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Research Letter In-Press Preview **Gastroenterology** **Immunology**

Selective IgA_2 deficiency in a patient with small intestinal Crohn's disease

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Conflict of interest: see supplementary material.

To the editor: The human IgA response is composed of two antibody subclasses, IgA_1 and IgA_2. With a shorter hinge region, IgA_2 is more resistant to bacterial proteases. Anecdotal evidence of IgA_2 deficiency is available (1-3), however no associations with clinical manifestations have been reported. Here, we describe a patient with selective IgA_2 deficiency (CD068) with concomitant small intestinal Crohn's disease (CD), with duodenal and ileal inflammation. To our knowledge, this is the first case of IgA_2 deficiency with a potential link to IBD, which might shed new insights into potential IgA_2-specific functions.

Ileal, colonic and peripheral blood mononuclear cells (PBMCs) were profiled from patient CD068, 19 healthy donors (HDs) and 15 IBD patients (Table S1). Total absence of IgA_2^+ plasma cells (PCs) (Figure 1A) and switched memory B (B_{mem}) cells (Figure 1B) was noted in CD068, but not in HD. The frequency of ileal and colonic IgA_1^+ PCs and B_{mem} cells was comparable in CD068,

IBD patients and HDs (Figures 1C, S1B). Conversely, a significant loss of IgA_2^+ PCs was detected in inflamed colonic areas from UC patients compared to HDs, while a trend towards loss of IgA_2^+ cells in inflamed ileal tissues from CD patients was noted (Figure 1C). We also found a significant expansion of IgG^+ PCs in inflamed ileum and colon of IBD patients compared to HDs. An increase in IgG^+ PCs (above HD interval estimate) was observed in both inflamed (ileum) and uninflamed (colon) tissues of CD068 (Figure 1C). In a subset of individuals, paired colon/ileum samples were analyzed in parallel (Figure S1C). Using tissue immunofluorescence (IF), we found no IgA_2^+ cells in the intestinal mucosa of CD068, while IgA_1^+ cells were readily detected (Figure 1D). To exclude a lack of detection by our primary antibody, we stained tissue for IgA_1 and total IgA, and obtained comparable results (Figure S1E).

In contrast to HDs, we could not detect IgA_2 in the serum or ileal biopsy culture supernatants in CD068, (Figure 1E). Levels of IgA_2 in circulation were comparable between HDs and CD patients (Figure 1F). The concentration of all antibody isotypes except IgA_2 were comparable between CD068 and HDs (Figure 1G). PBMCs stimulated with CD40L and IL-21 induced both IgA_1^+ and IgA_2^+ PCs in HD, but no IgA_2^+ PCs were induced in CD068 (Figure S1F). In an effort to identify a genetic basis for IgA_2 deficiency, we used a 407 gene panel for immune deficiency, but no homozygous alterations were detected (Table S2).

Next, we examined IgA-coated stool bacteria (Figure 1H) and found a complete lack of IgA_2 coating. Metagenomic sequencing of stool samples in 10 HDs, 7 CD patients and CD068 revealed a loss of alpha diversity in CD patients and CD068 compared to HDs. Further, gut bacteria from CD068 and CD samples clustered together (Figure 1I, S1G). Finally, a distinct expansion of Clostridiales and a loss of Bacteroidales was observed in CD068. To evaluate potential consequences of intestinal dysbiosis, we measured circulating zonulin as well as IgG and IgA to *Saccharomyces cerevisiae* (ASCA) at two different time points (Figure 1J). Zonulin levels were similar in HDs and CD patients (both n=10), but were elevated in CD068. In addition, ASCA IgA and IgG concentrations from CD068 were among the highest in a large database of HD and CD patients (n=367, n=806). These data demonstrate that in CD068, lack of IgA_2 was associated with gut dysbiosis, impaired gut barrier integrity, and exaggerated systemic antibody responses to commensals.

Given its higher resistance to proteolysis, IgA_2 may be particularly involved in immune exclusion and its lack could increase epithelial penetration by commensals. Accordingly, major B cell

perturbations in inflamed IBD tissue have been described (4) including a loss of IgA_2^+ PCs (5). The small intestine (SI) is the largest reservoir of IgA^+ PCs in the body and includes bacterial communities more heavily coated by IgA than those from the colon (6), which could render the SI more susceptible to tissue injury due to impaired IgA (or IgA_2) production. Although a causal relationship between the lack of IgA_2 and CD remains unproven, our study shows novel evidence that documents an association between IgA_2 deficiency and small bowel CD with duodenal inflammation. Further studies aimed at dissecting the specific function and reactivity of gut IgA_1 and IgA_2 could lead to a better understanding of the contribution of IgA subclasses to IBD pathogenesis.

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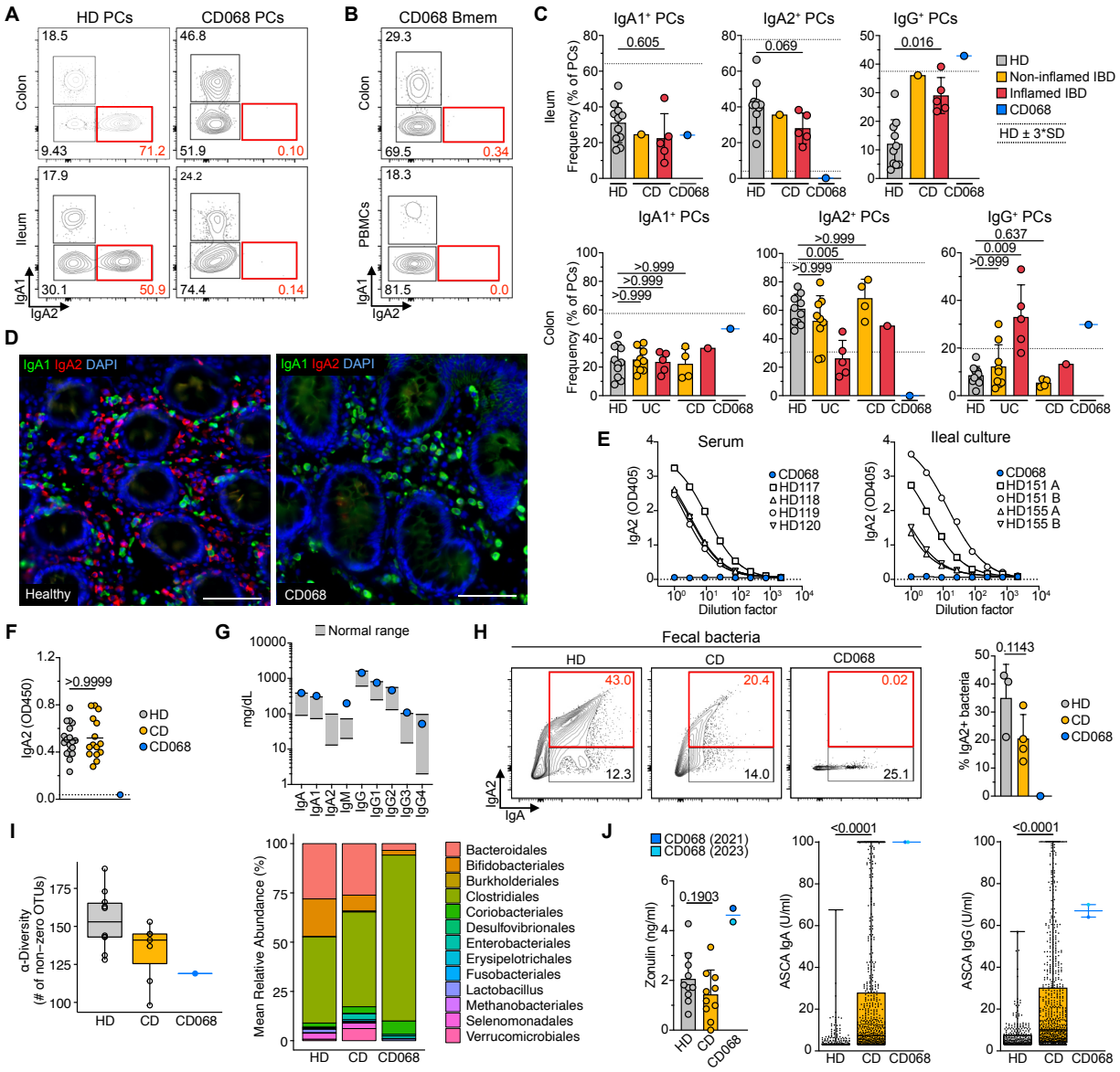

Figure 1. Selective IgA_2 deficiency in a patient with small intestinal CD. Flow cytometry staining of IgA_1 and IgA_2 on (A) intestinal PCs from a representative HD and patient CD068, and (B) memory B (B_{mem}) cells in colon and circulation from CD068. (C) Frequency of intestinal PCs from HDs (n=19), IBD patients (n=15), and CD068. Each point repsesents a sample. Mean, SD and Interval estimate are shown. (D) IF staining of IgA_1 and IgA_2 from a representative HD and CD068. (E) Secreted IgA_2 levels in the serum and ileal tissue culture supernatant of HDs and CD068. (F) Plasma IgA_2 in HDs (n=18), CD patients (n=15), and CD068. (G) Total serum immunoglobulins from HDs (grey) and CD068. (H) Representative flow cytometry plots and quantification of IgA^+ and IgA_2^+ microbiota in HDs (n=3), CD patients (n=4) and CD068. (I) Metagenomic sequencing analysis from fecal samples including alpha-diversity and relative

abundance (HD n=10, CD n=7). (J) Circulating zonulin and ASCA IgG and IgA in HDs (n=367), CD patients (n=806) and CD068. All comparisons done with Mann-Whitney test, except (C): Kruskal-Wallis test and Dunn's test, p values are shown.