**Science Advances**  
ado9455 Reviewer comments

**Reviewer: 4**

The authors have done much to address previous reviewer comments, and

to add substance to their proposed mechanism linking gut IgA to systemic

IgG responses. I believe in general they have made a strong case for their

conclusions from the IgA-deficient mouse model, though I would find it

valuable to provide a stronger link between their validation models (Pigr-/- and

ex-GF mice) and the overall mechanism they describe (excessive microbiotaspecific

IgG1 and exhaustion).

The fecal transfer experiments test whether a lack of IgA alters the microbiota

and if this altered composition attenuates vaccine responses in an IgA sufficient

host, presumably because of an enrichment of invasive bacteria

capable of translocating and causing exhaustion.

It would have been useful to perform culture studies on the MAT of these ex-GF mice.

Were tissues preserved and can they be subjected to 16S qPCR, or TLR ligand quantification

via cell lines (ex. InvivoGen)?

Was there more microbiota-specific IgG1 or total IgG1 in the blood of these mice?

**Similarly, was more anti-microbiota IgG1 observed in circulation of Pigr-/- mice, or increased total IgG1**, or increased PD-1 expression on T or B cells?

* Potentially add anti-microbiota IgG1 and total IgG1 in Pigr-/- [can be run within the next week] We already have the samples and ran fecal IgA on them.

The mechanism of attenuated vaccine responses in these mice is currently not well-linked to the mechanism investigated in Igha−/− mice.

Minor comments:

The authors state that “Wild type (WT) and Igha−/− breeders were set up from heterozygous Igha+/− parents to control for microbiota and genetic background variability between strains. WT mice used came from these breeders, unless indicated otherwise.” Use of the word “breeder” in the first sentence to refer to progeny may be confusing and interpreted in different

ways. **Do the authors mean that wild type (WT) and Igha−/− mice were**

**generated by mating heterozygous Igha+/− parents? Please consider revising**

**for clarity.**

**Reference to panels of Figure 5 are incorrect in the text (Lines 279-295); text**

**refers to Figure 5B when it presumably means Figure 5A, so on until 5F which**

**refers to 5E.**

**Moving information to Supplemental Figure legends or Methods may further**

**streamline the manuscript. For example, lines 100-103 can be moved to**

**Methods.**

**Reviewer: 5**

This paper combines analysis of human IgA deficient patients and detailed

immunology of the isotype-specific IgA knockout mouse. It is really interesting

that certain patterns of B cell responses are shared between humans and

the mouse model.

**But the authors emphasize a claim that the mechanism mediating these phenotypes is one acting through commensal microbiota (by highlighting this in the title, abstract, and throughout).**

While this is a reasonable hypothesis given what we know about IgA, only a small amount

of data in the paper relates to this claim, and this aspect of the paper is the

least thorough. There is interesting data here, particulary in the differences

in B cell responses, but this is **obscured by the focus on the microbiota as**

**mechanism**. Another strength of the paper is the human study of IgA deficient

patients, which is valuable for the field.

But there is no data related to human microbiota from these patients. I feel the authors have done some really wonderful experiments, but the **value is undermined by framing it around a**

**microbiota-related mechanism for which they do not show convicing evidence**.

Specific comments:

**As an example of what seems like a real mismatch in the text claims and data,**

**here is a sentence from the abstract: “IgA exerted this effect by constraining**

**the systemic translocation of gut commensal antigens, which caused chronic**

**immune activation, including T cell overexpression of programmed cell death-1**

**(PD-1).“**

No experiment tested whether MAT invasion or antigen translocation is what

caused the observed immune changes in IgA knockout mice.

**If the paper is supposed to focus on how IgA control of gut bacteria influences**

**B cell IgG responses, I would expect to see multiple experiments** that involve

manipulation of microbiota (doesn’t have to be germfree, could be antibiotics

treatment) followed by analyses of the various downstream effects proposed:

invasion of MAT, systemic IgG responses to commensal bacteria, changes in

B cells, and finally changes in antibody responses post-vaccination.

But the only data along these lines is Fig3J. Even **this experiment is hard to interpret**

**because of lack of method details and lack of any validation.**

**What strain are the germfree mice?**

**How old were they on colonization?**

**Were the cecal content donor mice co-housed or not? Did the donors exhibit the same microbiome difference shown in 3G?**

* Emilie will address these method clarifications

Is this difference maintained after colonizing germfree mice, even though they are (presumably) all IgA sufficient?

Though the result in the end looks remarkable, without validation, orthogonal approaches, or

measurement of any other phenotypes, it is hard to interpret this figure panel.

**Fig 3G, the microbiome sequencing and what exactly is being displayed here,**

**is not explained well in the legend, text, or methods. What is the z score?**

**What is this subset of ASVs (I assume they are ASVs because the methods**

**mentions ASVs) shown? It is unconventional to not show a more global view**

**as well (PCA or stacked bar charts).**

**The main text cites this panel and says the knockout mice have a depletion of Lachnospiraceae: this isn’t clear. Is the z score implying a single lachnospiraceae ASV was detected in 2/6 mice in**

**WT and 0/6 in knockout mice? That seems to be not great evidence. Methods**

**state the use of ANCOM and Lefse which are statistical models for differential**

**abundance: but the results of those are nowhere to be found.**

* Jose Clemente
* From KB:
* The Z-score corresponds to the ANCOM computed z-score of the centered-log ratio (CLR) of the ASV in any individual mouse. It does not refer to the comparison of Lachno present in 2/6 versus 0/6, but rather then significantly higher abundance of a given Lachno ASV in a given mouse relative to the other taxa present in that same mouse. LEfSe is not used in any of these results and can be deleted from methods (Supp page 11, reference 87). The new heatmap is updated to reflect this potential ambiguity, though it should be also stated in the figure legend (Lines 1029-1030).
* The original heatmap has the 10 most abundant and 10 least abundant ASVs; not sure why the least abundant 10 were used (I didn’t see any discussion in the text for Clostridia) so I used only the most abundant 10 for the new heatmap.
* I generated alpha (Shannon Entropy, p=0.015 via Kruskal-Wallis) and beta PCoA (Unweighted Unifrac, p=0.32 via PERMANOVA) in response to the more global view. The taxa barplot doesn’t seem to show any major differences in the two populations either so showing alpha, beta, and ANCOM I think offers the best concise summary.

Fig 3E: this is the main provided evidence of a barrier defect correlating with

alterations in IgG responses. But the way the figure is presented makes it hard

to distinguish how many mice were tested and how many were CFU positive in

the MLN or MAT because the circles are overlapping and figure is so narrow.

The supplemental figure S3G makes this clear: but its weird to need to look in

supplement for the same data graphed in a more interprettable way.

* Stay as it is

**Fig S6: This metabolomics data is super interesting, but its not clear how this**

**relates to the proposed microbiota mechanism**

The idea that there is more IgG1 reactivity against commensals in the knockout

mice is kind of undermined by the fact that there seems to be 10 times as

much total IgG1. It is not completely clear from the figure legend, but it

seems like the results in 3B are not normalized to the total IgG1 level. If thats

true, then normalizing to total IgG1 would likely show the opposite: that

microbiota reactivity is actually decreased as a proportion of the total IgG1

immunoreactivity. If so, I’m not sure what this means.

Fig 1C: IgA ko has less IgG3, more IgM: the steady state difference predicts

vaccine response

Fig 3A: IgA ko has way more IgG1, but this steady state difference did NOT

predict the results in Fig. 2

**Based on the paper as written, I dont really understand this seeming paradox.**

**“Next, we evaluated the effect of IgA on steady-state IgG1 production.” Why**

**the focus on IgG1 reactivity to commensals, and not other IgGs? Why is the**

**IgM data in supplement? The logic is not clear.**

Reviewer: 6

It is now established that the microbiota enhances antibody responses to

vaccination. In this excellent paper, the group of Andrea Cerutti provide data

on the role of IgA in amplifying antigen-specific IgG production to vaccination.

The authors found that anti-pneumococcal but not total IgG production was

impaired in mice with global or intraluminal IgA deficiency. The positive effect

of gut IgA on anti-pneumococcal IgG responses implicated gut bacteria, as

these responses were attenuated in germ-free recipient mice recolonized

with gut microbes from IgA-deficient donors. The authors further show that

IgA exerted this effect by constraining the systemic translocation of gut

commensal antigens, which caused chronic immune activation, including T

cell overexpression of programmed cell death-1 (PD-1). This immune inhibitory

receptor hindered anti-pneumococcal IgG production by mitigating B cell

functionality, which improved upon anti-PD-1 treatment. Thus, gut IgA is

functionally linked to systemic IgG via gut microbes.

Overall this paper provides important new mechanistic insight into how the

microbiome regulates immunity to vaccination, which is a topic of wide interest

to a broad readership. Therefore this paper should be published without delay.