Study Group. Child survival and BCG vaccination: a community based prospective cohort study in Uganda. *BMC Public Health*. 2015; 15: 175.
- [78] Larionova I, Kazakova E, Patysheva M, Kzhyshkowska J. Transcriptional, Epigenetic and Metabolic Programming of Tumor-Associated Macrophages. *Cancers*. 2020; 12: 1411.
- [79] Ishii M, Wen H, Corsa CAS, Liu T, Coelho AL, Allen RM, *et al.* Epigenetic regulation of the alternatively activated macrophage phenotype. *Blood*. 2009; 114: 3244–3254.
- [80] Zhang X, Liu L, Yuan X, Wei Y, Wei X. JMJD3 in the regulation of human diseases. *Protein & Cell*. 2019; 10: 864–882.
- [81] De Santa F, Narang V, Yap ZH, Tusi BK, Burgold T, Austenaa L, *et al.* Jmjd3 contributes to the control of gene expression in LPS-activated macrophages. *The EMBO Journal*. 2009; 28: 3341–3352.
- [82] Zhong Y, Ye P, Mei Z, Huang S, Huang M, Li Y, *et al.* The novel methyltransferase SETD4 regulates TLR agonist-induced expression of cytokines through methylation of lysine 4 at histone 3 in macrophages. *Molecular Immunology*. 2019; 114: 179–188.
- [83] Xia M, Liu J, Wu X, Liu S, Li G, Han C, *et al.* Histone methyltransferase Ash1l suppresses interleukin-6 production and inflammatory autoimmune diseases by inducing the ubiquitin-editing enzyme A20. *Immunity*. 2013; 39: 470–481.
- [84] Shinohara H, Kuranaga Y, Kumazaki M, Sugito N, Yoshikawa Y, Takai T, *et al.* Regulated Polarization of Tumor-Associated Macrophages by miR-145 via Colorectal Cancer-Derived Extracellular Vesicles. *Journal of Immunology (Baltimore, Md.: 1950)*. 2017; 199: 1505–1515.
- [85] Chang YC, Chen TC, Lee CT, Yang CY, Wang HW, Wang CC, *et al.* Epigenetic control of MHC class II expression in tumor-associated macrophages by decoy receptor 3. *Blood*. 2008; 111: 5054–5063.
- [86] Gao J, Wang D, Liu D, Liu M, Ge Y, Jiang M, *et al.* Tumor necrosis factor-related apoptosis-inducing ligand induces the expression of proinflammatory cytokines in macrophages and re-educates tumor-associated macrophages to an antitumor phenotype. *Molecular Biology of the Cell*. 2015; 26: 3178–3189.
- [87] Lauterbach MA, Hanke JE, Serefidou M, Mangan MSJ, Kolbe CC, Hess T, *et al.* Toll-like Receptor Signaling Rewires Macrophage Metabolism and Promotes Histone Acetylation via ATP-Citrate Lyase. *Immunity*. 2019; 51: 997–1011.e7.
- [88] Niu Y, Chen J, Qiao Y. Epigenetic Modifications in Tumor-Associated Macrophages: A New Perspective for an Old Foe. *Frontiers in Immunology*. 2022; 13: 836223.
- [89] Cheng C, Huang C, Ma TT, Bian EB, He Y, Zhang L, *et al.* SOCS1 hypermethylation mediated by DNMT1 is associated with lipopolysaccharide-induced inflammatory cytokines in macrophages. *Toxicology Letters*. 2014; 225: 488–497.
- [90] Tang RZ, Zhu JJ, Yang FF, Zhang YP, Xie SA, Liu YF, *et al.* DNA methyltransferase 1 and Krüppel-like factor 4 axis regulates macrophage inflammation and atherosclerosis. *Journal of Molecular and Cellular Cardiology*. 2019; 128: 11–24.
- [91] Chen YC, Young MJ, Chang HP, Liu CY, Lee CC, Tseng YL, *et al.* Estradiol-mediated inhibition of DNMT1 decreases p53 expression to induce M2-macrophage polarization in lung cancer progression. *Oncogenesis*. 2022; 11: 25.
- [92] García-Giménez JL, Romá-Mateo C, Pérez-Machado G, Peiró-Chova L, Pallardó FV. Role of glutathione in the regulation of epigenetic mechanisms in disease. *Free Radical Biology & Medicine*. 2017; 112: 36–48.
- [93] Zhang D, Tang Z, Huang H, Zhou G, Cui C, Weng Y, *et al.* Metabolic regulation of gene expression by histone lactylation. *Nature*. 2019; 574: 575–580.
- [94] Li J, DeNicola GM, Ruffell B. Metabolism in tumor-associated macrophages. *International Review of Cell and Molecular Biology*. 2022; 367: 65–100.
- [95] Geiger R, Rieckmann JC, Wolf T, Basso C, Feng Y, Fuhrer T, *et al.* L-Arginine Modulates T Cell Metabolism and Enhances Survival and Anti-tumor Activity. *Cell*. 2016; 167: 829–842.e13.
- [96] Su P, Wang Q, Bi E, Ma X, Liu L, Yang M, *et al.* Enhanced Lipid Accumulation and Metabolism Are Required for the Dif-

- ferentiation and Activation of Tumor-Associated Macrophages. *Cancer Research*. 2020; 80: 1438–1450.
- [97] Menjivar RE, Nwosu ZC, Du W, Donahue KL, Hong HS, Espinoza C, *et al.* Arginase 1 is a key driver of immune suppression in pancreatic cancer. *eLife*. 2023; 12: e80721.
- [98] Prima V, Kaliberova LN, Kaliberov S, Curiel DT, Kusmartsev S. COX2/mPGES1/PGE2 pathway regulates PD-L1 expression in tumor-associated macrophages and myeloid-derived suppressor cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2017; 114: 1117–1122.
- [99] Liu C, Chikina M, Deshpande R, Menk AV, Wang T, Tabib T, *et al.* Treg Cells Promote the SREBP1-Dependent Metabolic Fitness of Tumor-Promoting Macrophages via Repression of CD8<sup>+</sup> T Cell-Derived Interferon- $\gamma$ . *Immunity*. 2019; 51: 381–397.e6.
- [100] Cleophas MCP, Crişan TO, Lemmers H, Toenhake-Dijkstra H, Fossati G, Jansen TL, *et al.* Suppression of monosodium urate crystal-induced cytokine production by butyrate is mediated by the inhibition of class I histone deacetylases. *Annals of the Rheumatic Diseases*. 2016; 75: 593–600.
- [101] Li Z, Zheng W, Kong W, Zeng T. Itaconate: A Potent Macrophage Immunomodulator. *Inflammation*. 2023; 46: 1177–1191.
- [102] Liu TF, Vachharajani VT, Yoza BK, McCall CE. NAD<sup>+</sup>-dependent sirtuin 1 and 6 proteins coordinate a switch from glucose to fatty acid oxidation during the acute inflammatory response. *The Journal of Biological Chemistry*. 2012; 287: 25758–25769.
- [103] Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, *et al.* Five-year survival outcomes for patients with advanced melanoma treated with pembrolizumab in KEYNOTE-001. *Annals of Oncology: Official Journal of the European Society for Medical Oncology*. 2019; 30: 582–588.
- [104] Kremenovic M, Rombini N, Chan AA, Gruber T, Bärswyl L, Lee DJ, *et al.* Characterization of a Myeloid Activation Signature that Correlates with Survival in Melanoma Patients. *Cancers*. 2020; 12: 1431.
- [105] Wen Y, Wang X, Meng W, Guo W, Duan C, Cao J, *et al.* TNF- $\alpha$ -dependent lung inflammation upregulates PD-L1 in monocyte-derived macrophages to contribute to lung tumorigenesis. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*. 2022; 36: e22595.
- [106] Wang B, Cheng D, Ma D, Chen R, Li D, Zhao W, *et al.* Mutual regulation of PD-L1 immunosuppression between tumor-associated macrophages and tumor cells: a critical role for exosomes. *Cell Communication and Signaling: CCS*. 2024; 22: 21.
- [107] Shinichi Y, Ishizuka S, Komohara Y, Matsubara E, Mito R, Pan C, *et al.* The expression of PD-1 ligand 1 on macrophages and its clinical impacts and mechanisms in lung adenocarcinoma. *Cancer Immunology, Immunotherapy: CII*. 2022; 71: 2645–2661.
- [108] Wang J, Wu W, Yuan T, Wang L, Zang L, Liu Q, *et al.* Tumor-associated macrophages and PD-L1 in prostate cancer: a possible key to unlocking immunotherapy efficacy. *Aging*. 2024; 16: 445–465.
- [109] Tang Q, Chen Y, Li X, Long S, Shi Y, Yu Y, *et al.* The role of PD-1/PD-L1 and application of immune-checkpoint inhibitors in human cancers. *Frontiers in Immunology*. 2022; 13: 964442.
- [110] Zhao Y, Lee CK, Lin CH, Gassen RB, Xu X, Huang Z, *et al.* PD-L1:CD80 Cis-Heterodimer Triggers the Co-stimulatory Receptor CD28 While Repressing the Inhibitory PD-1 and CTLA-4 Pathways. *Immunity*. 2019; 51: 1059–1073.e9.
- [111] Wang L, Guo W, Guo Z, Yu J, Tan J, Simons DL, *et al.* PD-L1-expressing tumor-associated macrophages are immunostimulatory and associate with good clinical outcome in human breast cancer. *Cell Reports. Medicine*. 2024; 5: 101420.
- [112] Tabuchi M, Kikuchi S, Tazawa H, Okura T, Ogawa T, Mitsui E, *et al.* Functional remodeling of intraperitoneal macrophages by oncolytic adenovirus restores anti-tumor immunity for peritoneal metastasis of gastric cancer. *Molecular Therapy. Oncology*. 2024; 32: 200806.
- [113] Belgiovine C, Digifico E, Anfray C, Ummarino A, Torres Andón F. Targeting Tumor-Associated Macrophages in Anti-Cancer Therapies: Convincing the Traitors to Do the Right Thing. *Journal of Clinical Medicine*. 2020; 9: 3226.
- [114] Liu P, Zhao L, Kroemer G, Kepp O. PD-L1<sup>+</sup> macrophages suppress T cell-mediated anticancer immunity. *Oncoimmunology*. 2024; 13: 2338951.
- [115] Wang S, Wang J, Chen Z, Luo J, Guo W, Sun L, *et al.* Targeting M2-like tumor-associated macrophages is a potential therapeutic approach to overcome antitumor drug resistance. *NPJ Precision Oncology*. 2024; 8: 31.
- [116] Ochando J, Mulder WJM, Madsen JC, Netea MG, Duivenvoorden R. Trained immunity - basic concepts and contributions to immunopathology. *Nature Reviews. Nephrology*. 2023; 19: 23–37.
- [117] Dougan M, Luoma AM, Dougan SK, Wucherpfennig KW. Understanding and treating the inflammatory adverse events of cancer immunotherapy. *Cell*. 2021; 184: 1575–1588.
- [118] McQuade JL, Daniel CR, Hess KR, Mak C, Wang DY, Rai RR, *et al.* Association of body-mass index and outcomes in patients with metastatic melanoma treated with targeted therapy, immunotherapy, or chemotherapy: a retrospective, multicohort analysis. *The Lancet. Oncology*. 2018; 19: 310–322.
- [119] Hubler MJ, Kennedy AJ. Role of lipids in the metabolism and activation of immune cells. *The Journal of Nutritional Biochemistry*. 2016; 34: 1–7.
- [120] Machado MG, Patente TA, Rouillé Y, Heumel S, Melo EM, Deruyter L, *et al.* Acetate Improves the Killing of *Streptococcus pneumoniae* by Alveolar Macrophages via NLRP3 Inflammasome and Glycolysis-HIF-1 $\alpha$  Axis. *Frontiers in Immunology*. 2022; 13: 773261.
- [121] Kim KN, Yao Y, Ju SY. Short Chain Fatty Acids and Fecal Microbiota Abundance in Humans with Obesity: A Systematic Review and Meta-Analysis. *Nutrients*. 2019; 11: 2512.
- [122] Zhang X, Schalkwijk CG, Wouters K. Immunometabolism and the modulation of immune responses and host defense: A role for methylglyoxal? *Biochimica et Biophysica Acta. Molecular Basis of Disease*. 2022; 1868: 166425.
- [123] Prantner D, Nallar S, Richard K, Spiegel D, Collins KD, Vogel SN. Classically activated mouse macrophages produce methylglyoxal that induces a TLR4- and RAGE-independent proinflammatory response. *Journal of Leukocyte Biology*. 2021; 109: 605–619.
- [124] Hernandez-Castillo C, Shuck SC. Diet and Obesity-Induced Methylglyoxal Production and Links to Metabolic Disease. *Chemical Research in Toxicology*. 2021; 34: 2424–2440.
- [125] Kovats S. Estrogen receptors regulate innate immune cells and signaling pathways. *Cellular Immunology*. 2015; 294: 63–69.
- [126] Calippe B, Douin-Echinard V, Delpy L, Laffargue M, Lélou K, Krust A, *et al.* 17 $\beta$ -estradiol promotes TLR4-triggered proinflammatory mediator production through direct estrogen receptor  $\alpha$  signaling in macrophages in vivo. *Journal of Immunology (Baltimore, Md.: 1950)*. 2010; 185: 1169–1176.
- [127] Flemming A. Butyrate boosts microbicidal macrophages. *Nature Reviews. Immunology*. 2019; 19: 135.
- [128] Luu M, Weigand K, Wedi F, Breidenbend C, Leister H, Pautz S, *et al.* Regulation of the effector function of CD8<sup>+</sup> T cells by gut microbiota-derived metabolite butyrate. *Scientific Reports*. 2018; 8: 14430.
- [129] Monteiro LDB, Prodonoff JS, Favero de Aguiar C, Correada-Silva F, Castoldi A, Bakker NVT, *et al.* Leptin Signaling

Suppression in Macrophages Improves Immunometabolic Outcomes in Obesity. *Diabetes*. 2022; 71: 1546–1561.

- [130] Kiernan K, MacIver NJ. The Role of the Adipokine Leptin in Immune Cell Function in Health and Disease. *Frontiers in Immunology*. 2021; 11: 622468.
- [131] Obradovic M, Sudar-Milovanovic E, Soskic S, Essack M, Arya S, Stewart AJ, *et al.* Leptin and Obesity: Role and Clinical Implication. *Frontiers in Endocrinology*. 2021; 12: 585887.
- [132] Uehara T, Eikawa S, Nishida M, Kunisada Y, Yoshida A, Fujiwara T, *et al.* Metformin induces CD11b<sup>+</sup>-cell-mediated growth inhibition of an osteosarcoma: implications for metabolic reprogramming of myeloid cells and anti-tumor effects. *International Immunology*. 2019; 31: 187–198.
- [133] Kalliolias GD, Ivashkiv LB. TNF biology, pathogenic mechanisms and emerging therapeutic strategies. *Nature Reviews. Rheumatology*. 2016; 12: 49–62.
- [134] Winterbourn CC. Reconciling the chemistry and biology of reactive oxygen species. *Nature Chemical Biology*. 2008; 4: 278–286.
- [135] Rivera A, Siracusa MC, Yap GS, Gause WC. Innate cell communication kick-starts pathogen-specific immunity. *Nature Immunology*. 2016; 17: 356–363.