Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
TO THE EDITOR

Waning of SARS-CoV-2 RBD antibodies in longitudinal convalescent plasma samples within 4 months after symptom onset

Josée Perreault,1 Tony Tremblay,1 Marie-Josée Fournier,1 Mathieu Drouin,1 Guillaume Beaudoin-Bussières,2,3 Jérémie Prévost,1,3 Antoine Lewin,4,5 Philippe Bégin,4 Andrés Finzi,2,3,7 and Renée Bazin1

1Héma-Québec, Affaires Médicales et Innovation,Québec, QC, Canada; 2Centre de Recherche du Centre Hospitalier de l’Université de Montréal, Montréal, QC, Canada; 3Département de Microbiologie, Infectiologie et Immunologie, Université de Montréal, Montréal, QC, Canada; 4Héma-Québec, Affaires Médicales et Innovation, Montréal, QC, Canada; 5Faculté de Médecine et des Sciences de la Santé, Université de Sherbrooke, Sherbrooke, QC, Canada; 6Centre de Recherche du Centre Hospitalier Universitaire Sainte-Justine, Montréal, QC, Canada; and 7Département de microbiologie et d’immunologie, Université McGill, Montréal, QC, Canada

Transfusion of COVID-19 convalescent plasma (CCP) as a means to reduce the severity of the disease and help resolve the infection more rapidly is currently being investigated in more than 100 clinical trials. The beneficial effects of CCP transfusion in COVID-19 patients were recently reported, although most of the studies were not randomized controlled trials or they involved only a few patients.1,2 One of the main hypotheses to explain the potential clinical benefits of CCP is the presence of SARS-CoV-2-neutralizing antibodies (nAbs).3,4 Consequently, several groups have included nAb titers as a criterion for the selection of CCP units to be transfused.5,6,7 A good correlation between nAb and SARS-CoV-2 spike protein receptor binding domain (RBD) antibody titers has been reported,5,8 and therefore analysis of SARS-CoV-2 spike RBD antibodies using enzyme-linked immunosorbent assay (ELISA) represents a valuable tool for the initial characterization of CCP.

Héma-Québec, the agency responsible for the blood supply in Quebec, Canada, is involved in the collection and testing of CCP used in a randomized open-label trial of Convalescent Plasma for Hospitalized Adults with Acute COVID-19 Respiratory Illness (CONCOR-1, clinicaltrials.gov identifier #NCT04348656) designed to determine the effect of CCP at reducing the risk of intubation or death in adult patients hospitalized for COVID-19. Potential donors were recruited after at least 14 days of resolution of COVID-19 symptoms (see supplemental Methods for additional information, available on the Blood Web site). Initial diagnosis had been confirmed by public health authorities through either polymerase chain reaction or epidemiologic contact. All participants met the donor selection criteria for plasma donation in use at Héma-Québec and consented to the study. Seropositivity (presence of antibodies against SARS-CoV-2 RBD) was determined using a semiquantitative ELISA (supplemental Methods) adapted from previous work.15,16 Consistent with previous reports on the rate of seroconversion of COVID-19 patients,17-19 the overall proportion of our convalescent plasma donors (n = 282) that were tested seronegative at the time of donation was 6.9%. However, this proportion increased to about 15% when considering only donors who had waited for more than 11 to 12 weeks after symptom onset before donating (supplemental Table). This prompted us to perform a longitudinal analysis of the anti-RBD antibody response in CCP donors (11 males and 4 females; median age, 56 years; range, 20-67 years) who donated at least 4 times, during a time interval after symptom onset ranging from 33 to 77 days for the first donation to 66 to 114 days for the last donation. These donors reported symptoms of different intensity (from mild/moderate to severe), although none of them were hospitalized for COVID-19. Changes from baseline measurements were modeled with the use of a linear mixed-effects model for repeated measures based on a participant-level analysis with fixed effects for sex, age, and time since symptom onset (for more details, see supplemental Methods).

As shown in Figure 1A, the level of anti-RBD antibodies at the first donation varies greatly between donors. However, a decrease in anti-RBD antibody level between first and last donation was observed for all donors. To better illustrate the evolution of the anti-RBD antibody response over time, the relative level of anti-RBD antibodies was calculated at each time point using the first time point as reference (Figure 1B). In some donors, an increase was observed after their first donation, but this was always followed by a decline in anti-RBD antibodies at later time points. To rule out the possibility that the decline observed in all donors was a consequence of repeated donations, we determined the correlation between the number of donations and the overall decline in anti-RBD level, as defined using the maximal optical density (OD) and the OD at the last donation (ODlast donation/ODmax). As shown in Figure 1C, the decrease in anti-RBD levels did not correlate with the number of donations (r = .417, P = .1221). We then compared the decrease in anti-RBD level as a function of the time elapsed between the onset of symptoms and the time of the last donation (Figure 1D). The results revealed a significant correlation between these 2 parameters (r = .821, P = .0002), indicating that the anti-RBD response wanes over time of convalescence rather than because of repeated donations.

To get a more general picture of the decline in anti-RBD antibodies over time, we performed a repeated-measure analysis
with adjustment for donor age and sex. For group comparison, the time from onset of symptoms (33-114 days) was divided in quartiles containing similar numbers of samples (from 19 to 22 donor samples), and the data (OD values) in each of these quartiles were combined regardless of the donor identity. Figure 2 shows the distribution, median, and mean OD in each quartile. Overall, a significant decrease in OD value from baseline through last donation was observed (P < .0001). Pairwise comparisons showed that in the first and second quartiles (33-53 and 54-69 days after symptom onset, respectively), the median and mean OD were quite similar (mean, 1.499 ± 0.760 and 1.309 ± 0.710; median, 1.486 [interquartile range (IQR), 1.44] and 1.363 [IQR, 1.43], respectively, with P = .313), although a slight decrease in the mean values could be observed. This suggests that the anti-RBD response is relatively stable during the first 10 weeks after disease onset, in contrast with the recently reported decrease in neutralization activity in the plasma of convalescent patients a few weeks after symptom resolution.\textsuperscript{15,16,20-22} No significant decrease in median and mean OD values was observed between the second and third quartiles (54-69 and 70-84 days after symptom onset, respectively; mean, 1.309 ± 0.710 and 1.321 ± 0.720; median, 1.363 [IQR, 1.43] and 1.411 [IQR, 1.52], respectively, with P = .1221). However, the most striking observation comes from comparing the third and fourth quartiles (70-84 and 85-114 days after symptom onset, respectively), where a marked decrease in the mean OD values (significant mean OD decrease from 1.411 ± 0.720 to 0.835 ± 0.670, representing a 70.1% decrease with P = .0052) and an even more pronounced decrease in median values (median OD decreases from 1.411 [IQR, 1.52] to 0.411 [IQR, 1.15], representing a 70.1% decrease) were observed.

Interestingly, the decrease in OD values during a period of about 20 days (considering the mean and median of third and fourth quartiles, both of 76 and 95 days, respectively) is reminiscent of the plasma immunoglobulin G (IgG) half-life of 21 days,\textsuperscript{23} suggesting that de novo synthesis of anti-RBD antibodies stopped between the third and fourth quartiles in all CCP donors. This time frame is consistent with the first wave of a humoral immune response during which short-lived plasma cells actively secrete...
pathogen-specific antibodies until the antigen is eliminated.\textsuperscript{24} This is expected to be followed by the emergence of a cellular memory response that could play a major role in the long-term protection against reinfection, as recently proposed.\textsuperscript{25} The clinical significance of this in the event of re-exposure is therefore currently unknown.

Our study contains some limitations as only anti-RBD antibodies were measured in CCP from a limited number of different donors. Additional work including the characterization of our CCP donor plasma samples on other SARS-CoV-2 antigens (eg, full spike, nucleocapsid), the determination of the nAb titers, and contribution of antibody isotypes (IgA, IgG, and IgM) will permit extension of our initial observations on RBD antibodies to a broader humoral response to SARS-CoV-2 and help to better define its persistence. Nevertheless, the availability of sequential samples from the CCP repeated donors permitted to better pinpoint the time at which the anti-RBD response starts to significantly decline, regardless of the initial anti-RBD antibody level, which has been shown to correlate with disease severity.\textsuperscript{20,26,27} This observation has important implications for convalescent plasma collection especially because Xia et al\textsuperscript{28} recently provided data suggesting that the efficacy of CCP treatment in responder patients correlated with the antibody levels in CCP and for seroprevalence studies in the general population. Such studies should be performed close to the peak of infection, when most infected individuals (symptomatic or not) will still have easily detectable SARS-CoV-2 antibodies, to better estimate the true number of infections.

Acknowledgments

The authors are grateful to the convalescent plasma donors who participated in this study and the Héma-Québec team involved in convalescent donor recruitment and plasma collection. The authors also thank M. Gordon Joyce (US Military HIV Research Program) for the monoclonal antibody CR3022. All work was conducted in accordance with the Declaration of Helsinki in terms of informed consent and approval by an appropriate institutional board. Convalescent plasmas were obtained from donors who consented to participate in this research project at Héma-Québec (REB 2020-004).

This work was supported in part by Ministère de l’Économie et de l’Innovation du Québec, Programme de Soutien aux Organismes de Recherche et d’Innovation to A.F. A.F. is the recipient of Canada Research Chair on Retroviral Entry Grant RCHS2023 950-232424. G.B.-B. and J. Prévost are supported by Canadian Institutes of Health Research fellowships. P.B. is supported by a Fonds de Recherche du Québec-Santé Junior 2 Salary Award.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authorship

Contribution: A.L., A.F., and R.B. conceived the study; J. Perreault, T.T., M.-J.F., M.D., G.B.-B., and J. Prévost performed and interpreted the experiments; A.L., P.B., A.F., and R.B. analyzed the data; A.L. and R.B. wrote the manuscript; and all authors read, edited, and approved the final manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Renée Bazin, Héma-Québec, 1070 Ave des Sciences-de-la-Vie, Québec, QC G1V 5C3, Canada; e-mail: renee.bazin@hema-quebec.qc.ca.

Footnotes

Submitted 23 July 2020; accepted 17 August 2020; prepublished online on Blood First Edition 1 October 2020.

E-mail the corresponding author for original data.

The online version of this article contains a data supplement.

There is a Blood Commentary on this article in this issue.

REFERENCES

1. Duan K, Liu B, Li C, et al. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. Proc Natl Acad Sci USA. 2020;117(17):9490-9496.

2. Shen C, Wang Z, Zhao F, et al. Treatment of 5 critically ill patients with COVID-19 with convalescent plasma. JAMA. 2020;323(16):1582-1589.
TO THE EDITOR:

MPN patients with low mutant JAK2 allele burden show late expansion restricted to erythroid and megakaryocytic lineages

Ronny Nienhold,1 Peter Ashcroft,2 Jakub Zmajkovic,1 Shivam Rai,1 Tata Nageswara Rao,1 Beatrice Drexler,3 Sara C. Meyer,1,3 Pontus Lundberg,1,3 Jakob R. Passweg,3 Danijela Leković,4 Vladan Čokić,5 Sebastian Bonhoeffer,2 and Radek C. Škoda1

1Department of Biomedicine, University Hospital Basel and University of Basel, Basel, Switzerland; 2Institute of Integrative Biology, Eidgenössische Technische Hochschule (ETH) Zürich, Zürich, Switzerland; 3Division of Hematology, University Hospital Basel, Basel, Switzerland; 4Clinic of Hematology, Clinical Center of Serbia, Belgrade, Serbia; and 5Institute for Medical Research, University of Belgrade, Belgrade, Serbia

Myeloproliferative neoplasms (MPNs) are clonal hematopoietic stem cell (HSC) diseases characterized by increased proliferation of erythroid, megakaryocytic, and/or myeloid lineages.1 The JAK2-V617F mutation can be found in >95% of polycythemia vera (PV) patients, and also in approximately one-half of patients with essential thrombocythemia (ET) or primary myelofibrosis (PMF).2,3 Somatic mutations in exon 12 of JAK2 are found in 3% to 5% of PV patients.4 Quantification of the JAK2-mutant allele burden, also called variant allele frequency (VAF), in DNA from peripheral blood granulocytes is used to monitor the size of the mutant clone. ET patients have lower JAK2 VAF than PMF or PV patients.5 Interestingly, some MPN patients display very low VAF, which calls into question why they develop MPNs if the clone is apparently unable to expand. We therefore studied MPN patients with JAK2 VAF <20%.

In our cohort of 205 patients with JAK2-V617F+ MPNs, we identified 56 patients with a VAF <20% in purified granulocyte...