SUMMARY

The BCG (Bacillus Calmette-Guérin) vaccine, containing an attenuated strain of Mycobacterium bovis (M. bovis), is the only tuberculosis vaccine available worldwide. Despite more than a century of use, its effectiveness in protecting against Mycobacterium tuberculosis (M. tuberculosis) remains limited, especially in adults. In children, it provides effective protection against severe forms of tuberculosis, such as tuberculous meningitis and disseminated tuberculosis. In addition to specific protection against tuberculosis, the BCG vaccine has a significant effect on the immune system, inducing non-specific protective effects against pathogens other than mycobacteria. The mechanism underlying this phenomenon is known as trained immunity, resulting from the epigenetic reprogramming of innate immune cells, which can lead to their increased readiness to respond to subsequent pathogenic stimuli, including those unrelated to the original infectious agent that initiated these changes.

The aim of this doctoral dissertation was to evaluate the non-specific immune response in children aged 6-12 years who were vaccinated with BCG in the neonatal period, taking into account BCG-induced mechanisms of trained immunity verified on the basis of the response of peripheral blood leukocytes to a homologous (BCG bacilli) or heterologous (respiratory syncytial virus (RSV) and SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) antigens) in in vitro cultures. To achieve the set goal, an in vitro cell culture model was used, based on the stimulation of peripheral blood leukocytes of children with BCG vaccine bacilli or RSV or SARS-CoV-2 respiratory virus antigens, independently or in combination. The study took into account the serological status of children in terms of antibodies against RSV and/or SARS-CoV-2. The following were adopted as determinants of the non-specific immune response in the children studied: the ability of leukocytes to produce key pro-inflammatory cytokines (IL-1β, IL-2, IL-6, IL-8, TNF-α) and anti-inflammatory cytokines (IL-4, IL-10), the interferon response in terms of the production of IFN-α, IFN-β, IFN-γ, the intensity of surface receptor expression on monocytes, including CD14, CD11b, HLA-DR, CD206, and the NOD2 receptor, which acts as a sensor for mycobacterial cell wall components, among other things. The assessment of genetic variability of the NOD2 receptor, which may influence the course of the immune response in the studied children, was also included.

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Research results indicate that BCG vaccination in the neonatal period may lead to the development of trained innate immunity shaping the response profile of immunocompetent cells to infectious agents such as RSV and SARS-CoV-2. The nature of this modulation depends on the serological status of the child—in seronegative donors for SARS-CoV-2 and RSV antibodies, antiviral mechanisms characterized by increased expression of IFN-β and IL-2 mRNA predominated, while in seropositive donors, simultaneous activation of pro-inflammatory (increased expression of IL-1β, IL-6, TNF-α mRNA) and regulatory (increased expression of IL-4 and IL-10 mRNA) responses was observed, indicating synergistic interaction between trained immunity and acquired immune memory. In addition, an increase in NOD2 receptor expression was demonstrated in leukocytes, particularly after their in vitro costimulation with BCG bacilli and RSV/SARS-CoV-2 virus antigens, correlating with the level of both pro- and anti-inflammatory cytokine mRNA, suggesting that the NOD2 receptor may influence the course of the inflammatory response by modulating its nature depending on previous exposure to infectious agents such as RSV/SARS-CoV-2. In addition, increased expression of monocyte receptors (CD14, HLA-DR) was observed after stimulation with BCG bacilli and/or viral antigens, which may confirm the persistence of trained innate immunity effects. At the same time, the differentiation in the expression of CD11b and CD206 receptors suggests that BCG may modulate the direction of monocyte activation – from pro-inflammatory to regulatory, depending on previous exposure to viral antigens, which indicates the dynamic and complex nature of the immune response induced by BCG vaccination.

The results suggest that BCG bacilli not only strengthen the antiviral mechanisms of leukocytes, but also stimulate the development of a pro-inflammatory response and limit excessive inflammatory reactions, which manifests itself through the modulation of NOD2 expression compared to the expression of pro- or anti-inflammatory cytokines. Such an "immunological footprint" left after BCG vaccination emphasizes the importance of trained immunity as a mechanism supporting population immunity against various pathogens and indicates the potential of this vaccine as an immunomodulatory tool in the prevention of infectious diseases.

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