

## Immune memory: an evolutionary perspective

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### ABSTRACT

The innate immune system, through pattern recognition receptors, intercepts any kind of pathogen and reacts through chemotactic, phagocytosing, cytokines-secreting and cell-killing mechanisms in a very quick and effective way. Meanwhile, the adaptive immunity arm, through dendritic and T and B cells memory activation, is alerted and starts, more slowly, to produce antibodies, seen thanks to the progress of immunological investigations in comparative vertebrates, invertebrates, and vegetal models.

However, it has been stated that the innate immune system also displays adaptive potential in terms of reinfection resistance through immune memory, in addition to the modulation of responses against repeated low doses of lipopolysaccharides (Lps) or cross-immunization, starting from one pathogenic species and extending to others.

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### Introduction

The innate immune system is older than the adaptive one, the latter being developed 500 million years ago and the former being invertebrates' unique defense against infections and tissue and organ damage.<sup>1</sup> Innate immunity is expressed by monocytes generated by myeloid bone marrow stem cells, macrophages, dendritic cells, natural killer, and lymphocytes, through the induction of interferons and cytokines. Adaptive immunity is subdivided into immunoglobulin production through T gene cells activation and  $\beta$  lymphocyte clones producing immunoglobulins (which is characteristic of jawed vertebrates or the stomatognathic system), and lymphocytes-receptors selection by a gene rearrangement-based response (which is characteristic of jawless vertebrates).<sup>2</sup> With the progress of immunological investigations, comparing vertebrates, invertebrates and vegetal models, it has been stated that innate immune cells also display long-term adaptive potential rather than a particular transcription or functional programme in terms of reinfection resistance through immune memory.<sup>3</sup>

This property has recently been defined as 'trained immunity', a process that results in a more intense reaction to secondary infections, or none to infectious inflammatory agents.<sup>4–6</sup>

Essentially, this long-term adaptation of innate immune cells enables them, through PRRs, to react with stronger, more rapid or qualitatively different transcriptional responses when challenged with secondary noxious interaction acting through chemotactic, phagocytosing, cytokines-secreting and cell killing mechanisms in a very quick and effective way.<sup>2</sup> The immune system response is modulated by repeated low doses of lipopolysaccharides (Lps) and has the ability to cross-immunize from one pathogenic species to another.<sup>7</sup> This happens because different stimuli [for example,  $\beta$ -glucan, LPS or bacillus (BCG) vaccines] can induce differently

trained immunity programmes.<sup>8</sup> Meanwhile, the adaptive immunity arm, through dendritic and T&B cells memory activation, is alerted and starts the antibodies production more slowly.<sup>9</sup> The training process involves changes in chromatin organization at the site of the proper domains [a topologically associating domain (TAD)], transcription of long non-coding RNAs (lncRNAs), DNA methylation and the reprogramming of cellular metabolism.<sup>4</sup> Stimulation of innate immune cells is accompanied by the deposition of chromatin marks and changes in the DNA methylation status, leading to the unfolding of chromatin and facilitating the transcription and expression of proinflammatory factors.<sup>10</sup> All these changes are only partially removed after cessation of the stimulus. The trained immunity does not involve gene recombination like the adaptive one, but instead involves transcription reprogramming with a shorter memory if compared with the long-lasting and specific antibodies production process. This allows the quicker and more enhanced recruitment of transcription factors and gene expression after a secondary challenge. In such a way, it modulates the reactions to pathogenic invading agents or any other endogenous-exogenous noxa by increasing or reducing the intensity accordingly with environmental factors and timing: this pivotal role is accomplished by inflammatory and anti-inflammatory cascades induced by the activation of this immunity arm, in order to mitigate with 'tolerance' the damage to tissues and organs induced exceedingly from inflammatory defense. The trained immunity is generally reversible and shorter lived than classical epitope-specific adaptive immunological memory.<sup>3,11</sup> Importantly, however, recent studies have suggested transgenerational effects through the induction of trained immunity.<sup>12,13</sup> The immunological phenotype has been proven to last at least 3 months and up to 1 year, but heterologous protection against infections induced by live vaccines can last for up to 5 years.<sup>14</sup>

Although trained immunity is controlled by distinctive mechanisms and is less specific and of a shorter duration than adaptive immune memory,<sup>15</sup> both fulfil the same principal function: a quicker and stronger response against pathogens and improved survival of the host. In vertebrates, this provides many examples of cross protection against different pathogenic agents, starting with the individual sensitizing contact. We have summarized this in the following Table 1:

Trained immunity has quite an old historical background based in the last century, having been adopted to fight cancer through immune modulation. The Coley toxin, from the culture of streptococci, BCG and *C.parvum*, was significant in the field of immunotherapy, but only live attenuated BCG achieved a steady therapeutical role in surface-spreading bladder cancer, both alone and in combination with chemo and, even if not officially, also in treating melanoma and lymphoma.

Especially in the eastern countries,  $\beta$ -glucan is currently used to control cancer growth, added to chemotherapy to stimulate immunotherapy and also in association with check points inhibitors.<sup>30</sup> The better functioning of trained immunity to fight cancer is still a coveted goal in order to overwhelm immunosuppressive conditions and eventually to enhance the effectiveness of new vaccines and biological therapies.

Surprisingly, no author revisiting trained immunity throughout the history of immunotherapy has mentioned *Propionibacterium acnes* or *Corynebacterium parvum*, an historical milestone in the past for breast and colorectal cancer immune schedules. This puzzling inactivated bacterium, injected subcutaneously, intravenously and intrapleurally, or in the peritoneum in tumor-bearing patients, showed strong oncolytic activity and good control of the neoplastic growth, without impacting definitively the overall survival rate.

For this reason, after having been registered by Wellcome & Burroughs in 1970 under the Coryparv brand, it was abandoned, and no more was prescribed. Nevertheless, there had been a great number of preclinical literature contributions about the antiviral activity of *C. parvum* against at least 50 virus families challenged in the animal and veterinary pathology model, and we personally had a very positive experiences using *C.parvum* to interrupt severe and clinically complicated common viral infections in humans. For this reason, we believe that *C.parvum* is very appropriate as a training immunity modulator, specifically against overall viral infections.

We wondered why its antiviral potential has been ignored for such a long time, until the recent Covid-19 pandemic urgently required an immediate barrier against contagion and the very first infection stages, during the gap between virus insulation and the availability of effective vaccines. The trained immunity expresses its memory function by modifying epithelial stem cells and fibroblasts, but also promyeloblasts that generate monocyte and macrophages. The imprinted stem cells expressing receptors for several inflammatory mediators feedback their messages to the immune competent cells and to the epithelial defense barriers of the body. It is effective also upon bone marrow progenitor cells (central trained immunity), as well as in blood monocytes and tissue macrophages (peripheral trained immunity).<sup>4</sup>

Thus, the inflammatory memory of epidermal stem cells is strictly related to their regenerative role when the impaired epidermal barrier has to be quickly restored in defense against infectious damaging agents.

In the clinical setting, trained immunity gives some advantage in terms of infections protection and saving lives, and this has been verified with BCG, coley toxin,  $\beta$ -glucan and *C.parvum*, in immunotherapy, by activating macrophages and dendritic cells against pathogenic agents and cancer, and even in association with the modern biological therapies such as check point inhibitors.

In the current Covid-19 pandemic, we wanted to take advantage of the individual trained immunity mechanism because of its first-line role in virus access barriers and immediate virions destruction in the first infection stages, thus preventing their multiplication, viremia and major complications to death.

In this antiviral perspective, the choice of the most appropriate 'trigger' for setting up and deflagrating innate immunity is undoubtedly the *C.parvum* because of its well-trained property of taking control of viral infections in several experimental animals and veterinary models, which is reflected in the experience of Schindler et al. (1981) on mouse hepatitis coronavirus.<sup>31</sup>

Our pioneering, exciting clinical studies on abortions and herpes zoster were carried out in a very quick, safe and effective way not only on the skin vesicles, but also on the nociceptive system, and the subsequent experiences with some of the more complicated common viral infections (mumps, varicella, measles, influenza) definitely suggest this bacterium should be used to fight Covid-19 contagiousness and the very first stages of infection.

**Table 1.** Examples of cross protection against pathogenic agents.

The challenge with $\beta$ -glucan induces resistance to <i>Staphylococcus aureus</i>	16,17
Muramyl dipeptide is secondarily effective against <i>Streptococcus pneumoniae</i> and <i>Toxoplasma gondii</i> infections	18
Flagellin induces protection against <i>S. pneumoniae</i> and rotavirus	19,20
Vaccination with the BCG vaccine was shown to protect animals against secondary infections with <i>Candida albicans</i> , <i>Schistosoma mansoni</i> and <i>Mycobacterium tuberculosis</i> , such as yellow fever or malaria, and this was associated with an augmented proinflammatory activity of monocytes treatment of malignancies such as bladder cancer, melanoma, leukemia and lymphoma.	21–28
<i>Corynebacterium parvum</i> , 50 virus families or live humans vaccines commonly used in pediatric protocols help to overwhelm different types of recurrent infections, herpes-virus latency increases resistance to the bacterial pathogens <i>Listeria monocytogenes</i> and <i>Yersinia pestis</i> through enhanced production of IFN- $\gamma$ , and the systemic activation of macrophages	29

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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