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ADDENDUM

Immune/microbial interface perturbation in human IgA deficiency

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ABSTRACT
In a recently published article we report the metagenomic analysis of human gut microbiomes evolved in the absence of immunoglobulin A (IgA). We show that human IgA deficiency is not associated with massive quantitative perturbations of gut microbial ecology. While our study underlines a rather expected pathobiont expansion, we at the same time highlight a less expected depletion in some typically beneficial symbionts. We also show that IgM partially supply IgA deficiency, explaining the relatively mild clinical phenotype associated with the early steps of this condition. Microbiome studies in patients should consider potential issues such as cohort size, human genetic polymorphism and treatments. In this commentary, we discuss how such issues were taken into account in our own study.

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Introduction

In a recently published article we report the metagenomic analysis of human gut microbiomes evolved in the absence of immunoglobulin A (IgA). We first studied IgA-bound gut bacteria in healthy controls and surprisingly report poorly characterized *Coprococcus comes* as the main IgA target in the colon. In order to further get insights into the specific contributions of IgA to host/microbial symbiosis, we then explored patients deficient of IgA, but sufficient of all other antibody isotypes. We show that selective human IgA deficiency is not associated with massive quantitative perturbations of gut microbial ecology. While our study underlines a rather expected pathobiont expansion, we at the same time highlight a less expected depletion in some typically beneficial symbionts. We also show that IgM partially supply IgA deficiency, explaining the relatively mild clinical phenotype associated with the early steps of this condition.[1] Microbiome studies in patients should consider potential issues such as cohort size, human genetic polymorphism and treatments. In this commentary, we discuss how such issues were taken into account in our own study.

Selective IgA deficiency (IgAD) remains a bioclinical entity that is defined by serologic means, namely: serum IgA titers below 0.07mg/mL, with normal IgG levels. Selective IgAD is the most common form of primary immunodeficiency (PID) in the western world and affects approximately 1/600 individuals.[2] Selective IgAD was never until now associated with any kind of monogenetic PID, despite extensive research in the field. Common variable immunodeficiency (CVID) frequently combines IgA and IgG deficiency, CVID affects about 1/25000 Caucasians. Selective IgAD is associated with recurrent infections,[3] but also with inflammatory bowel disease (IBD) in a large case-control study (3.9% in patients versus 0.81% in healthy control).[4] In patients with Selective IgAD, sinopulmonary infections are primarily caused by encapsulated bacteria, including *Haemophilus influenzae* and *Streptococcus pneumoniae*, while intestinal infections are notably caused by the protozoan *Giardia intestinalis*. It was also suggested that IgG-deficient patients with particularly low IgA would be at risk for longstanding replication of gastrointestinal viruses.[5]

As underlined by Macpherson and Yilmaz in their comment of our published article,[6] IgA

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deficiency remains a paradox: how can we explain that loss of such an abundant antibody is frequently pauci-symptomatic ? What are the targets of IgA and the consequences of it?

In the absence of IgA, we observed at the same time preserved microbial diversity, and nevertheless significative and focused impact on particular bacterial species.

What is the clinical relevance of these findings?

Since secretory IgM (sIgM) is significantly elevated in stool from IgA deficient patients, in comparison to controls, *(IgM+ bacteria 6.26(0.625 to 45)% vs 0.05(0 to 2.4)%; $p < 0.0001$)*, we hypothesized that sIgM could at least partially replace sIgA and, in particular, might contribute to preserve healthy microbiota diversity, possibly by retaining highly diverse and putatively beneficial bacteria within gut mucus. This was our working hypothesis.

We therefore evaluated whether sIgM binding was associated with species diversity within each of the major phyla. As reported, we see a positive correlation between sIgM binding and Actinobacteria diversity in IgA deficient patients (*Spearman coefficient, $r = 0.716$; $p < 0.05$*), In the CVID patients we analyzed diversity loss was particularly pronounced in Actinobacteria *(Shannon diversity index, 1.688 (1.170 to 2.223) in HDs vs 1.342(0.319 to 1.827) in CVID patients; $p = 0.05$)*. It is therefore interesting to note that Actinobacteria diversity is preferentially decreased in CVID, while other phyla are comparatively less affected in this "IgM-deficient" model, while, allegedly, confounding factors might have similarly affected all phyla. Altogether, our data suggest that IgM can preserve healthy microbiota diversity, as it can be measured in the Actinobacteria phylum (Figure 1). There is clinical relevance for this concept as it is well established that patients lacking IgA selectively rarely develop IBD, which is instead more common and severe in those lacking both IgA and IgM.[7]

What are the limitations of this interpretation?

The positive correlation between IgM binding and Actinobacteria diversity, as well as the reduced diversity of Actinobacteria in CVID (in the absence of IgM), support the proposition that IgM preserves microbiota diversity. A larger CVID cohort is needed in order to tell whether the absence of IgM would in fact impact all phyla (not only Actinobacteria), which is probably the case.

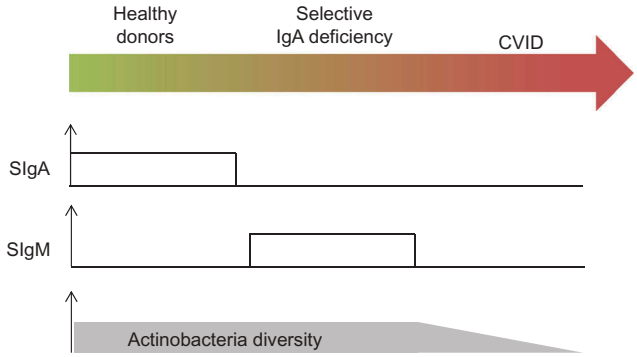

Figure 1. SIgM contribute to maintain actinobacteria diversity in the absence of IgA.

Considering microbiota complexity and inter-individual variations is it possible to conclude on a disease-associated signature from the study of arelatively limited number of patients?

We believe that the cohort size is sufficiently large to make conclusions regarding global gut microbiota composition. We found more similarities than differences between controls and patients with IgA deficiency. However, when we focus on IgA targets, there are clear differences in terms of prevalence of given species like *C. comes* or *E. coli*, which are respectively decreased and increased in selective IgAD. This explains why we are able to observe clear differences studying a relatively limited number of individuals. However, analysis of diversity and representation of the dominant phyla remain at low resolution level in the published study. We therefore can only conclude in this article that IgA deficiency does not strikingly alter global fecal microbiota composition in affected patients. Of note, our observations are limited to feces, and a more severe dysbiosis may be present in the small intestine, as shown in mouse models of IgA deficiency.[8] Nevertheless the combination of flow cytometry and metagenomic analysis proved

useful in order to increase the resolution of the analysis on human fecal samples by monitoring alterations of commensals specifically bound by secretory IgA in healthy controls.

How to conclude on CVID, a lot more complex condition than isolated IgA deficiency?

Indeed CVID is a more complex condition than selective IgAD. The clinical presentation of CVID is rather heterogeneous, various (PIDs) have been linked to CVID, and patients are usually heavily treated (Ig substitution, antibiotics). As we underline: the analysis of CVID patients in our paper is hampered by several confounders, such as previous antibiotic courses and small cohort size (although we took care to include CVID cases that did not receive antibiotics within 3 months prior to sampling). It is however interesting to note that Actinobacteria diversity is drastically decreased in CVID, an "IgM-deficient" model". We indeed show for the first time alterations in phylum diversity in CVID. Alterations are particularly affecting Actinobacteria and to a smaller extent Firmicutes. Jorgensen *et al.* recently published that CVID patients display dysbiotic gut microbiota with reduced alpha-diversity, reduced abundance of Actinobacteria and increased abundance of gamma-proteobacteria.[9] However, it was not discussed in this article whether diversity loss was phylum specific. Considering the trends observed in our own study, we would postulate this is not the case.

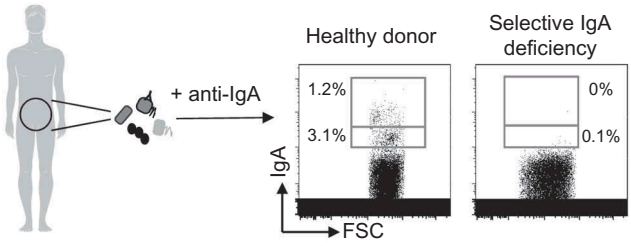

Figure 2. IgA binding to healthy and selective IgAD microbiota. Representative flow cytomery analysis of endogenous IgA colonic microbiota coating.

High and/or low affinity binding of IgA to microbiota. Could the IgA repertoire be split into high-versus low-affinity, binders?

Our approach allowed us to determine which bacterial consortia are *preferentially* bound by IgA using cytometry to detect bacteria-bound IgA.[10] Interestingly we detected IgA^{dim} and IgA^{bright} cells (Figure 2). The technique also allows to distinguish an $IgA^{very\ low}$ signal, detected as a shift in the main bacterial population, that is never observed in IgA deficient patients. Therefore $IgA^{very\ low}$ signal is not background noise, and, may be functional. More work will be necessary to determine whether IgA^{dim} and IgA^{bright} bacterial populations, correspond to bacteria bound by high versus low affinity IgA, respectively, and whether these subsets have overlapping bacterial repertoires or not. Very low affinity IgA interactions are also suggested and could account for the very discrete, but global, bacterial flow cytometry profile shift consistently observed in healthy donors, but not observed in selective IgAD patients. It should be emphasized that for metagenomics, we enriched for *all* IgA binders, using beads and not flow sorting of bright cells. Therefore all kinetic/avidity types of interactions should have been taken into account at this level of analysis.

Considering patient and control selection, what could have been a better study design?

We compared patients with unrelated healthy controls. Comparing IgAD patients with sibling or first degree relatives with normal IgA level could have represented another design option. However, besides unaffected twins living under the same roof, this option has its own limitations, especially when recruitment is driven by the inclusion of rare patients, like in our study. Siblings are rarely age-matched (rules out parents or children as controls), and sex-matched. We therefore focused on non-related age- and sex-matched healthy donors. The fact that we see no major, but only minor differences of gut microbiota composition suggests to us that the matching of healthy donors and patients is

representative and does not constitute a major source of error in the published study.

Should host genetics have been extensively explored as well?

IgA deficiency is of course observed in various PIDs of known genetic origin. In particular, CVID frequently combines IgA and IgG deficiency. [2] Indeed, there are now several CVID genes described.

However, selective IgAD occurring in a sporadic manner (as it is the case here) was never until now associated with any kind of monogenetic PID, despite extensive research in the field. Only gene polymorphisms were listed (http://omim.org/entry/137100). A single IgAD patient carrying a TACI mutation was described.[11] However, the patient presented with borderline low serum IgG level at time of diagnosis, was born from a mother suffering from CVID, and was himself probably CIVD, as subsequently acknowledged by the same authors.[12] Other TACI mutations were not found by the same group in 34 individuals with IgAD.[12] We have no such familial cases in this study, and, by definition, all IgAD patients included had normal systemic IgG levels (cf. Figure S1). More importantly, as heterozygous TACI mutations may also be found in healthy controls and were not functionally validated until now (only TACI -/- cells lack response to TACI ligands, [13] there is no genetic mutation directly responsible for a proven PID associated with any form of selective IgAD (neither sporadic, nor familial).

Altogether, and as stated above, selective IgAD remains a bio-clinical entity of unknown genetic origin that is defined by serologic means, namely undetectable seric IgA titers (< 0.07mg/mL) with normal IgG levels. The cohort we studied therefore cannot be stratified according to corresponding PID genes, because they have not been described in the literature.

Conclusion

Altogether we conclude that IgA plays a dual role. IgA not only protects from pathobionts and pathogens, but also preserves beneficial microbiota diversity. IgM can only partly compensate for the lack of IgA, as IgA deficient patients present with signs of altered microbiota composition and altered bacterial networks. Of note, polyclonal immunoglobulin preparation currently used in the clinic are IgA-depleted. We believe our data pave the way for trials based on oral IgA supplementation, not only in antibody PIDs, but also in other dysbiosis-associated conditions, such as IBD or graft-versus-host disease.

Acknowledgments

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