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NATURAL KILLER CELLS IN SARS-COV-2-VACCINATED SUBJECTS WITH INCREASED EFFECTOR CYTOTOXIC CD56DIM CELLS AND MEMORY-LIKE CD57+NKG2C+CD56DIM CELLS

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Introduction

The infection and negative effects of the SARS-CoV-2 virus are mitigated by vaccines [1]. Despite the presence of high affinity and persistent protective antibody responses indicate an efficient humoral immune response to vaccination [2,3], it is unknown whether vaccination might also elicit a robust protective innate immune responses with high affinity as well.

Aim

Evaluate the relevance of innate immune response to SARS-CoV-2 vaccines, particularly of Natural Killer (NK) cells.

Methods

Subjects: 20 healthy volunteers vaccinated with three doses of Comirnaty (Pfizer Australia Pty Ltd.), evaluated 9 months after the second vaccination and 1 month after the booster dose.
Samples: peripheral blood used plasma collection and PBMC
Stimulation: Spike antigen from Wuhan, Alpha B.1.1.7, Delta B.1.617.2 and Omicron B1.1.529 variants.
Granzyme ELISpot assay: on purified and stimulated NK cells.
FACS analysis: for CD3, CD56, CD26, NKG2A, NKG2D, NKG2C, CD69, CD127, CD57 and CD107a.

Results

Spike-binding and neutralizing antibody levels

We observed an increase in the spike-binding and neutralizing antibody levels 1 month after the booster dose (Fig. 1A, B) (Student t test, Fisher exact test, p= 0.001 respectively), demonstrating the success of the booster dosage in enhancing the humoral response towards SARS-CoV-2. (**Fig.1A and B**).

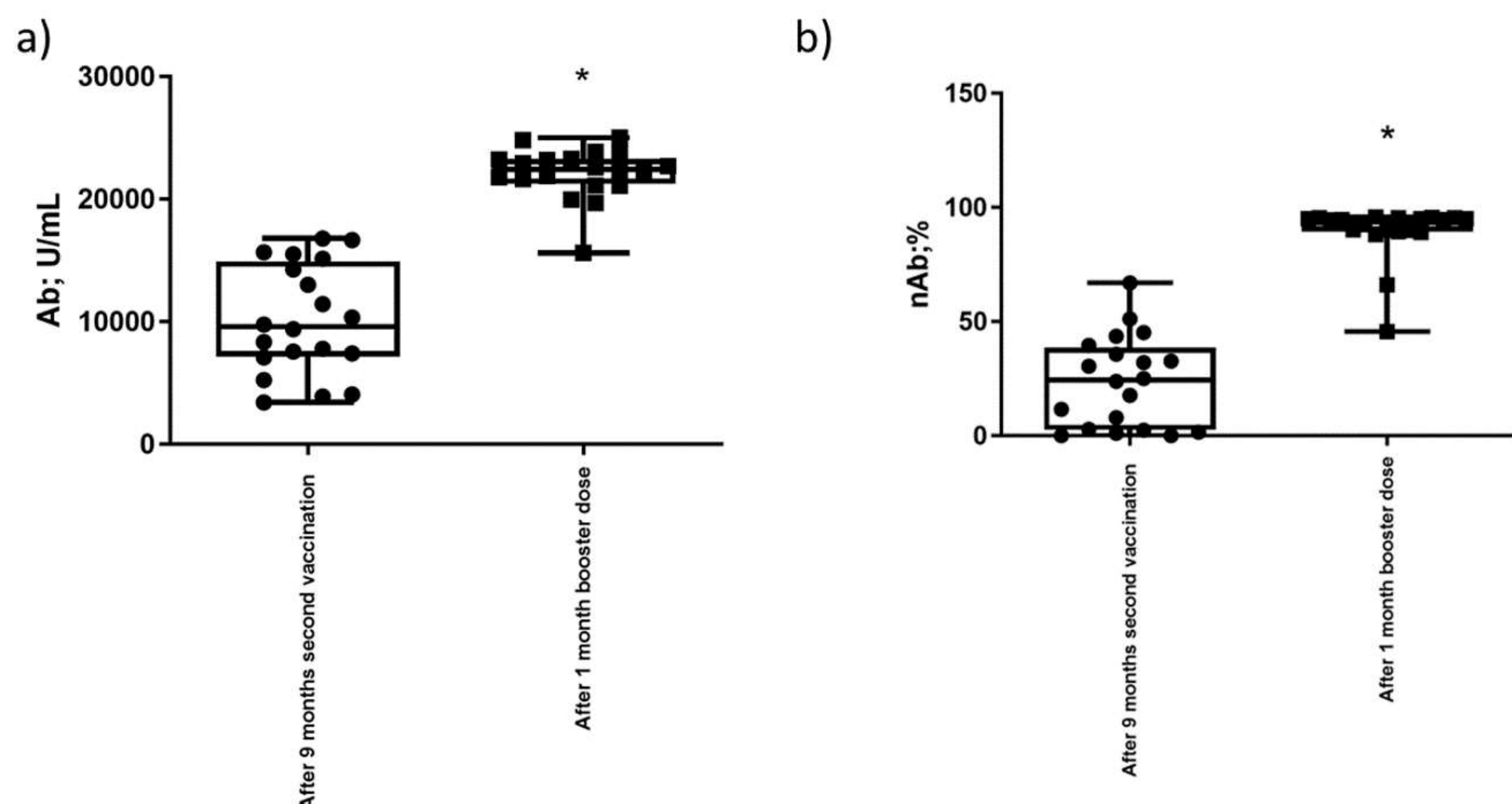

Figure 1. A) Anti-spike SARS-CoV-2 RBD IgG (Ab) plasma levels in 20 healthy individuals 9 mounths after the second vaccination and 1 month after the booster dose. Percentage of inhibition of SARS-CoV-2 infection of Calu3 in co-culture with plasma samples of 20 healthy individuals 9 mounths after the second vaccination and 1 month after the booster dose.

NK cell activation and immunophenotype

A considerable difference in NK surface markers expression 1 month after the booster dose.was found.
We reported the enrichment of CD16+CD56dim NK cells expressing NKG2A, NKG2C, and NKG2D (**Fig. 2A, B, and C;** (p=0.001; Fisher exact test), CD127, a differentiation marker, (**Fig 2D**; p=0.001; Fisher exact test) CD57, marker of maturation, (**Fig.2E** ; p=0.001; Fisher exact test) and CD69+. (**Fig.2F;** p=0.001; Fisher exact test),

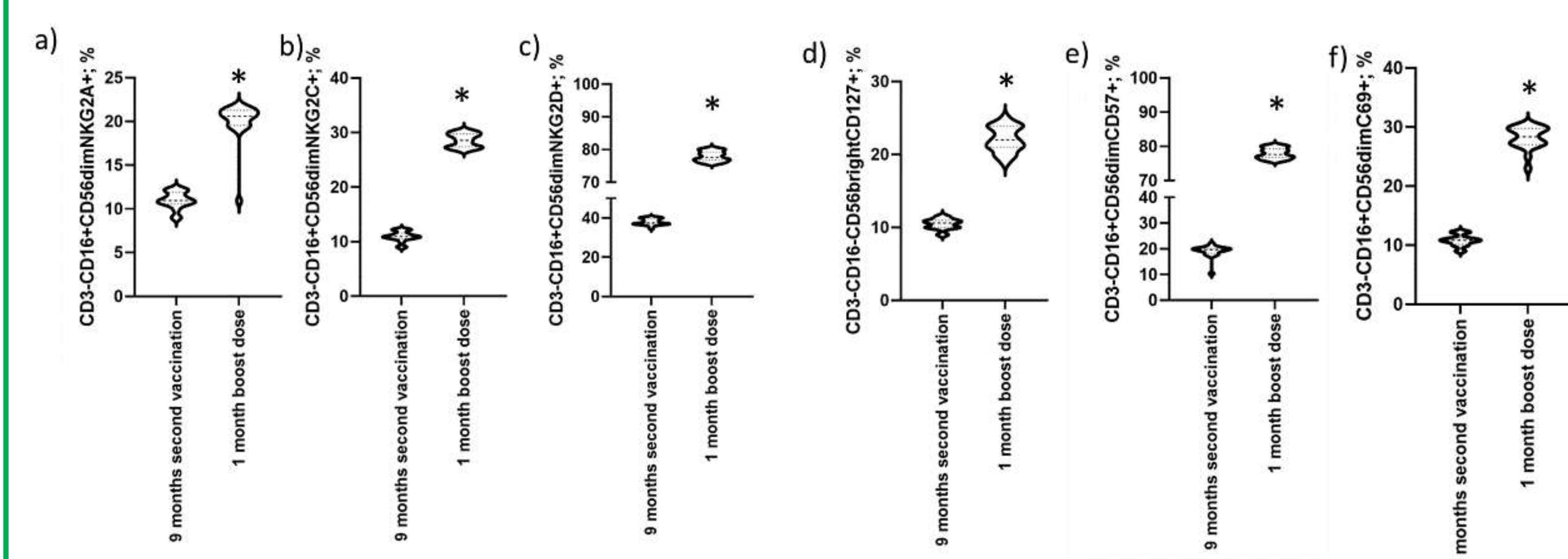

Figure 2. Differential profile of CD56dim and CD56bright NK cells 9 months after the second vaccination and 1 month after boost dose. The frequency of CD56dim NK cells expressing A) NKG2A, B) NKG2C, C) NKG2D, E) CD57 and F) CD69, and of CD56bright NK cells expressing D) CD127, in the peripheral blood of vaccinated subjects was assessed by flow cytometry.

Correlation between CD56dimCD57+ cells and neutralizing SARS-CoV-2 serology

In the 1-month post-booster dose group, the percentages of CD16+CD56dimNKG2C+ or CD16+CD56dimCD57+ cells (**Fig. 3A and B**) were significantly linked with the SARS-CoV-2 serology (r2: 0.88, 0.87, respectively; Spearman correlation test). **Figure 2C** shows a significant association between SARS-CoV-2 neutralizing antibody levels and CD16+CD56dimCD57+ cells nine months after the second vaccination (r2 = 0.93; Spearman correlation test).

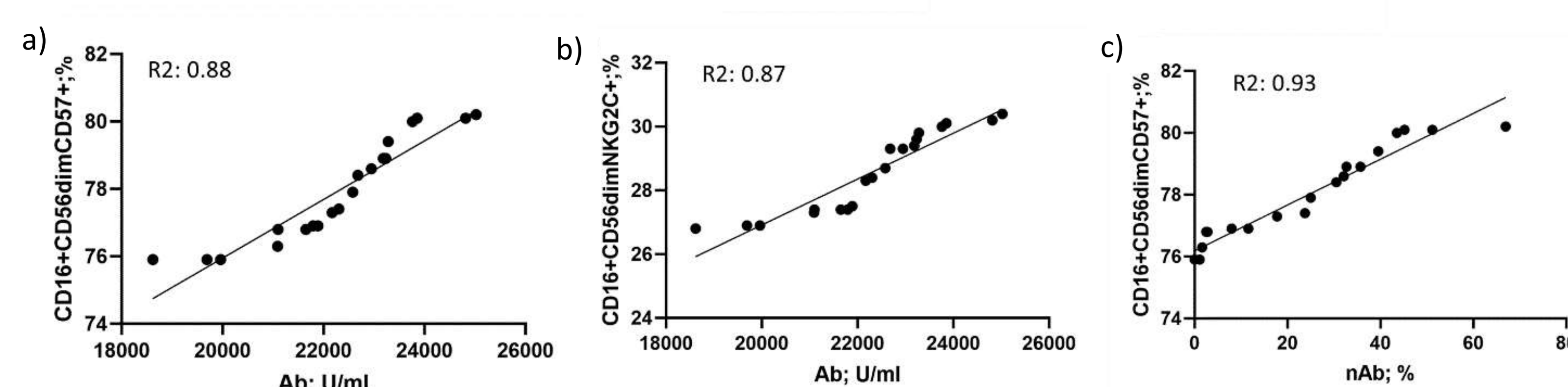

Figure 3 Correlation between A) CD56dimCD57+, B) CD56dimNKG2C+ cells and SARS-CoV-2 serology. C) Correlation between CD56dimCD57+ cells and neutralizing SARS-CoV-2 serology.

Activation markers in NK Subsets

We reported the presence of specific effector cytotoxic CD56dim, characterized by high levels of CD107a and granzyme production, and memory-like CD57+NKG2C+CD56dim phenotype of NK cells exposed to SARS-CoV-2 spike antigen (Wuhan, Alpha B.1.1.7, Delta B.1.617.2, Omicron B1.1.529 variants) (**Fig.3A**). Granzime-B+ NK cells increased after the booster dose when challenged with the Wuhan, Alpha B.1.1.7, Delta B.1.617.2, and Omicron B1.1.529 variants (**Fig. 3B and C**; p=0.001, Fisher exact test), with the Wuhan variant inducing the highest percentage of Granzime-B+ NK cells.

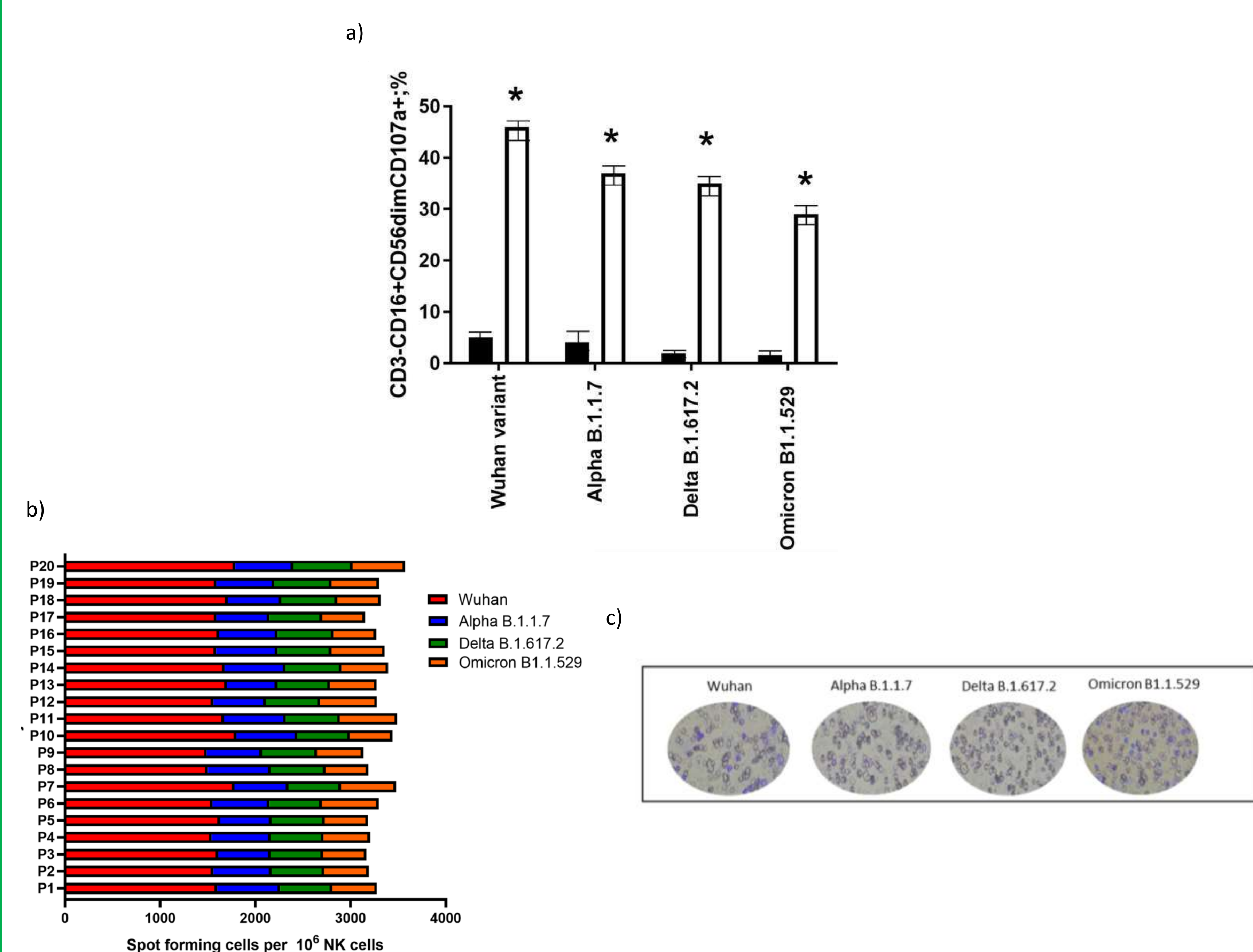

Figure 3. A) Number of NK cells expressing CD107a stimulated with Wuhan, Alpha B.1.1.7, Delta B.1.617.2, Omicron B1.1.529 variants. Black histogram: 9 mounths after the second vaccination; white histogram: 1 month after the booster dose. B) Spot forming cell per 10^6 NK cells secreting Granzime-B after stimulation with Wuhan, Alpha B.1.1.7, Delta B.1.617.2, Omicron B1.1.529 variants. 1 month after booster dose. C) Representative ELISpot results for Granzime-B secretion after stimulaton with Wuhan, Alpha B.1.1.7, Delta B.1.617.2, Omicron B1.1.529 variants.

Conclusions

We report the relevance of the innate immune response, especially NK cells, to SARS-CoV-2 vaccines to guarantee efficient protection against the infection following a booster dose.
In particular, the booster dose caused early NK CD56dim subset activation and memory-like phenotype, confirming the relevance of innate immune response in the efficacy of SARS-CoV-2 vaccination.

References
1. Olliaro P, Torreele E, Vaillant M. COVID-19 vaccine efficacy and effectiveness-the elephant (not) in the room. Lancet Microbe. 2021; 2: e279-e280.
2. Collier AY, McMahan K, Yu J, Tostanoski LH, Aguayo R, Ansel J, et al. Immunogenicity of COVID-19 mRNA Vaccines in Pregnant and Lactating Women. JAMA. 2021; 325: 2370-2380.
3. Rizzo R., Bortolotti D. et al. Humoral and adaptive immune responses to the SARS-CoV-2 vaccine. Int J Infect Dis. 2022, 412-414
4. Gentili V., Bortolotti D. et al. Natural Killer Cells in SARS-CoV-2-Vaccinated Subjects with Increased Effector Cytotoxic CD56dim Cells and Memory-Like CD57+NKG2C+CD56dim Cells. Fornt Biosci 2023; 28:156

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