



IgA deficiency alters systemic immune response to commensal gut microbes

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Abstract

Rationale: Selective IgA deficiency (SIgAD) is the most common primary immune deficiency. Symptomatic patients can experience increased atopy, recurrent infections or autoimmunity, though patients are frequently asymptomatic. Since IgA promotes homeostasis with commensal microbes, we investigated whether SIgAD impaired commensal microbe compartmentalization and altered systemic immune responses.

Methods

Blood and fecal samples were collected from 13 pairs of pediatric SIgAD patients and IgA sufficient siblings. Deep immunoprofiling using flow cytometry, CyTOF, cytokine analysis and ELISAs for Ig binding to fecal microbes was combined with metagenomic analysis of fecal microbiomes and microbial flow cytometry (mFLOW) of the IgA, IgG and IgM bound fecal microbiomes. mFLOW was performed by applying patient's serum antibodies to their fecal microbes, assessing binding of immunoglobulin isotypes and performing metagenomic sequencing of Ig-bound microbes.

Study Design

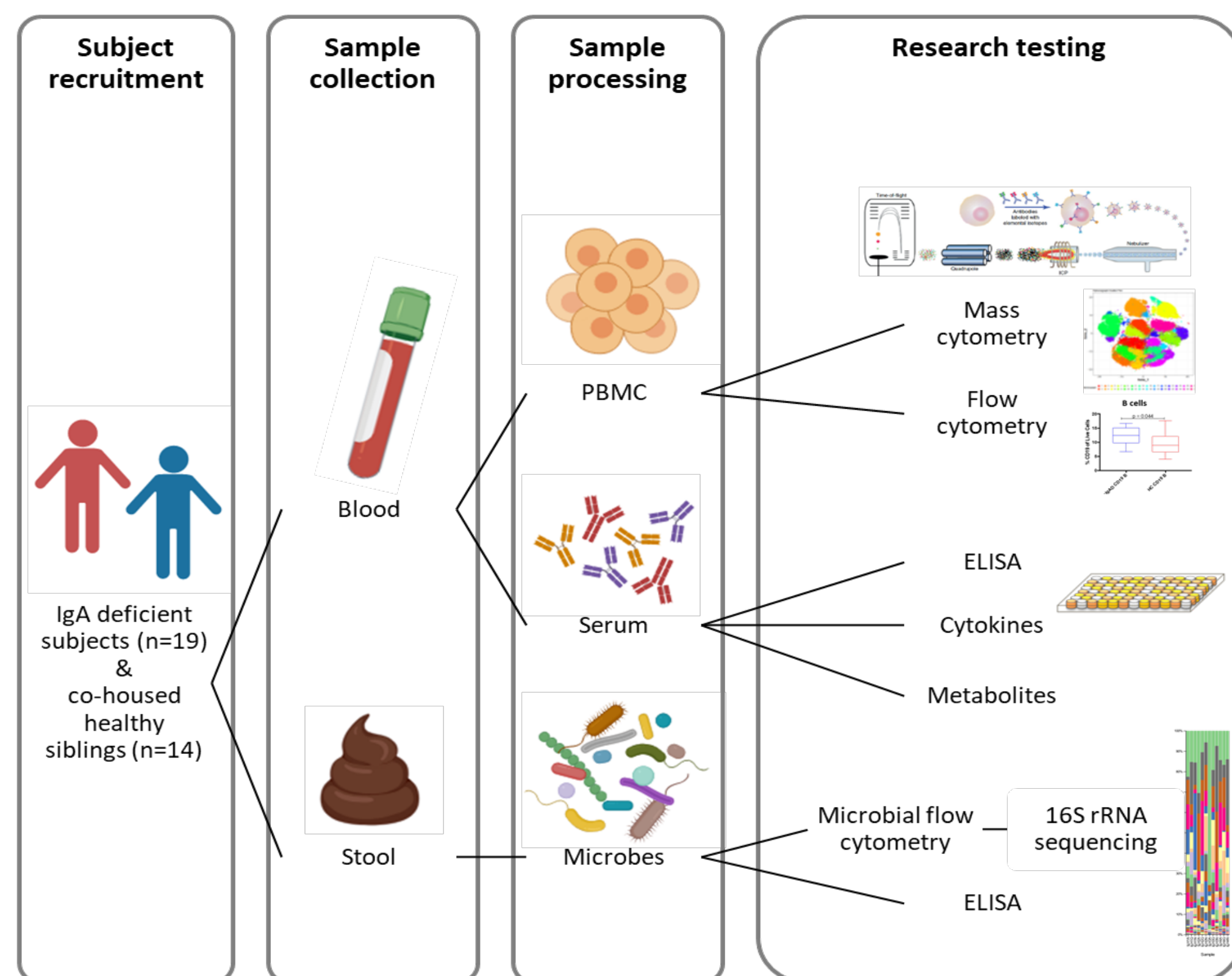


Figure 1: Experimental workflow.

Immunoprofiling

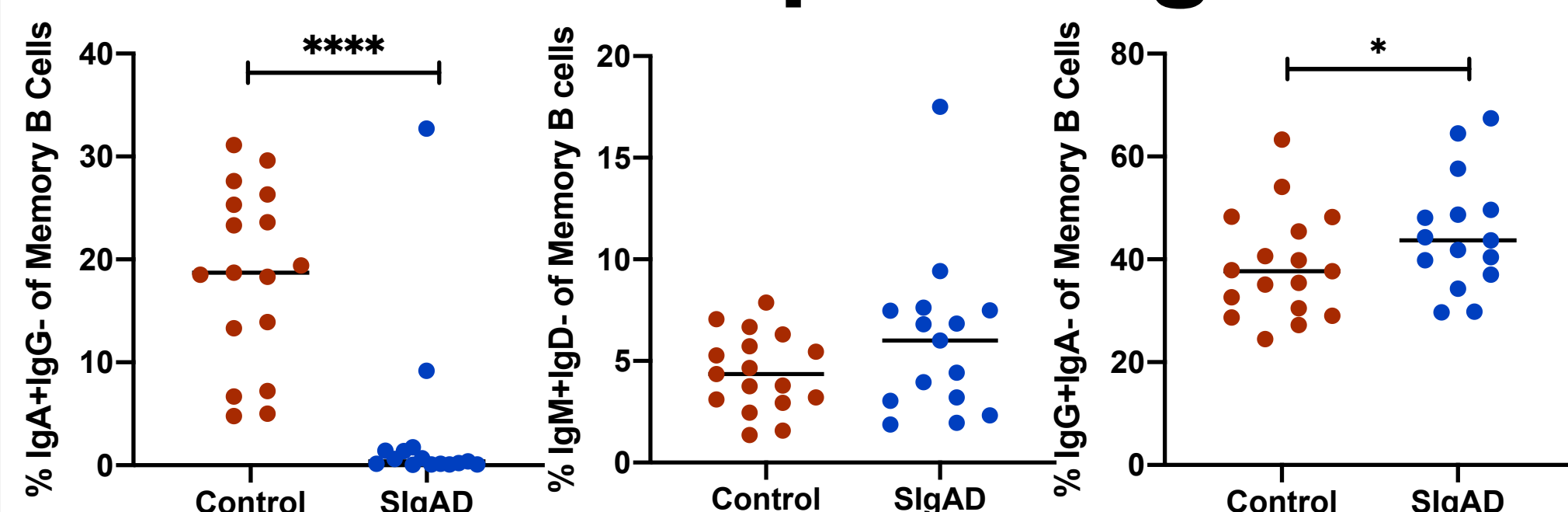


Figure 2: Elevated IgG-memory B cells in SIgAD.

Immunoglobulin (Ig) expressing memory B cells (CD19+CD27+) were detected by flow cytometry surface staining. Significant differences are indicated by * $p < 0.05$ and **** $p \leq 0.01$.

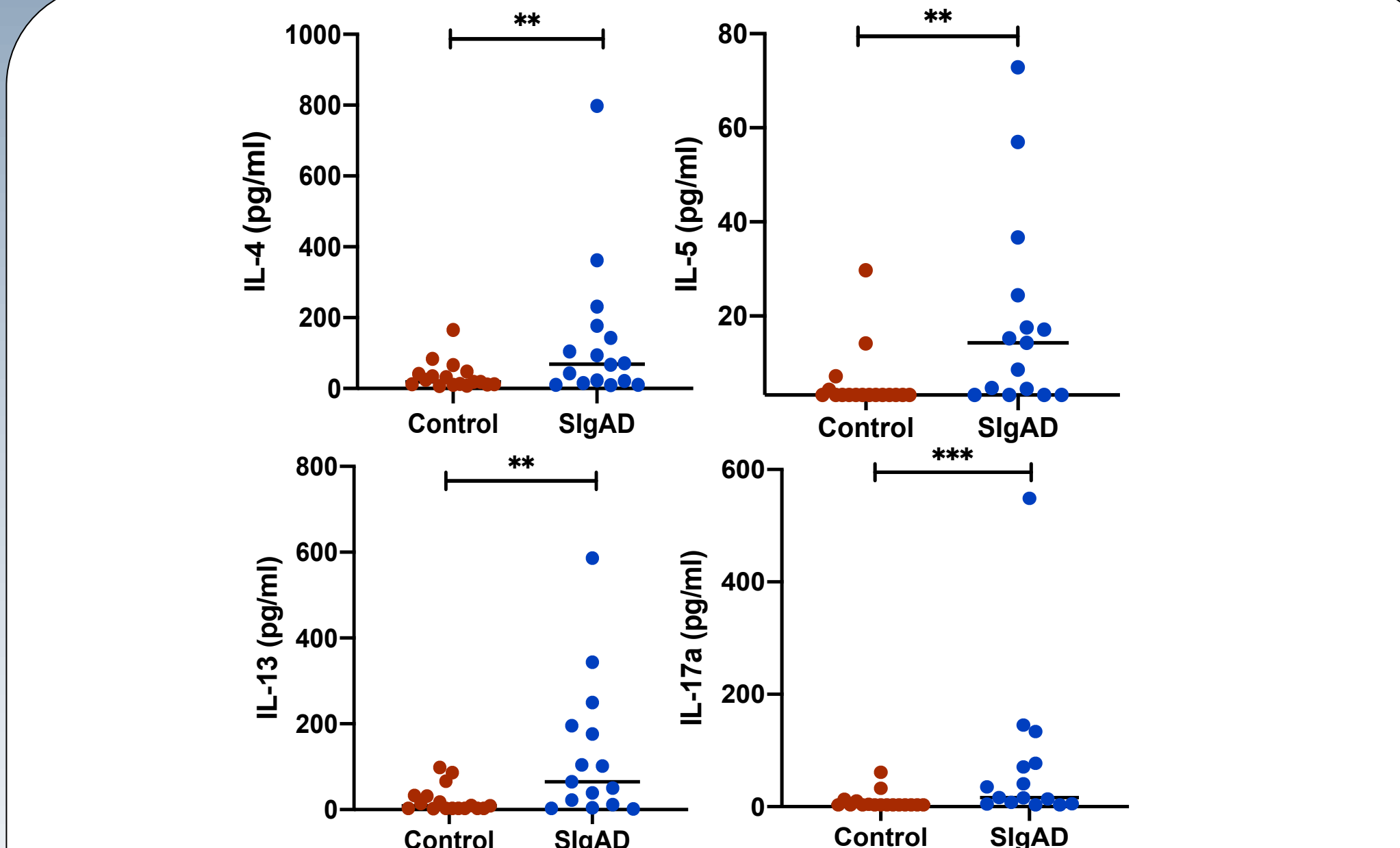


Figure 3: Elevated serum inflammatory cytokines in SIgAD. Significant differences are indicated by ** $p \leq 0.007$ and *** $p \leq 0.005$.

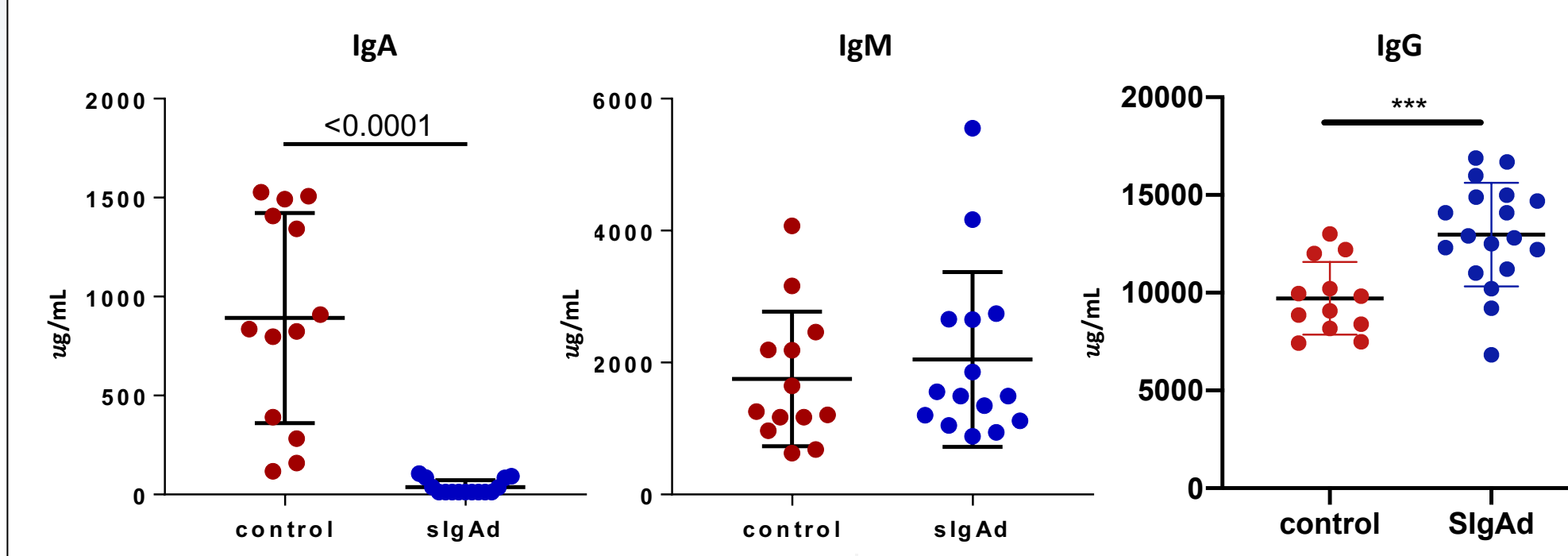


Figure 4: Elevated serum IgG in SIgAD. Significant differences are indicated by *** $p \leq 0.005$.

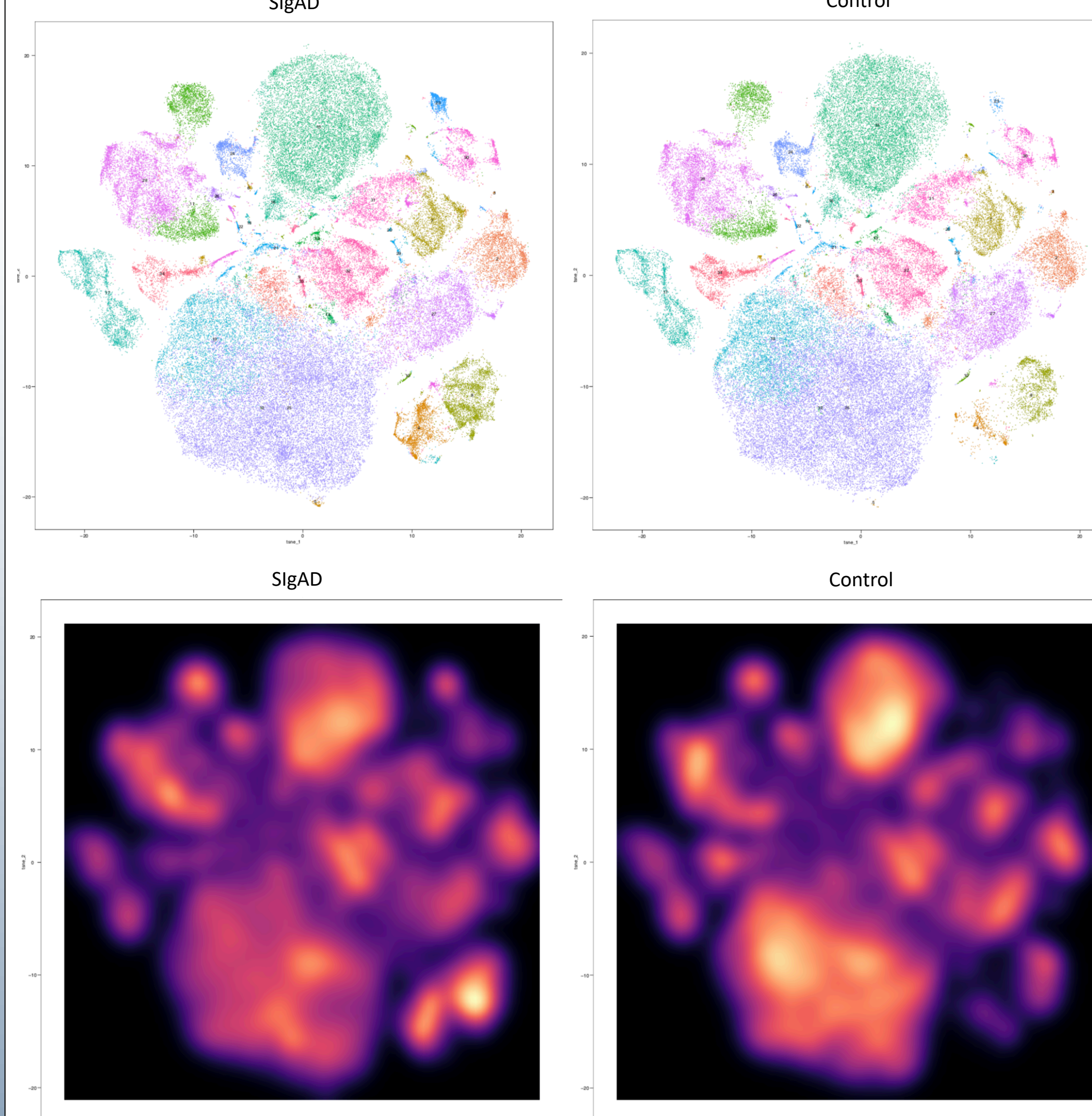


Figure 5: Deep immunoprofiling of CD3 T cells to identify potentially different cellular populations between conditions. PBMCs were labeled with metal-conjugated antibodies and detected by Mass Cytometry (CyTOF). A) Dimensionality reduction was performed using Phenograph and displayed as SIgAD on the left and control on the right. Each cell is represented by a dot and the cells contributing to a given cluster are a unique color. B) tSNE plot showing the topology of each cluster in SIgAD (left) and Controls (right). Yellow indicates an area of more cells and black or purple indicates fewer cells in each given cluster.

Microbial Flow Cytometry

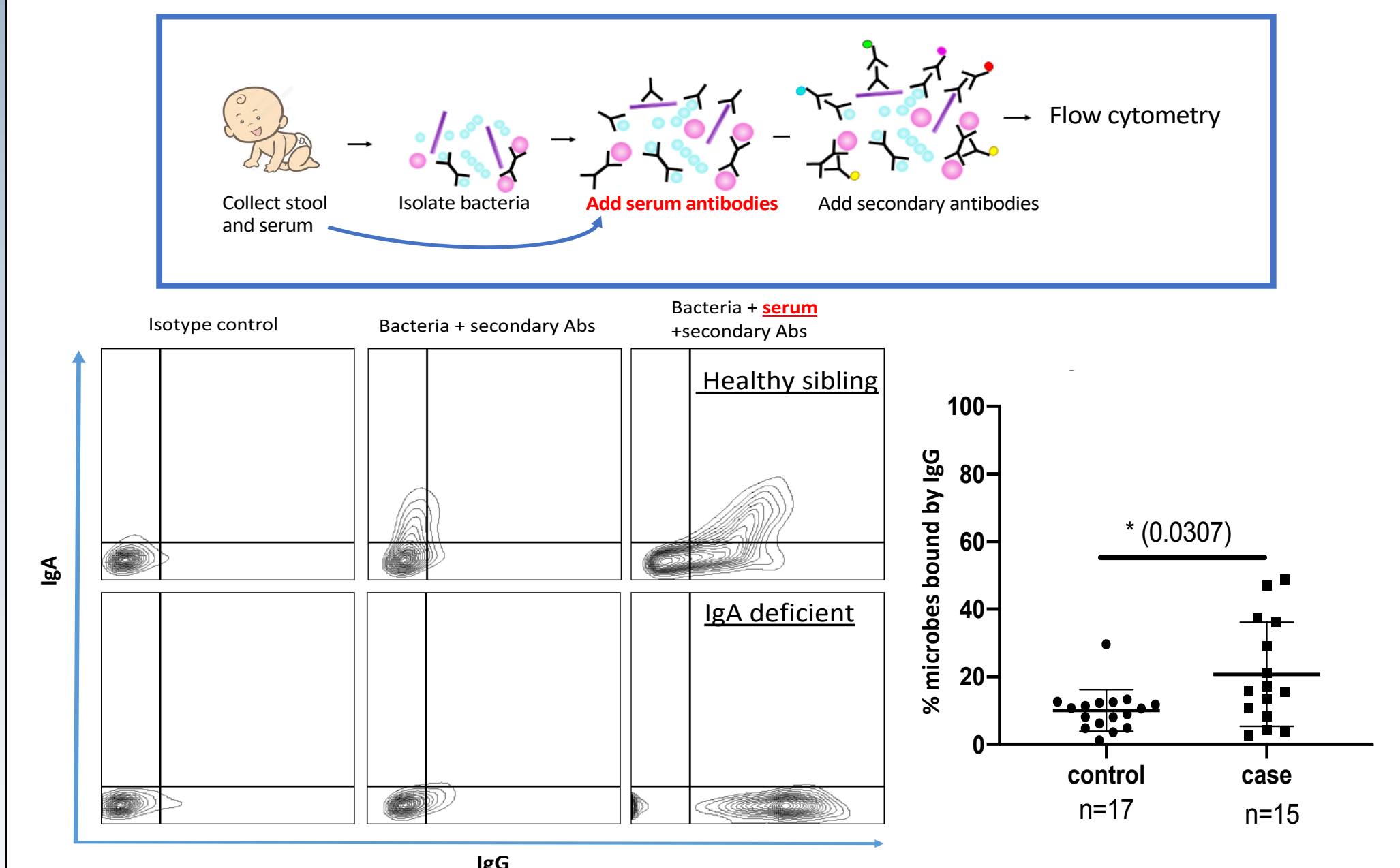


Figure 7: Microbial flow (mflow) analysis to characterize the antibody response to the microbiota.

Metagenomic Analysis

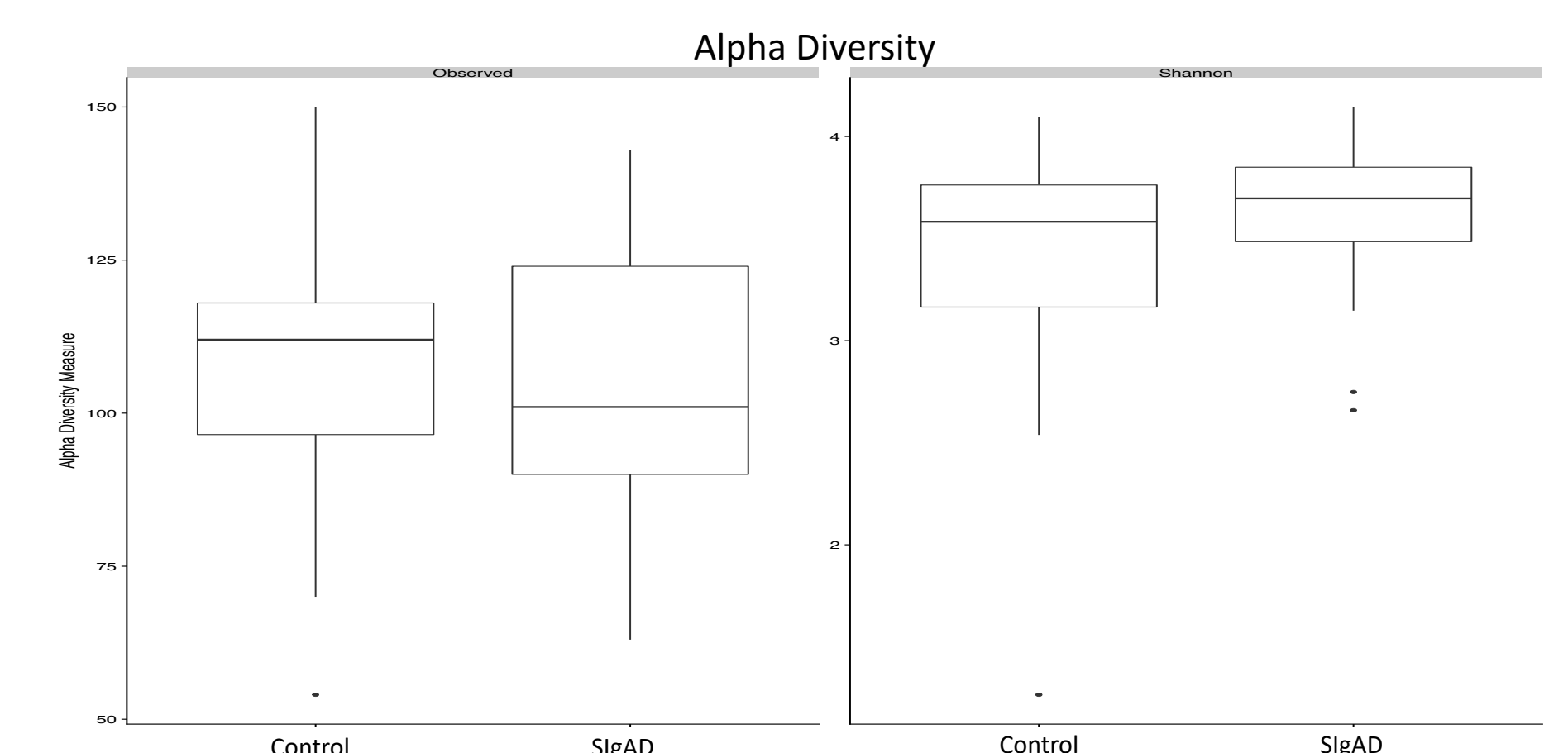


Figure 8: Fecal microbiome in SIgAD patients and sibling controls. Microbiome diversity similar among SIgAD patients and controls.

Results

- Higher frequency of fecal microbes targeted by serum IgG in SIgAD patients
- Many inflammatory cytokines including IL-4, IL-5, IL-13 and IL-17a are elevated in SIgAD
- Elevated serum IgG in SIgAD patients
- Elevated IgG+ circulating memory B cells in SIgAD
- Similar fecal microbiomes between SIgAD and control
- Sorted and sequenced IgG, IgA and IgM targeted microbes from healthy patients and IgG and IgM targeted microbes from SIgAD

Conclusions

- Developed cohort to study immune system and microbiome in SIgAD patients
 - Similar bulk fecal microbiome diversity in SIgAD and controls
 - Increased systemic inflammation observed in SIgAD patients
 - SIgAD has significant impacts on immunophenotype and access of the systemic immune response to commensal gut microbes
 - Increased commensal bound IgG+ in SIgAD patients
- These findings provide novel strategies for developing prognostic markers.