Exploring the Interplay Between COVID-19 Vaccination, Red Blood Cells, and Immune Function

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Introduction

Red blood cells (RBCs) are emerging as regulators of the innate immune response by interacting with inflammatory molecules, including cytokines/chemokines, nucleic acids, and pathogens.

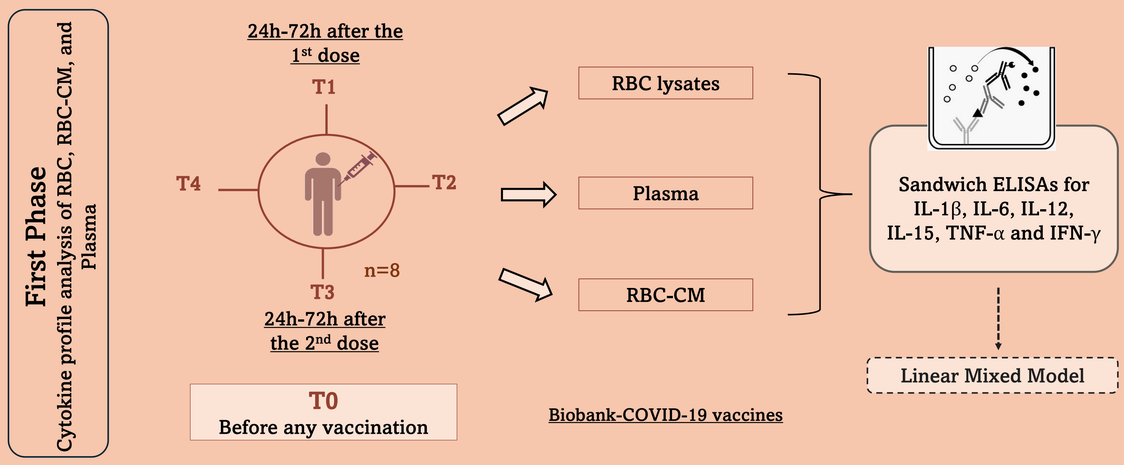
While the role of RBCs in the immune system is well documented, their impact on the immunization process by vaccines is not yet fully understood.

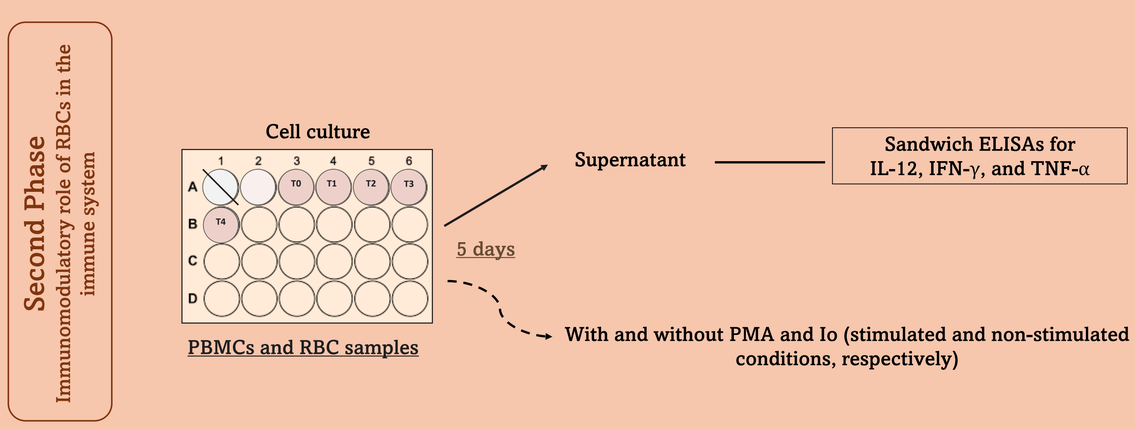
Objective

Taking as a model the emergent COVID-19 vaccines, the main aim of this project is to investigate whether vaccines induce modifications in the RBCs' cytokine profile capable of affecting the immune cells' activity and, therefore the immune response.

Materials and Methods

This study had two phases following sample preparation:





- For this study, RBC, RBC-CM, and Plasma samples from 8 individuals collected at five time points (T0, T1, T2, T3, and T4) following COVID-19 vaccination were selected and analyzed. Demographically, these patients exhibit similar characteristics, including gender, health status, and age.
- RBC samples were sourced from our pre-established Biobank comprising n=39 individuals who underwent COVID-19 vaccination in 2020.
- In sample preparation, RBC lysates were produced through the lysis of RBCs. RBC-CM denotes the conditioned media obtained from the culture of RBCs, containing vesicles and soluble molecules released by these cells into the medium.
- The rationale behind selecting these cytokines stemmed from their pivotal roles in the immune response, particularly their proinflammatory functions.

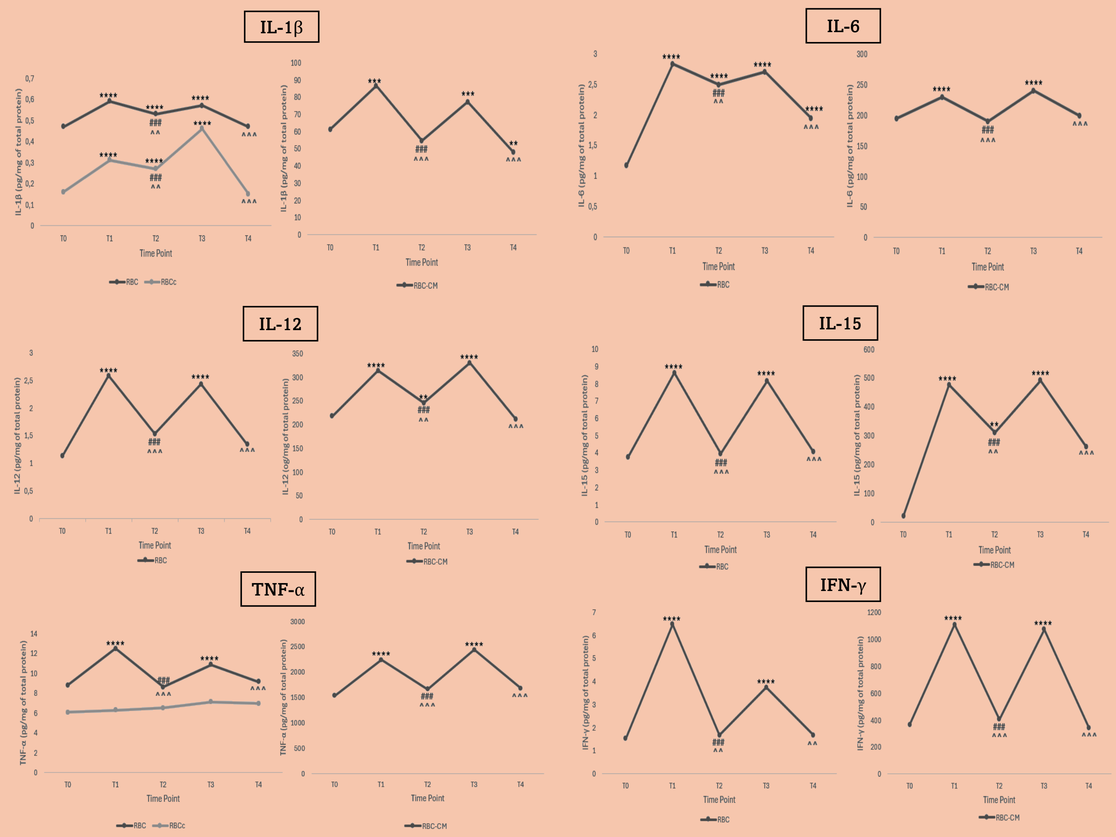
Results

Cytokine	Time Point	Plasma			RBC		
		Subjects for positive cytokine	pg/mL of whole blood		Subjects for positive cytokine	pg/mL of whole blood	
			Concentration	SD		Concentration	SD
IL-1β	T0	8/8	4,11	0,67	8/8	46,34	4,79
	T1	8/8	5,1	0,71	8/8	50,54	2,84
	T2	8/8	4,39	1,01	8/8	51,91	4,22
	T3	8/8	7,76	1,25	8/8	52,59	5,75
	T4	8/8	4,64	0,57	8/8	43	3,36
IL-6	T0	8/8	4,19	0,43	8/8	115,04	5,96
	T1	8/8	4,97	0,85	8/8	244,63	23,8
	T2	8/8	5,37	0,39	8/8	244,23	22,2
	T3	8/8	5,34	0,51	8/8	250,89	29,05
	T4	8/8	4,83	0,37	8/8	177,75	14,05
IL-12	T0	0/8	—	—	8/8	129,75	10,25
	T1	1/8	41,67	—	8/8	222,58	25,8
	T2	0/8	—	—	8/8	150,16	21,27
	T3	2/8	41,25	2,85	8/8	225,34	31,13
	T4	0/8	—	—	8/8	122,49	9,59
IL-15	T0	0/8	—	—	8/8	364,69	38,07
	T1	0/8	—	—	8/8	745,04	94,68
	T2	0/8	—	—	8/8	389,92	51,62
	T3	0/8	—	—	8/8	751,15	113,76
	T4	0/8	—	—	8/8	372,06	45,55
TNF-α	T0	8/8	7,58	0,9	8/8	85,84	4,18
	T1	8/8	9,57	0,55	8/8	107,47	11,84
	T2	8/8	9,02	0,5	8/8	84,47	8,56
	T3	8/8	10,06	1,07	8/8	100,35	10,58
	T4	8/8	5,82	0,57	8/8	83,18	3,89
IFN-γ	T0	8/8	15,14	4,31	8/8	148,95	53,81
	T1	8/8	27,5	5,72	8/8	555,41	165,15
	T2	8/8	16,7	10,84	8/8	165,17	38,29
	T3	8/8	26,86	8,77	8/8	344,11	68,37
	T4	8/8	12,51	3,03	8/8	150,91	63,86

Whole blood
Plasma vs RBC

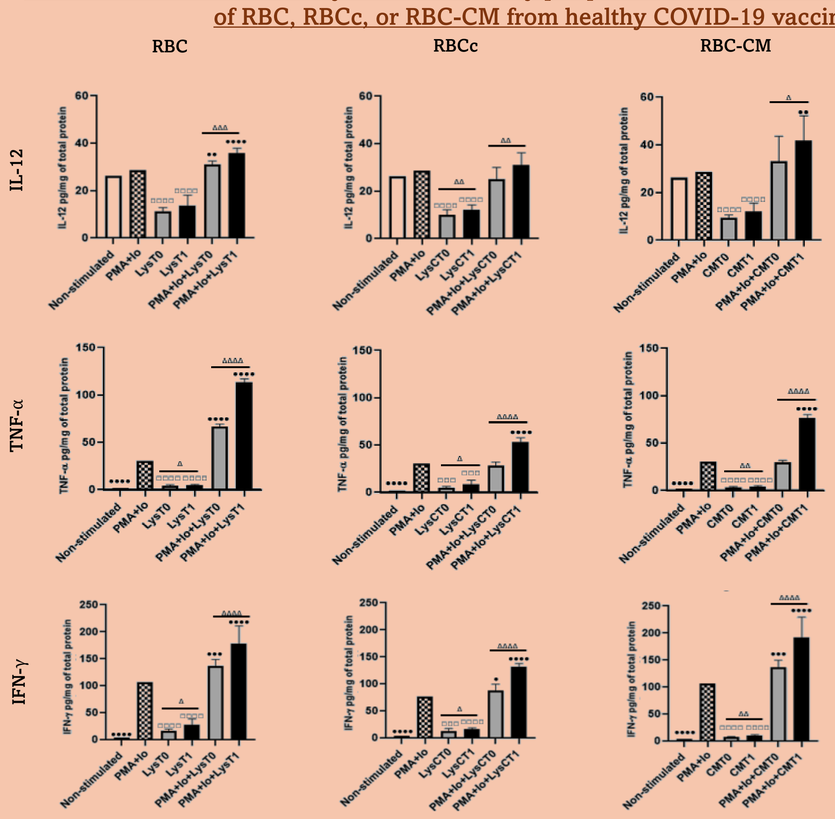
Cytokine concentration was shown to be 10 to 50 times higher in red blood cells than in plasma, depending on the specific type.

Cytokine profiles of red blood cells (RBC), RBC-conditioned media (RBC-CM), and RBC culture (RBCc) in response to COVID-19 vaccination across various time points



Cytokine profiles in RBCs and RBC-CM in response to COVID-19 vaccination. Sandwich ELISA analyzed RBC, RBCc and RBC-CM samples for the following cytokines: Interleukin-1β (IL-1β), Interleukin-6 (IL-6), Interleukin-12 (IL-12), Interleukin-15 (IL-15), Tumor Necrosis Factor-α (TNF-α), and Interefon-gamma (IFN-γ). The longitudinal time points considered during COVID-19 vaccine immunization were T0: before vaccination, T1: 24h-72h after the first vaccine dose, T2: before the second vaccine dose, T3: 24h-72h after the second vaccine dose, and T4: after one month of the last vaccine dose. Data was analyzed by statistical Linear Mixed Model (LMM). Statistically significant differences between pre-COVID-19 vaccination (T0) and post-vaccination time points (T1, T2, T3, and T4) are indicated by: * p< 0.05, ** p< 0.01, ***p<0.001, and **** p<0.0001. Between T1 and T2 these differences are denoted by: # p< 0.01, ## p<0.001, and ### p<0.0001. And between T3 and T2 or T4 by ^ p<0.01, ^^ p<0.001, and ^^^ p<0.0001. Holm-Bonferroni correction was applied to adjust p-values.

Differential Modulation of Cytokine Profiles by peripheral mononuclear blood cells (PBMCs) in the presence of RBC, RBCc, or RBC-CM from healthy COVID-19 vaccinated individuals



The statistical test employed for these findings was the Paired T-test. Statistically significant discrepancies between only the stimulated condition (PMA+Io) and the other conditions are denoted as follows: ● ● ● ● p<0.0001; ● ● ● p< 0.001; ● ● p<0.01; ● p<0.05. The same significant differences between time points from stimulated or non-stimulated conditions are denoted by: Δ. Finally, □ represents the significant discrepancies between the non-stimulated conditions. Holm-Bonferroni correction was applied to adjust p-values. As a note, RBCc refers to the RBC after culture.

Discussion and main conclusions

- RBCs show higher cytokine concentrations than Plasma;
- COVID-19 vaccination induces significant cytokine profile changes in RBCs and RBC-CM;
- RBC, RBCc, and RBC-CM lysates (pre- and post-vaccination) significantly decrease IL-12 but induce TNF-α and IFN-γ proinflammatory cytokine secretion by PBMCs in cell culture. After vaccination (T1), the negative effect of RBC lysates on IL-12 secretion is lesser while the positive effect on TNF-α and IFN-γ secretion is higher.
- Upon stimulation, the secretion of all cytokines under study, including IL-12, is greatly increased, especially in the presence of post-vaccination RBC lysates.
- Beyond cytokines, other immunomodulatory factors in RBCs may influence PBMC function and immune responses
- These findings could impact clinical diagnostics, therapy, and understanding of immune regulation.

Some references of other studies:

Dobkin, J., & Mangalmurti, N. S. (2022). Immunomodulatory roles of red blood cells. *Curr Opin Hematol.*, 29(6), 306–309. https://doi.org/10.1097/MOH.0000000000000734.

Karsten, E., Breen, E., & Herbert, B. R. (2018). Red blood cells are dynamic reservoirs of cytokines. *Scientific Reports*, 8(1), 3101. https://doi.org/10.1038/s41598-018-21387-w.

Acknowledgments

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