TRAINED IMMUNITY:
A DOUBLE-EDGED SWORD IN HEALTH AND DISEASE

Robbin Kramer[1]

[1]Master's student MSc Molecular Mechanisms of Disease, Radboud university medical center, Nijmegen, the Netherlands

Abstract

Trained immunity describes the long-term functional reprogramming of innate immune cells, leading to differences in the responsiveness of these cells against stimuli of different nature. These changes are characterised by modifications in the epigenetic and metabolic landscape of the cells. The term 'trained immunity' was first coined in 2011 by researchers at the Department of Internal Medicine at the Radboud University Medical Center. Since then, this one term has grown to encompass an ever-expanding line of research that spans different fields across the whole immunological spectrum. While the past decade has mostly focussed on elucidating the mechanisms behind trained immunity, the future of this field lies in our ability to find new ways of therapeutically targeting or harnessing trained immunity in different contexts. These contexts include vaccination strategies, immunotherapies, infectious diseases, and cardiovascular interventions, and promise to include many more in the next decade.

Introduction

The immune system is composed of various complex barriers, factors, and cell types, that work in concert to protect the organism against invading pathogens. Traditionally, the immune system of vertebrates is classified into two arms: the innate immune system and the adaptive immune system. The innate immune system represents the first line defence against pathogens, which must overcome this barrier to establish an infection. Innate immune cells detect 'self' and 'non-self' entities, after which they can attack anything deemed as 'non-self' [1]. In contrast, adaptive immunity is composed of cells that are highly specific against a certain antigen. Upon recognition of this antigen as 'non-self', these adaptive immune cells mount an attack and generate immunological memory against it [1]. The memory response enables the rapid and effective clonal expansion of cells during a subsequent encounter [1]. For a long time, the prevailing view was that only the adaptive immune system is capable of a memory response, whereas the innate immune system is just capable of rudimentary clearance of antigens.

However, a growing body of evidence over the past half a century opposes this view. This line of inquiry originated from Mackaness's seminal work in 1964, where he documented non-specific cell-mediated resistance to various pathogens [2]. He further described how this resistance was dependent on "an altered state of macrophages", which have a temporary heightened response after interferon-gamma release by lymphocytes [2]. Although this mechanism was still a by-product of the adaptive immune system, it provided early evidence for a heightened innate immune response following secondary infections. Subsequent research in plants and invertebrates, which lack an adaptive immune system, further corroborated the hypothesis of a memory-like response in the innate immune system. For instance, experiments performed in 1961 by Frank Ross revealed that infection by the tobacco mosaic virus also provides broad-spectrum protection that lasts for 20 days. This process is described as 'systemic acquired resistance', and is considered to be equivalent to immunological memory in vertebrates [3].

Lastly, epidemiological data demonstrated that live-attenuated vaccines reduce mortality that is not related to their primary target [4]. Vaccination trials in children further validated the existence of an innate memory-like response in humans. The full range of epidemiological data on this is beyond the scope of this article, but an extensive review by Aaby and Benn was published in 2019 [5]. The most extensively employed vaccine in the world is Bacillus Calmette-Guerin (BCG) vaccine, a live attenuated vaccine derived from Mycobacterium bovis. In addition to its protection against tuberculosis, multiple randomised trials have exhibited that the use of BCG reduces all-cause neonatal mortality, primarily through the prevention of various infections [6-8]. This rapid reduction in perinatal mortality could not be attributed to adaptive immunity, as the onset of the protection is too swift [9].

In 2011, Mihai Netea, Jessica Quintin, and Jos van der Meer at the Department of Medicine at the Radboud university medical center (Radboud University Medical Center) used the aforementioned research to postulate a new hypothesis, namely that of 'trained immunity'. In their paper titled "Trained Immunity: A Memory for Innate Host Defense", they define trained immunity as "a heightened response to a secondary infection that can be exerted both toward the same microorganism and a different one" [10]. A year later, they substantiated this hypothesis by showing that mice lacking B and T cells were protected against a Candida albicans reinfection in a monocyte-dependent manner [11]. Now, more than a decade later this hypothesis has grown into an ever-evolving research field which aims to further elucidate the concept of trained immunity. This review describes some of the accomplishments of the Radboud University Medical Center within the field of trained immunity, including the identification of the mechanisms behind this phenomenon. Furthermore, different fields which are impacted by trained immunity will be discussed. And lastly, the prospects and challenges of this new and exciting field will be covered.

Mechanisms of trained immunity

Conventional immunological memory arises from an interplay of different mechanisms. After antigen recognition, the production of lymphocyte clones leads to the formation of a large population of lymphocytes targeted against one antigen. Then, a subgroup of this population survives in the form of memory lymphocytes, which

upon restimulation quickly undergo clonal expansion for a stronger and quicker secondary immune response [1]. This memory can, depending on the antigen, result in life-long protection against a specific antigen. On the other hand, the lifetime of innate immune memory is still unclear, with timeframes ranging from 3 months to several years [12-14]. Although it is highly unlikely that trained immunity lasts as long as adaptive immunological memory, it is of note to mention that the lifetime of trained immunity does not match that of typical mononuclear cells [15]. Therefore, trained immunity must rely on other mechanisms than classical immunological memory to be effective.

Alternatively, two primary mechanisms control trained immunity, namely epigenetic reprogramming and metabolic alterations. In brief, epigenetics involves the regulation of gene function without modifying DNA sequence. This is typically achieved through changes in DNA methylation and histone modifications. These processes alter the accessibility of DNA to transcriptional machinery and thereby adjust the expression of the underlying genes [16]. In the case of trained immunity, epigenetic changes result in alteration in the inflammatory phenotype of innate immune cells. Upon initial stimulation, innate immune cells undergo rapid activation, marked by increases in gene expression of various pro-inflammatory genes. These shifts in gene expression are facilitated by increases in specific histone modifications, such as methylation or acetylation, as well as decreases in DNA methylation [17-19]. After the danger signal or pathogen has been effectively eliminated, some of the epigenetic changes remain. As a result, the upregulation of pro-inflammatory genes is quicker and stronger in the case of a subsequent (unrelated) stimulus [20].

One of the main ways by which epigenetics induces trained immunity is by modulating the metabolism of innate immune cells. Trained monocytes exhibit a shift from mostly oxidative phosphorylation, which is utilised in an inactive state, to more glycolysis [21]. This shift is evidenced by increases in glucose consumption, lactate production and the ratio between nicotinamide adenine dinucleotide (NAD(+)) and its reduced form (NADH). The mammalian target of rapamycin and its pathway can activate this metabolic reprogramming [22]. Furthermore, metabolic pathways can regulate epigenetics, by providing substrates or regulators for epigenetic enzymes [23]. As previously stated, the lifetime of trained immunity is generally longer than that of peripheral innate immune cells. This discrepancy is resolved by the concept of 'centrally trained immunity', where

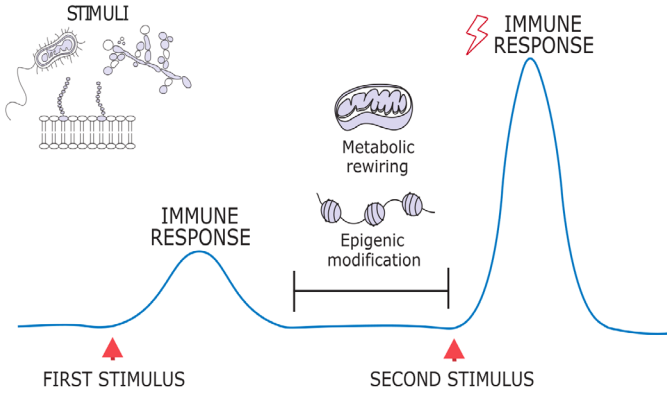

Figure 1 *- The mechanisms behind trained immunity. After a primary stimulus, epigenetic changes and metabolic alterations induce an altered state of activatability in innate immune cells. Because of this, these cells can bring about a stronger response after a second (unrelated) stimulus.*

training agents alter hematopoietic stem cells, which are the basis of the immune system. For example, the administration of β-glucan in an experiment done by Mitroulis et al. at the Radboud University Medical Center showed a pronounced increase in myelopoiesis, which coincided with alterations in the functional and transcriptional profile of bone marrow-derived progenitors [24]. This expansion ultimately provided protection against secondary lipopolysaccharide stimulation and chemotherapy-induced myelosuppression [24]. BCG, like other training agents, has also been shown to affect hematopoietic stem cells in a way that protected mice against a tuberculosis infection [25].

Applications of trained immunity

Since the initial introduction of trained immunity by Netea et al., researchers have utilised this newfound knowledge in different ways. Within the Radboud University Medical Center, different departments have picked up this knowledge and started applying it in their fields of interest. For example, the implications of trained immunity for vaccinations are immense. Therefore, the Department of Internal Medicine has continued its research into trained immunity in infectious diseases. Among others, they have further investigated the effects of BCG vaccination on protection against various non-specific infections. Here, promising, but ambiguous effects were reported.

For example, they found that vaccination with BCG protects against yellow fever viraemia and Influenza A, but not against severe acute respiratory syndrome coronavirus-2 [26-28]. Furthermore, in Denmark and Australia BCG vaccination of neonates did not significantly influence the number of hospitalisations until 15 months after birth or the occurrence of lower respiratory tract infections, respectively [29, 30]. On the other hand, BCG vaccination of elderly patients discharged from the Radboud University Medical Center did protect against new infections, most likely specifically against respiratory tract infections of probable viral origin [31]. Concurrently, BCG-vaccinated individuals showed more control over a controlled malaria infection performed at the Radboud University Medical Center [32]. More specifically, they demonstrated earlier signs of NK cell and monocytic activation, which was inversely correlated with parasitaemia. Because of this heterogeneity in response, an advisory board of the WHO has deemed the evidence to start channelling trained immunity in vaccinations inconclusive [33]. Accordingly, they have decided against altering vaccination strategies until more elaborate randomised trials are performed.

On the opposing of the spectrum, researchers have endeavoured to exploit trained immunity in immunotherapy against cancer. BCG immunotherapy has already been established as a routine treatment and prophylaxis of superficial bladder cancer [34]. At the Radboud University Medical Center, this treatment has also displayed induction of trained immunity, which reduced the likelihood of respiratory infections and conceivably had an effect on oncological outcomes [35]. Another intriguing prospect is the reversal of immunosuppressive phenotypes of tumour-associated immune cells. An instance of this phenomenon can be observed with tumour-associated macrophages (TAMs), whose presence is linked to poorer survival and prognosis in various cancers [34]. The ability of several therapeutics to repolarise these macrophages towards a pro-inflammatory phenotype is already being evaluated in clinical trials [36]. One approach that may prove beneficial in the modulation of phosphoinositide 3-kinase γ, a protein that is linked to both trained immunity and immunosuppressive phenotypes [27, 37].

A paper from 2020 resulting from a collaboration with several departments of the Radboud University Medical Center showed that a mouse melanoma model treated with a trained immunity-promoting

therapy experienced dose- and regimen-dependent tumour growth inhibition without changes in body weight [38]. This effect was induced by increased myelopoiesis due to epigenetic rewiring, which was thereby able to overcome the immunosuppression from the tumour microenvironment. Even more excitingly, the researchers propose combining their trained immunity therapeutic with other immunotherapies. This is attributed to the observation that the combination of their nano therapy with checkpoint inhibitors significantly inhibited tumour growth rate compared to the efficacy of each treatment administered in isolation [38].

Contribution to cardiovascular pathology

While trained immunity has shown some incredibly promising applications, there are also definite drawbacks to the presence of immunological memory. For example, trained immunity has been proposed to play a role in the development of cardiovascular disease, most notably in atherosclerosis. In the early stages of atherosclerosis, excessive lipids that have accumulated in vascular walls are taken up by macrophages, which become foam cells as a result [39]. This is a key step in the formation and deterioration of atherosclerosis, and it is therefore the subject of much research. In 2014, Siroon Bekkering, with the help of Jessica Quintin, Mihai Netea and others, was able to show that trained immunity plays a role in this process. They described how exposing monocytes to oxidised low-density lipoprotein instigates a proatherogenic phenotype in the resulting macrophages. Additionally, this phenotype was characterised by epigenetic histone modifications, increased proinflammatory cytokine production and foam cell formation [40].

This pivotal finding was then translated to in vivo research two years later, when the researchers saw the same pattern emerge in monocytes derived from symptomatic atherosclerosis patients [41]. Lastly, the bone marrow progenitors of patients with coronary artery disease due to severe atherosclerosis showed the tell-tale epigenetic and metabolic signs of trained immunity [42]. This has many implications for the treatment of people at risk of atherosclerosis and cardiovascular disease. Most notably because the standard treatment for this, statins, was unable to revert the trained phenotype [43].

Future directions and challenges

Although trained immunity is a widely observed phenomenon, its efficacy may be influenced by a variety of factors that require further investigation. Age, diet and exercise, among others, need to be thoroughly studied to determine their impact on trained immunity. This will become particularly important as trained immunity-based therapies are developed. Additionally, trained immunity is a complex system that involves various cell types, organs and pathologies. While it was beyond the scope of this review, much research is also conducted on the role of trained immunity in autoimmune diseases [21, 44, 45]. Notably, trained immunity is a systemic mechanism that involves reprogramming the immune system's core, the bone marrow. Therefore, research into trained immunity, at the Radboud University Medical Center and beyond, must be careful to consider potential off-target effects and safety concerns associated with these interventions. Such investigations are crucial for the safe and effective implementation of trained immunity-based therapies.

Conclusion

Trained immunity is definitely one of the astonishing discoveries that have come out of the Radboud in the last century. This field of research has shattered the long-existing belief that the adaptive immune system was alone in its ability for memory. While the beginning phases were focused on elucidating the mechanisms behind trained immunity, the research has now pivoted to look at ways to both utilise and suppress trained immunity in different contexts. Training agents like BCG could be promising in vaccination strategies and immunotherapies. On the other hand, innate immune memory can have a deleterious effect in a cardiovascular setting, so therapies targeting trained immunity should be considered here. All of this has led to many new research lines, both within and beyond Nijmegen, and there will surely be many more innovations in the future.

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References

1. Parkin, J. & Cohen, B. An overview of the immune system. Lancet 357, 1777-1789 (2001).
2. Mackaness, G.B. The Immunological Basis of Acquired Cellular Resistance. J Exp Med 120, 105-120 (1964).
3. Ross, A.F. Systemic acquired resistance induced by localized virus infections in plants. Virology 14, 340-358 (1961).
4. Mayr, A. Taking Advantage of the Positive Side-Effects of Smallpox Vaccination. Journal of Veterinary Medicine, Series B 51, 199-201 (2004).
5. Aaby, P. & Benn, C.S. Developing the concept of beneficial non-specific effect of live vaccines with epidemiological studies. Clinical Microbiology and Infection 25, 1459-1467 (2019).
6. Garly, M.L., et al. BCG scar and positive tuberculin reaction associated with reduced child mortality in West Africa. A non-specific beneficial effect of BCG? Vaccine 21, 2782-2790 (2003).
7. Roth, A., et al. BCG vaccination scar associated with better childhood survival in Guinea-Bissau. Int J Epidemiol 34, 540-547 (2005).
8. Stensballe, L.G., et al. BCG vaccination at birth and early childhood hospitalisation: a randomised clinical multicentre trial. Arch Dis Child 102, 224-231 (2017).
9. Parmar, K., et al. Bacillus Calmette-Guerin Vaccine and Nonspecific Immunity. Am J Med Sci 361, 683-689 (2021).
10. Netea, M.G., et al. Trained immunity: a memory for innate host defense. Cell Host Microbe 9, 355-361 (2011).
11. Quintin, J., et al. Candida albicans Infection Affords Protection against Reinfection via Functional Reprogramming of Monocytes. Cell Host & Microbe 12, 223-232 (2012).
12. Benn, C.S., et al. A small jab - a big effect: nonspecific immunomodulation by vaccines. Trends Immunol 34, 431-439 (2013).
13. Berendsen, M.L.T., et al. Maternal Priming: Bacillus Calmette-Guérin (BCG) Vaccine Scarring in Mothers Enhances the Survival of Their Child With a BCG Vaccine Scar. Journal of the Pediatric Infectious Diseases Society 9, 166-172 (2020).
14. Kleinnijenhuis, J., et al. Long-Lasting Effects of BCG Vaccination on Both Heterologous Th1/Th17 Responses and Innate Trained Immunity. Journal of Innate Immunity 6, 152-158 (2014).
15. Yona, S., et al. Fate Mapping Reveals Origins and Dynamics of Monocytes and Tissue Macrophages under Homeostasis. Immunity 38, 79-91 (2013).
16. Dupont, C., et al. Epigenetics: definition, mechanisms and clinical perspective. Semin Reprod Med 27, 351-357 (2009).
17. Arts, R.J.W., et al. Glutaminolysis and Fumarate Accumulation Integrate Immunometabolic and Epigenetic Programs in Trained Immunity. Cell Metabolism 24, 807-819 (2016).

18. Zhao, S., et al. H3K4 Methylation Regulates LPS-Induced Proinflammatory Cytokine Expression and Release in Macrophages. Shock 51(2019).
19. Keating, S.T. & El-Osta, A. Metaboloepigenetics in cancer, immunity and cardiovascular disease. Cardiovasc Res (2022).
20. Hu, S., et al. The mechanisms and cross-protection of trained innate immunity. Virology Journal 19, 210 (2022).
21. Netea, M.G., et al. Trained immunity: A program of innate immune memory in health and disease. Science 352, aaf1098-aaf1098 (2016).
22. Cheng, S.C., et al. mTOR- and HIF-1α-mediated aerobic glycolysis as metabolic basis for trained immunity. Science 345, 1250684 (2014).
23. Van Der Heijden, C.D.C.C., et al. Epigenetics and Trained Immunity. Antioxidants and Redox Signaling 29, 1023-1040 (2018).
24. Mitroulis, I., et al. Modulation of Myelopoiesis Progenitors Is an Integral Component of Trained Immunity. Cell 172, 147-161.e112 (2018).
25. Kaufmann, E., et al. BCG Educates Hematopoietic Stem Cells to Generate Protective Innate Immunity against Tuberculosis. Cell 172, 176-190.e119 (2018).
26. Leentjens, J., et al. BCG Vaccination Enhances the Immunogenicity of Subsequent Influenza Vaccination in Healthy Volunteers: A Randomized, Placebo-Controlled Pilot Study. The Journal of Infectious Diseases 212, 1930-1938 (2015).
27. Arts, R.J.W., et al. BCG Vaccination Protects against Experimental Viral Infection in Humans through the Induction of Cytokines Associated with Trained Immunity. Cell Host & Microbe 23, 89-100. e105 (2018).
28. Kaufmann, E., et al. BCG vaccination provides protection against IAV but not SARS-CoV-2. Cell Rep 38, 110502 (2022).
29. Stensballe, L.G., et al. BCG vaccination at birth and early childhood hospitalisation: a randomised clinical multicentre trial. Archives of Disease in Childhood 102, 224 (2017).
30. Messina, N.L., et al. Neonatal Bacille Calmette-Guérin Vaccination and Infections in the First Year of Life: The MIS BAIR Randomized Controlled Trial. The Journal of Infectious Diseases 224, 1115-1127 (2021).
31. Giamarellos-Bourboulis, E.J., et al. Activate: Randomized Clinical Trial of BCG Vaccination against Infection in the Elderly. Cell 183, 315-323.e319 (2020).
32. Walk, J., et al. Outcomes of controlled human malaria infection after BCG vaccination. Nat Commun 10, 874 (2019).
33. Higgins, J.P.T., et al. Association of BCG, DTP, and measles containing vaccines with childhood mortality: systematic review. BMJ 355, i5170 (2016).
34. Lamm, D.L., et al. Current perspectives on diagnosis and treatment of superficial bladder cancer. Urology 39, 301-308 (1992).
35. Van Puffelen, J.H., et al. Intravesical BCG in patients with non-muscle invasive bladder cancer induces trained immunity and decreases respiratory infections. J Immunother Cancer 11(2023).
36. Pathria, P., et al. Targeting Tumor-Associated Macrophages in Cancer. Trends Immunol 40, 310-327 (2019).
37. Kaneda, M.M., et al. PI3Kγ is a molecular switch that controls immune suppression. Nature 539, 437-442 (2016).
38. Priem, B., et al. Trained Immunity-Promoting Nanobiologic Therapy Suppresses Tumor Growth and Potentiates Checkpoint Inhibition. Cell 183, 786-801.e719 (2020).
39. Wang, T., et al. Atherosclerosis: pathogenesis and pathology. Diagnostic Histopathology 18, 461-467 (2012).
40. Bekkering, S., et al. Oxidized Low-Density Lipoprotein Induces Long-Term Proinflammatory Cytokine Production and Foam Cell Formation via Epigenetic Reprogramming of Monocytes. Arteriosclerosis, Thrombosis, and Vascular Biology 34, 1731-1738 (2014).
41. Bekkering, S., et al. Innate immune cell activation and epigenetic remodeling in symptomatic and asymptomatic atherosclerosis in humans in vivo. Atherosclerosis 254, 228-236 (2016).
42. Noz, M.P., et al. Reprogramming of bone marrow myeloid progenitor cells in patients with severe coronary artery disease. Elife 9(2020).
43. Bekkering, S., et al. Treatment with Statins Does Not Revert Trained Immunity in Patients with Familial Hypercholesterolemia. Cell Metab 30, 1-2 (2019).
44. Manz, R.A., et al. Immunological memory stabilizing autoreactivity. Curr Top Microbiol Immunol 305, 241-257 (2006).
45. Ochando, J., et al. Trained immunity — basic concepts and contributions to immunopathology. Nature Reviews Nephrology 19, 23-37 (2023).