CXCR4/IgG-expressing plasma cells are associated with human gastrointestinal tissue inflammation

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Background: We previously reported abnormalities in circulating B cells in patients with chronic granulomatous disease (CGD) and those with HIV infection. Gastrointestinal complications are common to both diseases and likely involve perturbation of immune cells, including plasma cells (PCs). IgA is the most abundant immunoglobulin in the human body, with roles in protection and maintenance of intestinal homeostasis. IgA is produced primarily by PCs residing in mucosal tissues that are also thought to circulate in the blood.

Objective: We sought to characterize and compare PCs in patients with infectious (HIV) and noninfectious (CGD and Crohn disease) diseases that have been associated with intestinal inflammation.

Methods: Phenotypic and transcriptional analyses were performed on cells isolated from the blood and colon.

Results: IgA-secreting CCR10-expressing PCs predominated in the guts of healthy subjects, whereas in patients with HIV, CGD, and Crohn disease, there was a significant increase in the proportion of IgG-secreting PCs. Where intestinal inflammation was present, IgG-secreting PCs expressed reduced levels of CCR10 and increased levels of CXCR4. The intensity of CXCR4 expression correlated with the frequency of IgG-expressing PCs and the frequency of $CXCR4^+/IgG^+$ PCs was associated with the severity of intestinal inflammatory disease yet distinct from PCs and plasmablasts circulating in the blood.

Conclusions: These findings suggest that regardless of the underlying disease, the presence of $CXCR4^+/IgG^+$ PCs in the gut is a strong yet localized indicator of intestinal inflammation. Furthermore, our findings suggest that $CXCR4^+/IgG^+$ PCs might play a role in immune cell homeostasis during inflammatory processes of the gut. (J Allergy Clin Immunol 2014;133:1676-85.)

Key words: *Plasma cells, primary and infectious immunodeficiencies, gastrointestinal inflammation, inflammatory bowel disease, homing receptors*

Chronic inflammation of the gut occurs in a wide array of disease settings, including infectious diseases, such as HIV, and noninfectious diseases, such as inflammatory bowel disease (IBD) and the primary immunodeficiency chronic granulomatous disease (CGD).[1-4] In patients with HIV infection, high levels of viral replication and cellular apoptosis decimate gut-associated lymphoid tissue $CD4^+$ T cells. This process occurs particularly during the acute phase of infection and is continued to a somewhat lesser degree during the chronic phase characterized by variable levels of persistent viremia. The end results are mucosal perturbations that cause loss of intestinal integrity and leakage of inflammatory microbial products that contribute to immune activation.[1] In patients with IBD, chronic inflammation is thought to involve disturbances in the interactions between immune cells and commensal bacteria of the gut, resulting in an increased proinflammatory response and damage to the mucosal and submucosal components of the gut.[2] Crohn disease (CD) is a form of IBD involving granuloma formation in subjects who are thought to have a genetic predisposition to CD but who otherwise are immune competent.[4] In contrast, CGD is a primary host defense deficiency characterized by a neutrophil disorder involving mutations in the NADPH oxidase pathway that cause defects in microbial killing and lead to increased susceptibility to life-threatening bacterial infections.[5] Inflammatory granuloma formation is frequent in patients with CGD, although colitis, with manifestations similar to colitis in IBD, is observed in approximately 30% of patients with CGD.[3]

Intestinal homeostasis is maintained by several interconnected, although not fully delineated, factors that include interactions between microbes, physical barriers, and the host immune system.[6,7] Among the many soluble factors involved in this delicate balance is secretory IgA, which provides a mucosal environment with protection against invading pathogens and helps to maintain a healthy diversified microbiota.[8] The induction of IgA-secreting PCs, occurring primarily in the gut-associated lymphoid tissue but also in the lamina propria, is itself controlled by complex interactions involving various immune competent cells and soluble factors that favor immunoglobulin class-switching to IgA.[9] IgA is traditionally referred to as a noninflammatory antibody for empiric reasons, but this might be related to the inability of IgA to activate complement and other proinflammatory factors,[6] the ability of IgA to restrict colonization of proinflammatory bacteria,[8] and the dendritic cell and T-cell pathways that suppress proinflammatory effector responses in part through

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Abbreviations used
APC: Allophycocyanin
CD: Crohn disease
CGD: Chronic granulomatous disease
DAPI: 4′-6-Diamidino-2-phenylindole dihydrochloride
HD: Healthy donor
IBD: Inflammatory bowel disease
MFI: Mean fluorescence intensity
PC: Plasma cell
PE: Phycoerythrin
UC: Ulcerative colitis

secretion of cytokines, such as TGF-β and IL-10, that favor IgA production.[7] Furthermore, IgA deficiencies in mouse models, as well as in the setting of human diseases, have been associated with gastrointestinal inflammation.[10,11]

One of the outcomes of gastrointestinal inflammation, whether genetic or acquired or infectious or noninfectious, is the skewing of gut B cells toward production of IgG.[6,12] Recent studies have also shown that intestinal inflammation caused by CD and ulcerative colitis (UC) is associated with alterations in chemokine receptor profiles of PCs in the gut and peripheral blood.[13,14] In the present study we investigated IgA-expressing and IgG-expressing PCs and chemokine receptor expression in the colons of healthy subjects, as well as patients with diseases associated with intestinal perturbations. Our findings reveal a spectrum of profiles unique to the gut environment, with CCR10 expression on IgA^+ and dual IgA^+/IgG^+ PCs predominating in the guts of healthy subjects with no evidence of inflammation, to increasing frequencies of CXCR4-expressing high-intensity IgG^+ PCs in the guts of subjects with diseases manifested by intestinal inflammation. Collectively, these findings identify an immune cell–based marker of intestinal inflammation that is observed in a broad spectrum of infectious and noninfectious disease settings.

METHODS

Participants and procedures

Demographics and clinical data for participants are detailed in Table I. Colonoscopies were performed after informed consent in accordance with the Institutional Review Board of the National Institute of Allergy and Infectious Diseases. A gastrointestinal symptom score based on the Harvey-Bradshaw Index, a validated CD clinical activity index, was created to assess the severity of symptoms in participants. Participants without any gastrointestinal symptoms were given a gastrointestinal symptom score of 1. The scores of patients with CD were assessed at the time of the initial visit. The score was applied retrospectively to the CGD colitis group based on information from clinical charts.[15] The histopathologic evaluation was done in a blinded fashion and given scores of 1 to 4, as detailed in Table I and the Methods section in this article's Online Repository at www.jacionline.org. Healthy donors (HDs) were selected based on age, sex, and absence of gastrointestinal symptoms.

Cell isolation from colonoscopic biopsy specimens

Approximately 30 tissue samples were obtained from each subject. The tissue samples were incubated with 0.5 mg/mL collagenase (Type IV; Sigma-Aldrich, St Louis, Mo) and 25 U/μL Benzonase (Novagen, Madison, Wis) in RPMI containing 20% FBS, HEPES, and penicillin/streptomycin at 37°C with shaking for 30 minutes. After further dissociation by means of frequent pipetting, cells were washed, enumerated, and stored on ice for use in phenotypic analysis.

Flow cytometry

Multicolor flow cytometric analyses were performed on gut-derived single cells. The following conjugated mAbs were used for cell staining: allophycocyanin (APC)–H7-CD20, V450-CD3, phycoerythrin (PE)–CD32, PE-Igκ, V450-Igλ, and APC-IgG (BD Biosciences, San Jose, Calif); PerCP-Cy5.5-CD19, PE-CD138, and PE-Cy7-CD27 (eBioscience, San Diego, Calif); PE-CCR10 and PE-CXCR4 (R&D Systems, Minneapolis, Minn); APC-IgM (BioLegend, San Diego, Calif); and fluorescein isothiocyanate–IgA (Dako, Carpinteria, Calif). Unless otherwise indicated, cells were permeabilized (Permeabilizing solution 2, BD Biosciences) before addition of anti-immunoglobulin antibodies. Samples were acquired on a FACSCanto II (BD Biosciences) and analyzed with FlowJo software (TreeStar, Ashland, Ore).

Fluorescence microscopy

Gut-derived single cells were stained with antibodies against CD3, IgG (Alexa Fluor 594), IgA (Alexa Fluor 488), CD20, CD27, and CD19. Cells were sorted into 3 fractions by using a FACSAria (BD Biosciences): a $CD3^+CD20^-CD19^-$ negative control fraction, $IgG^+CD19^+CD27^{++}CD20^-CD3^-$ PCs, and the remaining $CD19^+CD27^{++}CD20^-CD3^-$ PCs. Cells collected from each fraction were cytospun, fixed with 4′-6-diamidino-2-phenylindole dihydrochloride (DAPI)–containing antifade reagent (Invitrogen Life Technologies, Carlsbad, Calif), and visualized by using a fluorescence microscope (Evos; Advance Microscopy Group, Bothell, WV).

Immunoglobulin transcriptional analysis

Total RNA was extracted from sorted cells by using the RNeasy Micro kit (Qiagen, Valencia, Calif) and reverse transcribed with qScript cDNA SuperMix (Quanta, Gaithersburg, Md). The variable regions of recombined *IGH* genes were amplified, as described previously, by using the $5'V_H3$ leader primer (AAGGTGTCCAGTGTGARGTGCAG) with either the 3′Cγ CH1 primer (GGAAGGTGTGCACGCCGCTGGTC) for *IGG* or the 3′CaCH1 primer (TGGGAAGTTTCTGGCGGTCACG) for *IGA*.[16,17] The RNA moiety (H1 RNA) of human RNase P was amplified by using TaqMan RNase P Control probes (Applied Biosystems Life Technologies, Carlsbad, Calif). Semiquantitative analysis was performed with ImageJ software (http://imagej.nih.gov/ij/).

Statistical methods

Median values were compared by using Mann-Whitney or Wilcoxon matched-pairs signed-rank tests, where appropriate, with Prism software (GraphPad Software, La Jolla, Calif). Three-way analyses were compared simultaneously by using either Kruskal-Wallis or Friedman tests, and significant results prompted appropriate pairwise comparisons. The Spearman rank method was used to test for correlation, and the Fisher exact test was used to test for differences in sex. A P value of .05 or less was considered significant.

RESULTS

Description of study groups

Colon biopsies were performed on 39 subjects divided into 4 groups based on their specific disease diagnosis (Table I). There were no significant differences in age and sex among the groups, whereas mutation and treatment profiles were varied for CGD participants (see Table E1 in this article's Online Repository at www.jacionline.org). For the HIV group, all participants were antiretroviral therapy naive; the median $CD4^+$ T-cell count was 591 cells/μL, and the geometric mean plasma viremia was 7258 HIV RNA copies/mL (see Table E1). Two measures of gut disease and inflammation were reported, one based on histopathology (read as deidentified samples by a gastroenterology pathologist) and the other based on gastrointestinal symptoms, as described in the Methods section. All HDs, HIV-infected patients, and patients with nonsymptomatic CGD had absence of gastrointestinal

TABLE I. Participant profiles and clinical observations

Participant	Age (y)	Sex	Pathology score	GI symptom score	Combined score	Other pathologic findings
1-HD	34	M	2	1	3	
2-HD	50	F	1	1	2	Reactive lymphoid aggregate; hyperplastic polyp
3-HD	43	M	1	1	2	
4-HD	40	M	1	1	2	Reactive lymphoid aggregate
5-HD	36	M	1	1	2	
6-HD	24	M	1	1	2	
7-HD	43	M	1	1	2	Tubular adenoma
8-HD	22	F	1	1	2	
9-HD	28	M	1	1	2	Tubular adenoma
10-HD	30	M	1	1	2	Reactive lymphoid aggregate
11-HD	27	M	1	1	2	Lymphoid aggregate
1-HIV	21	M	1	1	2	Reactive lymphoid aggregates; tubulovillous adenoma
2-HIV	55	M	1	1	2	
3-HIV	36	M	1	1	2	Spirochetosis without inflammatory changes
4-HIV	30	M	1	1	2	Reactive lymphoid aggregate; pigmented macrophages
5-HIV	45	M	1	1	2	
6-HIV	48	M	1	1	2	
7-HIV	30	M	1	1	2	Reactive lymphoid aggregate
8-HIV	39	M	1	1	2	Lymphoid aggregate
9-HIV	52	M	1	1	2	Reactive lymphoid aggregate
10-HIV-A	23	M	2	1	3	Reactive lymphoid aggregate; poorly formed granuloma
1-CGD	29	M	4	3	7	
2-CGD	43	M	1	3	4	Pigmented macrophages; granulomas
3-CGD	37	F	4	4	8	
4-CGD	24	M	4	3	7	Pigmented macrophages; granulomas
5-CGD	22	M	4	3	7	
6-CGD	23	M	4	3	7	
7-CGD	20	M	4	3	7	Inflammatory pseudopolyp
8-CGD-N	19	M	1	1	2	Pigmented macrophages
9-CGD-N	29	M	1	1	2	Reactive lymphoid aggregate; pigmented macrophages
10-CGD-N	41	M	1	1	2	Tubular adenomas
11-CGD-N-A	28	M	1	1	2	
1-CD	25	F	4	3	7	
2-CD	47	M	4	3	7	Inflammatory pseudopolyp
3-CD	22	F	3	1	4	Granulomas
4-CD	23	F	3	1	4	Prominent eosinophilia
5-CD	25	M	4	2	6	Reactive lymphoid aggregate
6-CD	55	M	4	4	8	Reactive lymphoid aggregate
7-CD	30	M	1	1	2	

Pathology score: 1 = normal colon with no increase in inflammatory infiltrates; 2 = mild inflammation with mild increase in density of inflammatory cells and patchy involvement up to 25% of tissue; 3 = moderate inflammation with moderate increase in density of inflammatory cells and involvement of greater than 25% to 50% of tissue; and 4 = severe inflammation with marked increase in density of inflammatory cells and involvement of greater than 50% of tissue. Gastrointestinal symptom score based on the Harvey-Bradshaw Index (HBI): 1 = no symptoms/remission (HBI score, <5); 2 = mild disease (HBI score, 5-7); 3 = moderate disease (HBI score, 8-16); and 4 = severe disease (HBI score, >16). *-A*, IgA deficient; *GI*, gastrointestinal; *-N*, asymptomatic.

symptoms (score = 1, Table I). In the HD and HIV groups and the CGD with no gastrointestinal symptoms subgroup, all had a normal pathology score, with the exception of 1 participant in each of the HD and HIV groups, both of whom had mild acute inflammation. Patients with CGD with colitis had gastrointestinal symptom scores of 3 to 4, and 6 of the 7 patients had pathology scores of 4 (Table I), all of which were mixed (acute and chronic) inflammation. Six of 7 patients with CD had pathology scores of 3 or 4, all of which were mixed inflammation, and variable gastrointestinal symptom scores.

Analysis of gut PCs

Single-cell preparations were obtained by means of enzymatic digestion and limited mechanical manipulation of the gut tissue samples. The PCs were identified within the lymphocyte gate as $CD19^+$ cells that expressed high levels of CD27 in the absence of CD20 and CD3 (Fig 1, *A*). Similar results were obtained with an alternative strategy based on CD138 expression,[18] although this strategy was more difficult to apply to all samples because CD138 was not uniformly expressed on PCs nor was its expression restricted to PCs (data not shown), which is consistent with observations from previous studies on gut samples from HIV-infected patients.[19] Another plasma cell (PC) marker, CD38, also presents difficulties both in the gut[20] and in certain disease settings.[21] Furthermore, the gating strategy was verified by determining the immunoglobulin isotype distribution among the PCs. As shown in Fig 1, *B*, for a representative of the HD group, the sum of IgA-, IgG-, and IgM/IgD-secreting cells was close to 100% (IgE is rare in the gut), with a predominance of IgA.

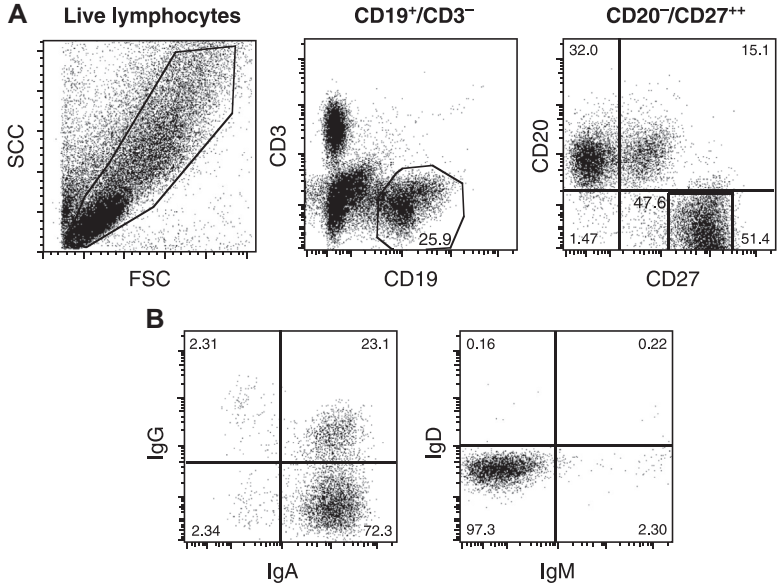


FIG 1. Identification of gut-derived PCs by using flow cytometry. **A,** PCs of a representative HD were defined as $CD19^{+}CD3^{-}CD20^{-}CD27^{++}$ within the lymphocyte gate. Each plot title identifies the gated cell population, and *numbers* indicate cell percentages in each quadrant or gate. *FSC*, Forward scatter; *SSC*, side scatter. **B,** The immunoglobulin isotype profile was determined with 2 separate stains: one for IgA and IgG and the other for IgM and IgD.

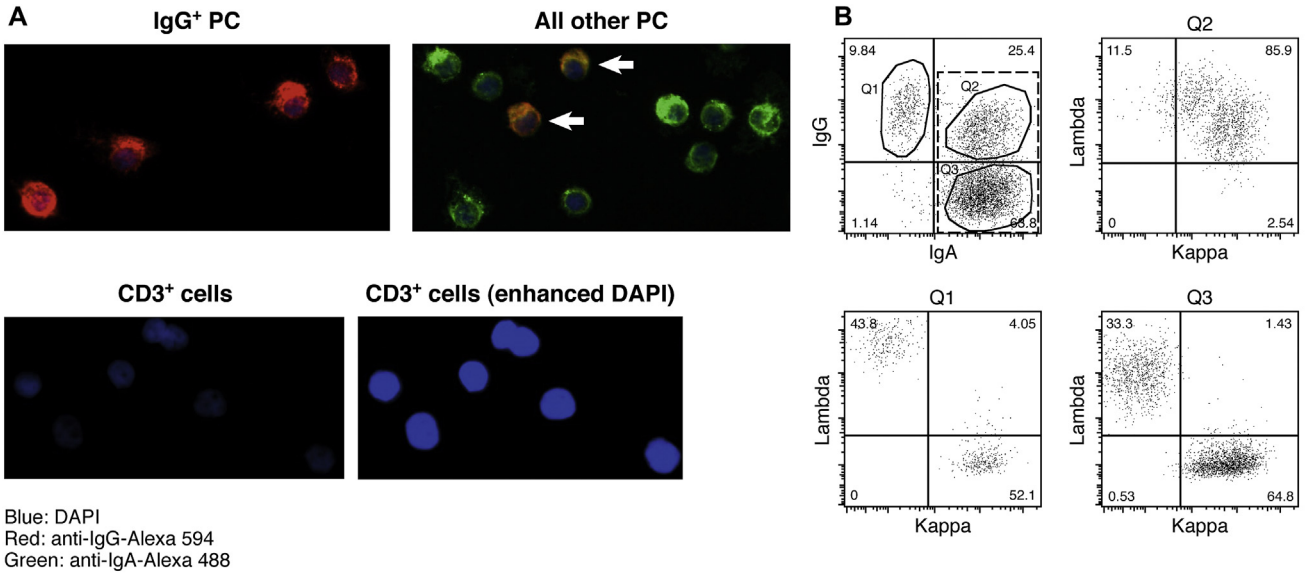
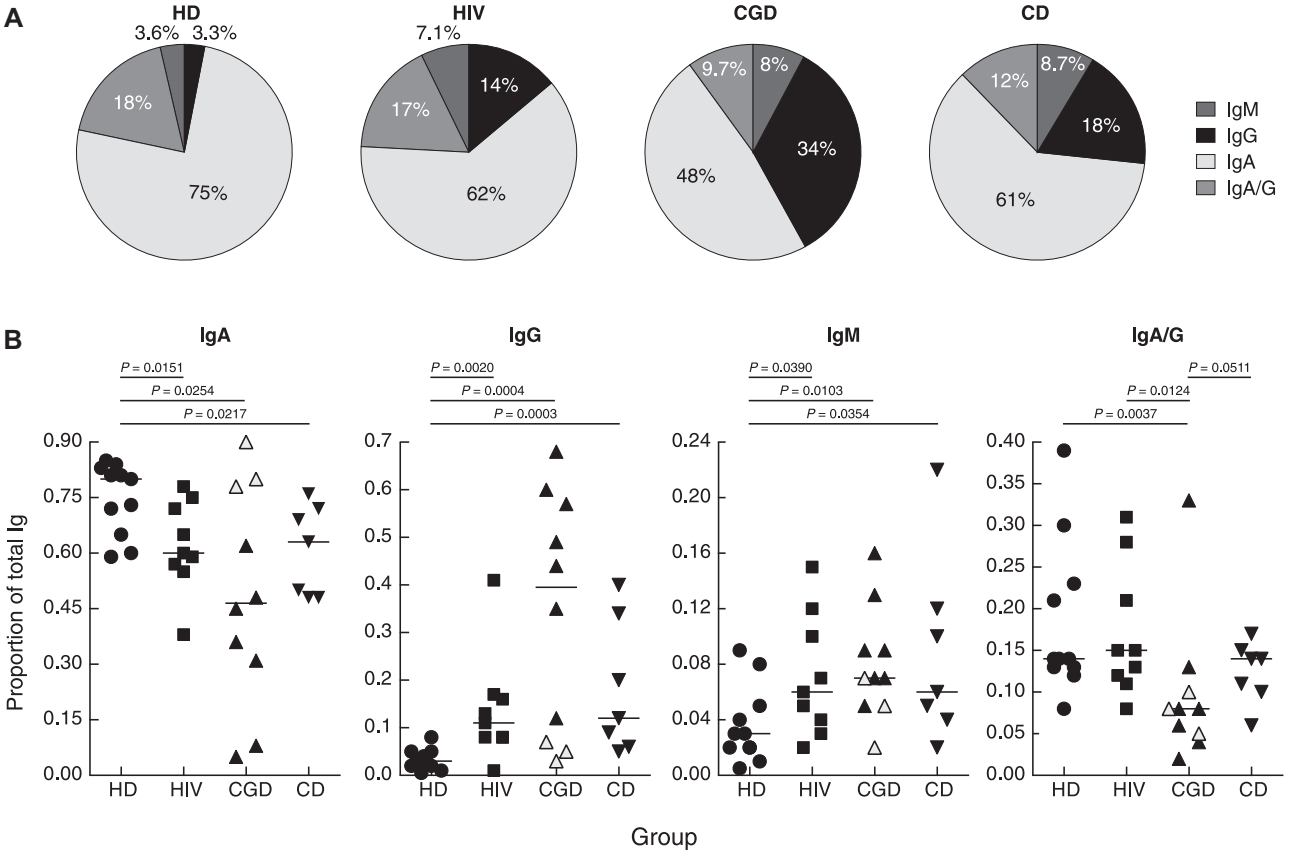
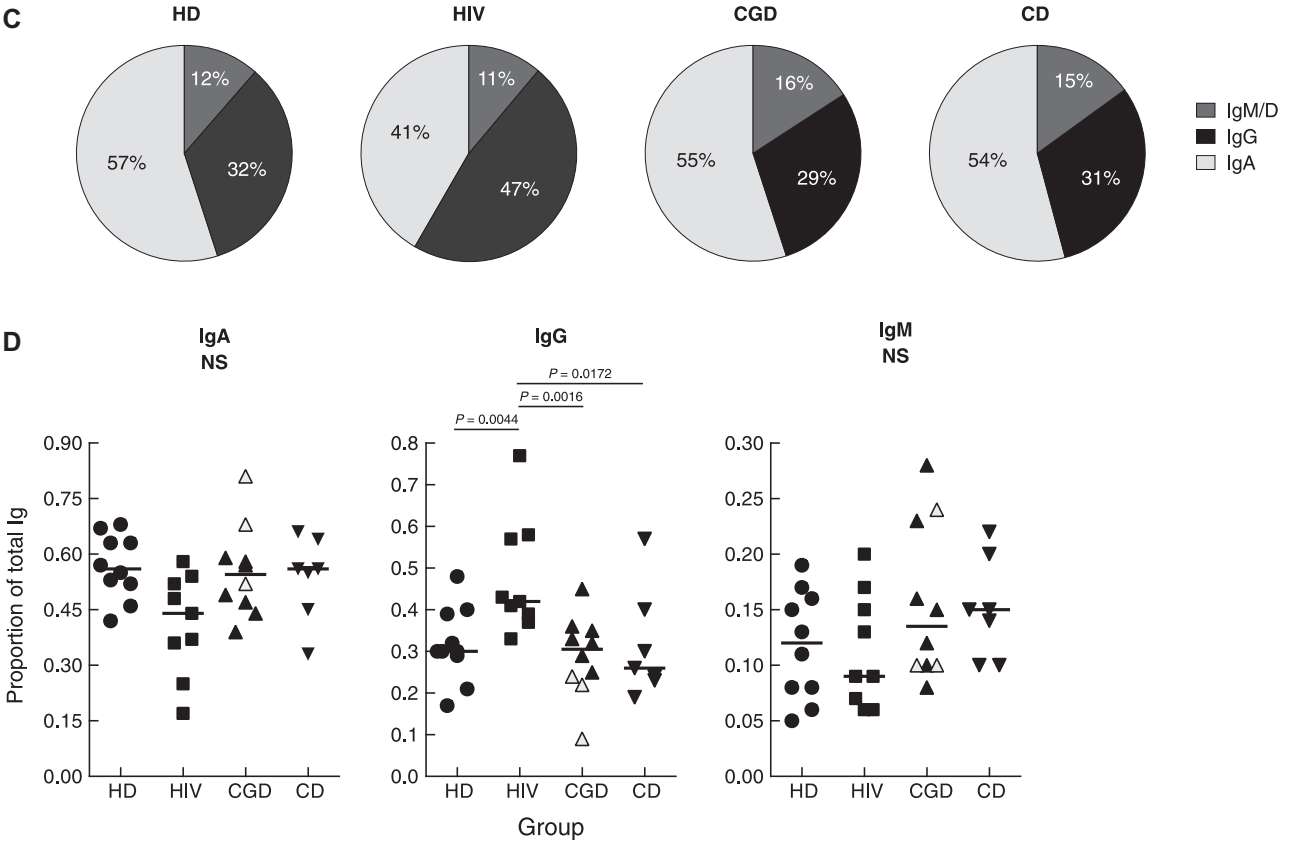


FIG 2. Characterization of dual IgA/IgG-expressing PCs in the gut. **A,** Microscopy of sorted PCs stained for IgA (Alexa Fluor 488, *green*) and IgG (Alexa Fluor 594, *red*). *Arrows* show dual positivity. **B,** Expression of immunoglobulin light chains for single IgG- or IgA-expressing and dual IgG/IgA-expressing PCs. *Numbers* indicate cell percentages in each quadrant. The *dotted box* identifies cells sorted for transcriptional analysis (see Fig E1).

In addition to IgA, there were minor fractions present comprised of PCs expressing only IgG and IgM/IgD-expressing PCs. Unexpectedly, we also observed a population of PCs that expressed both IgA and IgG. These dual IgA/IgG-expressing PCs were not observed among the other B-cell populations in the gut and did not appear to be doublets (data not shown), prompting us to further investigate the nature of these PCs using microscopy, flow cytometry, and transcriptional analysis (Fig 2 and see Fig E1 in this article's Online Repository at www.jacionline.org). A detailed description of the strategies used is presented in the Results section in this article's Online Repository at www.jacionline.org. As shown in Fig 2, *A*, sorted PCs expressing only IgG were characterized by strong single-color fluorescence in the cytoplasm, whereas the remaining PCs not expressing IgG only were composed of both single IgA-fluorescing and double IgA/IgG-fluorescing cells. The observation that both IgA and IgG were expressed on or in the same cell was confirmed by using immunoglobulin light chain distribution, as shown in Fig 2, *B*.


A
HD
3.6%
3.3%
18%
75%
HIV
7.1%
14%
17%
62%
CGD
9.7%
8%
34%
48%
CD
12%
8.7%
18%
61%
IgM
IgG
IgA
IgA/G
B
IgA
IgG
IgM
IgA/G
P = 0.0151
P = 0.0254
P = 0.0217
P = 0.0020
P = 0.0004
P = 0.0003
P = 0.0390
P = 0.0103
P = 0.0354
P = 0.0511
P = 0.0124
P = 0.0037
Proportion of total Ig
HD
HIV
CGD
CD
Group


C
HD
12%
57%
32%
HIV
11%
41%
47%
CGD
16%
55%
29%
CD
15%
54%
31%
IgM/D
IgG
IgA
D
IgA
NS
IgG
IgM
NS
P = 0.0172
P = 0.0016
P = 0.0044
Proportion of total Ig
HD
HIV
CGD
CD
Group

Whereas the patterns of expression for single IgG- and IgA-expressing PCs were mutually exclusive, as expected, dual expression of the Igλ and Igκ light chains was observed among the dual IgA/IgG-expressing PCs. The most likely explanation for B cells that are positive for 2 different immunoglobulin heavy and light chains (excluding IgD and IgM) is that one is produced by the cells whereas the other is in soluble form bound to a receptor expressed on the cell surface. On the basis of analyses presented in the Results section in this article's Online Repository, the most likely, although not conclusive, explanation is that the dual IgA/IgG-expressing PCs are primarily secreting IgG and binding IgA.

Increased frequency of IgG-expressing PCs in the guts of patients with symptomatic CGD and HIV-infected patients

Next we characterized the gut-derived PCs of subjects in Table I. As shown by the pie charts in Fig 3, *A*, and group analyses in Fig 3, *B*, PCs expressed predominantly IgA (alone) across all groups, although frequencies were highest for the HD group. For PCs expressing only IgG, the highest frequency was 34% for the CGD group (46% excluding nonsymptomatic patients), although frequencies were also significantly higher between the 2 other disease groups and the HD group (Fig 3, *B*). The pattern for IgM-expressing PCs was similar to that of PCs expressing IgG only, whereas for dual IgA/IgG-expressing PCs, frequencies were significantly lower for the CGD group when compared with the 3 other groups (Fig 3, *B*). Of note, when the 3 patients with CGD without colitis were excluded from the analyses, differences for IgA- and IgG-expressing PCs became significant when the CGD group was compared with the 2 other patient groups (see Fig E2, *A*, in this article's Online Repository at www.jacionline.org). Previous findings have suggested a link between PCs in the gut and terminally differentiated B cells (either PCs or plasmablasts) circulating in the peripheral blood.[14,22] Accordingly, we determined the immunoglobulin isotype distribution of PCs/plasmablasts of subjects in Table I. In contrast to the profiles in the gut, the only increased frequencies of circulating IgG^+ PCs/plasmablasts were those of the HIV-infected patients (Fig 3, *C* [pie charts] and *D* [group analyses]), which is consistent with previous findings.[23] Taken together, these data indicate that the increased frequency of PCs expressing only IgG in the gut was highest in patients with symptomatic CGD and restricted to this compartment, whereas in HIV-infected patients and patients with CD, numbers of PCs expressing IgG only were more modestly increased in the gut compared with those see in HDs, and in the case of patients with HIV, such an increase was also reflected in the peripheral blood.

Distinct expression of homing receptors on IgG-, IgA-, and dual IgA/IgG-expressing PCs

Given the association of intestinal PCs expressing IgG only with colitis/inflammation and distinctions between PCs expressing only IgG and dual IgG/IgA-expressing PCs, we sought to further characterize the phenotype of gut PCs. CCR10 is the principal homing receptor expressed on IgA-expressing PCs in the colons of healthy subjects, whereas CCR9 is more restricted to the small bowel.[24] Consistent with this pattern and recent findings,[13] CCR10 was expressed at high levels on both types of IgA-expressing PCs in the colons of HDs (Fig 4, *A* and *B*). Similar profiles were observed for the patient groups (Fig 4, *A* and *B*), although the mean fluorescence intensity (MFI) of CCR10 on both IgA-expressing PCs of patients with CGD was significantly lower compared with that of the HD and HIV groups (Fig 4, *B* and *C*). Of note, significant differences in Fig 4, *C*, were stronger when patients with nonsymptomatic CGD were excluded from the CGD group. Finally, levels of CCR10 expression on PCs expressing only IgG were approximately 10-fold lower than on IgA-expressing PCs, and there was no significant difference in the MFI between the groups (Fig 4, *C*).

Increased CXCR4 expression has been associated with intestinal inflammation[25] and, in particular, on gut IgG-expressing PCs of patients with CD and UC.[13] Accordingly, we investigated the expression of CXCR4 on gut PCs. CXCR4 was expressed at relatively low levels on PCs expressing only IgA and dual IgA/IgG-expressing PCs, although levels were significantly higher for the CGD group when compared with all other groups (Fig 5, *A* and *B*, for representative profiles and Fig 5, *C*, for group comparisons). In contrast, PCs expressing only IgG of patients with CGD and those with CD expressed levels of CXCR4 that were relatively high and significantly higher when compared with the other 2 groups (Fig 5). Of note, differences were even higher when patients with nonsymptomatic CGD were excluded from the CGD group and significantly higher when compared with the CD group (see Fig E2, *B*). Furthermore, we studied 2 IgA-deficient subjects with absent or mild gastrointestinal symptoms (Table I); CXCR4 levels were not increased on their PCs (Fig 5, *C*), which were primarily of the IgG isotype (data not shown).

We then considered differences in the expression of each homing receptor among the 3 PC populations. For CCR10 with all groups combined, the MFI was significantly higher on PCs expressing only IgA than on both dual IgA/IgG-expressing PCs and PCs expressing only IgG and, in turn, significantly higher on dual IgA/IgG-expressing PCs than on PCs expressing only IgG (see Fig E3, *A*, in this article's Online Repository at www.jacionline.org). The differences remained significant when groups were analyzed separately. Conversely, for CXCR4, with all groups combined, the MFI was significantly higher on PCs expressing only IgG than on both dual IgA/IgG-expressing PCs and PCs expressing only IgA and, in turn, significantly higher on dual IgA/IgG-expressing PCs than on PCs expressing only IgA (see Fig E3, *B*). Collectively, these data show that CCR10 is strongly associated with IgA expression, whereas CXCR4 is associated with increased IgG expression, and dual IgA/IgG-expressing PCs have intermediate homing receptor expression profiles.

FIG 3. Immunoglobulin isotype distribution among gut-derived and peripheral blood PCs/plasmablasts. **A** and **B,** Immunoglobulin isotype mean distribution (Fig 3, *A*) and median comparisons of gut-derived PCs (Fig 3, *B*) from the groups described in Table I. *Light gray triangles*, Patients with CGD with no gastrointestinal symptoms. **C** and **D,** Analyses were performed on peripheral blood PCs/plasmablasts from participants in Table I. *Horizontal bars* represent medians. *NS*, Not significant.

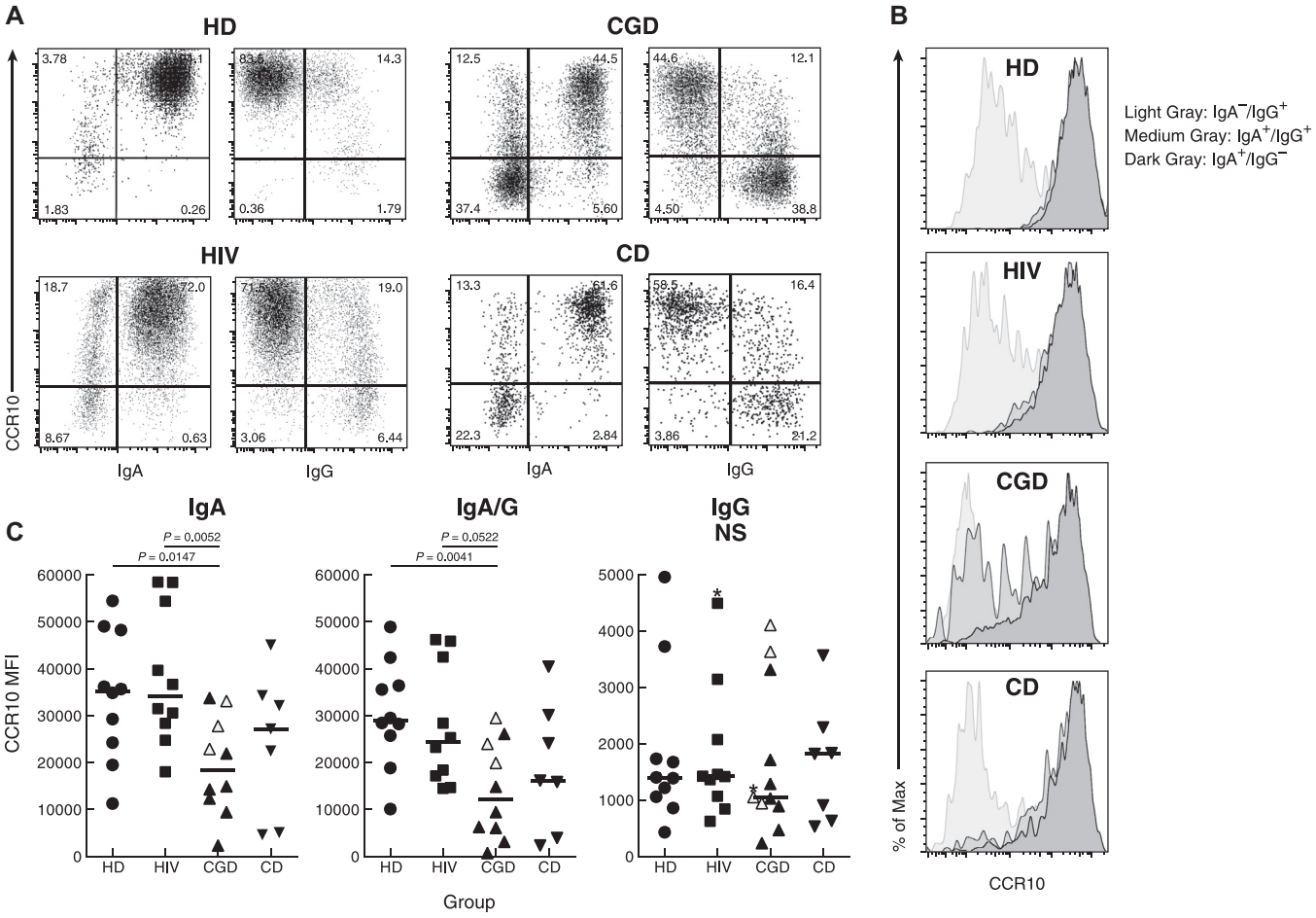


FIG 4. Expression of CCR10 on gut-derived PCs. **A,** Representative plots of CCR10 expression by IgA and IgG are shown by group. *Numbers* indicate cell percentages in each quadrant. **B** and **C,** Histograms from plots in Fig 4, *A* (Fig 4, *B*), and group comparisons of CCR10 expression (Fig 4, *C*) were obtained by gating on single IgG- or IgA-expressing and dual IgG/IgA-expressing PCs. *Horizontal bars* represent medians and *asterisks* and *light gray triangles* identify IgA-deficient subjects and patients with CGD with no gastrointestinal symptoms from Table I, respectively. *NS*, Not significant.

Finally, we assessed the association between CXCR4-expressing PCs expressing only IgG in the gut and various parameters for all groups combined. There was a direct and highly significant correlation between the fraction of gut PCs expressing only IgG and the MFI of CXCR4 on these cells (Fig 6, *A*). The groups also clustered together, with patients with symptomatic CGD at one end of the spectrum, HDs at the other end, and the nonsymptomatic CGD, CD, and HIV groups in between. There was also a strong association between the combined scores from the pathology and gastrointestinal indices and the frequency of $CXCR4^+$ PCs expressing only IgG among all gut-derived PCs (Fig 6, *B*). Taken together, these data suggest that the analysis of immunoglobulin isotype distribution and CXCR4 expression are strong indicators of active colonic inflammation, irrespective of the underlying disease.

DISCUSSION

In this study we investigated gut PCs across a spectrum of diseases involving varying degrees of inflammation and clinical intestinal manifestations. First, and in agreement with an extensive literature,[6,9,11,26] PCs expressing only IgA were the predominant population in the gut of HDs. Second, although not widely reported or appreciated, a substantial fraction of PCs in healthy gut tissue were dual IgA/IgG-expressing PCs. Both PCs expressing only IgA and dual IgA/IgG-expressing PCs expressed high levels of CCR10 and thus are likely to have followed similar pathways from inductive to effector sites. Third, PCs expressing only IgG constituted a very small fraction of PCs in the guts of HDs; however, this fraction increased significantly with increasing inflammation and was accompanied by increased expression of CXCR4 in patients with colitis. The highest frequencies of $CXCR4^+$ PCs expressing only IgG in the gut were observed in patients with active/chronic colitis (CGD and CD), with disease scores that were increased for both clinical and tissue pathology. Interestingly, in patients with nonsymptomatic CGD, PC profiles were very similar to those observed in HDs. In HIV-infected patients, in whom tissue examination and clinical evaluation revealed little evidence of active inflammatory gut disease, cellular profiles showed increased frequencies of PCs expressing only IgG without an increased intensity of CXCR4. These data are consistent with previous findings in gut cells of HIV-viremic patients,[12,27-29] although the effect of viremia *per se* and stage of disease on CXCR4-expressing and/or

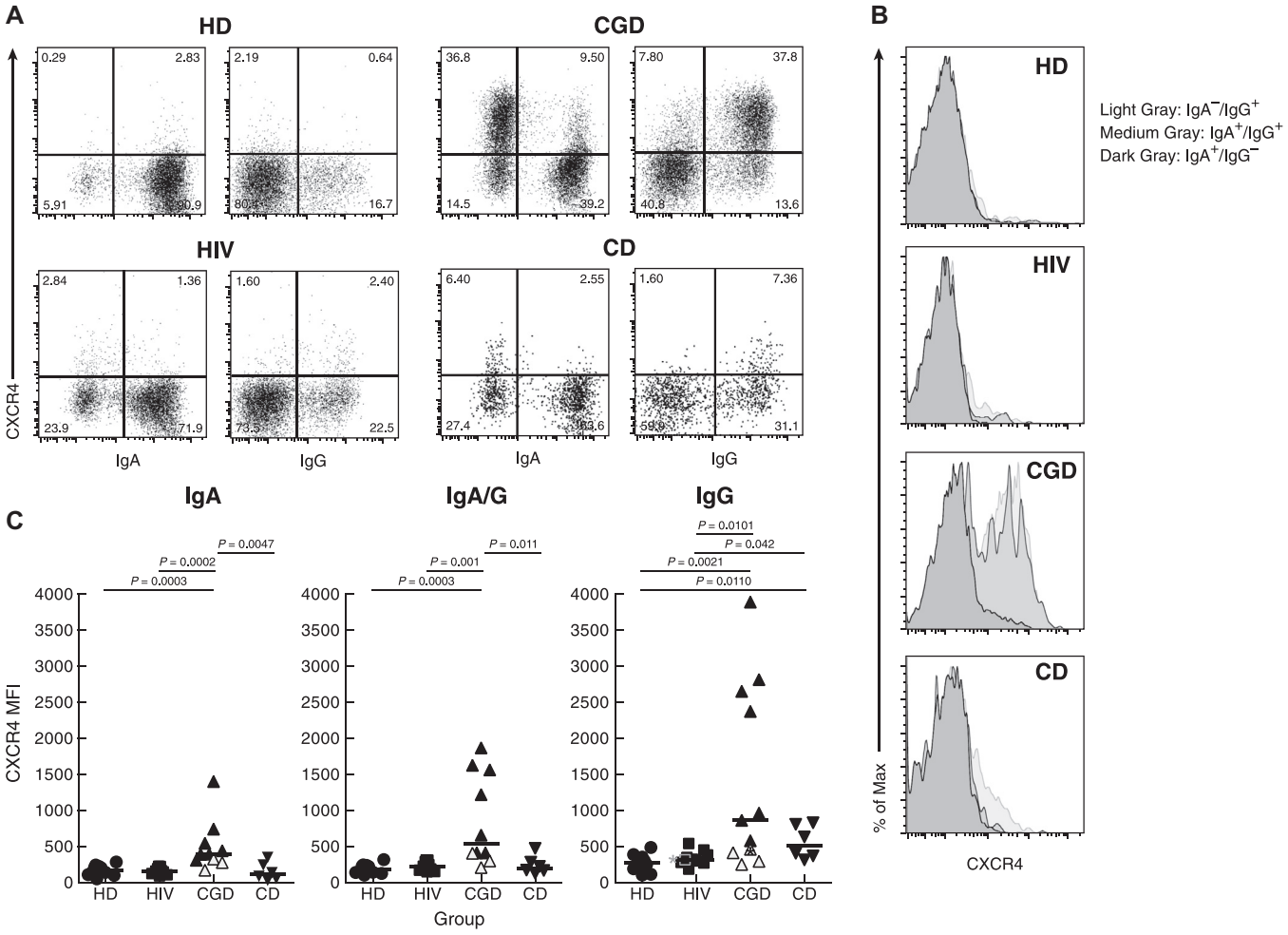


FIG 5. A-C, Expression of CXCR4 on gut-derived PCs. Analyses for CXCR4 were identical to those presented for CCR10 in Fig 4.

IgG-expressing PCs remains to be determined. Finally, patients with CD had an intermediate, although somewhat heterogeneous, profile both with regard to clinical and tissue manifestations of inflammatory disease and PC phenotypes. Collectively, these findings suggest that CCR10-expressing PCs expressing only IgA and dual IgA/IgG-expressing PCs reflect a healthy gut environment, whereas CXCR4-expressing PCs expressing only IgG are associated with both inflamed tissues and clinically active intestinal disease, which is consistent with recent findings in patients with UC.[13,14] One notable caveat to the current study is the paucity of female subjects investigated.

The importance of IgA and IgA-producing cells in maintaining intestinal homeostasis is widely reported, although its non-inflammatory properties are not as widely accepted or understood.[6,9,11,26] In human subjects IgA deficiencies have been associated with intestinal inflammatory conditions, although this is not an inevitable finding, and there appears to be a wide spectrum of manifestations.[30] Of note, there were 2 participants without active intestinal disease (1 patient with CGD and 1 HIV-infected subject, Table I) who were found to have an IgA deficiency based on serum analyses. Both had high frequencies of IgG-expressing PCs in their guts; however, neither had CXCR4 expression on their IgG-expressing PCs that was greater than levels observed for HDs, whereas CCR10 expression was either at or above average for their respective groups. Although more IgA-deficient subjects need to be investigated, these observations help solidify the notion that it is not the mere presence of IgG-expressing PCs in the gut that is associated with inflammatory conditions but rather those PCs expressing only IgG that also express CXCR4.

The gut PCs expressing only IgA and dual IgA/IgG-expressing gut PCs had phenotypes that were clearly distinct from those of the PCs expressing only IgG. Several factors in mucosal tissues, including both T cell–dependent and T cell–independent factors, have been shown to favor B-cell immunoglobulin class-switching to IgA.[9] However, few studies have addressed class-switching to IgG in the healthy intestinal mucosa. It is tempting to speculate that PCs expressing only IgA and dual IgA/IgG-expressing PCs are signatures of a healthy intestinal mucosa. In this regard IgA is the predominant isotype of PCs/plasmablasts that circulate, possibly from mucosal tissues,[22] in the blood of healthy subjects, and one recent study demonstrated increased frequencies of IgG/CXCR4/CXCR3-expressing PCs in the blood of patients with UC that mirrored changes in the gut.[14] However, alterations of plasmablasts/PCs in the blood did not extend to CD,[14] which is consistent with our findings; no clear associations were found for the 3 diseases studied between PCs in the gut and plasmablasts/PCs circulating in the blood.

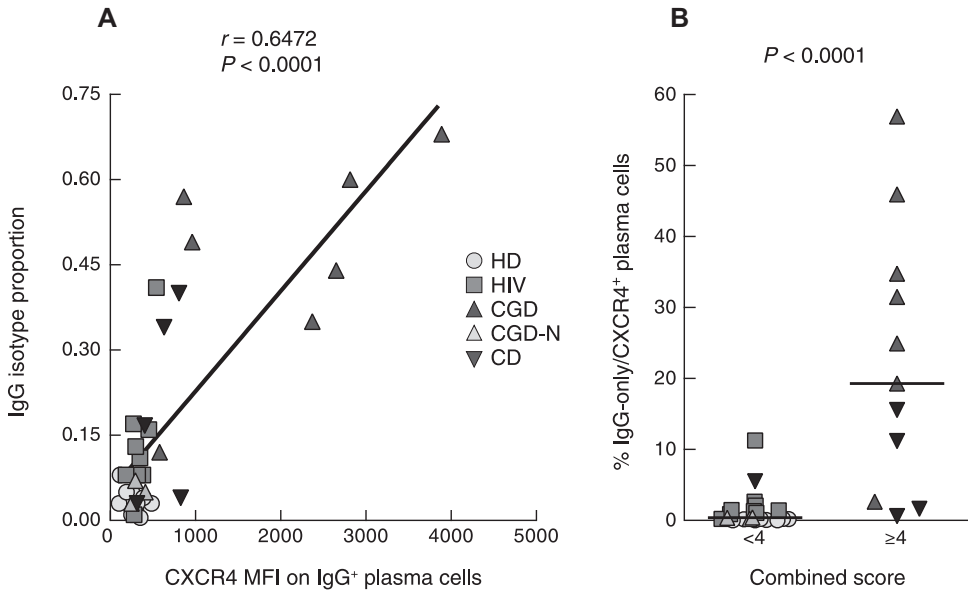


FIG 6. Correlation and association between CXCR4, IgG, and combined scores. Comparisons were performed between fraction of gut-derived PCs expressing only IgG and the intensity (MFI) of CXCR4 expression on PCs expressing only IgG **(A)** and the percentage of $CXCR4^+$ PCs expressing only IgG and the combined pathology and gastrointestinal symptom scores described in Table I **(B)**.

In summary, we have described 3 distinct populations of PCs in healthy and diseased intestinal tissues. In healthy subjects approximately 75% of PCs in the gut express only IgA, and another 18% express both IgA and IgG, the majority of which are likely producing IgG and binding IgA. In disease settings in which gastrointestinal perturbations are subclinical, such as in HIV-infected patients, there is an increase in frequencies of PCs expressing only IgG in the gut, but these cells do not express increased levels of CXCR4. Finally, in disease settings in which there is both tissue inflammation and symptomatic disease, such as in a subgroup of patients with CGD and patients with CD, there is an increase in frequencies of PCs expressing only IgG that is accompanied by increased expression of CXCR4. These findings suggest that IgG and CXCR4 expression by PCs in the gut can serve as an indicator of intestinal inflammation across a broad spectrum of diseases that cause intestinal disturbances.

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Key messages

- **There are at least 3 distinct populations of IgA- and IgG-expressing PCs in the guts of healthy subjects that are altered in patients with diseases associated with varying degrees of intestinal perturbations.**
- **The presence of CXCR4/IgG-expressing PCs is associated with gastrointestinal disease and tissue inflammation, and these PCs are observed in several disease settings.**
- **PCs in the gut are distinct from those circulating in the peripheral blood, at least in certain disease settings.**

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METHODS

Criteria used to establish pathology scores in Table I

Histologic examination showed tissues that ranged from normal to chronic (architectural distortion, intraepithelial lymphocytosis, expansion of lamina propria by lymphoplasmacytic infiltrate, and/or basal cell plasmacytosis), acute (neutrophils in the lamina propria, cryptitis, and/or crypt abscess), or mixed (acute and chronic) inflammation. Pigmented macrophages, granulomas, eosinophilia, and lymphoid aggregates were listed as additional findings and not included in the scoring of chronic/acute inflammation. Endoscopic examination was performed on the entire colon, and biopsy specimens were taken at obviously inflamed sites. Where there were multiple inflamed sites, pathology scoring was based on the most affected sample.

RESULTS

Details of phenotypic and transcriptional analyses of dual IgA/IgG-expressing PCs in Fig 2

To formally exclude the presence of doublets in the samples, we performed fluorescence microscopy using anti-immunoglobulin conjugated with fluorochromes that were not only optimal for this approach but also compatible with cell sorting. For microscopy, we sorted the stained gut cells into 3 fractions: PCs that expressed IgG alone, all other PCs, and CD3-expressing cells as a non–B-cell negative control. Of note, although the 2 antibodies used to stain for IgG and IgA shown in Fig 1, *B*, were adequate for flow cytometry, these antibodies were not suitable for microscopy. Conversely, the 2 antibodies that worked best for microscopy, namely anti-IgA and anti-IgG conjugated to Alexa Fluor 488 and Alexa Fluor 594, respectively, were only partially compatible in flow cytometry, explaining why only those PCs expressing IgG alone could be clearly distinguished and sorted from the other PCs. $CD3^+$ sorted cells served as a negative control; the blue fluorescence of DAPI was the only color visible among these cells.

The presence of dual-fluorescing cells in Fig 2, *A*, strongly suggests that both IgA and IgG can be detected on or in the same cell. To confirm this observation, we also assessed the immunoglobulin light chain distribution for single IgA- and IgG-expressing and dual IgA/IgG-expressing PCs by using flow cytometry. Although single IgG- and IgA-expressing PCs expressed λ or κ light chains, both light chains were detected among dual IgA/IgG-expressing PCs (Fig 2, *B*). The absence of single-immunoglobulin light chain expression within these dual IgA/IgG-expressing PCs strongly argues against doublets or allelic inclusion on the immunoglobulin heavy chain (at least a fraction would have been single-immunoglobulin light chain positive in either scenario) and argues strongly for binding of soluble antibodies that contain either immunoglobulin light chain but that collectively would result in the mixed pattern shown in Fig 2, *B*. To evaluate potential cell-surface receptors that might be binding soluble immunoglobulin, we had to consider that IgG is not expressed on the surfaces of IgG-producing PCs and can only be detected by means of intracellular staining, whereas IgA can be detected on the cell surfaces of IgA-producing PCs, although the intensity of expression is increased when cells are permeabilized (Fig E1, *A*). Given that the intensity of IgG expression tended to be higher on IgG-only PCs when compared with the IgG expressed by dual IgA/IgG-expressing PCs (Figs 1 and E1, *A*), we first surmised that the latter represented IgA-secreting PCs that had IgG bound to their surface. This was not the case; IgG staining was detected only when the cells were permeabilized, and this was observed despite cell-surface expression of CD32, the only known IgG FcR for B cells (Fig E1, *A*). Furthermore, when all IgA-expressing PCs, shown by the dashed box in Fig 2, *B*, were sorted for immunoglobulin transcriptional analysis, both IgG and IgA transcripts were detected (Fig E1, *B*). Of note, transcriptional analysis could not be performed on either cells expressing only IgA or only IgG because staining for IgG (either for inclusion or exclusion) would require permeabilization, a process that greatly diminishes the quality and quantity of the cellular RNA recovered. Nonetheless, the transcriptional analyses indicate that the IgG being detected in the dual IgA/IgG-expressing PCs came, at least in part if not completely, from IgG-producing cells.

These observations, in combination with the profiles of expression of immunoglobulin light chains in Fig 2, *B*, suggest that the most likely explanation for dual IgA/IgG-expressing PCs is that the majority are producing IgG and binding IgA. Several receptors for IgA have been described, although only a few would likely be expressed on PCs.[E1] Interestingly, CD71 was recently shown to bind secretory IgA in an intracellular endosomal compartment.[E2] Gut-derived PCs express CD71 (unpublished observations), and an intracellular localization of the IgA is most consistent with our observation that the intensity of IgA staining was uniformly increased with permeabilization.

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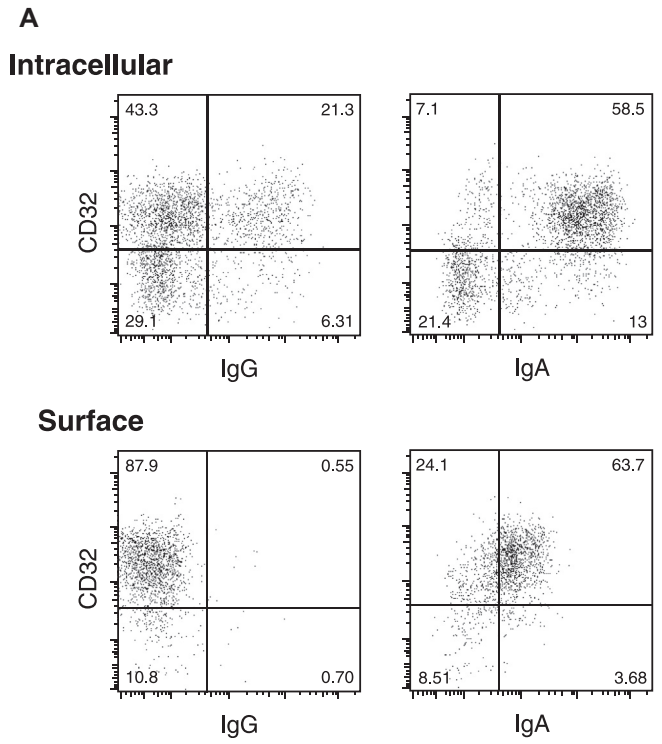

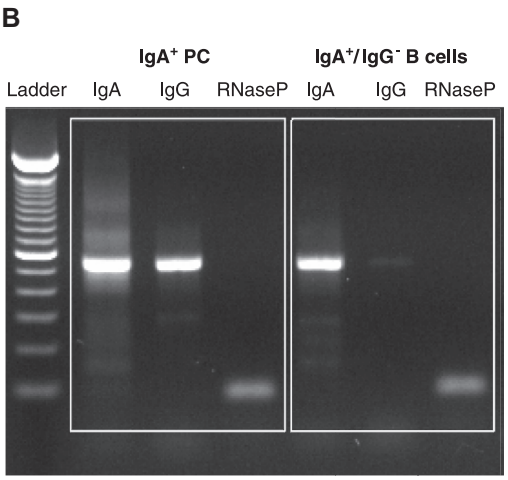

Sample	Target Gene	Band Relative Intensity (I)	$I_{IgX}/(I_{IgG} + I_{IgA})$ (%)
IgA^+ PC	IgA	3.23	54.1%
	IgG	2.74	45.9%
	RNaseP	1.00	
IgA^+/IgG^- B cells	IgA	2.60	96.2%
	IgG	0.10	3.8%
	RNaseP	1.00	

FIG E1. Properties of surface IgA^+/IgG^- gut-derived PCs. **A,** Flow cytometry with and without permeabilization of cells was performed to evaluate the expression of CD32, IgG, and IgA on *(Surface)* and within *(Intracellular)* gut-derived PCs. **B,** RT-PCR was performed to evaluate the expression of IgG and IgA in gut-derived PCs that were sorted by IgA. $CD19^+$ B cells from the peripheral blood and sorted by IgA^+/IgG^- were used as positive controls, and amplification of RNaseP was included as a housekeeping gene.

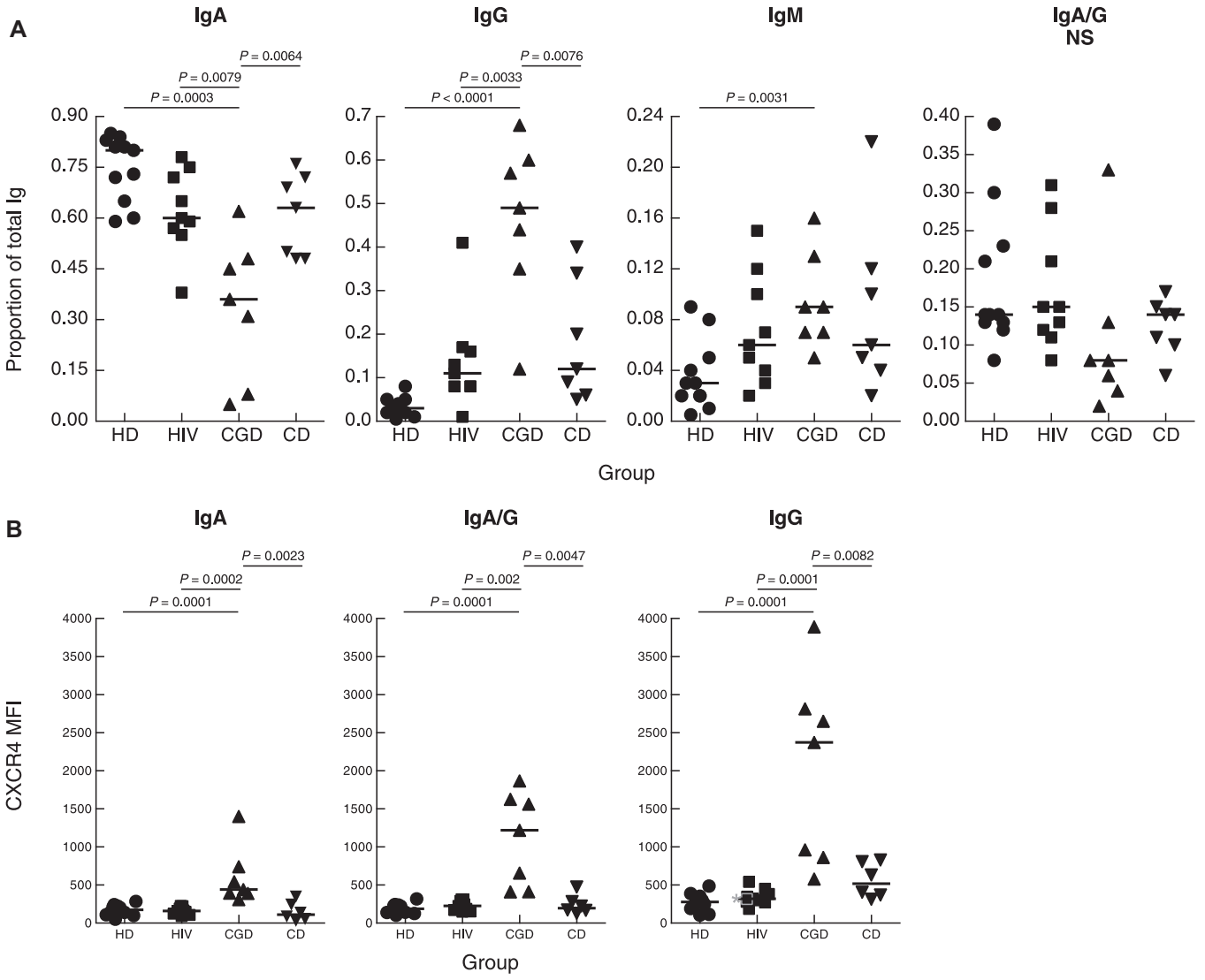

FIG E2. A, Graphs are identical to those of Fig 3, *B*, with the exception that the CGD group does not include patients without colitis. Only those *P* values that changed are shown. **B,** Graphs are identical to those of Fig 5, *B*, with the exception that the CGD group does not include patients without colitis. Only *P* values that changed are shown. *NS*, Not significant.

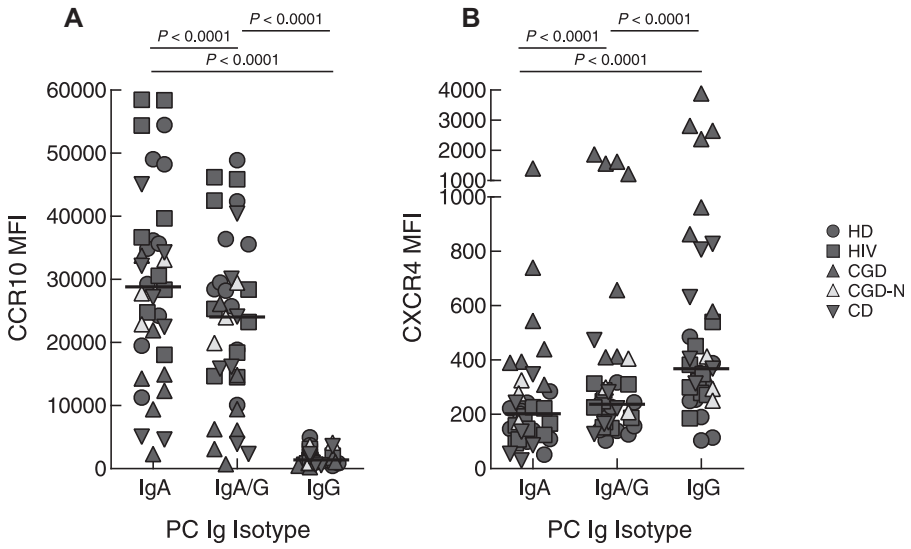

FIG E3. Comparison of homing receptor expression on gut-derived PCs by immunoglobulin isotype. Data are from Figs 4, *B*, and 5, *B*, with all groups combined. Intensities of CCR10 **(A)** and CXCR4 **(B)** were compared between PCs expressing only IgA, dual IgA/G-expressing PCs, and PCs expressing only IgG. *Horizontal bars* represent medians.

TABLE E1. Additional participant profiles and clinical observations

Participant	Mutated gene	Immune-modulating medication	$CD4^+$ T cells (count/μL)	HIV RNA (copies/mL)
1-HIV			337	2,901
2-HIV			459	12,592
3-HIV			599	5,114
4-HIV			615	10,089
5-HIV			583	40,702
6-HIV			483	9,722
7-HIV			1003	6,258
8-HIV			663	445
9-HIV			690	15,410
10-HIV-A			502	12,679
1-CGD	gp91phox	Adalimumab (Humira)		
2-CGD	p47phox			
3-CGD	p47phox	Prednisone, 10 mg/d; adalimumab (Humira)		
4-CGD	gp91phox	Adalimumab (Humira)		
5-CGD	p47phox	IFN-γ 3×/wk; adalimumab (Humira)		
6-CGD	gp91phox	Prednisone, 10 mg/d		
7-CGD	gp91phox	Adalimumab (Humira)		
8-CGD-N	p47phox	IFN-γ 3×/wk		
9-CGD-N	gp91phox	IFN-γ 3×/wk		
10-CGD-N	gp91phox			
11-CGD-N-A	p47phox			
1-CD		Infliximab every 6 wk		
2-CD		Hydrocortisone foam (Cortifoam)		
3-CD		Certolizumab		
4-CD		Adalimumab (Humira)		
5-CD				
6-CD				
7-CD				

-*A*, IgA deficiency; *CGD-N*, CGD with no gastrointestinal symptoms.