

Figure S1

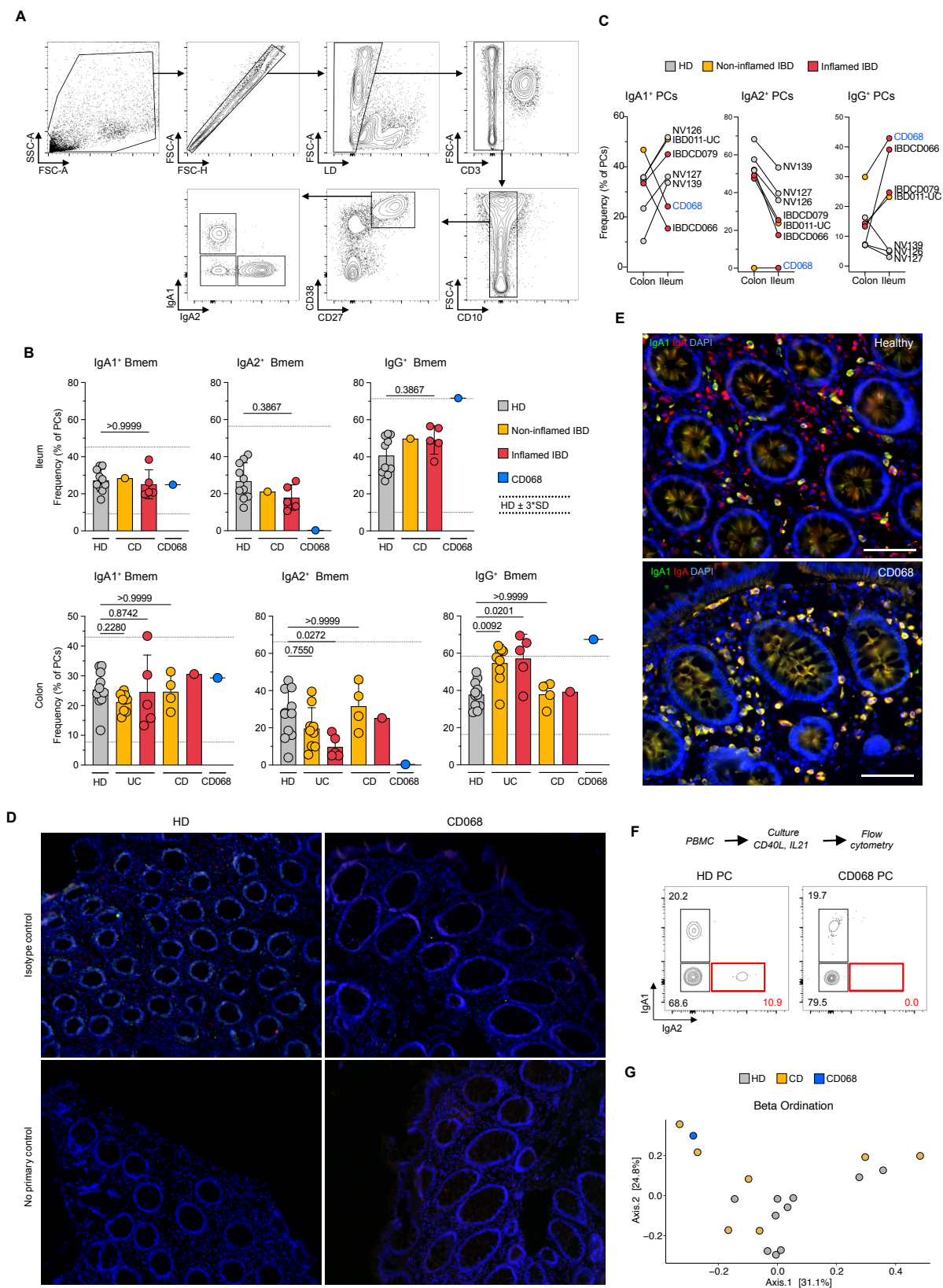


Figure S1. (A) Gating strategy used to identify viable CD3⁺CD10⁺CD27⁺CD38^{high} PCs within intestinal lamina propria mononuclear cells. (B) Frequency of intestinal B_{mem} cells from HDs, noninflamed IBD patients, inflamed IBD patients, and patient CD068. (C) Frequency of intestinal PC (Figure 1C) showing paired samples taken from the same individuals. (D) Immunofluorescence (IF) analysis of isotype controls or non-primary IgA₁ and IgA₂ controls in colonic tissue sections from a representative HD (left) or patient CD068. (E) IF staining for IgA₁ and total IgA from a representative HD and patient CD068. (F) Representation of IgA₁ and IgA₂ on PBMC-derived PCs stimulated *in vitro* for 10 days with CD40L and IL-21. (G) Metagenomic sequencing analysis from fecal samples showing beta ordination (from Figure 1I).

Table S1. Patient characteristics

Study number	Group	Gender 0-female 1-male	Age (years)	Duration of Disease	Previous IBD therapies	Concomitant IBD therapy	Non-IBD medications	Disease extent (Montreal classification)	Disease behavior (Montreal classification)	Inflammation	Assay	Race	Ethnicity
IBDCD066	CD	0	41	1 month	none	none	ocrelizumab (MS), off for 1 year due to COVID	L3	B1+perianal	T1	Flow cytometry	White	NA
IBDCD067	CD	0	20	2 years	none	none	none	L1+L4	B1	T1	Flow cytometry	White	NA
IBDCD068	CD	1	36	19 years	5ASA, infliximab, s/p ICR, adalimumab	ustekinumab	none	L1+L4	B2	T1	Flow cytometry	White	not hispanic or latino
IBDCD070	CD	1	25	6 months	none	none	none	L1	B1	T1	Flow cytometry	White	NA
IBDCD072	CD	1	29	1.5 years	none	ustekinumab	none	L1	B1	T1	Flow cytometry	White	not hispanic or latino
IBDCD078	CD	1	54	32 years	steroids, s/p ICR, and repeated SB surgeries	adalimumab	lamotrigine, cariprazine, sumatriptan, atorvastatin, propranolol, fenofibrate, eszopiclone, testosterone	L3	B2		Flow cytometry	White	not hispanic or latino
IBDCD079	CD	1	38	unknown	s/p ICR and colectomy > stoma reversal	infliximab	none	L3	B2	T1 + LC	Flow cytometry	Other	NA
IBDUC103	UC	1	53	18 years	5ASA, steroids, infliximab, adalimumab, MTX, vedolizumab	tofacitinib	statin, clopidogrel vitamin D	E2, currently on remission		LC	Flow cytometry	Asian Pakistani	NA
IBDUC104	UC	0	79	12 years	BMP	SASA	amlodipine, valsartan, thiazide, statin, montelukast, cetirizine	E3, currently on remission			Flow cytometry	Other	NA
IBDUC108	UC	1	39	4 years	prednisone	vedolizumab	atorvastatin, tamsulosin, PPI	E3, currently on remission			Flow cytometry	Other	NA
IBDUC114	UC	0	36	8 years	none	infliximab	none	E2, currently only mild proctitis			Flow cytometry	White	not hispanic or latino
IBDUC120	UC	1	38	1 year	steroids	SASA	none	E3, active moderate-severe in colon, the rest mild		LC	Flow cytometry	Other	NA
IBDUC124	UC	0	34	1 year	steroids 5ASA vedolizumab	infliximab	rivaroxaban	E3 pancolitis		LC	Flow cytometry	Asian Indian	NA
IBDUC128	UC	1	51	12 years	none	SASA	none	E3 pancolitis		LC	Flow cytometry	Asian	NA
UC316	UC	1	34	7 years	none	SASA	none	E2		LC	Flow cytometry	Other	NA
IBD011 (UC)	UC	1	25	6 years	5ASA, infliximab, MTX	infliximab	none	left sided colitis (m/p UC and not CD)			Flow cytometry	White	NA
NV126	HD	1	40	NA	NA	NA	none	NA	NA	NA	Flow cytometry	other	NA
NV127	HD	1	56	NA	NA	NA	losartan, thiazide, PPI (morbid obesity, OSA, HTN)	NA	NA	NA	Flow cytometry	White	hispanic or latino (Brazil)
NV128	HD	1	78	NA	NA	NA	aspirin, atorvastatin, losartan, thiazide, amlodipine (HTN, osteoarthritis, gait disorder)	NA	NA	NA	Flow cytometry	Other	NA
NV136	HD	0	62	NA	NA	NA	interferon beta1, metformin, sitagliptin, statin, losartan, aspirin (HTN hyperlipidemia, multiple sclerosis, diabetes, s/p CRC and hemicolectomy)	NA	NA	NA	Flow cytometry	African American	NA
NV137	HD	0	52	NA	NA	NA	bupropion, levothyroxine, statin	NA	NA	NA	Flow cytometry	White	NA
NV138	HD	0	46	NA	NA	NA	none (s/p breast cancer)	NA	NA	NA	Flow cytometry	African American	Not hispanic or latino (Jamaican)
NV139	HD	1	76	NA	NA	NA	atorvastatin, candesartan, metformin, tamsulosin, rivaroxaban, gabapentin	NA	NA	NA	Flow cytometry	Other	NA
NV140	HD	0	48	NA	NA	NA	none	NA	NA	NA	Flow cytometry	White	NA
NV143	HD	1	50	NA	NA	NA	amlodipine, metformin, ibesartan, statin	NA	NA	NA	Flow cytometry	Other	NA
NV144	HD	1	20	NA	NA	NA	PPI (eosinophilic esophagitis)	NA	NA	NA	Flow cytometry	White	NA
NV146	HD	0	38	NA	NA	NA	none	NA	NA	NA	Flow cytometry	White	NA
NV147	HD	0	50	NA	NA	NA	benztropine, aripiprazole (autism)	NA	NA	NA	Flow cytometry	African American	NA
NV148	HD	1	69	NA	NA	NA	aspirin preventive (no concomitant disease)	NA	NA	NA	Flow cytometry	White	Not hispanic or latino
NV168	HD	1	59	NA	NA	NA	PPI, atorvastatin, inhalers (dyslipidemia, GERD, OSA, COPD, prostate cancer)	NA	NA	NA	Flow cytometry	White	Not hispanic or latino
NV192	HD	1	43	NA	NA	NA	none	NA	NA	NA	Flow cytometry	Other	Not hispanic or latino (dominican republic)
NV193	HD	0	76	NA	NA	NA	aspirin, amlodipine, losartan, statin	NA	NA	NA	Flow cytometry	African American	NA
NV195	HD	0	63	NA	NA	NA	lisinopril, amlodipine, statin, PPI, thyroxine	NA	NA	NA	Flow cytometry	White	NA
NV196	HD	0	36	NA	NA	NA	none	NA	NA	NA	Flow cytometry	White	NA
NV197	HD	0	37	NA	NA	NA	s/p adenoid cystic carcinoma of hard palate 2018	NA	NA	NA	Flow cytometry	Asian Indian	Not hispanic or latino
IBDCD002	CD	0	49	24 years	MTX, steroids, 5ASA, antibiotics, s/p ICR, infliximab	none (off meds d/t pancreatic cancer)	none	L3+4	B3		Plasma/Serum	White	NA
IBDCD007	CD	1	42	18 years	5ASA, s/p surgery (ICR, segmental sigmoidectomy and takedown of vesical fistula), adalimumab, thiopurines, infliximab	ustekinumab (q4weeks)	none	L3	B3 + perianal fistula		Plasma/Serum	White	NA
IBDCD012	CD	1	30	4 years	none	COED nutrition, no medical therapy	none	L3	inflammatory + perianal fistula		Plasma/Serum	White	not hispanic or latino
IBDCD015	CD	1	37	24 years	5ASA, antibiotics, thiopurines, adalimumab, infliximab, previous surgical therapies	vedolizumab	none	L3	B2 stricturing + perianal		Plasma/Serum	White	not hispanic or latino
IBDCD016	CD	0	50	6 years	5ASA	SASA	none	L3	B2 stricturing + perianal		Plasma/Serum	White	not hispanic or latino
IBDCD020	CD	0	60	50 years		thiopurines +5ASA	PPI	L2	stricturing		Plasma/Serum	White	NA
IBDCD022	CD	1	34	15 years	surgery, certolizumab, infliximab, adalimumab, ustekinumab	adalimumab	none	L1+L4	Structuring + perianal		Plasma/Serum	Other	NA
IBDCD032	CD	1	25	22 years	5ASA	none	finasteride	L2	B1		Plasma/Serum	White	not hispanic or latino
IBDCD033	CD	1	33	3 years	s/p ICR and sigmoidectomy, BMP, infliximab	ustekinumab	none	L2	B3+perianal		Plasma/Serum	African American	not hispanic or latino
IBDCD035	CD	0	67	24years	5ASA, infliximab, s/p surgery (ileosigmoid fistula takedown, ileocolic resection, right colon resection, jejunocolic anastomosis, sigmoid resection)	adalimumab	levetiracetam, lorazepam, cholestyramine	L3+4	B3		Plasma/Serum	White	not hispanic or latino
IBDCD037	CD	1	31	22 years	s/p ICR, 5ASA, BMP, infliximab	adalimumab	none	L1	B2		Plasma/Serum	White	NA
IBDCD040	CD	0	64	19 years	5asa	none	risedronate	L2	B3+perianal		Plasma/Serum	Other	Hispanic
IBDCD041	CD	1	45	6 years	infliximab, BMP, mongersen trial	ustekinumab	losartan	L3	B1		Plasma/Serum	White	NA
IBDCD042	CD	0	34	6 years	infliximab, adalimumab, vedolizumab and tofacitinib	ustekinumab	none	L3	B2		Plasma/Serum	White	not hispanic or latino
IBDCD044	CD	0	38	18 years	5ASA, azathioprine	infliximab	none	L2	B1+perianal		Plasma/Serum	Other	NA
IBDCD005	CD	0	53	2 years	Steroids	vedolizumab	none	L1	B2		Plasma/Serum	White	NA
NV153	HD	0	66	NA	NA	NA	ACE inhibitors, Aspirin, thiazide	NA	NA	NA	Plasma/Serum	African American	NA
NV134	HD	1	59	NA	NA	NA	aspirin, probiotics	NA	NA	NA	Plasma/Serum	White	NA
NV163	HD	0	45	NA	NA	NA	Aspirin, insulin, gabapentin, nifedipine, statin	NA	NA	NA	Plasma/Serum	Other	NA

NV164	HD	0	51	NA	NA	NA	anti histamine, PPI, eltroxin, statin	NA	NA	NA	Plasma/Serum	African American	NA
NV060	HD	0	48	NA	NA	NA	none	NA	NA	NA	Plasma/Serum + Stool	African American	NA
NV171	HD	1	71	NA	NA	NA	insulin , jardiance, amlodipine, statin, ACE inhibitors, carvedilol, Aspirin, lipid	NA	NA	NA	Plasma/Serum	Other	NA
NV162	HD	1	25	NA	NA	NA	PPI, citalogram	NA	NA	NA	Plasma/Serum	Other	NA
NV172	HD	1	62	NA	NA	NA	insulin, metformin, statin, losartan	NA	NA	NA	Plasma/Serum	Other	NA
NV186	HD	0	48	NA	NA	NA	none	NA	NA	NA	Plasma/Serum	White	NA
NV174	HD	1	51	NA	NA	NA	Olmesartan, amlodipine, thiazide	NA	NA	NA	Plasma/Serum	American indian or Alaskan	NA
NV166	HD	0	50	NA	NA	NA	none	NA	NA	NA	Plasma/Serum	Asian	not hispanic or latino
NV206	HD	1	57	NA	NA	NA	statin, levothyroxine, metformin, ezetimibe	NA	NA	NA	Plasma/Serum	White	NA
NV205	HD	0	33	NA	NA	NA	spironolactone, montelukast	NA	NA	NA	Plasma/Serum	Asian	not hispanic or latino
NV207	HD	1	40	NA	NA	NA	none	NA	NA	NA	Plasma/Serum	White	NA
NV188	HD	1	66	NA	NA	NA	aspirin, PPI	NA	NA	NA	Plasma/Serum	Other	NA
NV189	HD	0	51	NA	NA	NA	Fluoxetine, cetrizine	NA	NA	NA	Plasma/Serum	Other	NA
NV208	HD	0	48	NA	NA	NA	Alprazolam, cetrizine, PPI, montelukast	NA	NA	NA	Plasma/Serum	Other	Latino
NV014	HD	1	42	NA	NA	NA	none	NA	NA	NA	Plasma/Serum + Stool	Asian	not hispanic or latino
NV004	HD	0	56	NA	NA	NA	PPI, amitriptyline, levothyroxine	NA	NA	NA	Plasma/Serum + Stool	White	NA
NV149	HD	0	65	NA	NA	NA	losartan, statin, letrozole	NA	NA	NA	Plasma/Serum	Other	NA
NV117	HD	1	40	NA	NA	NA	none	NA	NA	NA	Plasma/Serum	white	non-Hispanic
NV118	HD	1	72	NA	NA	NA	none	NA	NA	NA	Plasma/Serum	other	Hispanic
NV119	HD	1	24	NA	NA	NA	none	NA	NA	NA	Plasma/Serum	white	non-Hispanic
NV120	HD	1	36	NA	NA	NA	none	NA	NA	NA	Plasma/Serum	white	Hispanic
NV151	HD	0	51	NA	NA	NA	none	NA	NA	NA	Plasma/Serum	other	Hispanic
NV155	HD	0	60	NA	NA	NA	none	NA	NA	NA	Plasma/Serum	other	Hispanic
CD1140	CD	0	21	14	5ASA, corticosteroids, immunomodulatory drugs, metronidazole, ciprofloxacin	5ASA 6MP	DNA	L1	B1 + perianal		Stool	White	not hispanic or latino
CD1088	CD	0	31	21	5ASA, corticosteroids, integrin blockers, canasa, SCD	vedolizumab	DNA	L3	B1		Stool	White	not hispanic or latino
CD1039	CD	0	32	DNA	DNA	DNA	DNA	DNA			Stool	White	not hispanic or latino
CD1084	CD	0	35	29	5ASA, corticosteroids, metronidazole	DNA	DNA	L2	B1		Stool	White	not hispanic or latino
CD1050	CD	1	39	14	5ASA, corticosteroids, enteral nutrition, adalimumab, ustekinumab, infliximab, bowel resection	ustekinumab	Lansoprazole	L3	B2B3 + perianal		Stool	White	not hispanic or latino
CD1107	CD	1	40	28	5ASA	DNA	loratadine/pseudoephedrine, Fluticasone, Propionate	L2	B1 + perianal		Stool	White	not hispanic or latino
CD1003	CD	0	41	29	5ASA, corticosteroids, immunomodulatory drugs, metronidazole, ciprofloxacin	DNA	DNA	L1	B3		Stool	White	not hispanic or latino
CD1129	CD	0	42	DNA	DNA	DNA	DNA	DNA			Stool	White	not hispanic or latino
CD1002	CD	1	44	35	5ASA, immunomodulatory drugs, metronidazole, ciprofloxacin	adalimumab	DNA	DNA			Stool	White	not hispanic or latino
CD1105	CD	0	52	21	none	DNA	Synthroid	L3	B2		Stool	White	not hispanic or latino
CD1081	CD	1	54	35	none	Alltopurinol	Colcrys	L3	B1		Stool	White	not hispanic or latino
HD1124	HD	0	28	NA	NA	NA	DNA	NA	NA	NA	Stool	DNA	DNA
HD1186	HD	0	29	NA	NA	NA	none	NA	NA	NA	Stool	White	not hispanic or latino
HD1319	HD	1	29	NA	NA	NA	none	NA	NA	NA	Stool	White	not hispanic or latino
HD1065	HD	1	39	NA	NA	NA	none	NA	NA	NA	Stool	White	not hispanic or latino
HD1399	HD	1	43	NA	NA	NA	none	NA	NA	NA	Stool	White	not hispanic or latino
1001283B	HD	0	25	NA	NA	NA	none	NA	NA	NA	Stool	DNA	DNA
1001271B	HD	0	30	NA	NA	NA	none	NA	NA	NA	Stool	DNA	DNA
1001302B	HD	0	49	NA	NA	NA	none	NA	NA	NA	Stool	DNA	DNA
1001275B	HD	0	24	NA	NA	NA	none	NA	NA	NA	Stool	DNA	DNA
1001175B	HD	0	26	NA	NA	NA	none	NA	NA	NA	Stool	DNA	DNA

Table S1. Abbreviations: 5ASA (5-aminosalicylic acid), s/p (status post), ICR (Ileocolonic resection), MTX (methotrexate), 6MP (mercaptopurine), PPI (proton pump inhibitor), OSA (obstructive sleep apnea), HTN (hypertension), CRC (colorectal cancer), GERD (gastroesophageal reflux disease), COPD (chronic obstructive pulmonary disease), CDED (Crohn's Disease Exclusion Diet), PPI (proton pump inhibitor), OSA (obstructive sleep apnea), HTN (hypertension). TI: terminal ileum, LC: left colon. NA: not applicable, DNA: data not available.

Table S2. Genetic variants present in patient CD068

Gene Changes	In data bases	Role	Disease associated
TCIRG1 c.824_825del (p.Glu275Alafs*214)	no	Pathogenic	AR osteopetrosis
AP3B1 c.2389G>A (p.Val797Ile)	no	VUS	AR Hermansky-Pudlak syndrome
C7 c.2521G>C (p.Glu841Gln)	gnomAD 0.01%	VUS	AR complement component 7
CCBE1 c.373C>T (p.Arg125Trp)	gnomAD 0.02%	VUS	AR Hennekam lymphangiectasia-lymphedema syndrome
ITGB2 c.181C>T (p.Arg61Cys)	gnomAD 0.003%	VUS	AR leukocyte adhesion deficiency type 1
SAMD9 c.83T>C (p.Ile28Thr)	gnomAD 0.008%	VUS	AD myelodysplasia, infection, restriction of growth, adrenal hypoplasia and insufficiency
TCF3 c.23C>T (p.Ala8Val)	unclear	VUS	AD/ AR and autosomal recessive agammaglobulinemia
USB1 c.169G>A (p.Glu57Ly)	gnomAD 0.007%	VUS	AR poikiloderma with neutropenia

SUPPLEMENTAL MATERIAL

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Competing interests:

SM reports receiving research grants from Genentech and Takeda; receiving payment for lectures from Takeda, Genentech, Morphic; and receiving consulting fees from Takeda, Morphic, Ferring and Arena Pharmaceuticals.

JFC reports receiving research grants from AbbVie, Janssen Pharmaceuticals and Takeda; receiving payment for lectures from AbbVie, Amgen, Allergan, Inc. Ferring Pharmaceuticals, Shire, and Takeda; receiving consulting fees from AbbVie, Amgen, Arena Pharmaceuticals, Boehringer Ingelheim, BMS, Celgene Corporation, Eli Lilly, Ferring Pharmaceuticals, Galmed Research, Genentech, Glaxo Smith Kline, Janssen Pharmaceuticals, Kaleido Biosciences, Imedex, Immunic, Iterative Scopes, Merck, Microbia, Novartis, PBM Capital, Pfizer, Protagonist Therapeutics, Sanofi, Takeda, TiGenix, Vifor; and holds stock options in Intestinal Biotech Development.

METHODS

Patients

Patients were recruited between 2019 and 2022 in the Inflammatory Bowel Disease Center, the Gastroenterology Department and the Digestive Endoscopy Unit at Mount Sinai Hospital, NY. In accordance with the institutional review board, the protocol was approved and informed consent was obtained from all patients. Detailed patient characteristics are included in Table S1. Patients enrolled in the study were asked to donate intestinal biopsies and blood. Biopsies were collected during colonoscopies planned for regular care. When possible, ileal and colonic biopsies were obtained from the same individuals.

Patient CD068 is a 38-year-old male who had abdominal pain and bleeding at age 18, diagnosed with stricturing Crohn's disease. He had no medical history of respiratory tract infections, illnesses

or hospitalizations. His mother has a history of pemphigus vulgaris; there was no history of consanguinity. As part of our immunophenotyping efforts, intestinal biopsies were processed and characterized by flow cytometry. A 407 gene panel for immune deficiency was used to examine genetic alterations (Table S2). No homozygous alterations were noted. The patient was found to have 7 variants of unknown significance in *AP3B1*, *C7*, *CCBE1*, *ITGB2*, *SAMD9*, *TCF3*, and *USB1*, and one pathogenic recessive mutation in *TCIRG1* (associated with autosomal recessive osteopetrosis).

Intestinal tissue processing

During colonoscopy, 8-12 biopsies from non-inflamed and/or inflamed regions of the descending colon and terminal ileum were collected with forceps directly into ice-cold RPMI and transported to the laboratory. All biopsies were processed within 3h of collection. Two consecutive rounds of dissociation were performed to remove the epithelium, each consisting of 20 min incubation (37°C, 215 rpm) in 10ml HBSS (no calcium and magnesium) (Gibco) containing 5mM EDTA (Invitrogen), 100mM Hepes (Lonza) and 10% FBS, followed by 30s vortex and washing with HBSS. Biopsies were then transferred into 10ml of digestion media, made of RPMI (Gibco) containing 0.5mg/ml Collagenase IV (Sigma-Aldrich), 0.1mg/ml DNase-1 (Roche) and 1% FBS, and were incubated for 40 min (37°C, 215rpm). Biopsies were mechanically disrupted using a syringe needle, before two filtering and washing steps (RPMI) through 100µm and 40µm cell strainers. The single cell suspension was centrifuged (400G, 10 min) and resuspended in RPMI for downstream analysis.

Blood processing

Blood was drawn in EDTA tubes, transported to the lab at room temperature, and processed within 3h of collection. Blood was diluted with PBS 1X (Gibco) and gently overlaid on lymphocyte separation medium (MP Biomedicals). After centrifugation, mononuclear cells were recovered and washed two times with PBS. Serum was collected and immediately stored at -80°C.

Flow cytometry

After isolation from tissue, cells were incubated for 20min at 4°C in PBS containing a mix of staining antibodies. Cells were then washed with PBS and fixed using PBS with 2% formaldehyde (ChemCruz) for 20 min, before washing with PBS. Cells were resuspended in PBS and acquired within 24h using LSR Fortessa (BD Bioscience). Data was analyzed using FlowJo v10 (FlowJo). Antibodies used: anti-CD19 PE Cy7 (Abcam, SJ25C1), anti-CD27 PerCP Cy5.5 (Abcam, O323),

anti-CD10 APC Cy7 (Abcam, HI10a), anti-CD38 APC (Abcam, HB-7), anti-IgD PB (Abcam, IA6-2), anti-IgM AF700 (Abcam, MHM-88), anti-CD45 PE Dazzle 594 (Abcam, HI30), anti-CD3 BV605 (Abcam, UCHT1), anti-IgA1 AF488 (Abcam, B3506B4), anti-IgA2 PE (Abcam, IS11-21E11). Interval estimate calculated as 3 X SD around the mean of control HDs.

Tissue immunofluorescence

Immunofluorescence staining was performed on formalin-fixed, paraffin-embedded intestinal tissue sections of 5µm thickness. Samples were deparaffinized through two 5 min xylene baths, followed by consecutive washes in solutions of decreasing concentration of ethanol in distilled water (100, 90, 80 and 70%), and two 5 min baths in PBS. Antigen retrieval was done using Dako antigen retrieval solution (Dako) and 15 min heating in pressure cooker, before washing 3x with PBS. Samples were blocked using 10% goat serum (Life technologies) or donkey serum (Southern Biotech) for 1h at room temperature. Primary antibodies were then added to the tissue in serum, and samples were incubated overnight at 4°C. Slides were washed with PBS, before adding secondary antibodies and DAPI, which were incubated for 1h at room temperature. Slides were washed with PBS and mounted using Fluoromount G (Electron Microscopy Sciences). Tissue was visualized using a Nikon Eclipse Ni microscope and a Nikon DS-Qi2 camera.

Antibodies used: rabbit anti-human IgA1 (Abcam, ab193187), mouse anti-human IgA2 (Miltenyi, 130-093-081), goat anti-human IgA (Abcam, ab98550), goat anti-mouse AF594 (Abcam, ab150116), goat anti-rabbit AF488 (Abcam, ab150077), donkey anti-goat AF594 (Abcam, ab150132), donkey anti-rabbit AF488 (Abcam, ab150073).

Quantification of IgA₂ antibody levels with ELISA

Maxisorp ELISA plates (Fischer scientific) were coated with anti-human IgA₂ antibody (Miltenyi) 1µg/ml in Bicarbonate buffer, and incubated for 1.5h at room temperature. After washing with PBS-Tween (PBST) (Sigma), blocking was done using PBST with 3% BSA during 1.5h at room temperature. Before being added to the plate, plasma and serum samples were diluted 1:100 in PBST-BSA, while tissue supernatant was diluted 1:3 in PBST-BSA. Serial dilutions (1:3) were made through the plate, and samples were incubated overnight at 4°C. Plates were washed 3x with PBST, and secondary anti- IgA₂-HRP (Southern Biotech) antibody was added and incubated 1.5h at room temperature. Finally, plates were washed with PBST and TMB substrate was added. Absorbance was measured at 450nm using a plate reader.

Intestinal biopsy tissue culture

During colonoscopy, biopsies were taken with forceps directly into ice-cold RPMI with 10x Antibiotic-Antimycotic (RPMI-AA) (Gibco), and transported to the lab on ice. Under sterile conditions, biopsies were rinsed on a cell strainer using RPMI-AA 10x. Biopsies were placed in pairs into 48-well plates with 1 ml RPMI-AA 10X per well, and kept from this point at 37°C with 5% CO₂. Culture medium was replaced every 2 days using pre-warmed RPMI-AA 2X. Recovered culture medium was centrifuged 10min at 14000G and stored at -80°C.

Bacterial flow cytometry

Fecal pellets were dissolved in PBS at 100 mg/ml by vortexing. Fecal slurry was centrifuged 10min at 50G to remove large particles. Supernatant was filtered through a 40nm cell strainer and cells were then washed by centrifugation at 9000G and resuspension in washing buffer (PBS, 1% BSA, 2mM EDTA). Blocking was done using PBS +1% BSA +20% mouse serum, for 20min at 4°C. Cells were stained with anti-human IgA2-PE and anti-human IgA-APC for 30min at 4°C, before washing three times with washing buffer. Samples were stained with SYBR green before analysis by flow cytometry.

Immune globulin measurements

For patient CD068, serum immune globulins were IgG=1,435 (nl 603 - 1,613 mg/dl); IgA=387 (nl 90-386 mg/dl); and IgM= 197 (I n20 - 172 mg/dl). IgE=13 (6-495IU/ml). In terms of IgG subclasses, IgG1- 753 (248 - 810 mg/dL) IgG2 = 464(130 - 555 mg/dL) IgG3 = 108 (15 - 102 mg/dL); IgG4 = 52 (2 - 96 mg/dl). His IgA1 = 391.6 (73.2 - 301.2 mg/dL) mg /dl, and IgA2 = <0.5 (13.4 - 97.9 mg/dL).

Genetic Primary Immunodeficiency Panel

A primary immune deficiency panel of 407 genes was obtained with patient's consent (Invitae Corp, San Francisco CA).

In vitro B cell stimulation

For B cell activation, PBMCs were cultured in RPMI media (Gibco) supplemented with 10% FBS, the presence of CD40L (1µg/ml) (InvivoGen) plus IL-21 (100 ng/ml) (BioLegend) for 10 days. Cells were kept in 24-well plates at 37°C with 5% CO₂. After 10 days of culture, cells were analyzed by Flow cytometry, as indicated before.

Fecal collection, DNA extraction, and Shotgun metagenomic sequencing.

A stool sample from the index case, CD068 was obtained and compared with stool samples from a cohort of patients with CD and HD. All samples were processed at the Microbiome Translational Center (MTC) at the Mount Sinai Hospital. First, stool samples were aliquoted on liquid nitrogen or dry ice and stored at -80°C until DNA processing. Using previously described methods, DNA was isolated from fecal samples using bead beating in phenol chloroform and prepared using NEBNext® Ultra™ II DNA Library Prep kit before samples were pooled and sequenced with Illumina HiSeq 4000 with pair-end 150nt reads (Aggarwala et al, 2021). Metagenomic samples were quality trimmed with trimmomatic (Aggarwala et al, 2021) and subsampled to a median of 1.7M reads.

Microbiome analysis

MetaPhlAn v2.0 (Truong et al, 2015) was utilized for taxonomic assignment of sequencing reads. Abundance table output from MetPhlAn v2.0 was used in R Studio for downstream analysis and visualizations used ggplot2 (Wickham, 2016) and ggpubr (<https://github.com/kassambara/ggpubr>). The *Phyloseq* package (McMurdie & Holmes, 2013) was used to integrate sample, taxonomy, and abundance taxa as well as for characterization of microbial taxonomic community with alpha diversity and principal coordinate analysis (PCoA with Bray-Curtis distances). Taxa present in less than 3% of samples, not present above 0.1% relative abundance in at least one sample or annotated as “NA” were filtered out.

Antimicrobial antibody measurement

Patient CD068 plasma was processed at two different time points (2021 and 2023) for specific anti-Saccharomyces cerevisiae antibodies (ASCA IgA and IgG isotypes) as described before (Plevy et al, 2013). The Prometheus Laboratories Biobank of Autoimmune (AI) and Diagnostic Immunology Data Connected to Clinical Phenotypes and Disease Characteristics was interrogated to compare the serological profile from patient CD068 with those from CD patients (n=806), and normal healthy volunteers (NHV) (n=367).

Zonulin measurement

Patient CD068 plasma from two time points (2021 and 2023), as well as plasma from 10 CD patients and 10 Healthy donors were used. Zonulin levels from samples were measured using the Zonulin Serum ELISA (ALPCO) kit following the manufacturer's instructions. Plasma samples were diluted 1:20 and mixed with the biotinylated zonulin family peptide (ZFP) tracer. Samples were aliquoted into the anti-ZFP coated wells in duplicate and incubated for 1 hour at room

temperature while shaking. The plate was washed five times with a plate washer and incubated with peroxidase-labelled streptavidin for 1 hour at room temperature while shaking. The plate was washed five times and incubated with tetramethylbenzidine for 15 minutes at room temperature in the dark. The stop solution was added and absorbance was measured at 450nm.

Statistical methods

Unpaired comparisons were done using Mann-Whitney test. When applicable, non-parametric analysis were done using Kruskal-Wallis test and Dunn's multiple comparison test. p values are shown for all comparisons. Interval estimate was calculated as 3*SD around the mean of HD values.

Data availability

All microbiome data generated in this study is available in BioProject (<https://www.ncbi.nlm.nih.gov/bioproject/>) under PRJNA637878, PRJNA946744.

Supplementary References

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