

Peer Review Information

Journal: Nature Immunology

Manuscript Title: Recognition and inhibition of SARS-CoV-2 by humoral innate immunity pattern recognition molecules

Corresponding author name(s): Elisa Vicenzi, Alberto Mantovani, Cecilia Garlanda

Reviewer Comments & Decisions:

Decision Letter, initial version:
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Subject: Decision on Nature Immunology submission NI-A32363

Message: 29th Jul 2021

Dear Cecilia & Alberto,

Many thanks for providing your point-by-point response to the referees' comments on your manuscript entitled "Recognition and inhibition of SARS-CoV-2 by humoral innate immunity pattern recognition molecules". As noted previously, while they find your work of considerable potential interest, they have raised quite substantial concerns that must be addressed. In light of these comments, we cannot accept the current manuscript for publication, but would be very interested in considering a revised version that addresses these concerns along the lines proposed in your response.

We invite you to submit a substantially revised manuscript, however please bear in mind that we will be reluctant to approach the referees again in the absence of major revisions.

Specifically, the revision should include new experiments to address:

- (1) Provide additional genetic analysis on the effects of MBL2 variants among severe COVID-19 patient cases and controls
- (2) Repeat binding experiments using Ficolin-2, SP-D and collectin-10 and -11.
- (3) Discuss your findings in the context of the recently published work by Ali et al (Front. Immunol., 2021, 12, 714511) and Hsieh et al. (Front. Immunol., 2021, 12, 641360)
- (4) Perform binding experiments using recombinant S-protein from the the B.1.617.2 variant.

Please include the additional textual clarifications as indicated in your response letter.

When you revise your manuscript, please take into account all reviewer and editor comments, please highlight all changes in the manuscript text file in Microsoft Word format.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

When revising your manuscript:

* Include a "Response to referees" document detailing, point-by-point, how you addressed each referee comment. If no action was taken to address a point, you must provide a compelling argument. This response will be sent back to the referees along with the revised manuscript.

* If you have not done so already please begin to revise your manuscript so that it conforms to our Article format instructions at <http://www.nature.com/ni/authors/index.html>. Refer also to any guidelines provided in this letter.

* Include a revised version of any required reporting checklist. It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review. A revised checklist is essential for re-review of the paper.

The Reporting Summary can be found here:

<https://www.nature.com/documents/nr-reporting-summary.pdf>

When submitting the revised version of your manuscript, please pay close attention to our [href="https://www.nature.com/nature-research/editorial-policies/image-integrity">Digital Image Integrity Guidelines.](https://www.nature.com/nature-research/editorial-policies/image-integrity) and to the following points below:

- that unprocessed scans are clearly labelled and match the gels and western blots presented in figures.
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If you wish to submit a suitably revised manuscript we would hope to receive it within 6 months. If you cannot send it within this time, please let us know. We will be happy to consider your revision so long as nothing similar has been accepted for publication at

Nature Immunology or published elsewhere.

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Please do not hesitate to contact me if you have any questions or would like to discuss the required revisions further.

Thank you for the opportunity to review your work.

Kind regards,

Laurie

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Referee expertise:

Referee #1: Immunogenetics

Referee #2: Lectin and alternative complement pathways

Referee #3: Lectin and alternative complement pathways

Reviewers' Comments:

Reviewer #1:

Remarks to the Author:

In this manuscript, the authors have investigated the possible role of humoral pattern recognition molecules (PRM) in recognizing SARS-CoV-2 structural proteins and affecting virus infectivity and replication. Using a series of assays, they demonstrate that out of 10 PRM tested, only PTX3 and MBL were capable of binding to the nucleocapsid (N) and Spike (S) proteins of SARS-CoV-2, respectively. They further demonstrated that MBL binds to the S protein in a glycan-dependent manner. Two possible MBL-binding sites were identified on the S trimer, which in either case span across the S1 and S2 regions, suggesting that MBL might participate in inhibition of viral infectivity. Indeed, using appropriate cellular models, the authors demonstrated that MBL inhibits SARS-CoV-2 entry in susceptible cells in a concentration-dependent manner, and that inhibits viral

replication. The authors also demonstrated that interaction between MBL and the S proteins leads to complement activation, with deposition of terminal C5b-C9 components. Finally, they interrogated existing genomic databases from patients with COVID-19 and investigated the possible association between MBL2 polymorphisms and COVID-19 severity. While no significant association was identified for six MBL2 polymorphisms known to affect MBL levels and COVID-19 severity, a haplotype-based analysis revealed 7 different haplotypes associated with severe COVID-19, including one in a regulatory region containing an enhancer active in hepatocytes and macrophages. Overall, this is a well-conducted study that provides novel information on the role of humoral innate immunity in SARS-CoV-2 infection. An array of different techniques have been used to corroborate the authors' findings. The study has important potential implications, with activation of the MBL-dependent activation of the lectin pathway of complement activation playing a protective role early in the course of infection, and a possible detrimental role later in the course of the disease. Nonetheless, some points need to be addressed:

- 1) Statistical analysis of the results needs to be improved. In particular, too many experiments were performed only twice with only duplicate technical replicates.
- 2) Investigation of the genetic association between MBL2 variants and COVID-19 severity remains the least convincing aspect of the paper. Additional studies ought to be performed, including burden analysis based on the assumption of autosomal recessive inheritance of MBL deficiency. Previous experience from the literature clearly shows that only bi-allelic MBL2 variants (either in coding, or in coding plus regulatory regions) are associated with low levels of MBL protein and increased risk of infection. Several whole exome and whole genome sequencing studies have been conducted in COVID-19, and the relevant genomic data have been deposited and are publicly accessible. The authors should interrogate those datasets and assess whether there is an increased burden of bi-allelic MBL2 variants among patients with life-threatening COVID-19 compared to patients with mild disease and asymptomatic individuals. Such studies must be conducted by normalizing for ethnicity, as MBL deficiency has been reported with different incidence in different ethnic groups. Ideally, one would like to see that those bi-allelic variants (if found at higher frequency among patients with life-threatening COVID-19) do result in low MBL protein levels.
- 3) Similarly, efforts should be made to determine whether the rs10824845 polymorphism is associated with lower MBL transcript and protein levels
- 4) The quality of the Figures should be improved. It is often difficult to distinguish among different symbols in the same Figure. Use of color could help. Also, both in Fig. 1 and in Fig. 2, the legend of the various symbols included within panel A refers in fact to multiple panels (A to C in Fig. 1; A and B in Figure 2). This is not immediately obvious. The Figures should be modified to make this more intelligible. Also, the dotted line in Fig. 3A is barely visible.

Reviewer #2:

Remarks to the Author:

A. Summary of the key results

Authors demonstrated that SARS-CoV-2 nucleoprotein is recognized by long pentraxin

PTX3 while Spike glycoprotein (trimeric form, what important, from several mutant variants) by mannose-binding lectin (MBL). The latter leads to the activation of complement system via the lectin pathway. S-gp recognition depends on interaction of MBL lectin domain with glycans decorating viral antigen. These glycans were identified with the use of molecular modelling. Moreover, it was shown that MBL inhibits SARS-CoV-2. Certain polymorphisms of the MBL2 gene were found to be associated with disease severity.

B. Originality and significance

This is an original research, with high potential for application.

C. Data & methodology

Wide range of well-chosen methods was employed; data are interesting and well documented. However the size of some figures seems too small (like Fig. 3G, 5B-E or 6). Furthermore, Fig. 2B lacks legend. Some results are contradictory to those recently published by Dr Ali et al. (Front. Immunol., 2021, 12, 714511) who reported that ficolin-2 (similarly to MBL) recognizes both S-gp and N proteins and activates complement system. Furthermore, Dr Hsieh et al. (Front. Immunol., 2021, 12, 641360) found SP-D to interact with Spike protein. That discrepancies should be discussed.

D. Statistics

Statistical analysis is appropriate; the sample size (patients/controls) is sufficient.

E. Conclusions: robustness, validity, reliability

Conclusion is well documented and reliable

F. Suggested improvements

As mentioned, discussion of the own data on the background of reports by Dr Ali et al. and Dr Hsieh et al. will be interesting and important; additional experiments with the use of another ficolin-2 preparation (and possibly other preparations of ficolin-1 and -3) as well as SP-D are worth performing. It seems especially interesting due to known interaction between ficolin-2 and PTX-3. Afore-mentioned figures in a greater size could be much easier to follow.

G. References

Cited references are appropriate. An exception is lack of discussion of the mentioned papers although I realise that at least that by Dr Ali et al. was released very recently, probably when this manuscript was already completed. Anyway, it is important in regard to data presented here.

H. Clarity and context

It is a well-written report; data are clearly demonstrated; abstract, introduction, discussion and description of methods/materials are appropriate.

Reviewer #3:

Remarks to the Author:

This paper shows that PTX3 and MBL bind to SARS-CoV-2 nucleoprotein and Spike among 10 pattern recognition molecules (PRMs) tested by the authors. In this paper, the binding properties of MBL to recombinant SARS-CoV-2 Spike proteins prepared in various cultured cells were investigated, suggesting that the trimeric peplomer structure was recognized by MBL in the presence of calcium. It is interesting that the authors showed two possible MBL binding sites on the SARS-CoV-2 Spike protein. One of them spans across the S1 and S2 of the Spike protein, this may result in MBL binding specificity for the trimeric structure of the Spike protein. The authors showed that MBL also binds to variants of concern (VoCs) and activates the complement system through the lectin pathway. The authors also showed that MBL inhibits SARS-CoV-2 infection in cell lines and primary cells in a dose-

dependent manner. I think this is an important finding that suggests that MBL plays a protective role against SARS-CoV-2 infection. Finally, the authors examined the allele frequencies of the six SNPs that have been shown to be associated with serum MBL2 levels and found that there was a significant difference in allele frequency of rs5030737 between patients and controls (OR=1.43, P=0.049), which however did not survive the correction for multiple testing.

In summary, this paper has excellent novelty in that it investigated in detail the mechanism by which MBL binds to SARS-CoV-2, and that MBL inhibits SARS-CoV-2 infection in cell lines and primary cells. However, the author should consider the points described below.

1) There are two more PRMs involved in the activation of the lectin complement pathway, Collectin-10 (CL-10 or CL-L1) and Collectin-11 (CL-11 or CL-K1), in addition to MBL and ficolins. Similar to MBL, CL-10 and CL-11 have been shown to form complexes with MASPs and can activate the lectin complement pathway upon binding to carbohydrates. The authors tested interaction of Collectin-12 (CL-12 or CL-P1) with SARS-CoV-2 proteins. Similarly, CL-10 and CL-11 should be tested. There is a typo that the abbreviation for Collectin-12 is CL-P1 instead of CLP-1.

2) Ali et al. have recently reported that MBL, Ficolin-2 (FCN-2), and CL-11, which are PRMs of the lectin pathway, bind to SARS-CoV-2 Nucleoprotein and Spike, with subsequent activation of the lectin pathway (Ali YM, Ferrari M, Lynch NJ, Yaseen S, Dudler T, Gragerov S, Demopoulos G, Heeney JL, Schwaebler WJ. Lectin Pathway Mediates Complement Activation by SARS-CoV-2 Proteins. Front Immunol. 2021 Jul 5;12:714511. doi: 10.3389/fimmu.2021.714511. eCollection 2021.) In contrast, the authors have shown that Ficolin-2 did not bind to Nucleoprotein and Spike. It is necessary to discuss the discrepancy with the results of Ali et al.

3) The authors tested interaction of MBL with Spike proteins from VoC including B.1.1.7 (α), B.1.351 (β), and P.1 (γ) variants. The authors, however, did not test interaction of MBL with protein from B.1.617.2 (δ) which is currently a major concern worldwide. This experiment is not mandatory, but the author should discuss whether MBL can also interact with B.1.617.2 (δ).

Author Rebuttal to Initial comments

Re. Ms #NI-A32363 entitled "Recognition and inhibition of SARS-CoV-2 by humoral innate immunity pattern recognition molecules"

Point-by-point response to Reviewers' comments

Referee 1

Remarks to the Author:

In this manuscript, the authors have investigated the possible role of humoral pattern recognition molecules (PRM) in recognizing SARS-CoV-2 structural proteins and affecting virus infectivity and replication. Using a series of assays, they demonstrate that out of 10 PRM tested, only PTX3 and MBL

were capable of binding to the nucleocapsid (N) and Spike (S) proteins of SARS-CoV-2, respectively. They further demonstrated that MBL binds to the S protein in a glycan-dependent manner. Two possible MBL-binding sites were identified on the S trimer, which in either case span across the S1 and S2 regions, suggesting that MBL might participate in inhibition of viral infectivity. Indeed, using appropriate cellular models, the authors demonstrated that MBL inhibits SARS-CoV-2 entry in susceptible cells in a concentration-dependent manner, and that inhibits viral replication. The authors also demonstrated that interaction between MBL and the S proteins leads to complement activation, with deposition of terminal C5b-C9 components. Finally, they interrogated existing genomic databases from patients with COVID-19 and investigated the possible association between MBL2 polymorphisms and COVID-19 severity. While no significant association was identified for six MBL2 polymorphisms known to affect MBL levels and COVID-19 severity, a haplotype-based analysis revealed 7 different haplotypes associated with severe COVID-19, including one in a regulatory region containing an enhancer active in hepatocytes and macrophages.

Overall, this is a well-conducted study that provides novel information on the role of humoral innate immunity in SARS-CoV-2 infection. An array of different techniques have been used to corroborate the authors' findings. The study has important potential implications, with activation of the MBL-dependent activation of the lectin pathway of complement activation playing a protective role early in the course of infection, and a possible detrimental role later in the course of the disease.

Nonetheless, some points need to be addressed:

- 1) Statistical analysis of the results needs to be improved. In particular, too many experiments were performed only twice with only duplicate technical replicates.
- 2) Investigation of the genetic association between MBL2 variants and COVID-19 severity remains the least convincing aspect of the paper. Additional studies ought to be performed, including burden analysis based on the assumption of autosomal recessive inheritance of MBL deficiency. Previous experience from the literature clearly shows that only bi-allelic MBL2 variants (either in coding, or in coding plus regulatory regions) are associated with low levels of MBL protein and increased risk of infection. Several whole exome and whole genome sequencing studies have been conducted in COVID-19, and the relevant genomic data have been deposited and are publicly accessible. The authors should interrogate those datasets and assess whether there is an increased burden of bi-allelic MBL2 variants among patients with life-threatening COVID-19 compared to patients with mild disease and asymptomatic individuals. Such studies must be conducted by normalizing for ethnicity, as MBL deficiency has been reported with different incidence in different ethnic groups. Ideally, one would like to see that those bi-allelic variants (if found at higher frequency among patients with life-threatening COVID-19) do result in low MBL protein levels.
- 3) Similarly, efforts should be made to determine whether the rs10824845 polymorphism is associated with lower MBL transcript and protein levels.

4) The quality of the Figures should be improved. It is often difficult to distinguish among different symbols in the same Figure. Use of color could help. Also, both in Fig. 1 and in Fig. 2, the legend of the various symbols included within panel A refers in fact to multiple panels (A to C in Fig. 1; A and B in Figure 2). This is not immediately obvious. The Figures should be modified to make this more intelligible. Also, the dotted line in Fig. 3A is barely visible.

We thank Referee 1 for his/her appreciation of our work (“well-conducted study that provides novel information on the role of humoral innate immunity in SARS-CoV-2 infection. The study has important potential implications”).

Point 1 - Statistical analysis: we repeated most experiments (both binding assays and in vitro infections) strengthening our results. Concerning Figure 4C and D, we noticed a mistake in the legend on the number of experiments performed (2 experiments in duplicate instead of one), but further experiments have been performed. The figure legends have been modified accordingly.

Point 2, 3 - Genetic analysis

The Reviewer raises an important point, i.e. the effect of rare variants having a major effect on MBL levels. This approach is generally relevant for rare diseases caused by biallelic genetic alterations, a somewhat different perspective from our line of work. However, as requested by Reviewer 1, we performed further analyses to assess the distribution of subjects carrying biallelic variants among cases and controls. We focused on the 3 main coding variants (rs5030737, rs1800450, and rs1800451), already known to cause an important functional effect on the MBL protein, and on the promoter SNP rs7096206 that confers the strongest effect (among the promoter variants) on MBL levels. We considered only these variants since they have a MAF > 1% (allowing to identify a good number of carriers also in our cohort), and because they have been extensively studied in the literature as main genetic determinants causing MBL2 low levels/deficiency.

Moreover, we evaluated the contribution of rare variants to COVID-19 severity through a burden meta-analysis based on the Regeneron data.

With these new analyses, we found a significant predisposing effect in individuals carrying *MBL2* biallelic functional variants and significant results both grouping loss-of-function variants (OR=32.05), and clumping loss-of-function and damaging missense variants (OR=23.6).

We further analyzed MBL plasma levels at hospital admission in our cohort of patients and correlated them to MBL variants. We first focused on the three missense variants (functional SNPs rs5030737, rs1800450, rs1800451) and grouped individuals carrying at least one alternative allele (allele O) compared to those carrying the wild-type allele (A) and observed a significantly lower MBL plasma concentration in the first group ($p = 6.2 \times 10^{-8}$). Then we analyzed the impact of the rs10824845 polymorphism, by stratifying the same patients according to their genotype. We observed a reduction of MBL levels in heterozygous individuals, though not significant (1.2-fold decrease in heterozygous; $P = 0.10$). However, when considering the contribution of A/O alleles, a clear genotype-dependent modulation of MBL levels emerged ($P = 1.1 \times 10^{-6}$). These results have been included in the new Figure 6.

We integrated the details of these analyses in Material and Methods, paragraph “Statistical analysis”, and in the Results section “MBL2 haplotypes are associated with severe COVID-19”. Table 1 and figure 6 have been changed accordingly.

Point 4 – As suggested, we improved the quality of figures, introducing colors.

Reviewer #2:

Remarks to the Author:

A. Summary of the key results

Authors demonstrated that SARS-CoV-2 nucleoprotein is recognized by long pentraxin PTX3 while Spike glycoprotein (trimeric form, what important, from several mutant variants) by mannose-binding lectin (MBL). The latter leads to the activation of complement system via the lectin pathway. S-gp recognition depends on interaction of MBL lectin domain with glycans decorating viral antigen. These glycans were identified with the use of molecular modelling. Moreover, it was shown that MBL inhibits SARS-CoV-2. Certain polymorphisms of the MBL2 gene were found to be associated with disease severity.

B. Originality and significance

This is an original research, with high potential for application.

C. Data & methodology

Wide range of well-chosen methods was employed; data are interesting and well documented. However the size of some figures seems too small (like Fig. 3G, 5B-E or 6). Furthermore, Fig. 2B lacks legend. Some results are contradictory to those recently published by Dr Ali et al. (Front. Immunol., 2021, 12,

714511) who reported that ficolin-2 (similarly to MBL) recognizes both S-gp and N proteins and activates complement system. Furthermore, Dr Hsieh et al. (Front. Immunol., 2021, 12, 641360) found SP-D to interact with Spike protein. That discrepancies should be discussed.

D. Statistics

Statistical analysis is appropriate; the sample size (patients/controls) is sufficient.

E. Conclusions: robustness, validity, reliability

Conclusion is well documented and reliable

F. Suggested improvements

As mentioned, discussion of the own data on the background of reports by Dr Ali et al. and Dr Hsieh et al. will be interesting and important; additional experiments with the use of another ficolin-2 preparation (and possibly other preparations of ficolin-1 and -3) as well as SP-D are worth performing. It seems especially interesting due to known interaction between ficolin-2 and PTX-3. Afore-mentioned figures in a greater size could be much easier to follow.

G. References

Cited references are appropriate. An exception is lack of discussion of the mentioned papers although I realise that at least that by Dr Ali et al. was released very recently, probably when this manuscript was already completed. Anyway, it is important in regard to data presented here.

H. Clarity and context

It is a well-written report; data are clearly demonstrated; abstract, introduction, discussion and description of methods/materials are appropriate.

We thank Referee 2 for his/her appreciation of our work ("This is an original research, with high potential for application". "Well-written report; data are clearly demonstrated").

Reviewer 2 raised the point about potential contradictory results with two recent publications showing the interaction of Ficolin-2 with S- and N-proteins activating Complement or of SP-D with S-protein (Front. Immunol., 2021, 12, 714511 and Front. Immunol., 2021, 12, 641360, respectively). These papers appeared around the time of submission and for this reason they were not considered in our paper. To discuss these studies, we repeated binding experiments using Ficolin-2 and SP-D from other sources (in total, 4 for ficolin-2 and 2 for SP-D), confirming our original results (new figure 2 and Extended data Figure 1). We think that the experimental approaches used may explain the discrepancies with the study by Ali et al. (Front. Immunol., 2021, 12, 714511). We used commercially available and in house produced recombinant PRMs (pentraxins, C1q, MBL, ficolins, surfactant proteins and collectins) in the binding experiments to investigate the interaction with viral recombinant proteins, whereas Ali et al. used serum at different concentration as the source of PRMs. The discrepancy with our results might be due to indirect interaction of MBL or ficolin-2 with viral proteins mediated by a serum component. For instance, in their study, Ali et al. validated the results by Gao et al. (*medRxiv*, 10.1101/2020.1103.1129.20041962 (2020)), showing a direct interaction of

MASP-2 with N-protein. MASPs are normally present in plasma complexed with molecules of the lectin pathway, including MBL, ficolins and collectins. This could explain the interactions of MBL with N-protein, which we did not observe.

Concerning SP-D and the study by Hsieh et al. (Front. Immunol., 2021, 12, 641360), the authors observed an interaction between a home-made fragment of SP-D and S-protein, whereas with the recombinant full-length molecule from R&D they observed a very low affinity for S-protein. By conducting additional experiments with recombinant ficolin-2 and SP-D from different companies (new figure 2) and discussing the merit and limitations of these studies (page 15), we think we are providing the community with a solid, reliable picture of recognition of SARS-CoV-2 components by “ante-antibodies” (see also response to Reviewer 3).

As suggested, we improved the quality of figures.

Reviewer #3:

Remarks to the Author:

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In summary, this paper has excellent novelty in that it investigated in detail the mechanism by which MBL binds to SARS-CoV-2, and that MBL inhibits SARS-CoV-2 infection in cell lines and primary cells. However, the author should consider the points described below.

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abbreviation for Collectin-12 is CL-P1 instead of CLP-1.

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3) The authors tested interaction of MBL with Spike proteins from VoC including B.1.1.7 (α), B.1.351 (β), and P.1 (γ) variants. The authors, however, did not test interaction of MBL with protein from B.1.617.2 (δ) which is currently a major concern worldwide. This experiment is not mandatory, but the author should discuss whether MBL can also interact with B.1.617.2 (δ).

We thank Referee 3 for his/her appreciation of our work (“this paper has excellent novelty”).

Point 1 - We thank the Reviewer for raising this point. As suggested, we extended the analysis to Collectin-10 and Collectin-11. We tested both commercially available recombinant proteins, as well as in house produced recombinant Collectin-10 and -11 provided by Prof. P. Garred, an expert in the field, now included among authors. Results are reported in the new figure 2 and Extended Data Figure 1.

Point 2 - Reviewer 3 raised the point about a discrepancy with a recent publication showing the interaction of Ficolin-2 with S- and N-proteins activating Complement (*Front. Immunol.*, 2021, 12, 714511). This paper appeared around the time of submission and for this reason it was not considered in our paper. To discuss this study, we repeated binding experiments using Ficolin-2 from other sources, confirming our original results (new figure 2). We think that the experimental approaches used may explain the discrepancies with the study by Ali et al. We used commercially available and in house produced recombinant PRMs (pentraxins, C1q, MBL, ficolins, surfactant proteins and collectins) in the binding experiments to investigate the interaction with viral recombinant proteins, whereas Ali et al. used serum at different concentration as the source of PRMs. The discrepancy with our results might be due to indirect interaction of MBL or ficolin-2 with viral proteins mediated by a serum component. For instance, in their study, Ali et al. validated the results by Gao et al. (*medRxiv*, 10.1101/2020.1103.1129.20041962 (2020)), showing a direct interaction of MASP-2 with N-protein. MASPs are normally present in plasma complexed with molecules of the lectin pathway, including

MBL, ficolins and collectins. This could explain the interactions of MBL with N-protein, which we did not observe.

By conducting additional experiments with recombinant Ficolin-2 from 4 different companies (new figures 2) and discussing the merit and limitations of this study (page 15), we think we are providing the community with a solid, reliable picture of recognition of SARS-CoV-2 components by “ante-antibodies”.

Point 3 - Reviewer 3 asks to include the B.1.617.2 variant (δ) in the discussion. The mutations of this variant do not involve the glycosylation sites of S-protein as shown in Figure 3g, thus suggesting that the interaction with MBL would not be affected. As suggested by Referee 3, we performed binding assays and validated this hypothesis. Finally, we isolated the B.1.617.2 variant (δ) from a patient and included it in infection experiments, which showed that MBL retains its anti-viral potential also against this variant. The new experiments are shown in figures 3h and 4f, and presented and discussed at pages 8, 10 and 14. Given the global impact of the δ variant, we concur with the Referee that the formal demonstration that the spectrum of variants recognized and inhibited by MBL includes B.1.617.2 as well as possibly other (all?) Vol, has profound implications as to the significance of this report, which has the ambition to provide a rigorous, comprehensive assessment of ante-antibodies in COVID-19.

For completeness, the new Vol (ϵ , λ) have been included in the analysis shown in new Figure 3g, indicating that also for these variants, the interaction with MBL would not be affected. The text has been modified accordingly.

We thank the Editors and Reviewers for raising important and constructive points. We think that by addressing these issues, we ameliorated our study.

Decision Letter, first revision:

Subject: Your manuscript, NI-A32363A

Message: Our ref: NI-A32363A

22nd Nov 2021

Dear Dr. Garlanda,

Thank you for your patience as we've prepared the guidelines for final submission of your Nature Immunology manuscript, "Recognition and inhibition of SARS-CoV-2 by humoral innate immunity pattern recognition molecules" (NI-A32363A). Please carefully follow the step-by-step instructions provided in the attached file, and add a response in each row of

the table to indicate the changes that you have made. Please also check and comment on any additional marked-up edits we have proposed within the text. Ensuring that each point is addressed will help to ensure that your revised manuscript can be swiftly handed over to our production team.

We would like to start working on your revised paper, with all of the requested files and forms, as soon as possible (preferably within two weeks). Please get in contact with us if you anticipate delays.

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If you have any further questions, please feel free to contact me.

Best regards,

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On behalf of

Laurie A. Dempsey, Ph.D.
Senior Editor
Nature Immunology
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Reviewer #2:

Remarks to the Author:

Authors responded all my comments and corrected the manuscript. As I provided reply to A-H points to my previous review, there is no need to repeat it, as this work was generally highly scored. However, regarding "F" I would like to suggest one minor correction:
- Regarding MBL plasma concentrations - as relatively low number of patients (n=40) was tested and MBL levels are usually not normally distributed, the non-parametric (Mann-Whitney) test seems better choice than t test.

Reviewer #3:

Remarks to the Author:

As Reviewer #3 stated in the first review, this paper has excellent novelty in that it investigated in detail the mechanism by which MBL binds to SARS-CoV-2, and that MBL inhibits SARS-CoV-2 infection in cell lines and primary cells. However, reviewer #3 raised three points that the author should consider, and the authors made the following revisions.

1) In addition to MBL and ficolins, there are two more PRMs involved in the activation of the lectin complement pathway, Collectin-10 and -11, for which binding to SARS-CoV-2 should be tested. The authors tested both commercially available recombinant proteins, as well as in house produced recombinant Collectin-10 and -11 provided by Prof. P. Garred. They observed that in contrast with MBL, Collectin-10 and -11 did not bind to SARS-CoV-2 Spike protein. These results are included in the revised Figure 2 and Extended Data Figure 1.

2) Ali et al. have recently reported that MBL, Ficolin-2 (FCN-2), and CL-11, which are PRMs of the lectin pathway, bind to SARS-CoV-2 Nucleoprotein and Spike, with subsequent activation of the lectin pathway. In contrast, the authors have shown that Ficolin-2 did not bind to Nucleoprotein and Spike. In the revised manuscripts, the authors stated that the experimental approaches used may explain the discrepancies with the study by Ali et al. The authors used commercially available and in house produced recombinant PRMs (pentraxins, C1q, MBL, ficolins, surfactant proteins and collectins) in the binding experiments to investigate the interaction with viral recombinant proteins, whereas Ali et al. used serum at different concentration as the source of PRMs. The authors concluded that the discrepancy with the authors results might be due to indirect interaction of MBL or Ficolin-2 with viral proteins mediated by a serum component.

3) The authors tested interaction of MBL with Spike proteins from VoCs including B.1.1.7 (α), B.1.351 (β), and P.1 (γ) variants. The authors, however, did not tested interaction of MBL with protein from B.1.617.2 (δ) which is currently a major concern worldwide. The authors hypothesized that the mutations of B.1.617.2 (δ) do not involve the glycosylation sites of S-protein as shown in Figure 3g, thus suggesting that the interaction with MBL would not be affected. The authors performed binding assays and validated this hypothesis. The authors isolated the B.1.617.2 (δ) from a patient and included it in infection experiments, which showed that MBL retains its anti-viral potential also against this variant. The new experiments are shown in Figures 3h and 4f, and presented and discussed in the revised manuscript. In addition, the new VoIs (ϵ , λ) have been included in the analysis shown in revised Figure 3g, indicating that also for these variants, the interaction with MBL would not be affected.

In conclusion, the author has answered all concerns raised by Reviewer #3. The revised manuscript further highlights the importance of MBL binding to SARS-CoV-2, including VoCs and VoIs.

Author Rebuttal, first revision:

Point-by-point response to reviewer comments

Reviewer #2:

Remarks to the Author:

Authors responded all my comments and corrected the manuscript. As I provided reply to A-H points to my previous review, there is no need to repeat it, as this work was generally highly scored.

However, regarding "F" I would like to suggest one minor correction:

- Regarding MBL plasma concentrations - as relatively low number of patients (n=40) was tested and MBL levels are usually not normally distributed, the non-parametric (Mann-Whitney) test seems better choice than t test.

As specified in the methods and figure legend, we did not use a t test, because as hypothesized by the Reviewer, MBL concentrations were not normally distributed. For this reason, BoxCox transformation was used to normalize data.

We thank again the Editors and Reviewers for having provided a useful, critical evaluation of our work.

Final Decision Letter:

Subject: Decision on Nature Immunology submission NI-A32363B

Message: In reply please quote: NI-A32363B

Dear Dr. Garlanda,

I am delighted to accept your manuscript entitled "Recognition and inhibition of SARS-CoV-2 by humoral innate immunity pattern recognition molecules" for publication in an upcoming issue of Nature Immunology.

Over the next few weeks, your paper will be copyedited to ensure that it conforms to Nature Immunology style. Once your paper is typeset, you will receive an email with a link to choose the appropriate publishing options for your paper and our Author Services team will be in touch regarding any additional information that may be required.

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Kind regards & best wishes for the holidays!

Laurie

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