Advanced Journal of Graduate Research
ISSN:2456-7108
Volume 8, Issue 1, pp. 68-81, July 2020
DOI: https://doi.org/10.21467/ajgr.8.1.68-81

GRADUATE REVIEW ARTICLE

Trained Immunity at a Glance; A Review on the Innate Immune Memory and its Potential Role in Infections, Diseases and New Therapeutic Strategies

Silvia Incalcaterra[1*], Jorge Dominguez Andres[2]

[1] Faculty of Science, Radboud University, Nijmegen, The Netherlands
[2] Dept of Internal Medicine and Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, The Netherlands

* Corresponding Author email: incalcaterrasilvia@gmail.com

Article History
Received: 30 March 2020
Accepted: 21 April 2020
Published: 25 April 2020

Student(s)
- Silvia Incalcaterra

Academic Year: 2019-2020
Course Level: Master
Course Name: M.Sc. (Medical Biology with specialization in Human Biology)
Course year: 2nd Year

Mentor(s)
- Dr. Jorge Dominguez Andres

ABSTRACT

Despite the existence of two different branches of immunity, innate and adaptive, it has been described that both systems are characterized by the establishment of memory responses. Indeed, it has been shown that cells belonging to the innate immune system can express a so-called "trained" memory, although it has different features from the adaptive immune memory. Adaptive memory is a long-lasting specific memory whereas innate memory involves non-specific responses which enhance the immune response during a second reinfection. However, many aspects of the trained immunity are still unclear. Metabolic and epigenetic reprogramming have been pointed as the two processes responsible for the establishment of the innate memory. Trained immunity seems to be responsible for the heterologous effect of many vaccines such as BCG, thus giving insights for the development of new therapies. Although its potential beneficial role, trained immunity could also have detrimental effects that might worsen the progress of certain diseases. The purpose of this literature review is to provide an in-depth review on the major characteristics of trained immunity, describing the main pathways at the basis of the evolution and establishment of memory in innate cells. In addition, the present review assesses the modern evidence of the impact of trained immunity in health and disease, strengthening the hypotheses that this innate memory may be considered both in the formulation of new therapeutic strategies and in the current therapeutic approaches.

Keywords: Trained immunity, innate immunity, adaptive immunity, innate memory, vaccines, BCG

1 Introduction

Humans live in close contact with pathogens, allergenic substances and toxic compounds which could potentially disrupt the health of the individual [1]. Despite the enormous number of threats deriving from the environment, the homeostasis is continuously balanced through the action of the immune system [2, 3]. The immune system could be described as a complex interaction and networking among cells to produce an effective immune response against causative agents [4]. Host immune response is conventionally divided into innate immunity and adaptive immunity, although these two groups strictly interact with each other. The innate immunity is a rapid and non-specific response against pathogens, whereas adaptive immunity is a slower but antigen-specific response with the capability to establish immunological memory against the causative agents [5]. Different cell types and molecular substrates operate during the innate and the adaptive response; *Figure 1* [6] shows the main components belonging to the two different host immune defense mechanisms. The innate immunity acts as a second line of defense, in physical barriers such as skin, gut microbiota, gastric acids, mucous, saliva and tears [1, 7]. The cells characterizing the innate immunity are granulocytes (basophils, eosinophils and neutrophils), macrophages, dendritic cells (DCs), mast cells and natural killer (NK) cells [6]. NK cells and γδ T cells have a cytotoxic action, and these cells could be defined as either element of the innate or adaptive immunity [6]. In addition, innate immunity comprises a humoral part formed by the complement, a system of more than 30 soluble and surface-expressed proteins, mostly zymogens, and bioactive molecules such as defensins or ficolins [1, 5, 8]. The adaptive immune response, referred as the third line of defense, consists of lymphocytes, classified as B cells, $CD4^+$ T cells and $CD8^+$ T cells, and a humoral part formed by antibodies, which are heterotrimeric glycoproteins called immunoglobulins [6, 9]. The activation of the adaptive immunity is highly dependent on fundamental signals released by the previous activation of the innate immune response [10, 11]. Hence, although the mechanisms of action are different, the interaction between the adaptive and the innate immunity is necessary to achieve an efficient immune response [1].

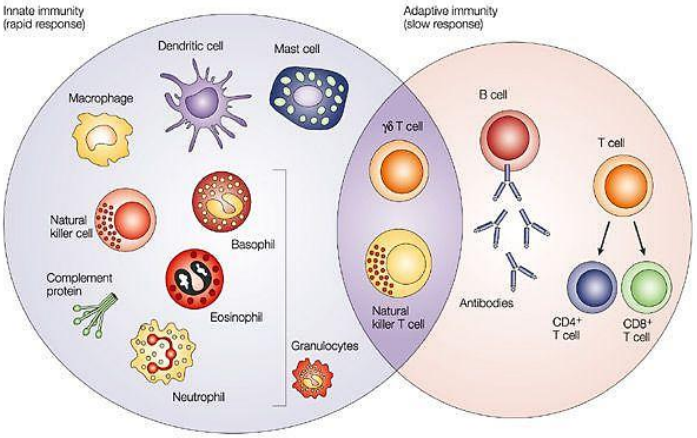

Figure 1: *Schematic drawing of the components acting during innate and adaptive immunity [6].*

2 Innate Immunity

In vertebrates, the innate immune response relies upon myeloid cells that are capable to phagocytose and destroy causative agents [12]. The detection of pathogens by the immune cells takes place via the pathogen-associated molecular patterns (PAMPs), which are components present in a variety of microorganisms but absent in the host [13]. PAMPs are recognized by pattern recognition receptors (PRRs) present on the surface of immune cells [13, 14]. PRRs are divided into five families, such as Toll-like receptors (TLRs), C-type lectin receptors (CLRs), Nacht-LRR receptors (NLRs), RIG-I-like receptors (RLRs), and the AIM2-like receptors (ALRs) [15]. The PRRs could be intracellular cytoplasmic or extracellular transmembrane receptors [11, 15], and their bond with the PAMPs triggers many processes including the actuation of the adaptive immune response. For instance, the detection of PAMPs by Dectin-1 CLR modulates the uptake of the microorganisms via phagocytosis, leading to the production of inflammatory cytokines such as IL-12 [15, 16], a fundamental cytokine involved in the activation of T Helper 1 (Th_1) cells [1]. Indeed, the activation of PRRs initiates signaling cascades that promote the transcription of targeted gene encoding for cytokines, interferons, pro-inflammatory or microbicide proteins [17, 18]. Macrophages, upon the

interaction between the TLR and the PAMPs, produces IL-8, a protein with chemotactic action that induces the recruitment of more immune cells in the site of infection [12]. The secretion of cytokines from the cells of the innate immunity (i.e. IL-12, IL-1 or type-1 Interferon) contributes to the activation of the adaptive immunity [12]. In addition to pro-inflammatory molecules, the action of the antigen presenting cells (APCs) could be considered another fundamental pillar for the synergism of innate and adaptive response. The APCs, such as DCs, monocytes and macrophages, express the MHC class II molecules involved in the presentation of antigen exogenous peptides to the $CD4^+$ T cells in the lymph nodes [12, 19]. Eventually, the activation of T cells enhances the adaptive immune response.

3 Adaptive Immunity

The innate immunity is a fast, non-specific primary response able to act against various pathogens. However, the high variability of antigenic structures and the capability of microorganisms to mutate has led to the implementation of the adaptive immune response [20]. Indeed, the specificity of the adaptive immune response against a certain antigen is the result of tailored receptors produced by a recombination of a large number of genes during B cells maturation [20-23]. Whereas $CD8^+$ T cells have mainly a cytotoxic action, the $CD4^+$ T naïve cells are activated via the presentation of processed antigens on MHC class II molecules by the APCs or the B cells [20, 22]. The release of cytokines by the T cells stimulates the clonal expansion of antigen-specific B cells and the consequent differentiation in plasma cells, which are responsible for the production of specialized antibodies able to opsonize the pathogen in the site of infection [20, 21]. It has been widely described the ability of the cells of the adaptive immunity to develop memory. This memory leads to a faster and more efficient response in the eradication of the pathogen if it establishes an infection in the host for a second time, even after many years [24, 25]. Memory cells also prevent the activation of naïve T and B cells during a second infection with the same antigen, thus increasing the promptness of the response [7]. For instance, T memory cells interact with APCs more rapidly due to the enhanced expression of adhesion molecules on their surface [26]. The concept of memory is the principle upon which vaccines has been designed, although thus far the most successful vaccines are those acting against invariant pathogens [25, 27]. Despite the memory was usually associate with the adaptive immunity, it has been recently shown the capability of the innate immune cells to generate a memory which results in an enhances responsiveness to a secondary challenge caused by either the same or a different pathogen [28, 29].

4 Evolutionary Perspective of Innate Immune Memory

From the appearance of the first eukaryotic cell, immune defense mechanisms have been recognized in every organism. For instance, amoeba could be considered as an ancient phagocyte [7]. It engulfs food recognized by specific receptors which can discriminate the self from the non-self, a principle at the basis of the phagocytosis and, furthermore, at the basis of immunity [7, 30]. It has been shown that the development of innate immunity occurred before the development of adaptive immunity during the evolution of vertebrates [31]. Moreover, adaptive immunity seems to be a unique feature of vertebrates, which comprise only the 1% of the whole living organisms, whereas invertebrates lean solely on innate immunity [23, 32]. Indeed, there is no evidence about the presence of antibodies in more ancestral organisms than in particular species of fish [33]. However, different studies have demonstrated that innate immunity in invertebrates may have the capability to establish a memory barely similar to the adaptive immune system [32]. B. Lemaitre et al. [34] showed that the production of selected antifungal peptides in *Drosophila* underlined an adaptive and specific immune response against fungal infections. Indeed, specificity is the main characteristics at the basis of the development of an adaptive immune response. The specificity of the adaptive immune system is related the somatic recombination of a large cluster of genes that results in a wide diversity of specific receptors, whereas the antigen receptors belonging to the innate immune cells are expressed in the same way as they are codified in the germ line [20, 35]. In the last years, it has been discovered an array of diversified receptors in many species of invertebrates such as echinoderms, mollusks

or crustacean, although these receptors do not generate a clonal selection [23]. In their study, J. Kurtz et al. [32] demonstrated that copepods produce a more successful response during a second infection of a parasite expressing already encountered antigens, thus suggesting that invertebrates might develop immune memory features [32]. This assumption has been consolidated by other findings on innate immune memory in invertebrates such as mosquitoes, tapeworms and *Bombus terrestris* [5]. The development of a memory has been broadly described also in plants. This memory is called defense priming, and it is established both in the harmed tissue and in the unharmed tissue of the plant, generating a stronger defense mechanism upon second stimuli [36]. The spread of the protection among tissues in plants has been termed Systemic Acquired Resistance (SAR), and it establishes a long-lasting protection against a wide range of pathogens [37]. Innate memory has been characterized also in mammals. For instance, mice vaccinated for a certain pathogen can be protected against other non-related causative agents [36]. J. C. Sun et al. [38] demonstrated the development of a memory in NK cells against a second reinfection with the *Cytomegalovirus* in a mouse model. Another strong evidence of the involvement of an innate immune memory is the heterologous effect of BCG vaccine against other pathogens beside the *Mycobacterium spp.* [37, 39]. For instance, BCG vaccine confers protection against secondary infections caused by *Candida albicans* or *Schistosoma mansoni*, and this protection seems to involve activated macrophages in the tissues [37]. In their study, J. Kleinnijenhuis et al. [40] showed that BCG vaccination in healthy volunteers enhanced the production of inflammatory cytokines by the NK cells, and the effect on NK cells appeared to be long-lasting.

The establishment of the innate immune memory relies on similar mechanisms in plants and mammals, i.e. epigenetic changes. The epigenetic modifications resulting from the DNA hypo methylation and histone methylation (H3K4me3) appear to generate long-lasting memory in the innate immune system which becomes more efficient in the context of a second challenge [36]. M. G. Netea et al. [5] suggested a two-steps evolutionary model which could explain the innate immune memory characterizing all the living organisms, but the exclusiveness of the further development of the adaptive immunity in vertebrates. A first evolutionary step is dominated by constant epigenetic modifications. These epigenetic modifications allowed the growth of a memory responsible for the increased efficacy of the immune response upon a second reinfection in all organisms [5]. A second step led to a higher immune response specificity in vertebrates through gene recombination and clonal selection [5]. *Figure 2* [5] shows the two-steps evolutionary hypothesis which could describe the creation of an innate immune memory in plants, invertebrates and invertebrates, giving also an insight on the similarities of the immune memory among all the living organisms. Nevertheless, the differences or the similarities behind the epigenetic modifications triggering a memory in the innate and adaptive immune response are still unclear.

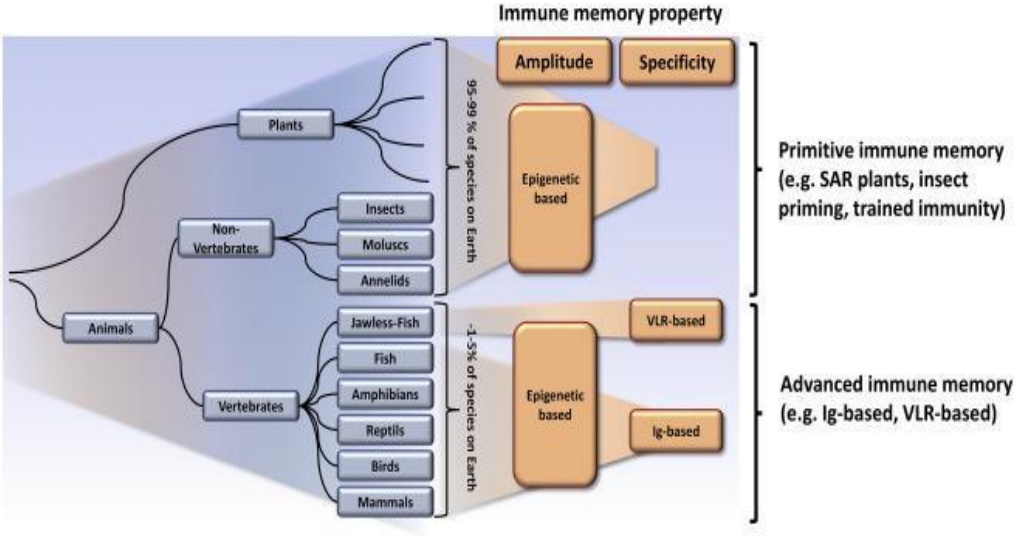

Figure 2: *Schematic representation of the two-steps evolutionary hypothesis of the innate and adaptive immune memory suggested by M. G. Netea et al. [5].*

5 Pillars of Trained Immunity

Netea et al. [41] named "trained immunity" the memory of the innate immune cells. Analyzing this definition, to a certain extent the innate immune cells are trained to remember and better respond to an infection. It is possible to underline two main characteristics of trained immunity that differ from the classical immunological memory of the adaptive immune cells. Firstly, the cells responsible for the trained immunity belong to the innate immune system, and these cells are indeed myeloid cells, NK cells and innate lymphoid cells (ILCs) [41]. Moreover, the memory of the trained immunity does not depend on gene recombination and antigen-specific reaction but it relies on a non-specific response against pathogens induced by epigenetic reprogramming [41]. It has been already discussed the influence of epigenetic changes in the development of the cell memory, which could consequently be considered as a pillar of trained immunity. In addition, beside the evident importance of metabolism in the functioning of the immune cells, it has been described the key role of metabolic pathways in the establishment of a memory both in the adaptive and innate immune system [41, 42]. Hence, metabolism could be regarded as the second pillar that supports trained immunity.

5.1 Metabolic Reprogramming

Regardless of their quiescent or activated status, cells need energy through ATP molecules to carry out their functions. The main substrate necessary for cell metabolism is glucose, which is employed as fuel within two distinct metabolic pathways, such as the cytoplasmic glycolysis and the mitochondrial oxidative phosphorylation (OXPHOS) [43]. Glucose is uptaken by the cell and transformed into pyruvate through glycolysis, generating 2 ATPs per molecule of glucose. Pyruvate could then be converted into lactate or introduced in the TCA cycle inside the mitochondria, hence starting the OXPHOS metabolic pathway. *Figure 3* [42] shows the main steps occurring after the uptake of glucose through the Glut-1 transporter on the cell membrane.

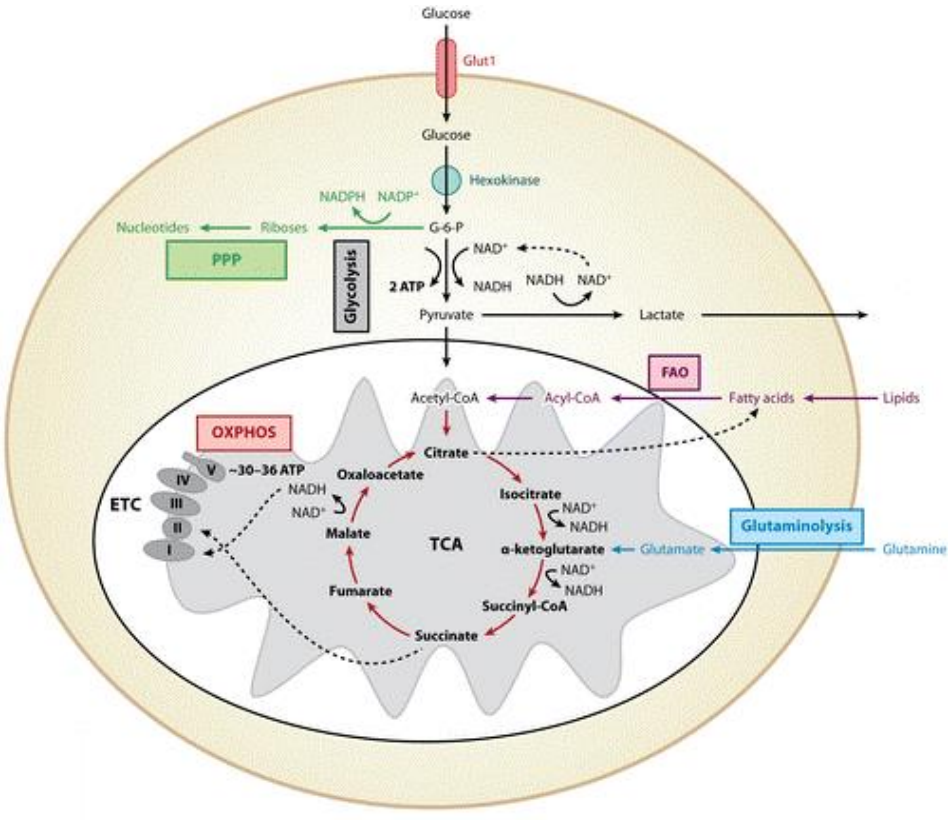

Figure 3: *Overview of the metabolic pathways and intermediate metabolites characterizing the cells [42].*

Via OXPHOS, 30-36 molecules of ATP per molecule of glucose are produced compared to the 2 molecules of ATP produced during glycolysis [42, 44], thus figuring the OXPHOS more favorable in terms of amount of energy. However, glycolysis appears to provide energy faster to the cells that need to promptly act during an early infection. As an example, neutrophils are the first responders to infections, and they utilize glycolysis both in the activated and quiescent status, presenting a very low mitochondrial density [42, 45]. Another example is depicted by macrophages. It has been observed a significant amount of glycolytic enzymes in activated macrophages, underlying a high glycolytic activity, and most of the glucose uptake by resting macrophages is converted into lactate [46]. Moreover, M1 macrophages involved in a prompt immune response rely on glycolysis, whereas M2 macrophages involved mainly in wound healing rely on OXPHOS [43, 46, 47]. Additionally, it has been demonstrated that DCs switch metabolic pathway into glycolysis upon activation with TLR agonists [43]. The activation of immune cells is characterized by an increased rate of glycolysis, resulting in a metabolic switch from OXPHOS [48]. This switching in metabolism is called the "Warburg effect", and it was firstly described as a main feature of the tumor cells which prefer a glycolytic pathway despite the presence of oxygen for a more productive oxidative phosphorylation. This metabolic switch hastens the immune cells activation, improving the capability of innate immune cell to act as "trained" cells. S. Cheng et al. [49] showed that upon stimulation with β-glucan, monocytes shifted the metabolism from OXPHOS to aerobic glycolysis via activation of the AKT-mTOR-HIF-1α pathway. Therefore, this metabolic change boosted the response of β-glucan-trained monocytes to further stimulation [49]. Moreover, the inhibition of mTOR and the glycolytic flux restrained monocyte training, both in mice models and humans [49, 50]. Notwithstanding the metabolic shift, the OXPHOS is still active, and the TCA cycle intermediates have an impact on epigenetic reprogramming which then lead to a trained immunity. High concentration of citrate has been described in inflammatory macrophages, and citrate has a fundamental role in the production of pro-inflammatory molecules such as prostaglandins, reactive oxygen species, and nitric oxide [51]. The metabolites produced during the TCA cycle, such as succinate and fumarate, appeared to be increased in trained macrophages and β-glucan-trained monocytes [48, 50]. Furthermore, the accumulation of succinate and fumarate seems to stabilize the effect of Hif-1α, increasing glycolytic activity [48]. These metabolites deriving from the TCA cycle are involved in the long-term reprogramming of trained immune cells [45]. Indeed, it has been largely described the correlation of metabolites from the glycolysis and the TCA cycle with the regulation of gene transcription, showing that these metabolites function as cofactors for enzymes involved in epigenetic changes, i.e. DNA and histone methyltransferases or histone acetyltransferases [50].

5.2 Epigenetic Reprogramming

Metabolic reprogramming seems to be strictly related to epigenetic changes. Thus, enzymes acting on histones exert metabolites in order to modify histone conformation [45], and the metabolic changes caused by an inflammatory stimulus might be responsible for the epigenetic reprogramming that lead to the "trained immunity" [52]. Genes expression is regulated via chromatin conformation, which makes DNA more or less accessible to transcription factors. Epigenetic chromatin markers have been recently described as dynamically influenced by external signals [53]. The chromatin is compartmentalized in units called nucleosomes, that are octamer structures formed by 2 copies of 4 core histones such as H2A, H2B, H3, and H4 [53, 54]. When nucleosomes are strictly bundled, genes are poorly accessible and the transcription is hindered. In contrast, when nucleosomes are unfolded, genes are easily accessible and the transcription is activated. Histone modifications such as acetylation and methylation enhance or inhibit gene expression through the activity of different enzymes. As an example, methylation at H3K27 and H3K9 with repressive marks and co-repressor enzymes prevents the activation of interferon-response genes in mouse bone-marrow-derived macrophages during the resting state of the cells in the absence of stimuli [55]. However,

histone modifications are reversible, and the inhibition of gene transcription can be counteracted and enhanced. Indeed, gene expression is activated upon external stimuli via the accumulation of epigenetic marks in promoters, which interacts with polymerase II to initiate gene transcription, and enhancers, which are recognized by transcription factors [53, 55]. Methylation at H3K4 and H3K36 induced upon stimulation with bacterial lipopolysaccharide (LPS) makes chromatin more accessible and permissive for the transcription of many genes [53, 55].

It has been described that enzymes involved in histones methylation and acetylation cooperate with intermediate metabolites deriving from the TCA cycle during glycolysis and OXPHOS. For instance, Acetyl-CoA is necessary for histone acetyltransferases, whereas α-ketoglutarate and flavin adenine dinucleotide (FAD) act as cofactors for DNA and histone demethylases [45, 54]. Encountering the pathogen promotes the modification of histones in monocytes and macrophages, altering the genes expression after a second stimulation [45]. Hence, histone modifications are considered the second fundamental building block of trained immunity together with the metabolic changes. In their study, J. Quintin et al. [56] underlined that the trimethylation of H3K4 (H3K4me3) is enhanced and stabilized in β-glucan-trained monocytes. The enrichment of H3K4me3 at promoter regions characterizes trained immunity, increasing the transcription of genes encoding for proinflammatory cytokines, and it has been described as a feature of the heterologous effect of the BCG vaccine [57]. Moreover, J. Quintin et al. [56] demonstrated that H3K4me3 and acetylation of H3K27 (H3K27ac) observed in monocytes after the first stimulus with β-glucan were correlated with a metabolic switch into glycolysis. Histone modifications such as H3K4me3 and H3K27ac have been pointed as a consequence of metabolic changes within the cells, suggesting fumarate as one of the major metabolites from the TCA cycle responsible for epigenetic reprogramming [50]. To consolidate the interplay between metabolic and epigenetic reprogramming, it has been described that by blocking glycolytic enzymes in trained monocytes, the histone marks H3K4me3 and H3K9me3 characterizing trained immunity are inhibited [28]. The stronger innate immune response upon second stimulation seems to be associated with the presence of latent or *de novo* enhancers [41, 57]. The scheme represented in *Figure 4* [41] describes the concept of *de novo* enhancers. After the first stimulation H3K4me3, the chromatin mark is only partially removed, with the establishment of a latent enhancer that allows a faster gene transcription and therefore a faster immune response during a reinfection. Indeed, the mono methylation of H3K4 (H3K4me1) kept slightly opened the chromatin, thus more accessible to transcription factors. Many studies have been done to characterized the mechanisms generating trained immunity. However, more insights are needed to deepen the knowledge on these mechanisms and further develop new treatments in diseases correlated with the immune system.

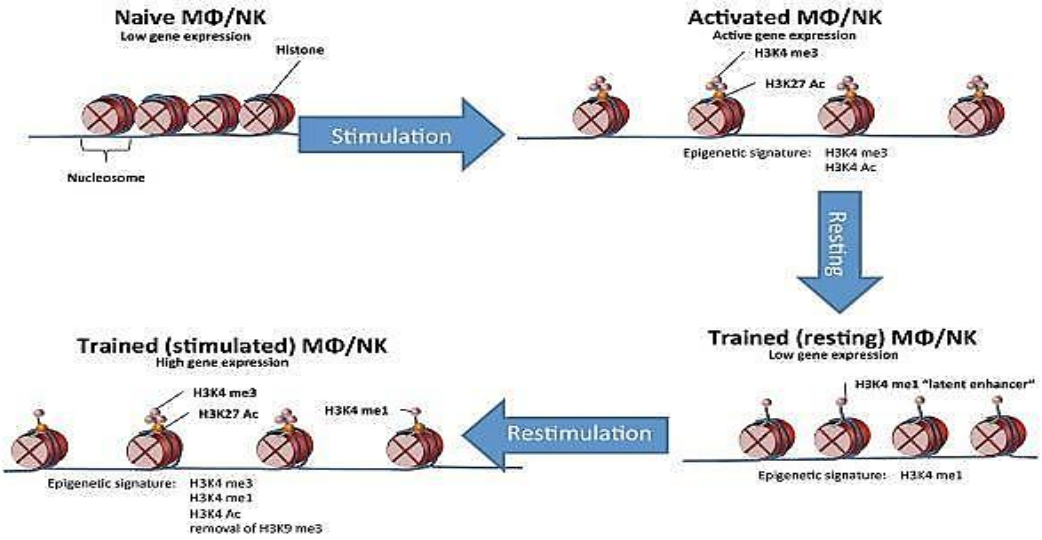

Figure 4: *Epigenetic changes of trained immunity characterized by the establishment of latent or de novo enhancers [41].*

5.3 Potential of Trained Immunity to Enhance Therapies

The establishment of trained memory enhances the innate immune response, thus resulting in a faster elimination of the infection. Additionally, this memory is characterized by cross-protection during a secondary challenge caused by either the same or a different pathogen [58]. The role of trained immunity in the enhancement of protection against infection could be identified in the non-specific protection induced by different vaccines. One of the most studied vaccines presenting non-specific protection is the Bacillus Calmette-Guérin (BCG) vaccine. The BCG vaccine is a live attenuated vaccine consisting of *Mycobacterium bovis*, and it is largely used against tuberculosis infection [59]. Different studies showed a decreased in neonatal mortality in infants vaccinated with BCG compared to non-vaccinated infants, data that could not be associated only as a result of protection against tuberculosis [49]. Therefore, it has been proposed an involvement of trained immunity which could be responsible for the cross-protection of vaccines against infections. In their study, J. Kleinnijenhuis et al. [60] reported a higher released of cytokines in response to unrelated pathogens in healthy volunteers after BCG vaccination. Moreover, they showed that Severe Combined Immunodeficiency (SCID) mice were totally protected against disseminated candidiasis after BCG vaccinations, with a 100% survival compared to 30% survival of non-vaccinated SCID mice [60]. They demonstrated that the mechanism through which BCG vaccine increases the innate immune response is a NOD2-mediated epigenetic change at the level of histone methylation (H3K4me3) [60]. M. Parra et al. [61] found that BCG vaccines induce expression of 4 host genes encoding for fundamental antimicrobial peptides, such as lactoferrin and cathelicidin-type peptide. These peptides reduce parasitemia in mice infected with the malaria parasite [61]. Another study supported the activity of BCG vaccine against malaria parasites and tegumentary leishmaniasis [62]. Furthermore, BCG vaccines seem to improve NK cells activity in humans [63]. In addition, peripheral blood mononuclear cells (PBMCs) revealed an upregulation in glycolysis after BCG vaccination [64]. BCG vaccine is also employed in the treatment of bladder cancer. In the guidelines of the European Association of Urology (EAU) BCG is recommended for intermediate-risk patients failed for intravesical chemotherapy and as the first treatment choice in high-risk patients [65].

The mechanism through which BCG vaccine stimulates trained immunity remaines unclear. However, a potential role of autophagy has been hypothesized. The autophagy of BCG by monocytes and macrophages might lead to a released of MDP that activates NOD2, which then induces epigenetic reprogramming of the cells [63]. It has been also shown that trained immunity impact hematopoietic progenitors and myelopoiesis, suggesting that trained immunity has an effect both on the mature myeloid cells and precursor cells [66]. Indeed, I. Mitroulis et al. [66] proved that β-glucan-induced trained immunity promotes hematopoietic stem cells expansion and myelopoiesis, thus conferring a protective response to a second stimulus.

Besides BCG vaccine, other vaccines have been reported to confer a non-specific protection. Vaccinia has been largely used to vaccinate against smallpox, an infectious disease declared eradicated by the World Health Organization (WHO) in 1980. Non-specific protection of vaccinia upon melanoma and non-Hodgkin lymphoma has also been described [63]. Studies conducted in Africa underlined a lower mortality risk in adults vaccinated against smallpox [58]. G. O. Gillard et al. [67] demonstrated that NK liver cells primed with vaccinia virus conferred protection in mice lacking of adaptive immune cells. Another example is influenza vaccine, which appears to induce secondary protection to respiratory infections in children [58]. Moreover, influenza vaccine might be promising in the reduction of post-operative metastatic diseases via the enhancement of NK cells [68].

Trained immunity has recently been studied for the development of Trained Immunity-based Vaccines (TIbVs). *Figure 5* [58] represents how TIbVs could result in a broader protection against infections through the stimulation of both trained and adaptive immunity. TIbVs are composed by TI inducers and Antigens (Ags). TI inducers could be defined as a mixture of PAMPs recognized by a variety of PPRs. Once activated,

PRRs trigger epigenetic and metabolic reprogramming, upregulation of glycolysis and production of pro-inflammatory cytokines, leading to trained immunity, which confer a non-specific protection against pathogens. Ags are specific antigens related to the pathogen which trigger the activation of adaptive immunity, hence resulting in a specific immune response.

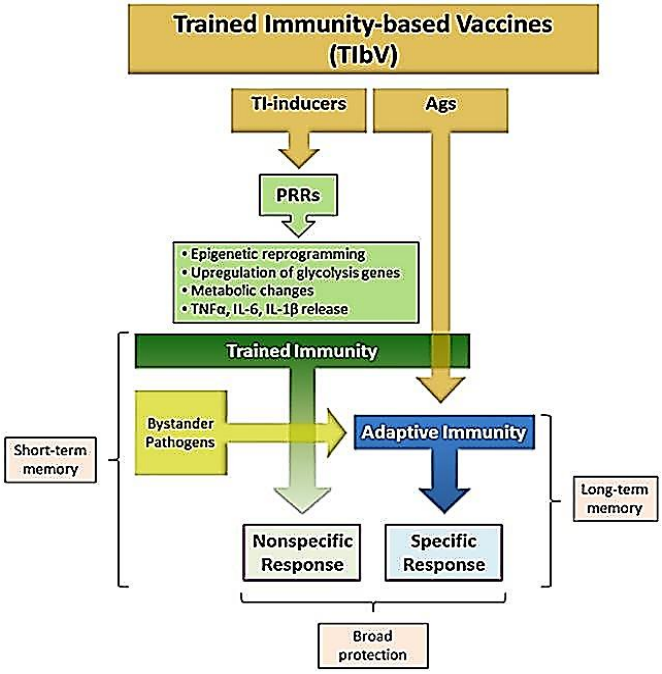

Figure 5: *Overview of Trained Immunity-based Vaccines composition and functioning [58].*

A variety of PRRs seems to be promote trained immunity inducing a change in cellular metabolism by acting on CLRs or NLRs [58]. Therefore, new combinations of PRRs as TIbVs formulas could lead different trained immunity effects and a more efficient therapeutic result. Moreover, the primed functional state of trained cells appears to last long, and it could provide protection to causative agents that comes into contact with the host during this period of time [58]. As a matter of the fact that enhanced innate immune response to reinfection has been described in organisms lacking adaptive immunity and in SCID mice [60, 69], one could speculate that TIbVs could be useful for the treatment of patients with adaptive immunodeficiency. Moreover, these vaccines could be useful against infections that do not have a conventional prophylaxis yet, or they could be used to prevent illnesses derived from virus and bacteria co-infections [58]. However, there is still uncertainty about the lasting of the innate immune memory compared to the lifelong memory and consequent immunization provided by the adaptive immune cells. Indeed, it is claimed that the effect of trained immunity may last for maximum few months rather than many years [70].

5.4 Detrimental Activity of Trained Immunity

The beneficial role of trained immunity in the development of new therapeutic strategies is a new field of study that could improve treatments against infections and diseases. However, the enhanced response of the immune cells has been also described as potentially detrimental. The first scenario that underlines detrimental effects mediated by trained innate immunity is represented by organ rejection after transplantation. The innate immune system is able to discriminate self and non-self molecules. The recognition of non-self molecules initiates a fast inflammatory response and the differentiation of APCs, which present the non-self antigen to the adaptive T cells [71]. Moreover, studies in mammals showed the so called alloimmunity, such as the triggering of the immune response to cells or tissues belonging to members the same species, and it might occur during organs and bone marrow transplantation or even during pregnancy [71]. Hence, to prevent organ rejection, the main suppressive immune therapies target the T cells activation by inhibiting essential signals such as antigen presentation, co-stimulation or cytokine

productions [72]. However, the long-term survival rate is still unsatisfactory [72]. M. H. Oberbanscheidt et al. [71] demonstrated that allogeneic grafts trigger proliferation and differentiation of monocytes into DCs in lymphoid-cell deficient mice, thus suggesting a fundamental role of innate immune system in organ transplantation. Another finding supported the monocytes alloimmune response which is independent from T, B and NK cells [73]. In addition, the monocytes manifests memory and they need prior priming with allogeneic non-self [73]. According to additional data, infiltration of monocytes and macrophages characterize the rejection in T-cell depleted human renal allograft recipients, reaffirming the involvement of the monocytes in the initiation of the alloimmune response [72]. A role of macrophages in alloimmunity has been described. The epigenetic reprogramming of macrophages and the consequent establishment of a nonpermanent innate memory appears to be triggered not only by PAMPs but also by damage-associated molecular patterns (DAMPs), usually released during tissue damage and after organ and tissue transplantation [71, 72].

Atherosclerosis is a chronic inflammatory disease in the artery wall, and it consists in a sub-endothelial accumulation of plasma lipoproteins which results in infiltration of macrophages and T_{h1} cells to form a plaque [74]. Macrophages constantly phagocyte the lipoprotein, thus becoming the so-called foam cells. Risk factors are identified in diabetes, smoke, hypertensions, chronic kidney disease (CKD) and a role of the immune system in the progression of the disease is emerged [74, 75]. New evidences linked trained immunity as a potential contributor to the chronic inflammation status of the blood vessels wall. S. Bekkering et al. [76] showed that exposure of modified LDL to monocytes-derived macrophages induced a long-lasting pro-inflammatory phenotype due to epigenetic reprogramming. Trained immunity as a potential cause of the chronic inflammatory status could be a novel pharmacological target in the treatment of the disease [76]. The same epigenetic reprogramming in monocytes-derived macrophages has been described upon stimulation with Lp(a) [77]. Hence, modified LDL and Lp(a) induce the epigenetic reprogramming and training of macrophages which then intensify their inflammatory response, resulting in a worsen inflammation [78].

Sepsis is the consequence of an exaggerated release of inflammatory cytokines in response to an infection, and it may lead to a septic shock [79]. In physiological situations, the infection induces a pro-inflammatory response to remove the causative agent and afterwards an anti-inflammatory response to avoid hyper inflammation and consequent tissue damage. The pro and anti-inflammatory response are balanced. However, if the pro-inflammatory response is excessive, the balance is disrupted and the responsiveness of the anti-inflammatory pathways becomes abnormal [79]. Therefore, after 24-48 hours of hyper inflammation, there is an excessive activation of anti-inflammatory responses which leads to an immunosuppression known as immunoparalysis [45, 79]. A major threat for patients in the immunoparalysis state is represented by secondary infections that may lead to death. There are divergent findings on the suppressive action of sepsis on hematopoiesis [80, 81]. However, K. Bomans et al. [80] underlined an enhanced hematopoiesis in post-septic mice. Moreover, they demonstrated that monocytes isolated from the bone marrow showed a higher responsivity upon second stimulation, thus indicating a trained immune state after sepsis at the bone marrow level [80]. Nevertheless, immunoparalysis after sepsis is related to innate immune tolerance, and this phenomenon has been largely studied via the endotoxin tolerance with lipopolysaccharides (LPS) [82]. In this latter case, one could speculate that inducing a trained immune state might avoid an exaggerated anti-inflammatory response.

Trained immunity increases the responsiveness of the innate immune system to secondary stimuli [45], resulting in a positive and faster eradication of infections or in aggravated conditions. The detrimental roles of trained immunity in different medical conditions are becoming more and more evident. Further studies are required to deepen the knowledge of the innate immune memory. Consequently, new therapeutic approaches based on metabolic, epigenetic and immunological pathways targeting trained immunity could be developed to increase and promote the health of patients.

6 Conclusions

The immune system comprises innate and adaptive immunity. The innate and the adaptive immunity have always been described as two different systems with specific characteristics and functions. However, in the past decades the immune system has been redrawn as the result of sophisticated interactions among innate and adaptive cells, and these cells may share a common feature, such as memory. Memory has always been related to adaptive immunity, leading to a specific response to a reinfection caused by the same pathogen. This principle drove to the development of vaccines, which improved the immunization against certain pathogens and, in some cases, allowed the eradication of them. In recent years, the discovery of innate immune memory opened new perspectives in research and medicine. Indeed, innate immune cells undergo through metabolic and epigenetic reprogramming that enhance a non-specific response to a second infection caused by either the same or a different pathogen. Trained immunity seems to be responsible for the heterologous, non-specific effect of some vaccines, such as BCG vaccine, influenza vaccine or vaccinia. As an example, BCG vaccine is currently used as a therapy for bladder cancer, it has been described to enhance protection against protozoan infections such as malaria or tegumentary leishmaniasis and it reduces neonatal mortality. Therefore, these discoveries led to the formulation of new potential Trained Immunity-derived Vaccines, enhancing both the innate and adaptive memory for a broader immunity. TIbVs could be beneficial in the therapy of the adaptive immunodeficiency and could guarantee a protection to co-infections or against pathogens that has no conventional vaccines yet. However, many aspects of trained immunity need to be deepened. The long-lasting protective effect of trained immunity is still on debate, and it seems to be no longer than few months compared to the lifelong immunization of the adaptive immune cells. Moreover, many detrimental side effects of trained immunity have been described. Macrophages undergo to epigenetic reprogramming in atherosclerosis, worsen the inflammation status. Trained monocytes and macrophages seem to be responsible for the alloimmune response resulting in the rejection of allografts in organ and tissue transplantation. The role of trained immunity during sepsis could be either beneficial or detrimental. Indeed, it has been shown an increased hematopoietic rate and a trained immune status in monocytes isolated from the bone marrow after sepsis. However, the implement of trained immunity might avoid the immunoparalysis, thus reducing the risk of mortality after sepsis due to secondary infections. Further studies are needed to achieve more insights on the role of trained immunity in infections and diseases. Manipulation of trained immunity through immunologic, metabolic and epigenetic pathways could improve the efficacy and duration of therapies, and it could reduce the mortality rates and worsening of certain immune-related diseases.

7 Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

How to Cite this Article:

S. Incalcaterra and J. Dominguez, “Trained Immunity at a Glance; A Review on the Innate Immune Memory and its Potential Role in Infections, Diseases and New Therapeutic Strategies”, *Adv. J. Grad. Res.*, vol. 8, no. 1, pp. 68-81, Apr. 2020. https://doi.org/10.21467/ajgr.8.1.68-81

References

[1] Chaplin, D.D., *Overview of the immune response.* The Journal of allergy and clinical immunology, 2010. 125(2 Suppl 2): p. S3-S23. https://doi.org/10.1016/j.jaci.2009.12.980.

[2] Chaplin, D.D., *1. Overview of the human immune response.* Journal of Allergy and Clinical Immunology, 2006. 117(2, Supplement 2): p. S430-S435. https://doi.org/10.1016/j.jaci.2005.09.034

[3] Accolla, R., *Host Defense Mechanisms against Pathogens.* 2006. 7 (supplement 2): p.s5-s7. https://doi.org/10.1089/sur.2006.7.s2-5

[4] Furman, D. and M.M. Davis, *New approaches to understanding the immune response to vaccination and infection.* Vaccine, 2015. 33(40): p. 5271-5281. https://doi.org/10.1016/j.vaccine.2015.06.117

[5] Netea, M.G., et al., *Innate and Adaptive Immune Memory: an Evolutionary Continuum in the Host's Response to Pathogens.* Cell Host & Microbe, 2019. 25(1): p. 13-26. https://doi.org/10.1016/j.chom.2018.12.006

[6] Dranoff, G., *Cytokines in cancer pathogenesis and cancer therapy*. Nature Reviews Cancer, 2004. 4(1): p. 11-22. https://doi.org/10.1038/nrc1252

[7] Janeway CA Jr, T.P., Walport M, et al., *The front line of host defense.* Immunobiology: The Immune System in Health and Disease, 2001. 5th edition. https://www.ncbi.nlm.nih.gov/books/NBK10757/

[8] Mathern, D.R. and P.S. Heeger, *Molecules Great and Small: The Complement System.* Clinical journal of the American Society of Nephrology : CJASN, 2015. 10(9): p. 1636-1650. https://doi.org/10.2215/CJN.06230614

[9] Schroeder, H.W., Jr. and L. Cavacini, *Structure and function of immunoglobulins.* The Journal of allergy and clinical immunology, 2010. 125(2 Suppl 2): p. S41-S52. https://doi.org/10.1016/j.jaci.2009.09.046.

[10] Schenten, D. and R. Medzhitov, *Chapter 3 - The Control of Adaptive Immune Responses by the Innate Immune System*, in *Advances in Immunology*, F.W. Alt, Editor. 2011, Academic Press. p. 87-124. https://doi.org/10.1016/B978-0-12-387664-5.00003-0.

[11] Noah W. Palm, R.M., *Pattern recognition receptors and control of adaptive immunity.* Immunological Reviews, 19 December 2008. https://doi.org/10.1111/j.1600-065X.2008.00731.x

[12] Beutler, B., *Innate immunity: an overview.* Molecular Immunology, 2004. 40(12): p. 845-859. https://doi.org/10.1016/j.molimm.2003.10.005

[13] Mushegian, A. and R. Medzhitov, *Evolutionary perspective on innate immune recognition.* The Journal of cell biology, 2001. 155(5): p. 705-710. https://doi.org/10.1083/jcb.200107040

[14] Tang, D., et al., *PAMPs and DAMPs: signal 0s that spur autophagy and immunity.* Immunological reviews, 2012. 249(1): p. 158-175. https://doi.org/10.1111/j.1600-065X.2012.01146.x.

[15] Brubaker, S.W., et al., *Innate immune pattern recognition: a cell biological perspective.* Annual review of immunology, 2015. 33: p. 257-290. https://doi.org/10.1146/annurev-immunol-032414-112240.

[16] Romagnolo, A.G., et al., *Role of Dectin-1 receptor on cytokine production by human monocytes challenged with Paracoccidioides brasiliensis.* 2018. 61(4): p. 222-230. https://doi.org/10.1111/myc.12725

[17] Lamkanfi, M. and Vishva M. Dixit, *Mechanisms and Functions of Inflammasomes.* Cell, 2014. 157(5): p. 1013-1022. https://doi.org/10.1016/j.cell.2014.04.007.

[18] Ulevitch, R.J., *Regulation of Receptor-Dependent Activation of the Innate Immune Response.* The Journal of Infectious Diseases, 2003. 187(Supplement_2): p. S351-5. https://doi.org/10.1086/374605

[19] Goldman AS, P.B., *Immunology Overview.* Medical Microbiology, 1996. 4th edition. https://www.ncbi.nlm.nih.gov/books/NBK7795/

[20] Bonilla, F.A. and H.C. Oettgen, *Adaptive immunity.* Journal of Allergy and Clinical Immunology, 2010. 125(2, Supplement 2): p. S33-S40. https://doi.org/10.1016/j.jaci.2009.09.017

[21] Nutt, S.L., et al., *The generation of antibody-secreting plasma cells.* Nature Reviews Immunology, 2015. 15(3): p. 160-171. https://doi.org/10.1038/nri3795.

[22] Evavold, C.L. and J.C. Kagan, *How Inflammasomes Inform Adaptive Immunity.* Journal of Molecular Biology, 2018. 430(2): p. 217-237. https://doi.org/10.1016/j.jmb.2017.09.019.

[23] Gourbal, B., et al., *Innate immune memory: An evolutionary perspective.* 2018. 283(1): p. 21-40. https://doi.org/10.1111/imr.12647.

[24] Ratajczak, W., et al., *Immunological memory cells.* Central-European journal of immunology, 2018. 43(2): p. 194-203. https://doi.org/10.5114/ceji.2018.77390

[25] Sallusto, F., et al., *From Vaccines to Memory and Back.* Immunity, 2010. 33(4): p. 451-463. https://doi.org/10.1016/j.immuni.2010.10.008.

[26] Ahmed, R. and D. Gray, *Immunological Memory and Protective Immunity: Understanding Their Relation.* 1996. 272(5258): p. 54-60. https://doi.org/10.1126/science.272.5258.54

[27] Campos, M. and D.L. Godson, *The effectiveness and limitations of immune memory: understanding protective immune responses.* International Journal for Parasitology, 2003. 33(5): p. 655-661. https://doi.org/10.1016/s0020-7519(03)00066-3

[28] Mulder, W.J.M., et al., *Therapeutic targeting of trained immunity.* Nature Reviews Drug Discovery, 2019. 18(7): p. 553-566. https://doi.org/10.1038/s41573-019-0025-4.

[29] Netea, M.G. and J.W.M. van der Meer, *Trained Immunity: An Ancient Way of Remembering.* Cell Host & Microbe, 2017. 21(3): p. 297-300. doi: 10.1016/j.chom.2017.02.003.

[30] Buchmann, K., *Evolution of Innate Immunity: Clues from Invertebrates via Fish to Mammals.* Frontiers in immunology, 2014. 5: p. 459-459. https://doi.org/10.3389/fimmu.2014.00459.

[31] Medzhitov, R. and C.A. Janeway, *An ancient system of host defense.* Current Opinion in Immunology, 1998. 10(1): p. 12-15. https://doi.org/10.1016/s0952-7915(98)80024-1

[32] Kurtz, J., *Specific memory within innate immune systems.* Trends in Immunology, 2005. 26(4): p. 186-192. https://doi.org/10.1016/j.it.2005.02.001

[33] Boman, H.G., I. Nilsson, and B. Rasmuson, *Inducible Antibacterial Defence System in Drosophila.* Nature, 1972. 237(5352): p. 232-235. https://doi.org/10.1038/237232a0

[34] Lemaitre, B., J.M. Reichhart, and J.A. Hoffmann, *Drosophila host defense: differential induction of antimicrobial peptide genes after infection by various classes of microorganisms.* Proceedings of the National Academy of Sciences of the United States of America, 1997. 94(26): p. 14614-14619. https://doi.org/10.1073/pnas.94.26.14614

[35] Milutinović, B. and J. Kurtz, *Immune memory in invertebrates.* Seminars in Immunology, 2016. 28(4): p. 328-342. https://doi.org/10.1016/j.smim.2016.05.004.

[36] Reimer-Michalski, E.-M. and U. Conrath, *Innate immune memory in plants.* Seminars in Immunology, 2016. 28(4): p. 319-327. https://doi.org/10.1016/j.smim.2016.05.006

[37] Netea, Mihai G., J. Quintin, and Jos W.M. van der Meer, *Trained Immunity: A Memory for Innate Host Defense.* Cell Host & Microbe, 2011. 9(5): p. 355-361. https://doi.org/10.1016/j.chom.2011.04.006.

[38] Sun, J.C., J.N. Beilke, and L.L. Lanier, *Adaptive immune features of natural killer cells.* Nature, 2009. 457(7229): p. 557-561. https://doi.org/10.1038/nature07665.

[39] Netea, M.G. and R. van Crevel, *BCG-induced protection: Effects on innate immune memory.* Seminars in Immunology, 2014. 26(6): p. 512-517. https://doi.org/10.1016/j.smim.2014.09.006.

[40] Kleinnijenhuis, J., et al., *BCG-induced trained immunity in NK cells: Role for non-specific protection to infection.* Clinical Immunology, 2014. 155(2): p. 213-219. https://doi.org/10.1016/j.clim.2014.10.005

[41] Netea, M.G., et al., *Trained immunity: A program of innate immune memory in health and disease.* Science, 2016. 352(6284): p. aaf1098. https://doi.org/10.1126/science.aaf1098

[42] Ganeshan, K. and A. Chawla, *Metabolic Regulation of Immune Responses.* 2014. 32(1): p. 609-634. https://doi.org/10.1146/annurev-immunol-032713-120236.

[43] Pearce, E.L. and E.J. Pearce, *Metabolic pathways in immune cell activation and quiescence.* Immunity, 2013. 38(4): p. 633-643. https://doi.org/10.1016/j.immuni.2013.04.005.

[44] du Plessis, S.S., et al., *Oxidative phosphorylation versus glycolysis: what fuel do spermatozoa use?* Asian journal of andrology, 2015. 17(2): p. 230-235. https://doi.org/10.4103/1008-682X.135123

[45] Dominguez-Andres, J. and M.G. Netea, *Long-term reprogramming of the innate immune system.* 2019. 105(2): p. 329-338. https://doi.org/10.1002/JLB.MR0318-104R.

[46] Kelly, B. and L.A.J. O'Neill, *Metabolic reprogramming in macrophages and dendritic cells in innate immunity.* Cell research, 2015. 25(7): p. 771-784. https://doi.org/10.1038/cr.2015.68

[47] O'Neill, L.A.J. and E.J. Pearce, *Immunometabolism governs dendritic cell and macrophage function.* The Journal of experimental medicine, 2016. 213(1): p. 15-23. https://doi.org/10.1084/jem.20151570.

[48] Arts, R.J.W., L.A.B. Joosten, and M.G. Netea, *Immunometabolic circuits in trained immunity.* Seminars in Immunology, 2016. 28(5): p. 425-430. https://doi.org/10.1016/j.smim.2016.09.002

[49] Cheng, S.-C., et al., *mTOR- and HIF-1α–mediated aerobic glycolysis as metabolic basis for trained immunity.* 2014. 345(6204): p. 1250684. https://doi.org/10.1126/science.1250684.

[50] Arts, R.J.W., et al., *Glutaminolysis and Fumarate Accumulation Integrate Immunometabolic and Epigenetic Programs in Trained Immunity.* Cell Metabolism, 2016. 24(6): p. 807-819. https://doi.org/10.1016/j.cmet.2016.10.008.

[51] Domínguez-Andrés, J., L.A.B. Joosten, and M.G. Netea, *Induction of innate immune memory: the role of cellular metabolism.* Current Opinion in Immunology, 2019. 56: p. 10-16. doi: 10.1016/j.coi.2018.09.001.

[52] Yahya Sohrabi, R.G., Hannes M. Findeisen, *Altered Cellular Metabolism Drives Trained Immunity.* Trends in Endocrinology and Metabolism, 2018. 29 (9): p. 602-605. https://doi.org/10.1016/j.tem.2018.03.012.

[53] Chen, S., et al., *Epigenetic regulation of macrophages: from homeostasis maintenance to host defense.* Cellular & Molecular Immunology, 2020. 17(1): p. 36-49. https://doi.org/10.1038/s41423-019-0315-0

[54] Samuel T. Keating, A.E.-O., *Epigenetic and Metabolism.* Circulation Research, 2015. 116: p. 715-736. https://doi.org/10.1161/CIRCRESAHA.116.303936

[55] Rodriguez, R.M., B. Suarez-Alvarez, and C. Lopez-Larrea, *Therapeutic Epigenetic Reprogramming of Trained Immunity in Myeloid Cells.* Trends in Immunology, 2019. 40(1): p. 66-80. https://doi.org/10.1016/j.it.2018.11.006.

[56] Quintin, J., et al., *Candida albicans Infection Affords Protection against Reinfection via Functional Reprogramming of Monocytes.* Cell Host & Microbe, 2012. 12(2): p. 223-232. https://doi.org/10.1016/j.chom.2012.06.006.

[57] van der Heijden, C.D.C.C., et al., *Epigenetics and Trained Immunity.* Antioxidants & redox signaling, 2018. 29(11): p. 1023-1040. https://doi.org/10.1089/ars.2017.7310.

[58] Sánchez-Ramón, S., et al., *Trained Immunity-Based Vaccines: A New Paradigm for the Development of Broad-Spectrum Anti-infectious Formulations.* 2018. 9(2936). https://doi.org/10.3389/fimmu.2018.02936

[59] Oliveira, T.L., et al., *Recombinant BCG strains expressing chimeric proteins derived from Leptospira protect hamsters against leptospirosis.* Vaccine, 2019. 37(6): p. 776-782. https://doi.org/10.1016/j.vaccine.2018.12.050.

[60] Kleinnijenhuis, J., et al., *Bacille Calmette-Guérin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes.* 2012. 109(43): p. 17537-17542. https://doi.org/10.1073/pnas.1202870109

[61] Parra, M., et al., *Molecular Analysis of Non-Specific Protection against Murine Malaria Induced by BCG Vaccination.* PLOS ONE, 2013. 8(7): p. e66115. https://doi.org/10.1371/journal.pone.0066115

[62] dos Santos, J.C., et al., *Non-specific effects of BCG in protozoal infections: tegumentary leishmaniasis and malaria.* Clinical Microbiology and Infection, 2019. 25(12): p. 1479-1483. https://doi.org/10.1016/j.cmi.2019.06.002

[63] Blok, B.A., et al., *Trained innate immunity as underlying mechanism for the long-term, nonspecific effects of vaccines.* 2015. 98(3): p. 347-356. https://doi.org/10.1189/jlb.5RI0315-096R

[64] de Bree, L.C.J., et al., *Non-specific effects of vaccines: Current evidence and potential implications.* Seminars in Immunology, 2018. 39: p. 35-43. https://doi.org/10.1016/j.smim.2018.06.002

[65] Babjuk, M., et al., *EAU Guidelines on Non-Muscle-Invasive Urothelial Carcinoma of the Bladder.* European Urology, 2008. 54(2): p. 303-314. https://doi.org/10.1016/j.eururo.2008.04.051

[66] Mitroulis, I., et al., *Modulation of Myelopoiesis Progenitors Is an Integral Component of Trained Immunity.* Cell, 2018. 172: p. 147-161.e12. https://doi.org/10.1016/j.cell.2017.11.034

[67] Gillard, G.O., et al., *Thy1+ Nk Cells from Vaccinia Virus-Primed Mice Confer Protection against Vaccinia Virus Challenge in the Absence of Adaptive Lymphocytes.* PLOS Pathogens, 2011. 7(8): p. e1002141. https://doi.org/10.1371/annotation/b29086ef-e08d-444c-8113-18a6dd429a7c

[68] Tai, L.-H., et al., *Perioperative Influenza Vaccination Reduces Postoperative Metastatic Disease by Reversing Surgery-Induced Dysfunction in Natural Killer Cells.* 2013. 19(18): p. 5104-5115. https://doi.org/10.1158/1078-0432.CCR-13-0246

[69] Gyssens, I.C. and M.G. Netea, *Heterologous effects of vaccination and trained immunity.* Clinical Microbiology and Infection, 2019. 25(12): p. 1457-1458. https://doi.org/10.1016/j.cmi.2019.05.024

[70] Gardiner, C.M. and K.H.G. Mills, *The cells that mediate innate immune memory and their functional significance in inflammatory and infectious diseases.* Seminars in Immunology, 2016. 28(4): p. 343-350. https://doi.org/10.1016/j.smim.2016.03.001

[71] Oberbarnscheidt, M.H., et al., *Non-self recognition by monocytes initiates allograft rejection.* The Journal of clinical investigation, 2014. 124(8): p. 3579-3589. https://doi.org/10.1172/JCI74370

[72] Ochando, J., et al., "Trained immunity in organ transplantation*" Am J Transplant.* 20: 10– 18, 2020. https://doi.org/10.1111/ajt.15620

[73] Zecher, D., et al., *An Innate Response to Allogeneic Nonself Mediated by Monocytes.* 2009. 183(12): p. 7810-7816. https://doi.org/10.4049/jimmunol.0902194

[74] Gisterå, A. and G.K. Hansson, *The immunology of atherosclerosis.* Nature Reviews Nephrology, 2017. 13(6): p. 368-380. https://doi.org/10.1038/nrneph.2017.51

[75] Libby, P., et al., *Atherosclerosis.* Nature Reviews Disease Primers, 2019. 5(1): p. 56. https://doi.org/10.1161/CIRCRESAHA.116.308334

[76] Siroon Bekkering , J.Q., Leo A.B. Joosten , Jos W.M. van der Meer , Mihai G. Netea , Niels P. Riksen, *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: p. 1731–1738. https://doi.org/10.1161/ATVBAHA.114.303887

[77] van der Valk, F.M., et al., *Oxidized Phospholipids on Lipoprotein(a) Elicit Arterial Wall Inflammation and an Inflammatory Monocyte Response in Humans.* Circulation, 2016. 134(8): p. 611-624. https://doi.org/10.1161/CIRCULATIONAHA.116.020838

[78] van Tuijl, J., et al., *Immunometabolism orchestrates training of innate immunity in atherosclerosis.* Cardiovascular Research, 2019. 115(9): p. 1416-1424. https://doi.org/10.1093/cvr/cvz107

[79] Wentowski, C., N. Mewada, and N.D. Nielsen, *Sepsis in 2018: a review.* Anaesthesia & Intensive Care Medicine, 2019. 20(1): p. 6-13. https://doi.org/10.1016/j.mpaic.2018.11.009

[80] Bomans, K., et al., *Sepsis Induces a Long-Lasting State of Trained Immunity in Bone Marrow Monocytes.* Frontiers in immunology, 2018. 9: p. 2685-2685. https://doi.org/10.3389/fimmu.2018.02685

[81] Zhang, H., et al., *Sepsis Induces Hematopoietic Stem Cell Exhaustion and Myelosuppression through Distinct Contributions of TRIF and MYD88.* Stem cell reports, 2016. 6(6): p. 940-956. https://doi.org/10.1016/j.stemcr.2016.05.002

[82] van der Meer, J.W.M., et al., *Trained immunity: A smart way to enhance innate immune defence.* Molecular Immunology, 2015. 68(1): p. 40-44. https://doi.org/10.1016/j.molimm.2015.06.019