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CORRESPONDENCE

Inflammatory and senescence-associated mediators affect the persistence of humoral response to COVID-19 mRNA vaccination in transfusion-dependent beta-thalassemic patients

To the Editor:

Survival and complication-free survival in patients with transfusion-dependent β-thalassemia (TDT) continue to improve in settings with adequate access to care, but several frailty traits persist and remain to be fully elucidated.[1] TDT patients are characterized by an impaired innate and adaptive immune response, mainly due to chronic transfusions and iron overload.[2] This favors the shift towards lymphocyte Th2 phenotype and the restriction of both T and B-cell receptor repertoires.[2–5] We recently reported a rapid decline of antibody against the region-binding domain (RBD) in TDT patients exposed to anti-SARS-CoV-2 mRNA vaccine (BNT162b2) when compared to health-care workers (HCW), similarly to that reported in healthy elderly subjects.[6] Taking advantage of the previously characterized cohort of TDT patients ($n = 154$), we evaluated whether we might identify immunomodulating factors before vaccination associated with a reduced anti-RBD antibody persistence. To this aim, we planned a nested retrospective study within the clinical trial (NCT05157256) approved by the Ethical Committee of the National Institute for Infectious Diseases "L. Spallanzani," as National Review Committee Board for COVID-19 pandemic in Italy.[6] A total of 64 TDT patients (41.5% of the whole cohort of vaccinated patients) were included in this analysis; they were selected according to the random availability of serum samples before vaccination (T0) and of data about anti-RBD response after 2 (T1) and 12 (T2) weeks from the second dose of vaccine. A group of HCW ($n = 10$) was also included. Demographic and clinical patients' characteristics are summarized in Table S1. No significant differences in age and gender between TDT and HCW were observed (Table S1).

In TDT patients, the anti-RBD titer was lower than in HCW at T2 (TDT: 344.2 BAU/mL [IQR: 203.6–532.6] vs. HCW: 534.7 BAU/mL [IQR: 307.2–830.2], $p < .0012$), confirming a more rapid decline of humoral response observed in the whole TDT cohort.[6] Accordingly, the fold of reduction, measured as the ratio between the anti-RBD Abs titer at T1 and at T2 (T1/T2 ratio), was higher in TDT patients than in HCW (Figure S1A, $p < .0001$). We assessed a possible impact of clinical variables on the extent of antibody decrease in TDT patients. No impact of age ($p = .193$) and splenectomy ($p = .112$) on T1/T2 ratio was observed in TDT patients. By contrast, a slightly faster anti-RBD decrease was observed in male than in female TDT patients, and in patients treated with deferiprone (DFP) as compared to deferasirox (DFX) (Figure S1B,C).

To identify marker(s) associated with a different durability of the anti-RBD vaccine response, senescent-Associated Secretory Phenotype (SASPs), including inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α) and TIMP-1 and MMP-9, were quantified in sera of TDT and HCW at T0 by an automated ELISA assay (Biotechne). As shown in Figure S2A, before vaccination, a higher expression of SASPs factors was observed in TDT patients, with higher levels of IL1-β, TNF-α, IL-6, IL-8, MMP-9, TIMP-1 as compared to those observed in HWC ($p < .001$ for all comparison), this finding confirming previous reports.[7–10] Noteworthy, MMP-9 and TIMP-1 play pleiotropic activities, from modulation of inflammatory network to suppression of the immune response.[11] Specifically, their expression can be induced by pro-inflammatory stimuli (e.g., IL-1β and TNF-α) and, on the other hand, they can modulate the inflammatory response.[11] No impact of age, gender and chelation therapy on these markers was observed in TDT patients, but higher serum levels of IL-6, IL-8 and TIMP-1 were observed in splenectomized patients. This might be possibly related to absence of the spleen, which is important in both coordination of pro-inflammatory (e.g., IL1β, TNFα) and anti-inflammatory (e.g., IL10) cytokines (Figure S2B), and in B-cell maturation.[12,13]

To define a possible contribution of the basal (pre-vaccination) profile of those markers in shaping the subsequent persistence of the immune response to SARS-CoV2 vaccination, we divided the TDT patients into four groups based on the extent of anti-RBD reduction over time (four quartiles of the variable anti-RBD decrease T1/T2, Figure S3A). Specifically, TDT patients were divided in: (i) slow decrease (1st quartile, 0–25th percentile); (ii) slow/middle decrease (2nd quartile, 25th–50th percentile); (iii) middle/fast decrease (3rd quartile, 50th–75th percentile) and (iv) fast decrease (4th quartile, 75th–100th percentile). Principal component analysis (PCA) was therefore performed, in order to identify the major trends inherent to the SASP factor profile (Figure 1, left panel). PCA efficiently segregated the first (red) and the fourth (green) group of TDT patients, characterized respectively by the slowest and the fastest reduction of anti-RBD Abs overtime (Figure 1, left panel). The factors mainly responsible for this segregation were the inflammatory cytokines (IL-1β, IL-8) and MMP-9. Accordingly, before vaccination,

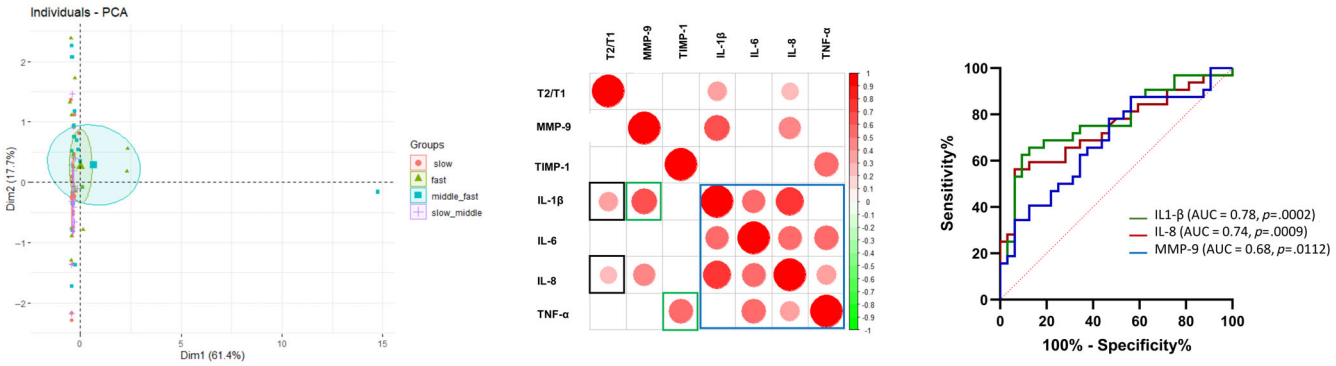

FIGURE 1 Principal component analysis of the soluble mediators (measured by immunoassay) in the four groups of patients (slow, slow/medium, middle/fast, and fast); Score plot of different groups are marked, respectively, in red, purple, light blue, and green colors. Confidence ellipses are drawn to better appreciate the differences between groups ($n = 4$). Spearman correlation matrices among IL1-β, TNF-α, IL-6, IL-8, MMP-9, TIMP-1, and T1/T2 ratio. Only statistically significant ($p < .05$) parameters are shown: red = positive correlation, green = negative correlation. Receiver operating characteristic (ROC) curve for the IL-1β (green curve), IL-8 (red curve), and MMP-9 (blue curve) as marker of COVID-19 reduction of anti-region-binding domain overtime (slow + slow/middle vs. middle/fast + fast).

TDT patients with a fast antibody decrease showed higher levels of IL1-β, IL-8, and MMP-9 when compared to those in the slow and slow/middle group (Figure S3B).

To define possible associations among SASP factors and anti-RBD reduction, a multiple correlation analysis was performed (Figure 1, middle panel). The inflammatory markers correlated with each other (blue square), suggesting a coordinated inflammatory profile in TDT patients. Moreover, we found positive correlation between TNF-α and TIMP-1, between IL-8 and MMP-9 and between IL-1β and MMP-9 (green square). This observation supports the proposed strict relationship between inflammation and MMP-9, thus linking cell senescence to inflammaging.[14,15] Of note, this plasmatic environment impacts on the durability of anti-RBD response overtime, since a positive correlation between the anti-RBD fold decrease (T1/T2) and IL-1β, IL-8 has been reported (black square). Finally, to formally prove a predictive value of SASPs factors on the anti-RBD durability, we performed a ROC analysis (Figure 1, right panel). Results showed that IL-1β and IL-8 are the best factors able to distinguish between TDT patients with a T1/T2 ratio below (slow + slow/middle) or above (middle/fast + fast) the median (IL-1β-AUC: 0.78, $p = .0002$; IL-8-AUC: 0.74, $p = .0009$).

An effective primary immune response should be characterized by a good potency in terms of specific antibody titer early after vaccination/infection and by a persistence overtime of both antibody and memory B cells. Here, we show for the first time that IL1-β, IL-8, and MMP-9 before vaccination significantly impact the kinetics of decay of humoral response to SARS-CoV2 vaccination in TDT patients. Notably, interactions between pro-inflammatory cytokines and B-cell response have been described, contributing to the downregulation of the E47 transcription factor and AID enzyme, which are required for class-switch recombination in B cells and memory cells.[16] This mechanism might account for a reduced or impaired humoral response in the context of "inflammaging" or age-related immune impairment,[17] as in TDT patients in view of their immunological similarities. Indeed, Russel Knode et al. have recently suggested that only specific subsets of B cells have intrinsic age-related defects in class switching.[17] In our population, higher pro-inflammatory cytokines might associate with a larger subset of defective B cells, explaining the different humoral response to vaccination according to the specific cytokine profile expression. Further investigations are needed in order to define the impact of these markers on the differentiation of specific memory B- and T- cells. The faster decrease of anti-RBD antibodies observed in TDT patients after vaccination together with the lower frequency of Spike-specific memory B and T cells defines an immune frailty that could benefit of specific vaccination schedule with closer/additional booster doses, mainly in splenectomized patients or in those with more expressed SASPs.

In conclusion, our data identify inflammatory and senescence-associated soluble mediators before vaccination able to predict the durability of the humoral response to SARS-CoV2 mRNA vaccination. A similar observation helps characterize the specific immune deficit in patients with TDT and highlights the key role of the plasmatic environment in modulating the effectiveness of the immune response to vaccination, opening new interesting perspectives for studies and interventions.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

PATIENT CONSENT STATEMENT

All patients signed the informed written consent.

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