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The role of neutrophils in trained immunity

Lydia Kalafati[1] | Aikaterini Hatzioannou[1] | George Hajishengallis[2] | Triantafyllos Chavakis[1]

[1]Institute for Clinical Chemistry and Laboratory Medicine, University Hospital and Faculty of Medicine, Technische Universität Dresden, Dresden, Germany

[2]Department of Basic and Translational Sciences, School of Dental Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

Correspondence
Triantafyllos Chavakis, Institute for Clinical Chemistry and Laboratory Medicine, University Hospital and Faculty of Medicine, Technische Universität Dresden, Fetscherstrasse 74, 01307 Dresden, Germany.
Email: triantafyllos.chavakis@ukdd.de

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Summary

The principle of trained immunity represents innate immune memory due to sustained, mainly epigenetic, changes triggered by endogenous or exogenous stimuli in bone marrow (BM) progenitors (central trained immunity) and their innate immune cell progeny, thereby triggering elevated responsiveness against secondary stimuli. BM progenitors can respond to microbial and sterile signals, thereby possibly acquiring trained immunity-mediated long-lasting alterations that may shape the fate and function of their progeny, for example, neutrophils. Neutrophils, the most abundant innate immune cell population, are produced in the BM from committed progenitor cells in a process designated granulopoiesis. Neutrophils are the first responders against infectious or inflammatory challenges and have versatile functions in immunity. Together with other innate immune cells, neutrophils are effectors of peripheral trained immunity. However, given the short lifetime of neutrophils, their ability to acquire immunological memory may lie in the central training of their BM progenitors resulting in generation of reprogrammed, that is, "trained", neutrophils. Although trained immunity may have beneficial effects in infection or cancer, it may also mediate detrimental outcomes in chronic inflammation. Here, we review the emerging research area of trained immunity with a particular emphasis on the role of neutrophils and granulopoiesis.

KEYWORDS
bone marrow, cancer, emergency myelopoiesis, granulopoiesis, hematopoietic stem and progenitor cells, inflammation, innate immune memory, trained immunity

1 | INTRODUCTION: TRAINED INNATE IMMUNITY—INNATE IMMUNE MEMORY

Immune defense and immune responses in vertebrates comply to the classical dichotomy of immunity, the innate and the adaptive immune systems. Innate immune responses, which are mediated by a variety of cells, including neutrophils, monocytes, and macrophages, develop rapidly and are thought to be non-specific. Stimulus-induced innate immune functions include, among others, cell migration, phagocytosis, and cytokine production.[1] Adaptive immunity involves a specialized set of cells, such as T and B lymphocytes that react slower, but acquire high antigen specificity and can

Lydia Kalafati and Aikaterini Hatzioannou contributed equally to this work.

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develop long-term specific immunological memory.[2] However, this traditional dichotomy may not be able to explain the full complexity of immune responses.

After the discovery of pattern recognition receptors (PRRs) in innate immunity evidence accumulated for the existence of protection mechanisms against infection and potentially reinfection that could not be solely attributed to the adaptive branch of immunity.[3,4] PRRs, such as Toll-like receptors (TLRs), NOD-like receptors (NLRs), RigI-helicases, and C-type lectin receptors, are expressed in a plethora of innate immune cells, allowing them to recognize and respond to multiple pathogen-expressed molecules, that is, pathogen-associated molecular patterns (PAMPs), and damage-associated molecular patterns (DAMPs).[5–7] Importantly, studies in plants and invertebrates that do not have adaptive immunity, as well as significant recent work in vertebrates, and epidemiological studies in humans suggest that innate immune cells may also experience long-lasting alterations that may confer increased protection against reinfection. This property has been designated trained innate immunity (or trained immunity) and is fundamentally based on innate immune memory.[8–11]

Unlike the classical immunological memory of adaptive immunity that requires gene rearrangement and clonal expansion of antigen-specific lymphocytes, the enhanced innate immune responses to future homologous or even heterologous challenges seen in trained immunity depend on sustained epigenetic modulation of innate immune cells that modify their transcriptional program and function and increase their immune preparedness.[9,10,12] Therefore, innate immune memory refers to an altered functional state of the cell that persists from weeks to months; however, there are studies showing also heterologous protection induced by live vaccines even after longer periods, for example, five years.[9,13] In other words, trained innate immune cells display an acquired capacity to react qualitatively different, mostly stronger, upon being challenged by secondary stimuli, as compared to untrained cells. This altered responsiveness is attributed to the differential regulation of the expression of several inflammatory genes. The mechanisms governing this differential gene expression are only partially understood and may involve alterations in chromatin remodeling, histone modifications, and rewiring of cellular metabolism,[9] as will be outlined below.

2 | INDUCERS OF TRAINED IMMUNITY

Initially, it was thought that trained immunity is induced mainly by exogenous microbial stimuli. Currently, it is appreciated that nonmicrobial and even endogenous stimuli may induce trained immunity.[9] Microbial stimuli that are well known to mediate innate immune training are live attenuated vaccines, such as the Bacille Calmette-Guerin (BCG) vaccine,[14] the oral polio vaccine,[15] the measles vaccine,[16] the smallpox vaccine,[17] and the new vaccine against tuberculosis MTBVAC.[18] Additionally, prototype and most studied trained immunity agonists are β-glucans,[9] which are highly conserved glucose polymers from the wall of various fungi and yeast and act as immunostimulatory agents.[19] Moreover, lipopolysaccharide (LPS) has also been implicated in mediating trained immunity.[20,21] Hepatitis B virus[22] and the pathogen *Plasmodium falciparum*[23] have also been described to induce innate immune training.

In addition to the aforementioned microbial stimuli and microbiota-derived immunostimulatory factors,[24] trained immunity effects have been also described upon exposure to endogenous nonmicrobial factors such as lipoproteins,[25,26] uric acid,[27,28] aldosterone,[29] catecholamines,[30] S100-alarmins,[31] heme,[32] activators of the liver X receptor pathway,[33] or interferons.[34] Essentially, the list of trained immunity agonists is likely very wide, since several factors that act as PAMPs or DAMPs might bear the potential to induce innate immune training.[11] However, the inflammatory effector function of each stimulus and the underlying pathways involved may vary substantially, thereby differentially affecting the capacity of distinct stimuli to induce trained immunity-associated long-term changes in cells. Hence, trained immunity-related programs may display substantial versatility and complexity.

3 | EPIGENETIC AND IMMUNOMETABOLIC REGULATION OF TRAINED IMMUNITY

Pro-inflammatory gene loci in quiescent innate immune cells are mostly found to be repressed.[35] Several findings suggest that the memory-like phenotypes in innate immunity are associated with altered chromatin accessibility in trained immune cells or their BM progenitors, as assessed by assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-seq)[36,37] and may also involve sustained stimulus-induced histone modifications at the level of inflammatory genes, thereby altering their transcription and the responsiveness of the cells upon future challenges.[9,12] Some important epigenetic marks at distal regulatory elements or at the promoter of genes have been related to trained immunity programs: the histone 3 lysine 27 acetylation (H3K27ac), the histone 3 lysine 4 monomethylation (H3K4me1), and the histone 3 lysine 4 trimethylation (H3K4me3). The H3K27ac is found at distal enhancers that carry a H3K4me1 and the H3K4me3 marks promoters of activated genes.[9] The collision of transcription factors on these regions is described to be random.[38] However, this stochastic regulation of gene expression is not optimal in cases that require immediate responses to stimuli. Studies performing analysis of chromatin interactions have reduced this notion of stochasticity in gene transcription by showing that multiple rapidly responding genes in innate immune cells are organized in topologically associated domain (TAD) structures and interact within these complexes.[9,39] These structures may enable immune-related genes to interact with some long noncoding RNAs (lncRNAs), the so-called "immune-gene priming lncRNAs" (IPLs) and prime their transcriptional activation.[40] IPLs are located in the same TAD as the immune genes they regulate, thereby directing histone modulating enzyme complexes to the promoters of this set of co-regulated genes, and allowing for their epigenetic reprogramming.[41] The prototypic IPL, UMLILO (Upstream Master LncRNA

of the Inflammatory chemokine Locus) forms chromosomal contacts with the ELR+ CXCL chemokines (Interleukin [IL]-8, CXCL1, CXCL2, and CXCL3) and acts in order to facilitate their H3K4me3 epigenetic priming.[40] It is also suggested that other cytokines, such as IL-6 and IL-1β, that are involved in trained immunity, are similarly regulated in an IPL-dependent manner.[42] In addition, β-glucan-mediated innate immune training induces increased nuclear factor of activated T cells (NFAT)-dependent transcription of IPLs that subsequently results in innate immune gene epigenetic rewiring.[42] Furthermore, priming with β-glucan or BCG can upregulate IPLs in BM progenitor cells that in turn results in greater deposition of H3K4me3 marks on immune gene promoters.[42] Hence, lncRNA contributes to induction of trained immunity and may serve as an entirely new druggable target for therapeutic immunomodulation.

Epigenome modification is intimately linked with cellular metabolism, as several metabolic pathways produce metabolites that may modify chromatin and the activity of epigenetic factors.[43,44] Given the relevance of epigenetic reprograming in trained immunity, it is not surprising that changes in cell metabolism may also mediate the establishment of innate immune memory.[45] Changes in cellular metabolic pathways, for example, glycolysis, cholesterol biosynthesis, amino acid and fatty acid metabolism and oxidative phosphorylation, can dictate innate immune cell plasticity, regulate the activity of chromatin-modifying enzymes, and have been implicated in trained innate immunity.[45–47] For example, an increase in mechanistic target of rapamycin (mTOR)- and hypoxia-inducible factor-1α (HIF1α)-mediated aerobic glycolysis is found in both β-glucan and BCG trained monocytes.[48] In addition, BCG-induced trained immunity requires a strong increase in glycolysis that is responsible for histone modifications and functional changes in monocytes.[49] Induction of trained immunity in human monocytes is also linked to elevated glutaminolysis and accumulation of the tricarboxylic acid (TCA) cycle metabolite, fumarate, that regulates epigenetic changes by inhibiting KDM5 histone demethylases.[50] Studies in human monocytes revealed that β-glucan-dependent training results in increased mevalonate levels, thereby promoting trained immunity through activation of the insulin-like growth factor 1 receptor and mTOR pathway.[51] Furthermore, another TCA cycle metabolite, alpha-ketoglutarate promotes an anti-inflammatory M2-like polarization of macrophages; reducing the ratio of alpha-ketoglutarate to succinate in turn supports a pro-inflammatory M1-like phenotype in macrophages.[52] The metabolite itaconate that is produced from a TCA metabolite, cis-aconitate, and has been ascribed multiple anti-inflammatory actions in mouse and human macrophages,[53–55] has been implicated as a target of β-glucan-induced trained immunity in monocytes. Specifically, β-glucan-induced trained immunity down-regulates expression of the enzyme that regulates itaconate production, immune-responsive gene 1 (IRG1) in monocytes, thereby also counteracting development of immune tolerance.[56] Together, there is a close link between immunometabolic and epigenetic pathways during establishment of trained immunity; the better understanding of this link and its regulation requires substantial future investigation. Of note, research on the immunometabolic regulation of trained immunity has mostly focused on monocytes and macrophages, whereas neutrophils have been poorly studied in this regard so far.

4 | BONE MARROW HEMATOPOIESIS AND CENTRAL TRAINED IMMUNITY

Initially, trained immunity was studied in mature myeloid cells. However, the short lifetime of these cells, especially monocytes and neutrophils, in the circulation was in disagreement with the long-term effects of innate immune memory persisting for months to years.[9] This discrepancy could be reconciled by findings that trained immunity does not only occur in mature myeloid cells within peripheral tissues and organs (peripheral trained immunity) but may in fact be initiated in progenitors of these cells in the BM (central trained immunity)[9,57] (Figure 1).

All mature blood cells, including innate immune cells, are generated in the BM by a hierarchically organized process called hematopoiesis.[58] The hematopoietic stem cells (HSCs) are on the top of the hematopoiesis pyramid and display the unique capacity to self-renew. Moreover, HSCs have the ability to differentiate to all blood lineages. When HSCs are activated, they differentiate into multipotent progenitors (MPPs). HSCs and MPPs together form the pool of hematopoietic stem and progenitor cells (HSPCs) and are the main two populations within the lineage$^-$Sca1$^+$c-kit$^+$ (LSK) cells in the BM.[59,60] For the generation of myeloid cells, HSPCs will subsequently differentiate into more committed progenitors, such as common myeloid progenitors (CMPs) and granulocyte-macrophage progenitors (GMPs).[61] Under infectious or inflammatory conditions with increased demand for myeloid cells in the periphery, myeloid progenitors in the BM expand; in addition, HSCs also respond with expansion and enhanced myeloid differentiation; this process giving rise to new myeloid cells upon stress conditions is designated emergency myelopoiesis.[57,60,61] When the aforementioned process leads to predominantly increased numbers of neutrophils (e.g., in the context of an acute bacterial infection), it is specifically termed emergency granulopoiesis.[61] In this regard, it is important that HSCs and HSPCs are capable to recognize and respond to a variety of soluble factors reaching the BM microenvironment during peripheral infection or inflammation.[57,62] HSPCs express PRRs, such as TLRs that recognize PAMPs, as well as receptors for pro-inflammatory cytokines, such as IL-1, IL-6, tumor necrosis factor (TNF), type I and II interferons (IFN) and receptors for growth factors, such as granulocyte macrophage-colony stimulating factor (GM-CSF) or macrophage-colony stimulating factor (M-CSF), enabling them to recognize systemic inflammatory challenges and acutely respond.[57,63–65] The HSPC response to such PAMPs, cytokines, or growth factors commonly results in emergency myelopoiesis with an increase in the GMP pool and subsequent myeloid cell production.[61,66] Importantly, however, although HSPC reactions are critical for the response to infections as well as for restoration of hematopoiesis after irradiation,

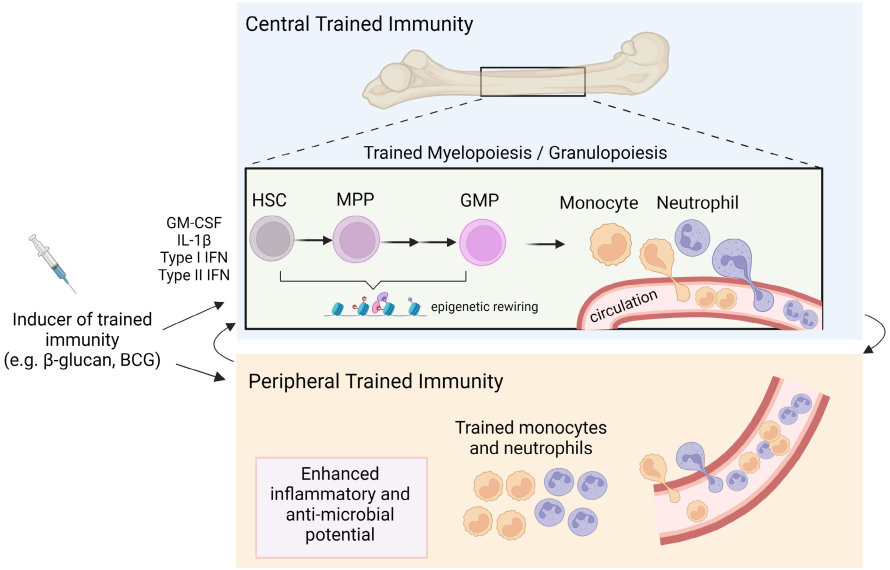

FIGURE 1 Central and peripheral trained immunity. Central trained immunity defines that the process of myelopoiesis is trained by epigenetic rewiring of HSPCs (HSCs and MPPs) as well as of GMPs, thereby giving rise to more mature myeloid cells (neutrophils and monocytes) and importantly to myeloid cells with higher inflammatory potential. Agonists, such as the β-glucan or the BCG vaccine, may promote central trained immunity in a manner that involves signaling by factors, such as GM-CSF, IL-1β, type I, or type II IFNs. Induction of trained immunity also involves the direct modulation of the inflammatory preparedness of mature myeloid cells in the bloodstream or peripheral organs (peripheral trained immunity). BCG, Baccillus Calmette-Guerin; GMP, granulocyte-macrophage progenitor; HSC, hematopoietic stem cell; MPP, multipotent progenitor.

excessive and chronic activation may lead to impaired function, or even exhaustion, of HSPCs resulting in chronic pathologies.[67–69]

In the context of trained innate immunity and its early stages, our group and others have provided evidence that different agonists of trained immunity can induce responses in HSPCs that resemble emergency myelopoiesis processes. Via engaging signaling triggered by different pro-inflammatory factors, such as IL-1, IFNs, and growth factors, such as GM-CSF, agonists of trained immunity, such as BCG or β-glucan, may induce long-lasting alterations in HSPCs, at the metabolic and epigenetic level, which lead to sustained increase of myelopoiesis and granulopoiesis.[20,36,37,70–75] In addition, this central trained immunity leads to generation of trained myeloid cells, that is, cells with increased inflammatory responsiveness (Figure 1). The existence of central BM-mediated trained immunity as a major arm of innate immune memory was established by performing BM transplantation studies in mice.[37,71] Increased myelopoiesis and granulopoiesis as well as generation of myeloid cells with enhanced inflammatory potential are both integral components of central BM-mediated trained immunity that results in sustained elevation of systemic innate immune responses upon future challenges.[20,36,37,70–75]

5 | BENEFICIAL VERSUS MALADAPTIVE INNATE IMMUNE TRAINING

A large body of published work demonstrates the beneficial role of trained immunity in mice and humans especially during the response to infectious pathogens.[9] Epidemiological findings report that several vaccines are able to promote "non-specific" immunity by protecting the immunized individual not only against the target pathogen but also against a broad variety of unrelated infections.[76] As an example of the off-target beneficial effects of vaccines, BCG vaccination in children of West Africa resulted in overall reduced mortality, due to protection against other infections in addition to tuberculosis.[77] Other live vaccines, such as the oral polio vaccine, the measles vaccine, and the smallpox vaccine, have been also shown to induce heterologous protective effects.[76] Trials performed in children[78] and adults[14,79] demonstrated an elevated activation of innate immune cells following BCG vaccination. Accordingly, BCG vaccination in humans resulted in increased pro-inflammatory function of monocytes that was accompanied by partial protection against experimental malaria[80] and yellow fever.[81] In mice, BCG vaccination provides protection against secondary infection with *Schistosoma mansoni*,[82] *Candida albicans*,[83] and *Mycobacterium tuberculosis*.[75] BCG administration protected SCID mice that do not have adaptive immune responses from lethal candidiasis,[14] thereby further underlining the functional relevance of innate immune memory in this process.

Another commonly used trigger of trained immunity, β-glucan, also confers protection against future infectious challenges. Specifically, mild infection with *Candida albicans* or simply pretreating with β-glucan could protect against subsequent lethal candidiasis; this phenomenon relied on induction of trained immunity and was independent of adaptive immunity, as shown by the use of Rag1-deficient mice lacking lymphocytes.[84] Moreover, *Candida albicans* gut colonization induced trained immunity that led to enhanced immune responses against multiple, systemic infections.[85]

Additionally, β-glucan-induced training has been reported to exhibit a protective effect against systemic *Streptococcus pneumoniae* infection[86] and *Mycobacterium tuberculosis* infections.[74]

In addition, there is evidence supporting a possible anti-tumor effect of trained immunity agonists BCG[87] and β-glucan.[88,89] BCG has been reported as a potential therapeutic strategy for several malignancies, such as bladder cancer, where it has been an approved therapy against non-muscle-invasive bladder cancer forms,[90] melanoma,[91] leukemia,[92] and lymphoma.[93] With regard to β-glucan, it is currently being used in a series of clinical trials as an adjuvant immunotherapy against different forms of cancer.[89] In this regard, and as it will be outlined in detail below, we could recently show that the anti-tumor effects of β-glucan are, at least partially, mediated by the induction of central trained immunity, specifically trained granulopoiesis, that led to generation of neutrophils with potent anti-tumor activity.[37] Taken together, multiple studies support that induction of trained immunity may have beneficial effects in the context of heterologous infections or may activate anti-cancer programs.

While innate immune memory involves changes that improve the hosts' protection against infection or cancer, trained innate immunity may have also a dark side. The elevated inflammatory responsiveness associated with trained immunity-induced innate immune cell rewiring may also result in maladaptive or detrimental effects during chronic inflammation and tissue damage. A cardinal example is sterile inflammation caused by Western diet that may induce trained immunity and chronic cardiometabolic diseases.[57,94] In addition, innate immune memory-induced augmented immune cell activation could provide mechanistic underpinnings for the epidemiological observations linking infections with atherosclerosis occurrence.[95,96] Several endogenous stimuli have been demonstrated to trigger trained immunity, such as oxidized low-density lipoprotein (oxLDL), thereby eliciting increased inflammatory cytokine production, which contributes to the pathogenesis of atherosclerosis.[25,26] Moreover, Western diet-induced NLRP3 inflammasome-dependent epigenetic and transcriptomic reprogramming of GMPs results in augmented inflammatory responses even after switching mice back to normal diet.[36] Accordingly, monocytes obtained from individuals with hypercholesterolemia exhibit enhanced inflammatory properties consistent with trained immunity, which persisted even after statin treatment.[97] Furthermore, monocytes in the circulation of patients with symptomatic atherosclerosis exhibit increased production of cytokines upon future challenge, accompanied by metabolic and epigenetic rewiring.[98]

Interestingly, we could recently show that maladaptive BM-mediated trained immunity may provide a mechanistic link for the development of inflammatory comorbidities. Specifically, experimental periodontitis triggered IL-1-dependent maladaptive central trained immunity, associated with epigenetic rewiring of HSPC and sustained generation of monocytes and neutrophils with enhanced inflammatory responsiveness that could thereby exacerbate inflammatory arthritis,[72] which is a frequent periodontitis comorbidity.[99] Importantly, periodontitis-driven maladaptive myelopoiesis training was transmissible by BM transfer to recipient mice, which also exhibited increased susceptibility to inflammatory arthritis.[72] Since trained immunity lacks specificity to the initial stimulus, maladaptive training of myelopoiesis may underlie the emergence not only of the periodontitis-arthritis comorbidity but also of several inflammatory comorbidities.[60,99,100] Collectively, trained innate immunity is a double-edged sword that can promote both beneficial and deleterious effects in a context-dependent manner.[60,72,99–101]

6 | NEUTROPHILS AND GRANULOPOIESIS

Neutrophils, also known as polymorphonuclear (PMN) leukocytes, comprise the majority of circulating leukocytes in humans. Neutrophils have an enormous turnover ratio with roughly 0.5–1 x 10^{11} neutrophils being daily generated under steady-state conditions.[102] The de novo production of neutrophils occurs in the BM by myeloid progenitors, GMPs through a process termed as granulopoiesis.[61,103–106] As neutrophils are the first cells to respond in infection or injury, the demand for these cells in the periphery is constantly high. Thus, adaptation of myeloid progenitors in such cases underlines the process of emergency granulopoiesis, which leads to production of approximately 10^{12} neutrophils daily.[61] Given these large amounts of neutrophils, it is not surprising that approximately 60% of the BM hematopoiesis is dedicated to producing these cells.[107] Recent studies revealed further details regarding the differentiation paths from GMPs to neutrophils. Three subpopulations were found in the BM, neutrophil precursors that differentiate into immature neutrophils and then mature neutrophils. The differentiation of GMPs to neutrophil precursors was dependent on C/EBPε transcription factor and involves progenitors within the GMP population.[105] Novel research has recently shed more light into the different subpopulations of neutrophil precursors found in humans, such as proNeus,[104] preNeus,[105] early neutrophil progenitors,[108] human neutrophil progenitors,[109] and neutrophil-committed progenitors.[110] Interestingly, such committed progenitors and neutrophil precursors expand in infection and cancer.[104,105]

Although neutrophils comprise a terminally differentiated population,[111] single-cell analytical studies have revealed substantial heterogeneity within neutrophils.[106] The general notion is that these cells are short lived; however, their lifespan may vary in different tissues. Initially, ex vivo survival assays and experiments utilizing adoptive transfer demonstrated that neutrophils live 8–12 h in the bloodstream and 1–2 days in the tissue,[102,112,113] whereas in vivo methods indicate that the neutrophil lifetime in humans may reach 5 days.[114]

At the end of their lifetime, neutrophils undergo apoptosis and they subsequently get phagocytically cleared by macrophages through a process named efferocytosis.[115] Aged neutrophils may return to the BM and get cleared by BM macrophages.[116,117] Despite their impressive turnover rate, the number of neutrophils in the circulation remains constant. This is owing to the well-orchestrated

balance between production and elimination.[118] Their multiple phenotypic changes and properties suggest existence of great neutrophil functional heterogeneity.[119] The functional heterogeneity of neutrophils in the periphery may be shaped by the tissue microenvironments. For instance, neutrophils obtain in peripheral tissues, for example, in the lung, the capacity to contribute to vessel repair or to promote the integrity of hematopoiesis, hence facilitating the restoration of hematopoiesis upon irradiation.[103] Moreover, neutrophils display a great repertoire of functions, such as degranulation, phagocytosis, antigen-presentation, formation of neutrophil extracellular traps (NETs), release of cytokines and other inflammatory and anti-microbial factors,[119,120] thereby actively regulating inflammation and its resolution,[121] and participating in several pathologies, including cancer.[122,123] Moreover, as it was recently demonstrated and will be outlined in the next paragraphs, neutrophils are integral components of innate immune memory (Figure 2).[37,72,124]

7 | NEUTROPHILS AND GRANULOPOIESIS IN TRAINED INNATE IMMUNITY

As alluded to above, neutrophils have been recently recognized as effectors of trained immunity.[37,124] The BCG vaccine, a prototypical agonist of trained immunity, provides broad protection against infection by multiple unrelated pathogens.[9] Although these off-target protective effects of BCG are typically attributed to innate immune training of monocytes, recent studies suggest a prominent role of neutrophils in this regard.[124,125] A few weeks following BCG vaccination of healthy humans, the absolute number of neutrophils in the blood increases.[124,125] Nevertheless, 3 months after vaccination, the neutrophil counts re-equilibrate back to normal; however, neutrophils maintain qualitative changes and particularly an activated/trained phenotype with increased expression of CD11b, CD66b and diminished expression of CD62L and PDL1.[124] This highly activated phenotype is accompanied by enhanced capacity of BCG-trained

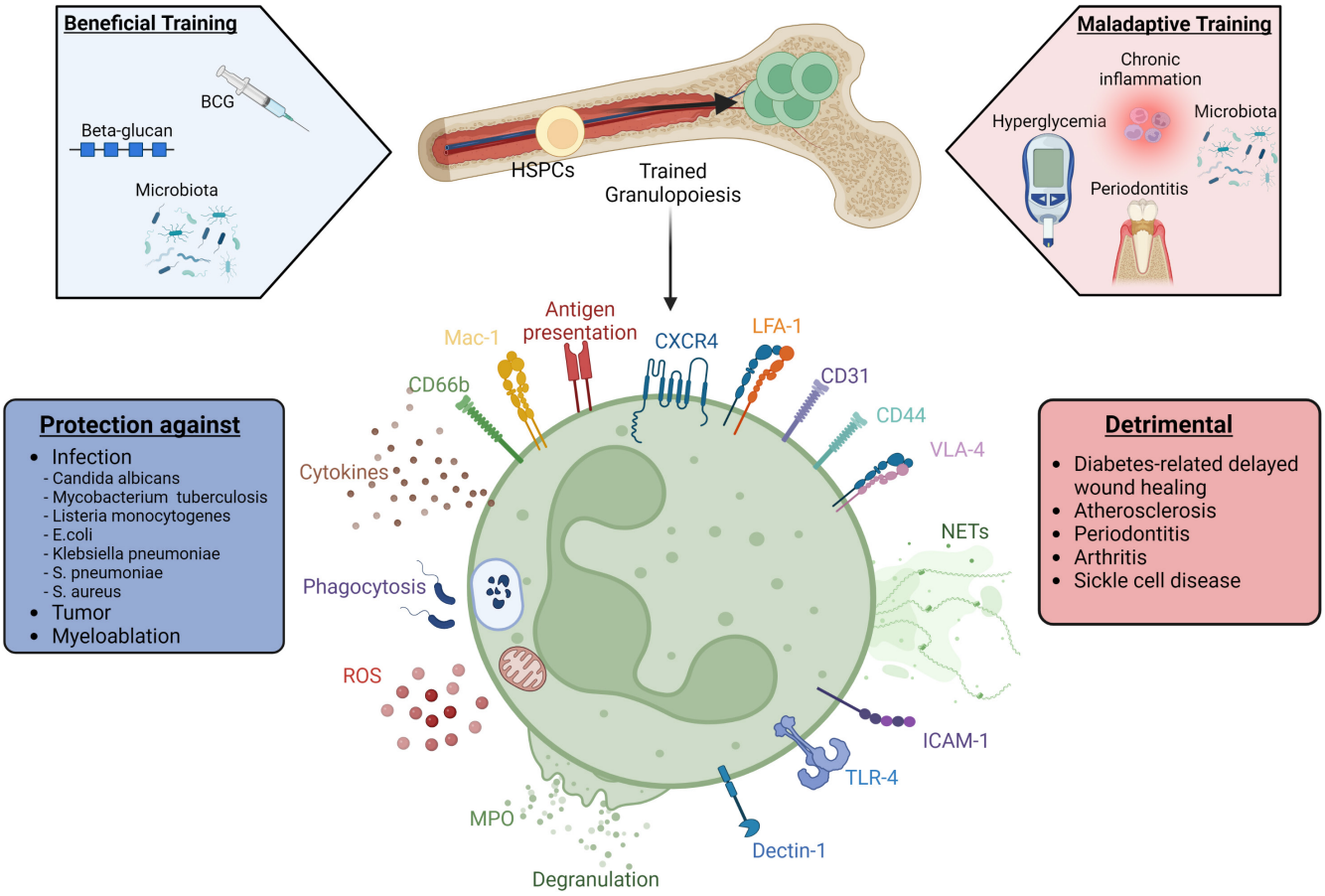

FIGURE 2 Neutrophils in protective and maladaptive trained immunity. Trained innate immunity and particularly trained granulopoiesis may exert both beneficial and detrimental effects. Trained immunity leads to sustained reprogramming of granulopoiesis with generation of trained neutrophils that display more potent effector mechanisms such as degranulation, ROS production, phagocytosis, antigen presentation, release of cytokines and other immune mediators, and increased expression of adhesion molecules and activation markers. Trained neutrophils may provide protection from infections or tumor or enhance tissue damage in the context of cardiometabolic or inflammatory diseases. BCG, Baccillus Calmette-Guerin; CXCR-4, C-X-C chemokine receptor type 4; ICAM-1, intracellular adhesion molecule 1; MPO, myeloperoxidase; NETs, neutrophil extracellular traps; ROS, reactive oxygen species; TLR-4, toll-like receptor 4; VLA-4, very late antigen-4.

human neutrophils to kill *Candida albicans* in vitro resulting from their improved degranulation and phagocytic capacity, enhanced production of IL-8, elastase and reactive oxygen species (ROS) upon a secondary stimulation. Importantly, neutrophils from BCG-vaccinated individuals present several hallmarks of trained immunity, including epigenetic and metabolic changes.[124,125] Specifically, increased levels of H3K4me3 are observed at promoter sites of genes participating in JAK–STAT signaling, or of genes encoding pro-inflammatory cytokines or glycolysis-related proteins in neutrophils from vaccinated persons, as compared to controls.[124] Consistent with the epigenetic data, the transcription of genes implicated in the PI3K-AKT pathway as well as hexokinase 1 (HK1), which is one of the rate-limiting enzymes of glycolysis, are significantly upregulated in BCG-trained human neutrophils three months after the vaccination. Nevertheless, most of the BCG-induced transcriptomic changes of neutrophils are evident mainly following their secondary re-stimulation, while epigenetic changes are persistently present following the initial stimulus,[124] similar to innate immune training of monocytes.[81,126,127] The long-term effects of BCG-induced training on neutrophil function despite their short life span imply that BCG may induce central trained immunity by modifying neutrophil progenitor cells in the BM. Intravenous administration of the BCG vaccine in mice results in an increase of HSPCs and significant transcriptional reprogramming of HSCs and MPPs without these cells being infected by BCG.[75] Enhanced expression of myeloid-lineage-specific markers, such as *Cebpe*, *Cebpa*, *Irf8*, *Csf1r*, and *Csf2rb*, with a concomitant decrease in the expression of lymphoid lineage-specific markers, such as *Lck*, *Rag1*, *Rag2*, *Pax5*, and *Irf4*, in MPPs indicates an increase of myelopoiesis and reciprocal decrease of lymphopoiesis; increased myelopoiesis is dependent on IFNγ signaling.[75] These data imply that BCG vaccination trains the mouse BM hematopoietic progenitor cells to obtain a myeloid bias resulting in numeric (quantitative) and functional (qualitative) alterations of neutrophils, monocytes, and macrophages in the periphery. Nevertheless, studies of BCG-mediated trained immunity have addressed the anti-mycobacterial capacity of mouse BM-derived monocytes and macrophages but not of neutrophils.[75] In accordance with the aforementioned work demonstrating BCG-induced central trained immunity in mice, transcriptomic analysis of HSPCs from BM aspirates of individuals 90 days following intradermal vaccination with BCG showed increased expression of *CX3CR1*, *MPEG1*, *IRF4*, *CEBPD*, *MARCO*, *IL1R1*, *IL1R2*, *S100A8*, *S100A9*, *S100A12*, and *SERPINA1*, hence revealing elevated myelopoiesis and granulopoiesis.[125] BCG vaccine-induced central trained immunity in humans is mediated by the transcription factors HNF1A and HNF1B.[125] Another prototype agonist of trained immunity, β-glucan, which derives from the fungal cell wall, also promotes central trained immunity with long-lasting effects in neutrophils. Administration of β-glucan to mice leads to proliferation of HSPCs, including myeloid-biased subpopulations thereof, such as $CD41^+$ LT-HSCs, or MPP3 cells, indicating an enhancement in myelopoiesis that is dependent on IL-1 signaling and signaling downstream of the common beta subunit of the IL-3- / GM-CSF-receptor (CD131).[71,74] β-glucan-mediated central trained immunity is also associated with metabolic reprogramming of mouse BM progenitor cells, which exhibited increased glycolysis and cholesterol biosynthesis, as assessed by transcriptomics and lipidomics analysis.[71] Consistently, blockade of mechanisms mediating cholesterol efflux, such as ATP-binding cassette transporters ABCA1 and ABCG1 or apolipoprotein E, also confers an increased myeloid bias to HSPCs via enhanced CD131-dependent signaling leading to elevated myelopoiesis and granulopoiesis.[128–130] Importantly, training of mice with β-glucan protects HSCs from both LPS- and chemotherapy (5-FU)-induced DNA damage. As a consequence, β-glucan-trained mice are protected against neutropenia in the circulation following chemotherapy-induced myelosuppression.[71] Moreover, β-glucan-induced central trained immunity protects mice from *Mycobacterium tuberculosis* infection.[74]

An additional outcome of β-glucan-induced central trained immunity is potent anti-tumor activity. Specifically, mouse mature neutrophils deriving from β-glucan-mediated trained granulopoiesis in the BM display a tumor-suppressive phenotype, including increased ROS production, phagocytosis, and antigen presentation.[37] This process involves epigenetic rewiring of granulopoiesis in a fashion that required type I IFN, as evidenced by single-cell epigenomic analysis of GMPs and neutrophils.[37] Systemic delivery of neutrophils from β-glucan-trained mice to tumor-bearing mice diminishes tumor growth. These findings indicate a potential novel therapeutic option, in which trained neutrophils could be used in conjunction with available immunotherapy drugs targeting primarily the adaptive branch of immunity.[37]

Furthermore, β-glucan-induced trained immunity may result in neutrophils that protect against infections as well. Zymosan, which is a fungal cell wall preparation rich in β-glucan, can also induce trained immunity, accompanied by increased numbers of neutrophils in the BM and periphery and protection against *L. monocytogenes* infection. The neutrophils of zymosan-trained mice produce increased amounts of IL-6 following in vitro re-stimulation with LPS and display increased production of myeloperoxidase (MPO) and a better killing capacity against *L. monocytogenes*.[131] Moreover, the metabolism of neutrophils from zymosan-trained mice is rewired, exhibiting higher glycolytic rate and mitochondria respiration upon stimulation with *L. monocytogenes*. Zymosan-induced innate immune memory effects on neutrophils result from central BM-mediated trained immunity, as these effects are sustained for a prolonged time (9–12 weeks following zymosan administration). Indeed, zymosan induces a myeloid bias, as evidenced by increased numbers of MPP3 (myeloid-skewed MPPs) and GMPs in the BM of trained mice.[131] Nevertheless, the protective effect of zymosan-induced trained immunity against listeriosis may not be mediated solely by neutrophils and may require the participation of further innate immune cells, as assessed by neutrophil depletion studies by Ciarlo et al. who found that the absence of neutrophils does not alter the protection of trained mice against listeriosis. Nevertheless, these authors also observed a similar effect of zymosan-induced training on promoting myelopoiesis and increasing neutrophil numbers in the periphery.[132]

Symbiotic microbial communities (microbiota) that reside on the mucosal surfaces and skin of vertebrates[24] may also be involved in establishment of trained immunity, associated with the recognition of microbiota-derived PAMPs by PRRs of innate immune cells or their BM precursors.[133,134] The function of neutrophils in this context as well as how antibiotic-mediated killing of microbiota affects granulopoieisis and the number of peripheral blood neutrophils is not entirely clear. The application of a combination of antibiotics either before birth and at the neonatal period[135] or during adulthood[136] in mice results in decreased neutrophil numbers in the BM and blood. Furthermore, Karmarkar et al. showed that zymosan-induced neutrophil infiltration into the peritoneum was impaired in germ-free or antibiotics-treated mice.[133] The reduced numbers of neutrophils associated with the absence of microbiota result in profound changes in the protection of neonatal mice against common infections.[135] The deletion of microbiota by antibiotics resulted in increased susceptibility of mice to *E. coli* or *Klebsiella pneumoniae* infection, and this was correlated with the diminished counts of neutrophils in the periphery.[135] Apart from the defects in the number of neutrophils caused by the absence of microbiota, there is also an important alteration to their function. The potential role of microbiota to inducing trained immunity in neutrophils is evident by experiments, in which neutrophils from the BM of antibiotic-treated or germ-free mice show reduced phagocytic capacity and as a consequence reduced killing of *S. pneumoniae* and *S. aureus* in vitro.[134] Neutrophils can sense microbiota either directly through the PRRs they express, like TLR2 and TLR4,[137] or indirectly by recognizing cytokines produced by other immune cells in response to microbiota products.[138,139] For instance, the microbiota-induced activation of mouse neutrophils is mediated by the TLR-MyD88 signaling axis.[133] The intracellular PRR NOD1 is also implicated in training of neutrophils by the microbiota. In vitro training of neutrophil-like HL-60 cells with a NOD1 ligand, specifically a fragment of peptidoglycan (MurNAcTriDAP), increases the killing capacity of these cells against *S. pneumoniae*. Moreover, neutrophils from *Tlr4*$^{-/-}$ and *Nod2*$^{-/-}$ mice lose their enhanced in vitro killing capacity for *S. pneumoniae* and *S. aureus* only after treatment of these mice with antibiotics.[134] Therefore, microbiota-derived PAMPs are directly sensed by neutrophils through TLR and NOD receptors, although future studies engaging neutrophil-specific deletion of these PRRs are required to further demonstrate the cell-intrinsic importance of these pathways in neutrophil responses. Additionally, non-direct sensing of microbes by neutrophils may also exist. For instance, following recognition of microbes by TLR4, group 3 innate lymphoid cells (ILC) increase the production of IL-17A and granulocyte-colony stimulating factor (G-CSF) that promote granulopoiesis in the BM, thereby increasing the numbers of peripheral neutrophils in mice. This IL17A/G-CSF-dependent enhancement of neutrophils is a critical factor providing protection against *E. coli* and *Klebsiella pneumoniae* sepsis.[135]

Moreover, the microbiota may promote an aged phenotype in mouse neutrophils that is characterized by decreased expression of CD62L, increased expression of CXCR4,[136] and decreased proliferation.[140] Compared to young neutrophils, aged neutrophils display increased expression of factors involved in adhesion and migration, such as lymphocyte function-associated antigen-1 (LFA-1), macrophage-1 antigen (Mac-1), PECAM-1/CD31, CD44, very-late-activation antigen-4 (VLA-4), ICAM-1, as well TLR-4.[140] Consistent to a more active phenotype, microbiota-induced aged neutrophils stimulated with LPS produce increased levels of ROS and NETs.[136] The hyperactive phenotype of aged neutrophils and the increased NET production contribute to exacerbated organ damage of mice in the context of endotoxin-induced inflammation.[136] Additionally, aged neutrophils promote the pathology of sickle cell disease in an animal model; accordingly, the depletion of microbiota limits the expansion of aged neutrophils and improves disease outcome.[136] In another study, mouse-aged neutrophils, exposed to PAMPs (LPS or lipoteichoic acid) or DAMPs (e.g., high-mobility group protein 1; HMGB1), upregulate Mac-1 and TLR-4 and present a significantly higher phagocytic potential, as compared with non-aged neutrophils.[140]

Recently, it was demonstrated that COVID-19 infection can also induce central trained immunity with increased granulopoiesis. Four to 12 months after COVID-19 infection, single-cell transcriptomic and epigenomic analysis of the peripheral blood mononuclear cells enriched in CD34^{+} HSPCs of affected individuals revealed that that progenitor cells retain their granulopoiesis bias.[141] Specifically, HSPCs from COVID-19-recovered patients demonstrate higher expression of S100A8/A9 and CSF3R and increased chromatin accessibility of these loci. Moreover, mature monocytes retain the altered chromatin accessibility found in progenitor cells and present an increased Type I IFN signature.[141] Whether COVID-19-induced epigenetic imprinting in innate immune cells or their progenitors can affect the outcome of future infections with SARS-COV-2 or heterologous pathogens needs to be addressed.

As outlined above, innate immune training may also be induced by nonmicrobial DAMPs besides PAMPs.[9] For instance, heme, which acts as a DAMP, may trigger trained immunity. Administration of heme to mice promotes a myeloid bias by increasing the frequency of myeloid-biased multipotent progenitors (MPP3) and decreasing the lymphoid-biased multipotent progenitors (MPP4), associated with higher neutrophil numbers in the BM.[32] Furthermore, heme induces long-lasting epigenetic changes in the LSK compartment.[32] Heme-induced trained immunity results in higher neutrophil and monocyte infiltration into the peritoneum following a secondary LPS challenge.[32] Trained immunity induced by heme is protective against polymicrobial sepsis when heme is given 7 but not 28 days prior to the secondary sepsis challenge.[32] These findings indicate that many not yet understood factors may regulate the responses of trained innate immune cells to subsequent challenges.

As neutrophils also exert a pathologic role in various inflammatory processes, such as thrombosis, atherosclerosis, periodontitis, or autoimmunity,[142,143] it is conceivable that maladaptive trained immunity may facilitate such pro-inflammatory and pathological functions of neutrophils in several contexts.

Nonmicrobial triggers are frequently linked with induction of maladaptive trained immunity in innate immune cells, including

neutrophils. Tumors may hijack neutrophils to adopt a tumor growth-promoting phenotype.[144] Along this line, the in vivo exposure of mouse neutrophils to the microenvironment of mammary tumors increases, via a G-CSF-mediated mechanism, the in vitro and in vivo production of NETs upon re-stimulation with LPS, thereby promoting a pro-thrombotic effect.[145] Another important nonmicrobial training signal is hyperglycemia, the cardinal symptom of diabetes. Neutrophils isolated from blood of patients with type 1 or type 2 diabetes produce more NETs following ex vivo activation with phorbol-12-myristate-13-acetate (PMA) compared to those of healthy individuals and this is linked with the exposure of neutrophils to increased concentrations of glucose.[146] The hyperglycemia-induced training of neutrophils contributes to the delayed wound healing, a major clinical problem in diabetes. Indeed, diabetic mice display NET-dependent impaired wound healing capacity that is reversed upon deletion of PAD4, a calcium-dependent enzyme that is a key factor in mediating NETosis.[146] Based on the increased NET production of neutrophils when pre-exposed to hyperglycemia and the important role of NETs in the elimination of infectious microorganisms, one might hypothesize that hyperglycemia may have a beneficial role in the protection against infections. Nevertheless, this is not the case as neutrophils isolated from patients with type 2 diabetes produce more NETs (when activated in vitro with PMA or A23187) with diminished capacity to kill carbapenem-resistant and hypervirulent *K. pneumoniae* strain, compared to controls.[147] These findings potentially explain the increased susceptibility of type 2 diabetes patients to infection with such virulent bacteria.[147] The mechanisms underlying such maladaptive effects of hyperglycemia on neutrophils and whether they involve trained immunity-related epigenetic programs has not been elucidated yet and requires further investigation. Neutrophils are, however, central in a diabetes/hyperglycemia-mediated feed-back loop to the BM. Specifically, in diabetic mice, the numbers as well as the proliferation of GMPs and CMPs in the BM are increased. Hyperglycemia skews BM progenitor cells to preferentially differentiate toward granulocytes and monocytes, resulting in higher numbers of neutrophils.[148] Neutrophil-derived S100A8/A9 are in turn involved in the activation of CMPs and the production of colony stimulating factors that further potentiate GMP proliferation and consequently the augmented production of mature myeloid cells.[148]

Chronic low-grade inflammation is considered a factor promoting the development of inflammatory comorbidities. Repetitive injections of low dose of LPS are an animal model of low-grade inflammation results in neutrophil-dependent exacerbation of atherosclerosis.[149] Specifically, repetitive transfusion of neutrophils trained in vitro with low dose LPS-treatment into high fat diet fed $ApoE^{-/-}$ mice leads to increased plaque size, enhanced lipid content and reduced plaque stability.[149] LPS-mediated low-grade inflammation increases the number of neutrophils in blood, spleen, and atherosclerotic plaques of treated mice and promotes their activation phenotype with enhanced expression of CD11b, dectin-1 and reduced expression of CD62L. Neutrophils chronically exposed to low-dose LPS adopt a non-resolving inflammatory phenotype with elevated ROS production and increased expression of leukotriene B4 (LTB4), matrix metallopeptidase 9 (MMP9) and miR24 and reduced expression of homeostatic resolving mediators such as leucine-rich repeat containing 32 (LRRC32) and miR-126.[149]

Chronic low-grade inflammation has also been linked with modulation and potentially training of myelopoiesis.[60] Coronary artery disease (CAD) leads to increased MPPs and pre-monocytes ($CD45RA^{+}CD123^{-}$) in the BM of patients.[150] Transcriptional studies of HSCs, MPPs, and GMPs show a clear myeloid lineage bias with upregulation of pathways related to neutrophil and macrophage activation, cytokine synthesis and elevated expression of genes participating in production and activation of neutrophils, such as insulin-like growth factor two receptor (IGF2R), S100A11 and TNFRSF1B. This myeloid bias does not result in higher neutrophil numbers in the circulation of CAD patients but likely alters their function since total myeloid cells from CAD patients produced increased amount of IL-8 upon in vitro LPS re-stimulation.[150] Moreover, myocardial infarction in mice promotes atherosclerosis progression via a systemic mechanism that involves a feed-back to the BM, IL-1- and/or GM-CSF-dependent enhanced myelopoiesis and production of inflammatory myeloid cells that accelerate cardiovascular inflammation.[151–153] Along the same line, western diet feeding of mice induces central trained immunity and increases the capacity of BM cells and splenic myeloid cells to respond to secondary stimuli, for example, to LPS ex vivo.[36] Western diet induces epigenetic and transcriptomic changes in GMPs toward a more activated phenotype; additionally, increased numbers and a more activated phenotype of monocytes and granulocytes are observed in the spleen of western diet-fed mice. The aforementioned effects of western diet are sustained even after switching mice to normal diet, clearly suggesting induction of detrimental trained immunity.[36] Feeding mice with a high-fat diet increases the numbers of LT-HSC, MPP, GMPs, as well as the potential for granulocyte and macrophage differentiation. Nevertheless, only increased levels of pro-inflammatory macrophages are detected following a switch to normal diet while neutrophils are unaffected.[154]

As alluded to above, besides metabolic pathologies, inflammatory diseases, such as periodontitis, a dysbiosis-related inflammatory disease of the tooth-supporting tissues[155] or arthritis, may also induce detrimental central trained immunity involving neutrophils.[72] Periodontitis and associated bacteremias may cause systemic inflammation in humans with increased levels of inflammatory cytokines, such as IL-1 or IL-6, as well as elevated blood neutrophils, which display a hyper-responsive phenotype with increased generation of inflammatory mediators or ROS upon ex vivo re-stimulation.[100,156–158] This hyper-responsive neutrophil phenotype may persist even after successful treatment of periodontal disease, thus indicative of innate immune memory.[159] Additionally, in humans periodontal inflammation positively correlates with BM hematopoietic activity, hence, suggesting increased myelopoiesis.[160] Interestingly, in patients with rheumatoid arthritis hematopoietic activity may remain higher in the BM despite clinical remission.[161] These observations imply development of maladaptive BM-mediated innate immune memory with perpetuated myelopoiesis and granulopoiesis in periodontitis

and arthritis that may promote their chronicity as well as their comorbidity. Indeed, we could recently show that both experimental ligature-induced periodontitis (LIP) and collagen antibody-induced arthritis (CAIA) promote myelopoiesis and granulopoiesis with enhanced numbers of HSPCs and particularly myeloid-biased CD41^{+} LT-HSCs and MPP3, as well as GMPs and neutrophils in the mouse BM. Although the quantitative expansion of HSPCs, GMPs, and neutrophils is reversed upon resolution of inflammation by ligature removal, LIP-trained mice display a more robust response to secondary systemic LPS administration.[72] In addition, neutrophils and monocytes of LIP-exposed mice produce increased amounts of IL-6 and TNF upon in vitro re-stimulation with LPS.[72] LIP-induced central trained immunity is mediated by IL-1-signaling in HSPCs and characterized by epigenetic rewiring of HSPCs with enhanced accessibility of genes related to myeloid differentiation, granulocyte differentiation, and inflammation.[72] Transplantation of BM from LIP-trained mice to naïve recipient mice not only exacerbates periodontitis but also arthritis development in the latter, as compared to BM transfer from non-trained donors.[72] Thus, neutrophils are effectors of periodontitis-induced maladaptive central trained immunity leading to chronicity of inflammation and inflammatory comorbidities (Figure 3). Intriguingly, BM neutrophils may also act as mediators of the periodontitis-induced central inflammatory memory. Specifically, enhanced gingival production of G-CSF due to periodontitis reaches the circulation and stimulates BM neutrophils to secrete IL-1β, which in turn acts on BM HSPCs to induce their training.[72] It becomes evident that the potential role of neutrophils as both effectors and mediators of beneficial or maladaptive innate immune memory induced by different microbial or host-derived triggers, including metabolic or inflammatory conditions, requires detailed mechanistic investigation in the future.

8 | FACTORS MODULATING THE OUTCOME OF TRAINED INNATE IMMUNITY

It is obvious from the aforementioned examples that trained immunity in general and neutrophil training in particular may be beneficial for the defense against infectious agents or against tumors but can also lead to increased inflammation and cardiometabolic diseases.[70] The factors that regulate this fine balance between beneficial and maladaptive inflammatory memory and neutrophil training remain largely unexplored. The strength of the training signal may play a critical role in the outcome of neutrophil training, as is implied by in

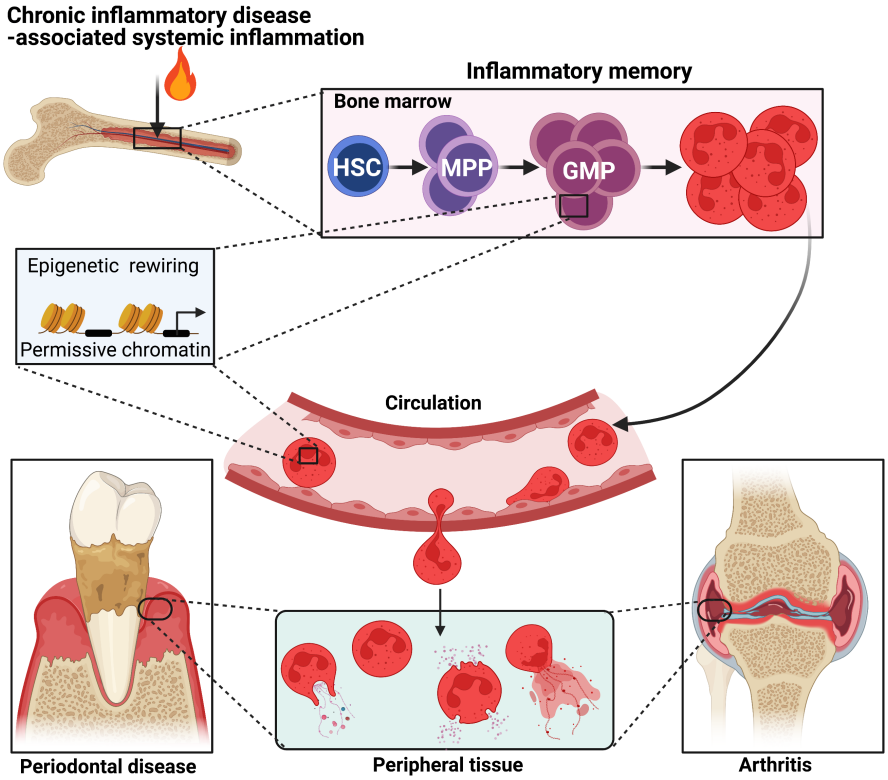

FIGURE 3 Maladaptive training of neutrophils in the BM contributes to the emergence of inflammatory comorbidities. Systemic inflammation induces epigenetically based inflammatory memory in HSPCs in the BM. Inflammation-adapted (trained) progenitors give rise to elevated production of neutrophils (and myeloid cells in general) with enhanced immune responsiveness to subsequent infectious or inflammatory challenges. These trained or hyper-responsive neutrophils infiltrate peripheral tissues, such as the periodontium and the joints, and exacerbate inflammatory disorders, such as periodontitis and arthritis. Because both periodontitis-induced and arthritis-induced systemic inflammation lead to inflammatory memory in the BM and increased susceptibility to either disease, it is concluded that maladaptive innate immune training of BM hematopoietic progenitors links distinct inflammatory comorbidities. GMP, granulocyte-monocyte progenitor; HSC, hematopoietic stem cell; MPP, multipotent progenitor.

vitro studies inducing training of mouse BM neutrophils with a big range of LPS concentrations,[162] with the component of gram-positive bacteria lipoteichoic acid[163] and with isolated small gut microbiota-derived extracellular vesicles.[164] The aforementioned studies demonstrate that exposure of neutrophils to low concentrations of the training agent enhances the production of pro-inflammatory molecules, such as TNF, IL-6, MCP-1 or IL-1β, generation of ROS, and their phagocytic and migratory capacity upon restimulation, while high concentrations of the training agent result in increased production of anti-inflammatory agents, such as IL-10, as well as impaired phagocytosis and migration in vitro.[162–164] The route of administration of the training factor may also determine the subsequent responses and outcomes. For instance, intravenous administration of BCG vaccine to mice directs the accumulation of BCG in the BM and promotes expansion of HSPCs, while subcutaneous administration of the same vaccine does not affect HSPC numbers.[75] Interestingly, the duration of the training effect induced by a COVID-19 infection was proportional to the disease severity.[141] In humans, the training outcomes may be modulated by genetic predisposition as well. Polymorphisms in *HNF1A*, *HNF1B*, *GATA2/3*, *GFI1/1B*, *HOXA4*, and visual system homeobox 1 (*VSX1*) alter the responses of previously BCG or β-glucan ex vivo trained monocytes to a subsequent LPS-stimulated production of IL-6 and TNF. A detailed analysis for the identification of predictive biomarkers that could forecast the efficiency of the innate immune training response was also conducted. The elevated plasma levels of CCL20 and CCL23 correlated with the elevated production of IL-1β and IL-6 by BCG vaccine-trained peripheral blood mononuclear cells re-exposed in vitro to *C. albicans*.[125]

9 | INNATE IMMUNE TRAINING INDUCERS IN THERAPEUTIC APPLICATION AGAINST CANCER

The training agonist BCG is used as an adjuvant therapy for bladder cancer for over 40 years now[165]; BCG immunotherapy is successfully used for the therapy of non-muscle invasive bladder cancer.[166,167] The therapeutic effect of BCG is attributed, at least in parts, to neutrophils since their deletion abrogates the BCG-mediated anti-tumor function in mice.[168] Neutrophils are the first cells that infiltrate the bladder after BCG treatment where they produce high amounts of chemokines and cytokines and direct trafficking of T cells and other immune cells to the bladder.[168,169] Moreover, human and mouse neutrophils exhibit direct tumor killing capacity by releasing increased amounts of TRAIL and upregulate NETs production after BCG treatment.[170,171]

β-glucans are also used for cancer treatment in experimental models for decades now, long before their characterization as innate immune training agonists.[172] In most cases, β-glucan-based regiments are currently combined with other immunotherapies, anti-angiogenic or tumor cell-targeting therapeutics.[173] Based on the success of such combinatorial approaches in syngeneic mouse tumor models[174,175] and human xenograft models,[176,177] clinical trials are underway using β-glucan in combination with further anti-cancer therapies, such as immunotherapies or anti-angiogenic antibodies targeting vascular endothelial growth factor.[178,179] Neutrophils play a central role in anti-tumor-immunity induced by the β-glucan-related therapies.[37,180,181] For instance, adoptive transfer of neutrophils from β-glucan-trained mice inhibits tumor growth, suggesting that β-glucan-trained neutrophils may represent an adjuvant cancer immunotherapy.[37] Treatment with β-glucan results in upregulation of complement receptor 3 (CR3, alternative name for the Mac-1 integrin) on human and mouse neutrophils in the tumor microenvironment; neutrophils thereby mediate killing of iC3b-opsonized tumor cells.[182,183] Moreover, β-glucan primes mouse neutrophils to efficiently kill tumor cells and produce inflammatory cytokines that will further promote T-cell-mediated anti-tumor immune responses.[184]

Recently, Priem et al. developed a novel anti-cancer nanobiologic based on the apolipoprotein A-1 component of HDL and muramyl tripeptide phosphatidylethanolamine (MTP10-HDL).[185] This nanobiologic presents high BM avidity and induces central trained-immunity that protects mice from melanoma growth and increases immune checkpoint inhibitor anti-tumor efficacy. MTP10-HDL treatment increases myelopoiesis concomitantly with the numbers of neutrophils in the BM and tumor. MTP10-HDL-induced trained immunity results in transcriptomic and epigenetic changes of BM progenitor cells and enhanced in vitro responses of mature myeloid cells upon re-activation with LPS.[185]

10 | CONCLUSIONS AND OPEN QUESTIONS

Neutrophils contribute to both acute inflammation and chronic inflammatory diseases.[143,186] Akin to other myeloid cells, for example, monocytes/macrophages, neutrophils are also an integral component of innate immune memory. In this context, generation of trained neutrophils with enhanced inflammatory preparedness may be the result of central BM-mediated trained immunity and epigenetic rewiring of granulopoiesis.[60] Innate immune training of myeloid cells, including neutrophils, may elicit both beneficial as well as detrimental responses in a context-dependent manner. For instance, inflammatory memory can enhance neutrophil-mediated pathogen defense or anti-tumor actions whereas it may also promote tissue injury and inflammation chronicity.[60] At this point, several questions are open and require experimental investigation in the future. For instance, it is currently uncertain whether neutrophils are solely effectors or also mediators of the enhanced immune responses associated with trained immunity. Moreover, can specific training agents preferentially result in neutrophil-related trained immunity, as opposed to broader effects on several myeloid cells? Given the role of neutrophils and other myeloid cells in tissue homeostasis and repair,[187] can trained immunity affect tissue repair processes? Can trained immunity affect tissue homeostasis? What mechanisms underlie the development of protective versus maladaptive trained immunity? Ostensibly, microbial

products may induce trained immunity in neutrophils associated with protective and beneficial outcomes, while sustained chronic inflammation seemingly promotes maladaptive inflammatory memory in granulopoiesis.[37,72,124] In this regard, the conceivable question is whether the duration and strength of the training signal determine the training outcome in a quantitative and qualitative fashion. Delineating the factors, variables, and mechanisms that favor the protective versus the detrimental actions of neutrophil-related trained immunity are of great importance for the design of potential future therapeutic interventions.

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CONFLICT OF INTEREST

The authors have no conflict to disclose.

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ORCID

George Hajishengallis https://orcid.org/0000-0001-7392-8852
Triantafyllos Chavakis https://orcid.org/0000-0002-1869-5141

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