Coronavirus disease 2019 (COVID-19) is a clinical syndrome caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). BNT162b2 (Pfizer, USA) and mRNA 1273 (Moderna) vaccines were the first messenger RNA (mRNA)-based vaccines ever approved. In both vaccines, a mRNA sequence determines the structure and assembly of the immunogen, the SARS-CoV-2 spike (S) glycoprotein. In this study, the Australian authors investigated cytokine responses to heterologous pathogens, Toll-like receptor agonists, and SARS-CoV-2 antigens in children aged 5 to 11 years vaccinated with two doses of the BNT162b2 mRNA COVID-19 vaccine.

A recent study that investigated subclasses of immunoglobulin (Ig)G specific for the subunit 1 (S1) and receptor-binding domain (RBD) of the SARS-CoV-2 S protein in children 5 to 11 years of age has found increased levels of IgG4 specific for S1 and RBD one year after the BNT162b2 vaccination. IgG4 is the least abundant IgG subclass in humans. Because of some unique structural and functional features, it is described as a “blocking” and “anti-inflammatory” antibody that cannot activate antibody-dependent immune effector response. https://discovermednews.com/elevated-igg4-children-after-mrna-bnt162b2-vaccination/

SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus. Its genome encodes four structural proteins, namely the spike (S), envelope (E), nucleocapsid (N), and membrane (M) protein. The S protein is a glycosylated homotrimer with each monomer composed of subunits S1 and S2, separated by host cell proteases. The S1 domain comprises the N-terminal domain (NTD), the RBD with a receptor binding motif (RBM), and two C-terminal domains.

About the study

The study included 29 children from a clinical trial called COSI BAIR, a part of Melbourne Infant Study: BCG for Allergy and Infection Reduction (MIS BAIR). MIS BAIR is a randomized controlled trial investigating whether neonatal BCG vaccination can protect children from infections, allergies, and asthma. Neonates were randomly assigned to receive or not receive the BCG vaccine in the first ten days of life. Participants from both groups were recruited for the COSI BAIR study. All participants in the COSI BAIR trial received two doses of the BNT162b2 vaccine, 8 weeks apart.

The age limit was five to eleven years. The exclusion criteria were hypersensitivity to the BNT162b2, previous COVID-19 vaccination, previous COVID-19 confirmed by polymerase chain reaction, clinically significant illness, and receipt of BCG vaccine outside the MIS BAIR trial.

The paired blood samples were taken from participants, the first was taken immediately before the first BNT162b2 vaccination, and the second 28 days after the second vaccination. The samples from eight children were taken 6 months after the second BNT162b2 vaccination. *In vitro* cytokine responses to heterologous stimulants, such as SARS-CoV-2 antigens, Toll-like receptor agonists, and killed pathogens (*Haemophilus influenzae* type B, *Listeria monocytogenes*, BCG, *Staphylococcus aureus, Escherichia coli*, *Candida albicans,*

and hepatitis B virus surface antigen) were assessed by whole blood stimulation assay. The SARS-CoV-2 S protein, the S1 and S2 subunits, and RBD were quantified by ELISA.

A cytokine assay was employed to analyze supernatant levels of 27 pro-inflammatory and anti-inflammatory cytokines and chemokines, including eotaxin, basic fibroblast growth factor basic (FGF), granulocyte-colony stimulation factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ), interleukin (IL)-1β, IL-1rα, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p70), IL-13, IL-15, IL-17, IFN-γ-induced protein 10 (IP-10), monocyte chemoattractant protein (MCP-1), macrophage inflammatory protein (MIP)-1α, MIP-1β, platelet-derived growth factor-BB (PDGF-BB), RANTES, tumor necrosis factor-α (TNF-α) and vascular endothelial growth factor (VEGF).

Results

Cytokine responses one month after the second BNT162b2 vaccination

28 days after the second BNT162b2 vaccination, cytokine and chemokine responses to stimulation with pathogens (bacterial, fungal, viral) and Toll-like receptor agonists showed a general decrease compared to responses before the vaccination. IFN-γ and MCP-1 showed the largest decreases.

A stimulation with BCG, *H. influenzae*, *S. aureus*, and hepatitis B antigen decreased the responses of IL-6, IL-15, and IL-17. Stimulation with *L. monocytogenes* decreased the responses of IL-15, TNF-α, and IP-10, whereas *H. Influenzae* and *S. aureus* decreased the response of IL-8. RANTES was the only analyte that increased in response to heterologous stimulants (*L. monocytogenes* and *C. albicans*).

In contrast, cytokine and chemokine responses to stimulation with SARS-CoV-2 antigens increased compared to the responses before the vaccination. IL-6, IL-15, GM-CSF, IL-10, IL-12p70, IL-2, IL-13, MIP-1β, and RANTES showed the largest increase to stimulation with irradiated S1 and S2 subunits of SARS-CoV-2. The responses of TNF-α, G-CSF, PDGF-BB, VEGF, FGF-basic, IL-4, IL-17, and IP-10 also increased after stimulation with the S1 and S2 subunits. MCP-1 was the only analyte that decreased after the stimulation with SARS-CoV-2 N protein.

The BCG status had a negligible or no effect on cytokine responses.

Cytokine responses up to six months after the second BNT162b2 vaccination

This analysis included blood samples from eight children before the first vaccination and 6 months after the second BNT162b2 vaccination who remained negative for the SARS-CoV-2 N protein.

Six months after the second BNT162b2 vaccination, cytokine responses showed a sustained decrease to viral but not to bacterial pathogens compared to the responses before the vaccination. After stimulation with hepatitis B antigen and Poly (I: C), the responses of IL-6, IL-15, TNF-α, GM-CSF, PDGF-BB, VEGF, FGF-basic, IL-10, IFN-γ, IL-2, IL-4, IL-5, IL-9, IL-13, and eotaxin decreased. Hepatitis B antigen stimulation decreased the responses of IL-1β, IL-12p70, IL-17, and MIP-1β, whereas Poly (I: C) stimulation decreased the responses of IL-1ra, IL-7, and MIP-1α. *C. albicans* stimulation decreased the responses of MCP-1 and eotaxin but increased the responses of IL-1β, IL-1ra, IL-8, FGF-basic, IL-12p70, IL-8, and MIP-1.

Stimulation with bacterial pathogens BCG, *E. coli, H. influenza,* and *L. monocytogenes* increased the response of IL-8. Stimulation with *E. coli* and *H. influenzae* increased the responses of TNF-α and G-CSF. BCG stimulation increased the response of RANTES but decreased the response of IP-10.

The responses of most cytokines and chemokines, like IL-1β, IL-1ra, IL-6, G-CSF, GM-CSF, VEGF, FGF-basic, IFN-γ, IL-2, IL4, IL-5, IL-17, MIP-1α, and MIP-1β to stimulation with

SARS-CoV-2 antigens remained increased six months after the second BNT162b2 vaccination.

Conclusion

This study demonstrated changes in cytokine responses to heterologous pathogens, Toll-like receptor agonists, and SARS-CoV-2 antigens in children aged 5 to 11 years vaccinated with two doses of the BNT162b2 mRNA COVID-19 vaccine.

28 days after the second BNT162b2 vaccination, cytokine and chemokine responses to stimulation with pathogens (bacterial, fungal, viral) and Toll-like receptor agonists showed a general decrease compared to responses before the vaccination. These effects persisted, and six months after the second BNT162b2 vaccination, cytokine responses showed a sustained decrease to viral but not to bacterial pathogens compared to the responses before the vaccination.

Since a decrease in immune response to other pathogens makes children more susceptible to other infections, the authors concluded that these findings highlight the need for further investigation and consideration of vaccination policy.

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Journal Reference

Noé A, Dang TD, Axelrad C, Burrell E, Germano S, Elia S, Burgner D, Perrett KP, Curtis N and Messina NL (2023) BNT162b2 COVID-19 vaccination in children alters cytokine responses to heterologous pathogens and Toll-like receptor agonists. *Front. Immunol.* 14:1242380. (Open Access) https://doi.org/10.3389/fimmu.2023.1242380