European Journal of Immunology

Scarcity of Autoreactive Human Blood IgA+ Memory B cells

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Review Timeline:

Submission date:	04-Apr-2016
First Editorial decision:	03-May-2016
Revision received:	17-Jun-2016
Second Editorial decision:	14-Jul-2016
Accepted:	26-Jul-2016

Handling Executive Committee member: Prof. Hans-Martin Jack

Please note that the correspondence below does not include the standard editorial instructions regarding preparation and submission of revised manuscripts, only the scientific revisions requested and addressed.

First Editorial Decision 03-May-2016

Dear Dr. Mouquet,

Manuscript ID eji.201646446 entitled "Scarcity of Autoreactive Human Blood IgA+ Memory B cells", which you submitted to the European Journal of Immunology, has been reviewed. The comments of the referee) are included at the bottom of this letter.

Although the referees have recommended publication, some revisions to your manuscript have been requested. Therefore, I invite you to respond to the comments of the referees and revise your manuscript accordingly.

You should also pay close attention to the editorial comments included below. **In particular, please edit your figure legends to follow Journal standards as outlined in the editorial comments. Failure to do this will result in delays in the re-review process.**

European Journal of Immunology

If the revision of the paper is expected to take more than three months, please inform the editorial office. Revisions taking longer than six months may be assessed by new referee(s) to ensure the relevance and timeliness of the data.

Once again, thank you for submitting your manuscript to European Journal of Immunology. We look forward to receiving your revision.

Yours sincerely,
Nadja Bakocevic

on behalf of Prof. Hans-Martin Jack

Dr. Nadja Bakocevic
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Reviewer: 1

Comments to the Author
This is a descriptive, yet interesting study, using the now well-established single cell PCR-based expression cloning of paired IgH and IgL cDNA from healthy B cell subsets and assessing their reactivity by ELISA and immunofluorescence. The novelty of the study resides in assessing the reactivity profile of a significant number of IgA+ B cell clones from the peripheral blood of healthy donors. Arguably, the reactivity of blood, i.e. non-mucosal IgA+ cells is largely unknown. The authors report a low frequency of polyreactive and autoreactive clones within the IgA subset. The data are well presented and convincing. The main weaknesses of the study are the limited number of healthy subjects assessed (only 4) and the limited comparison with IgG clones (only available for 2 subjects). Because of this limited number, the difference between IgG and IgA reactivity profiles does not appear very strong. Additional critiques are listed below.

- Typo page 5, line 110: Jk?
- The version of Figure 2 I reviewed does not have colored doted lines (cf legends)

- Figure 2, the grey dots for polyreactive clones are barely visible
- Figure 3, 4 and text: The authors propose that the frequency of polyreactive IgA+ B cells is lower than that of IgG+ cells. However, this is based on 2 subjects (for IgG) and historical data. This interpretation should be toned down. Even the difference between the frequency of poly/autoreactive IgG+ between the two subjects tested in the study and historical data suggests a high level of variability.
- I could not find the age of the donors.
- Figure 3, D, G. The font is too small.
- Figure 5A and text: The authors brush aside the differences between IgG and IgA versions of the same mab. They appear to be significant to this reviewer. This should be discussed as such and not dismissed.

Reviewer: 2

Comments to the Author
This is a very interesting study which characterize for the first time the IgA memory repertoire in healthy humans. The study is well written and the results are highly convincing.

Major point: It will be important before publication to demonstrate the quality of the IgA produce in the used expression system. What is the ratio of polymeric vs monomeric IgA when put in a IgG backbone? Is the level of glycosylation the same (lectin reactivity for example) in the recombiant IgA? All these factors could be associated to the reduction of IgA autoreactivity against self-antigens.

First Revision – authors' response 17-Jun-2016

Reviewer 1

We thank the reviewer for her/his careful reading of our paper, and many useful suggestions. We have revised the paper as follows:

The main weaknesses of the study are the limited number of healthy subjects assessed (only 4) and the limited comparison with IgG clones (only available for 2 subjects). Because of this limited number, the difference between IgG and IgA reactivity profiles does not appear very strong.

Thank you for raising these points. Although we agree that 4 healthy individuals might appear as a limited number, a considerable amount of antibodies were cloned and characterized per individual, and our data are very consistent between donors in all the analyses performed (immunoglobulin gene, poly- ans

self-reactivity analyses). Moreover, the difference observed for the self-reactivity frequency between the two memory B-cell populations is highly statistically significant ($p < 0.005$). Threferore, we are more than confident regarding the robustness of the reactivity differentials that we show.

Typo page 5, line 110: Jk?

The typo originating from the pdf conversion has been corrected and now reads “Jk1”.

The version of Figure 2 I reviewed does not have colored doted lines (cf legends)

We apologize for this omission, the dotted green line corresponding to the reactivity of the negative control antibody mGO53 is now included in Fig. 2I.

Figure 2, the grey dots for polyreactive clones are barely visible

Thank you for this suggestion; we have substituted the grey dots by red dots, more visible, and changed the legend accordingly.

Figure 3, 4 and text: The authors propose that the frequency of polyreactive IgA+ B cells is lower than that of IgG+ cells. However, this is based on 2 subjects (for IgG) and historical data. This interpretation should be toned down. Even the difference between the frequency of poly/autoreactive IgG+ between the two subjects tested in the study and historical data suggests a high level of variability.

We agree that the difference between the two B-cell populations evidenced in this study regarding the frequency the polyreactive clones is more modest compared to the one observed for self-reactivity. We have therefore moderated this difference in the revised version of the manuscript when necessary (lines 160 and 267). However, despite some interindividual variabilties, the frequencies that we calculated for both types of reactivity are strinkingly very similar to the ones determined in previoulsy published works (as highlighted in the discussion). Thus, again, we are very confident regarding the robustness of the reactivity differentials presenting here.

I could not find the age of the donors.

The age of the donors at the time sampling are provided in the table of the Supporting Information Fig. 1.

Figure 3, D, G. The font is too small.

According to the reviewer’s recommendation, fonts size has been increased for a better visibility.

Figure 5A and text: The authors brush aside the differences between IgG and IgA versions of the same mab. They appear to be significant to this reviewer. This should be discussed as such and not dismissed.

We do not consider that we “brush aside the differences”, and therefore strongly disagree with this comment. Indeed, we clearly described and highlighted the variations that we observed in the paragraph from line 210 to 217. But more importantly, as clearly stated in the manuscript, the “status” for being poly- and self-reactive clones remained unchanged whatever the isotype used to express the antibodies.

Reviewer 2

We thank the reviewer for recognizing that “This is a very interesting study … well written, and the results are highly convincing” and for her or his input that will help the manuscript to be improved. She or he requests that we address the following points:

It will be important before publication to demonstrate the quality of the IgA produce in the used expression system. What is the ratio of polymeric vs monomeric IgA when put in a IgG backbone?
Is the level of glycosylation the same (lectin reactivity for example) in the recombinant IgA? All these factors could be associated to the reduction of IgA autoreactivity against self-antigens.

These are indeed important points, we thanks the reviewer for raising them out. It is undoubtely demonstrated that HEK293T derived cells (as the Freestyle cells used in our study) are among the more reliable eucariotic protein-expression sytem for the production of recombinant proteins and in this case of antibodies (Schirrmann T, Antibody Engineering vol. 2, 2010; Frenzel, Front Immunol, 2013). This is particularly true regarding co- and post-translational modifications such as glycosylation, which is critical for antibody structure and function. This is especially important for the Fc region of IgAs that possess more N- and O-glycan moities than IgGs, and therefore of IgA Fc-dependent functions. To adress all the reviewer’s requests, we perfomed a new set of experiments for which the results are now presented in a new supplementary figure (Supporting Information Fig.3) :

As expected based on the litterarure (Morton, J Immunol, 1993; Hendrickson, J Exp Med, 1995; Sorensen, Int Immuno, 2000; Lorin, J Immunol Methods, 2015), recombinant IgAs are produced as monomers having the right molecular weight but also naturally assembled non-covalently (because of the absence of J chain) as dimers and multimers as we confirmed here by SDS-PAGE experiments (SupFig. 3E). Purified IgA fractions contain in average 19% of polymeric immunoglobulins as determined by size-exclusion chromatography (SupFig. 3F). Importantly, all IgA antibodies produced with the IgG backbone were

monomeric (15 IgG tested) exept 4-170 that forms 34% of polymers (SupFig. 3E and), which could be due to his high level of polyreactivity.

As mentioned above, the level of glycosylation of the Fc region of IgG and IgA is not comparable, IgAs being heavily glycosylated. Although, we did not performed mass spectrometry analyses to characterize the glycans composition of the recombinant IgAs, what we did it to ensure that the variable domains, especially for IgH, would be properly glycosylated in IgA memory B-cell antibodies expressed as recombinant IgGs by performing lectin immunoblot experiments (as suggested by this reviewer). Indeed, we show now that for both native IgG (used as control) and IgG-expressed IgAs, a clear correlation between the number of putative N-glycosylation sites (PNGS) identified by sequence analysis and the level of reactivity detected by lectin binding (SupFig. 3D). Moreover, perfoming analyses of the number of PNGS in IgH and IgL variable domains across the different populations and groups, we did not find any difference between IgG and IgA (SupFig. 3A), as well as between reactive (poly- and self-reactive) and non-reactive IgA or IgG antibodies (SupFig. 3B and 3C).

Second Editorial Decision 14-Jul-2016

Dear Dr. MOUQUET,

It is a pleasure to provisionally accept your manuscript entitled "Scarcity of Autoreactive Human Blood IgA+ Memory B cells" for publication in the European Journal of Immunology. For final acceptance, please follow the instructions below and return the requested items as soon as possible as we cannot process your manuscript further until all items listed below are dealt with.

Please note that EJI articles are now published online a few days after final acceptance (see Accepted Articles: http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1521-4141/accepted). The files used for the Accepted Articles are the final files and information supplied by you in Manuscript Central. You should therefore check that all the information (including author names) is correct as changes will NOT be permitted until the proofs stage.

We look forward to hearing from you and thank you for submitting your manuscript to the European Journal of Immunology.

Yours sincerely,
Nadja Bakocevic

European Journal of Immunology

on behalf of Prof. Hans-Martin Jack

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