

Expression of immunoglobulin D is increased in chronic rhinosinusitis



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ABSTRACT

Background: Immunoglobulin (Ig) D is largely localized to the upper airway and reacts with colonizing respiratory pathogens.

Objective: To determine whether chronic rhinosinusitis (CRS) is associated with increased IgD expression.

Methods: We performed immunofluorescent staining for cytoplasmic IgD, IgA, IgM, and surface plasma cell marker CD138 (syndecan-1) in sinus tissue of patients with CRS with and without nasal polyps (CRSwNP and CRSsNP, respectively) and control subjects without CRS (n = 6 each). Sinonasal mucus antibody levels of patients with CRSwNP or CRSsNP and control subjects were measured by enzyme-linked immunosorbent assay (n = 13, 11, and 9 subjects, respectively). Cells per square millimeter and antibody levels were compared by analysis of variance. Histopathology was performed with sinus tissue from subjects in the 3 groups (n = 6, 8, and 13 subjects respectively).

Results: Cells expressing cytoplasmic IgD exceeded those with cytoplasmic IgA and IgM and represented most CD138⁺ plasma cells in the lamina propria. The frequencies of IgD⁺ plasma cells were significantly higher in patients with CRSsNP and CRSwNP compared with control subjects ($P < .01$). Only patients with CRSwNP showed increased frequencies of IgM and IgA plasma cells ($P < .01$). In contrast to high plasma cell frequencies in tissues, the levels of secreted IgD were lower than those of IgA, IgM, and IgG but were highest in the CRSwNP group compared with the other groups ($P < .05$).

Conclusion: IgD plasma cells are prominent in sinus tissues and are increased in CRS. That IgD protein also shows the lowest concentration of antibodies in secretions suggests that its activity might be targeted to the tissue rather than secretions.

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Introduction

Chronic rhinosinusitis (CRS) is a common but heterogeneous disease process defined by local inflammation of sinuses and the upper airway with persistent symptoms for 12 weeks.¹ CRS affects

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10.9% of populations in Europe and 12.5% in the United States.² Symptoms include, but are not limited to, nasal blockage, congestion, rhinorrhea, smell dysfunction, and facial pain or pressure. CRS is clinically categorized into 2 major phenotypes based on endoscopic findings: CRS with nasal polyposis (CRSwNP) and CRS without nasal polyposis (CRSsNP). Different T-cell–dependent mechanisms are proposed to underlie CRSsNP (predominantly T-helper cell type 1 inflammation with high levels of interferon- γ and transforming growth factor- β) and CRSwNP (T-helper cell type 2 inflammation with high levels of interleukin-5 and immunoglobulin [Ig] E).³ In addition to the putative role of IgE in some CRS subtypes, the contributions of other antibody isotypes, particularly IgD, are less well characterized.

Humans express 5 classes of immunoglobulins (IgM, IgD, IgG, IgA and IgE). Unlike the other isotypes, the anatomic distribution and function of IgD and the plasma cells that produce this isotype are incompletely understood. The evolutionary origins of IgD

extend back to teleost fishes.⁴ IgD is expressed in primates, dog, mouse, and rat but not in several other species^{5,6} and was initially identified in humans in 1965.^{7,8} Recognized primarily for its expression on the surface membrane of naive and several low-prevalence B-cell subsets, the functions of the secreted protein are not well defined.⁹

Clues to the specificity and activity of IgD derive from its relatively selective expression in plasma cells in the upper respiratory mucosa (eg, tonsil and nasal mucosa)⁹ and its increased levels with chronic tonsillitis.¹⁰ Moreover, the binding of surface IgD on naive B cells by proteins from respiratory pathogens (eg, *Haemophilus influenzae* and *Moraxella catarrhalis*) drives T-cell-independent polyclonal B-cell activation.^{11–13} Such nonspecific activation could serve as a bacterial strategy to subvert antigen-specific antibody responses that might otherwise help control these organisms to nonspecific polyclonal responses.¹¹ Secreted IgD binds to viruses (rubella and measles), bacteria (*Escherichia coli*, specific surface proteins on *H influenzae* and *M catarrhalis*), and selected bacterial virulence factors (eg, streptolysin O from *Streptococcus pyogenes*, diphtheria toxin).^{14–16} Allergic inflammation also can promote binding of IgD to granulocytes such as neutrophils and eosinophils in the skin.^{17,18}

The IgD expressed as a transmembrane receptor on mature naive B cells is generated by alternative splicing of pre-mRNA containing IgM and IgD heavy-chain constant regions. IgD also is produced by plasma cells and secreted into mucosal fluids or serum.⁶ Most class switching from IgM⁺IgD⁺ naive cells to IgG, IgM, IgA, or IgE occurs in the germinal centers of lymphoid follicles. However, whether naive B cells differentiate into IgD-producing plasma cells without typical class switching is less clear and could involve somewhat different mechanisms.¹⁹ Whether germinal centers are present in the sinus mucosa to support the classic class switch mediated by activation-induced cytidine deaminase and other related mediators also is controversial.^{9,20}

In summary, IgD is increased in tonsillar tissue of subjects with chronic tonsillitis,¹⁰ but whether these findings extend to effector sites, such as the sinuses, during chronic disease is unknown. Upper respiratory pathogens can selectively activate IgD⁺ B cells in vitro,¹¹ and IgD might induce antimicrobial and proinflammatory responses in basophils.⁹ In addition, the 2 forms of CRS likely result from the changing inflammatory milieu in the mucosa.³ Therefore, we studied the differential expression of IgD plasma cells in sinus tissues and protein in secretions of adults with CRSsNP and CRSwNP and control subjects to evaluate the expression of IgD during CRS using human sinus tissue and secretions.

Methods

Human Subjects

Written informed consent was obtained from patients as approved by the Colorado Multi-Institution Review Board (COMIRB 11-1442). Ethmoid sinus tissue and middle meatal swabs were obtained from patients diagnosed with CRSwNP and CRSsNP and control subjects (n = 6 each) during endoscopic sinus surgery performed for standard clinical indications (Table 1A, B).²¹ Control subjects were undergoing endoscopic approaches for skull base surgery or dacryocystorhinostomy for epiphora, without endoscopic or radiographic evidence of inflammation. Computed tomograms and nasal endoscopic results were scored according to the Lund-McKay and Lund-Kennedy scoring criteria, respectively.^{22,23} Nasal swabs (BD CultureSwabs, Franklin Lakes, New Jersey) of the middle meatus were tested for total immunoglobulins by enzyme-linked immunosorbent assay from 9 control subjects, 11 patients with CRSsNP, and 13 patients with CRSwNP.

Table 1A

Clinical Characteristics of Control Subjects and Patients With CRSwNP and CRSsNP Tested for Plasma Cells in Sinus Tissue by Immunohistochemistry

	Control	CRSsNP	CRSwNP
Subjects, n	6	6	6
Age (y), mean ± SD	51.2 ± 14.9	51 ± 13.8	42.8 ± 20.0
Men/women, n	2/4	3/3	1/5
Aspirin sensitivity, n (%)	0 (0)	1 (16.7)	2 (33.3)
Allergic rhinitis, n (%)	1 (16.7)	3 (50)	4 (66.7)
Asthma, n (%)	0 (0)	3 (50)	5 (83.3)
CT score ^a , mean ± SD	0 ± 0	9.67 ± 6.8	18.33 ± 4.6
Endoscopy score ^b , mean ± SD	0 ± 0	3.67 ± 3.3	7.8 ± 2.8

Abbreviations: CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps; CT, computed tomographic.

^aThe Lund-Mackay score²² is a widely used method for radiologic staging of CRS. A score of 0 (no abnormality), 1 (partial opacification), or 2 (complete opacification) is assigned to each sinus, with a maximum score of 24. The ostiomeatal complex is assigned a score of 0 (not obstructed) or 2 (obstructed).

^bThe Lund-Kennedy endoscopic scoring system²³ quantifies the pathologic states of the nose and paranasal sinuses, focusing on the presence of polyps, discharge, edema, scarring, or adhesions and crusting. Scores range from 0 to 20. Polyps are graded as absent (0), present in the middle meatus (1), or present beyond the middle meatus (3). Discharge is graded as not present (0), thin (1), or thick and purulent (2). Edema, scarring, and crusting are each graded as absent (0), mild (1), or severe (2).

Immunohistochemistry

Immunohistochemistry was performed as recently described.²⁴ Briefly, tissue specimens were embedded in paraffin and fixed onto glass slides in 4-μm sections. Deparaffinization was performed using mixed xylenes, followed by rehydration using decreasing concentrations of ethanol (EtOH). Then, high-temperature antigen retrieval was performed using a citric acid buffer (pH 6.5). Slides were blocked with 10% normal goat serum for 1 hour, stained with a mixture of directly labeled F(ab')₂ goat anti-human IgD (Alexa Fluor 647 conjugated; 1:100), IgA (Alexa Fluor 488; 1:100), and IgM (Alexa Fluor 594; 1:100; Jackson Immunochemistry, West Grove, Pennsylvania). After 4',6-diamidino-2-phenylindole (DAPI) nuclear staining, mounting medium was applied with coverslips. Separate slides were costained for IgD and CD138 (Alexa Fluor 594-conjugated AffiniPure F(ab')₂ fragment goat anti-human CD138; Jackson Immunochemistry) to verify the presence of plasma

Table 1B

Clinical Characteristics of Control Subjects and Patients With CRSwNP and CRSsNP Tested for Immunoglobulin Levels in Sinus Secretion by Enzyme-linked Immunosorbent Assay

	Control	CRSsNP	CRSwNP
Subjects, n	9	11	13
Age (y), mean ± SD	40.7 ± 16.1	49 ± 11.2	
Men/women, n	4/5	6/5	7/6
Aspirin sensitivity, n (%)	0 (0)	0 (0)	5 (38.4)
Allergic rhinitis, n (%)	4 (44.4)	2 (18.2)	9 (69.2)
Asthma, n (%)	3 (33.3)	3 (27.3)	10 (76.9)
CT score ^a , mean ± SD	1.1 ± 3.2	3.7 ± 2.7	9.6 ± 6.7
Endoscopy score ^b , mean ± SD	0.3 ± 0.7	4.6 ± 3.5	13.9 ± 6.4

Abbreviations: CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps; CT, computed tomographic.

^aThe Lund-Mackay score²² is a widely used method for radiologic staging of CRS. A score of 0 (no abnormality), 1 (partial opacification), or 2 (complete opacification) is assigned to each sinus, with a maximum score of 24. The ostiomeatal complex is assigned a score of 0 (not obstructed) or 2 (obstructed).

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cells. For hematoxylin and eosin staining to identify the presence of epithelium, white blood cells, cilia, and lymphoid aggregates or follicles, cryostat sections were prepared and mounted on frosted glass slides in 4- μ m sections and stored at -40°C until staining.

Image Processing and Cell Counting

Slides were scanned using an Olympus VS120 scanner outfitted with an OrcaR2 camera (Olympus, Center Valley, Pennsylvania). Image processing was performed using Olympus VS-ASW FL2.7 software (2004–2013). We identified 10 random regions of interest containing epithelium and lamina propria in each slide, each 0.1735 mm^2 in area. Image channels were separated into images depicting only DAPI, cyanine-5, fluorescein isothiocyanate, and tetramethylrhodamine and uploaded into CellProfiler 2.1.1 cell image analysis software (Broad Imaging Institute, Cambridge, Massachusetts). The frequencies of DAPI⁺, IgD⁺, IgM⁺, and IgA⁺ cells and the proportions of each cell types were calculated per square millimeter by dividing the cell count by the area of the region of interest and dividing the isotype by the total number of DAPI⁺ cells, respectively. Manual confirmation of these counts was performed using ImageJ, a freely available JAVA-based public-domain image processing and analysis program developed at the National Institutes of Health (Bethesda, Maryland).

Statistical Analysis

Values for the 3 groups were compared by 1-way analysis of variance of counts per square millimeter. Those with significant differences were compared by 2-group comparisons with unpaired *t* tests using GraphPad Prism 6.00 (GraphPad Software, La Jolla, California). Data are presented as scatterplots of average counts per square millimeter per subject, with bars representing mean values. Statistical significance was set at an α value equal to .01.

Total Antibody in Secretions by Enzyme-Linked Immunosorbent Assay

We coated Nunc Maxisorp microtiter plates (eBioscience, San Diego, California) with goat anti-human IgA, IgG, IgM (Jackson Immunology), and IgD (Southern Biotech, Birmingham, Alabama) in carbonate buffer (pH 9.6) overnight at 4°C . Nasal swabs were diluted in 400 μL of phosphate buffered saline and serially diluted with standards in duplicates for 2 hours at room temperature. We detected levels of antibodies with horseradish peroxidase–labeled affinity purified goat anti-human IgA, IgG, IgM, and IgD at 1 hour at room temperature. 3,3',5,5'-Tetramethylbenzidine was used as the developer and plates were read at 450 nm. Total protein levels were determined by the Pierce bicinchoninic acid protein assay (ThermoFisher Scientific, Waltham, Massachusetts). Then, we coated the microtiter plates with rabbit anti-human albumin (Dako, Santa Clara, California) in carbonate buffer (pH 9.6) overnight at 4°C . Plates were blocked with 10% rabbit serum for 2 hours. Nasal swabs and standards were diluted in duplicates and incubated for 2 hours at room temperature. We detected levels of antibody with rabbit anti-human horseradish peroxidase (Dako) at 1 hour at room temperature. 3,3',5,5'-Tetramethylbenzidine was used as the developer and plates were read at 450 nm.

Histologic Lymphoid Structure Analysis

Six control, 8 CRSsNP, and 13 CRSwNP slides stained with hematoxylin and eosin were independently evaluated by a board-certified hematopathologist (Q.N.). Surfaces lined by ciliated, pseudostratified, columnar epithelium including identifiable mucocytes (goblet cells) were referred to as the Schneiderian membrane with normal histology. CRSsNP was defined by submucosal edema with mixed inflammatory cells, including mature

lymphocytes with variable plasma cells, eosinophils, histiocytes, and neutrophils. CRSwNP was characterized by respiratory epithelium with variable squamous metaplasia, basement membrane thickening, edematous stroma with mixed chronic inflammatory infiltrate predominantly composed of eosinophils, plasma cells and lymphocytes, and, characteristically, an absence of submucosal seromucous glands. Lymphoid aggregates were considered present when there was a dense and sizable (>50 lymphoid cells) aggregate with predominantly lymphoid cells (ie, chronic inflammatory cells including plasma cells, eosinophils, histiocytes, where neutrophils constitute only a minor subset within the aggregate). All other lymphoid patterns were considered part of the chronic inflammatory infiltrate. Germinal centers were considered present when the conspicuous presence of centroblasts, centrocytes, small lymphocytes, tangible body macrophages, and follicular dendritic cells was identified within a lymphoid nodule. To determine whether there was a significant correlation between IgD⁺ plasma cells and degree of inflammation, the slides were graded on a 4-point scale of 0 to 3, with 0 being no inflammation and 3 being highly inflamed.²⁵ The amount of inflammation was judged based on the observed density of inflammatory cells, which include variable proportions of plasma cells, eosinophils, histiocytes, and neutrophils. The grade of inflammation was correlated with the density of IgD⁺ plasma cell using Pearson correlation analysis.

Results

Patient Characteristics

Disease-related variables (presence of allergies, polyps, computed tomographic and endoscopy scores), but not age or sex, differed among the 6 control subjects, 6 patients with CRSsNP, and 6 patients with CRSwNP from whom sinus tissue samples were obtained (Table 1A). Similarly, the demographics of the 3 groups from whom secreted antibodies were studied were comparable (Table 1B).

Localization of Plasma Cells in Sinus Tissues

We identified discrete individual cells staining for cytoplasmic IgD, IgA, and IgM dispersed throughout the tissues (Fig 1A and eFig 1). No cells coexpressed multiple intracytoplasmic isotypes (eg, cytoplasmic IgD and cytoplasmic IgM). The cytoplasmic localization of IgD and coexpression of IgD in the cytoplasm with CD138 (syndecan-1) on the cell surface confirmed that these IgD⁺ cells were consistent with plasma cells, rather than naive B cells expressing surface IgD (Fig 1B). By manual counts, CD138⁺ cells in the lamina propria (nonepithelial cells) constituted a median of 29.4% (range 21.5–40.6%) of more than 2,500 DAPI⁺ cells from 4 random samples from 3 subjects with CRSwNP samples (609–742 cells/sample). Of these CD138⁺ lamina propria cells, most (median 70.0%, range 61.9–81.7%) expressed cytoplasmic IgD and virtually all cells with cytoplasmic IgD coexpressed CD138.

We excluded the possibility that IgD⁺ cells expressed only surface IgD rather than cytoplasmic IgD. Simultaneous staining of control tonsil tissue from adults showed a clear discrimination of surface IgD-staining cells surrounding germinal centers, whereas cells with cytoplasmic IgD were identified in the extrafollicular areas (eFig 2). The surface IgD⁺ cells also expressed IgM, consistent with naive B cells, whereas, as noted, no cell with cytoplasmic IgD expressed another isotype.

A unique feature of plasma cells with cytoplasmic IgD was their selective presence in the epithelium, whereas those with cytoplasmic IgA⁺ and IgM⁺ cells were absent in this venue (Fig 1C). We observed intraepithelial IgD⁺ cells consistent with plasma cells in 3 of 6 control subjects, 4 of 6 subjects with CRSsNP, and 3 of 6 subjects with

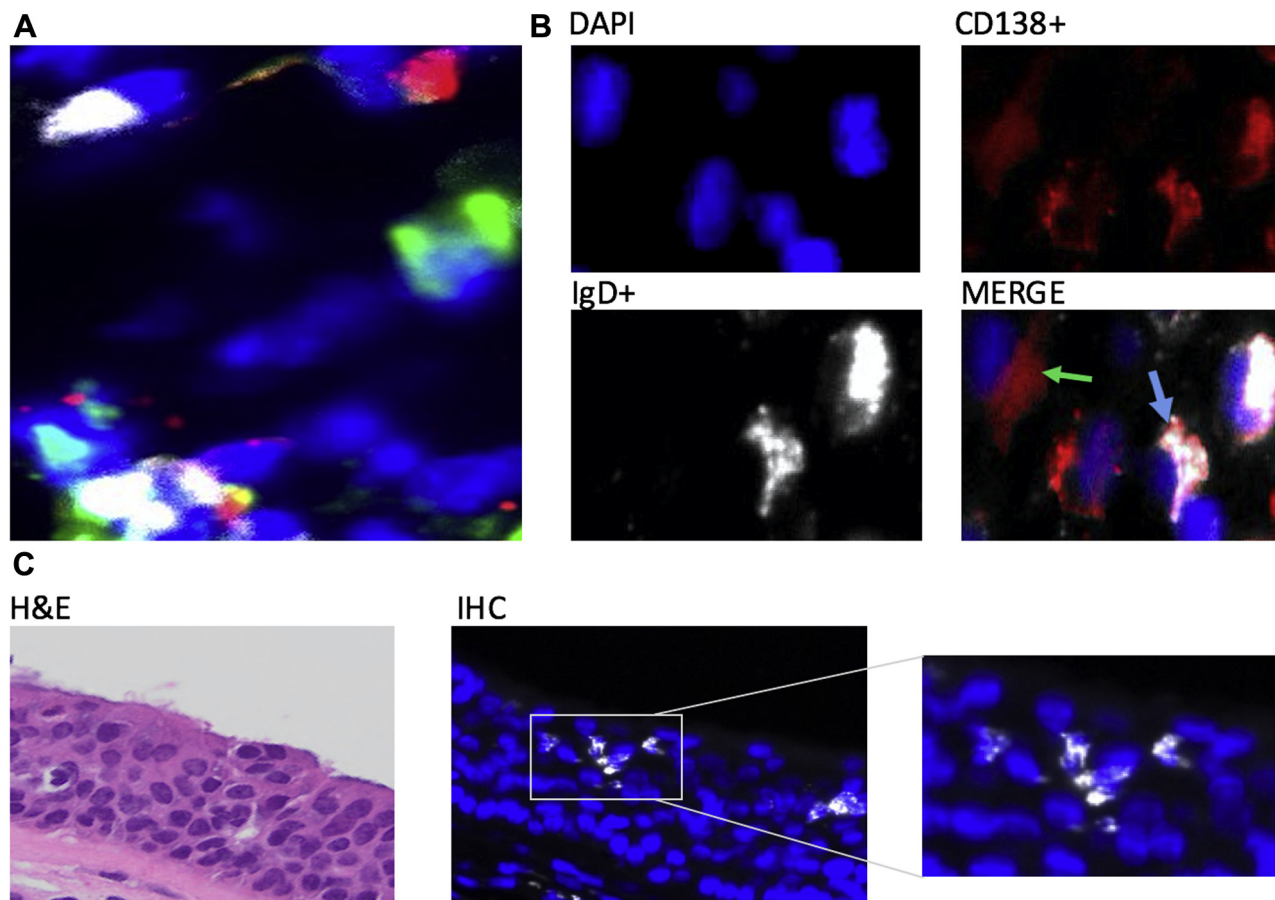


Figure 1. (A) Immunofluorescent triple staining for cells expressing cytoplasmic immunoglobulin (Ig) D (white), IgM (red), and IgA (green) in sinus tissue. Nuclei are stained with 4',6-diamidino-2-phenylindole (DAPI; blue). Results are shown from a representative patient with chronic rhinosinusitis without nasal polyps (original magnification 400 $\times$). (B) Immunofluorescent staining showing coexpression of cytoplasmic IgD (white), plasma cell marker CD138 (red), and nuclear staining (blue). Blue arrow, IgD and CD138 co-staining; green arrow, CD138 without IgD staining. Results shown are representative of 18 samples (original magnification 400 $\times$). (C) Localization of IgD plasma cells in the sinus epithelium. IgD⁺ plasma cells (white) DAPI nuclear stain (blue). (Inset) Higher magnification showing morphology of IgD⁺ plasma cells (original magnification 400 $\times$). H&E, hematoxylin and eosin; IHC, immunohistochemistry.

CRSsNP. Intraepithelial IgD⁺ plasma cells could not be stained with antibody to CD138 because of the coexpression of syndecan-1, the CD138 ligand for the antibody, on plasma cells and epithelial cells.²⁴

Plasma Cell Frequencies

The densities of cells expressing cytoplasmic IgD in the sinus lamina propria exceeded those with IgM and IgA in each of the 3 patient groups (Fig 2C). The frequency of IgD⁺ plasma cells per square millimeter was higher in the 2 CRS groups compared with controls ($P < .01$; Fig 2A). Unlike results with IgD, IgM⁺ and IgA⁺ plasma cells were higher in those with CRSsNP compared with controls and patients with CRSwNP ($P < .01$). Values for the latter 2 groups were similar (Fig 2B, C). Thus, IgD⁺ plasma cells most consistently distinguished patients with CRS from controls.

Mucus Antibody Levels

Secreted IgD was detected in the mucus of all subjects (Fig 3A). Unlike with plasma cells, IgG and IgA constituted most locally secreted antibodies, rather than IgD, which was present in the smallest amounts of any isotype (Fig 3E). Consistent with results with plasma cells, levels of IgD, IgM, and IgA were significantly higher in patients with CRSwNP than in controls. In contrast to the relative distribution of plasma cells, levels of IgD protein were not increased but were very similar in secretions from subjects with CRSsNP and controls, as were IgM and IgA. Secreted IgM and IgA were not significantly higher in patients with CRSwNP compared

with those with CRSsNP (Fig 3B, C). We found no differences in IgG, which constituted most secreted antibodies, among the 3 groups (Fig 3D, E).

Lymphoid Structure by Histology

We surveyed sinus tissues for histologic evidence of lymphoid follicles and active germinal centers. Of 27 tissues reviewed by a hematopathologist (Q.N.), fully organized germinal centers were not histologically identified in any sinus tissue, regardless of clinical diagnosis. However, unorganized lymphoid aggregates, which are typically collections of B cells without follicular structure, were identified in sinus tissues from a minority of subjects (1 of 6 control subjects [16.7%], 1 of 8 with CRSsNP [12.5%], and 6 of 13 with CRSwNP [46.2%]; $P = .19$ by χ^2 test; Fig 4D). We observed a significant correlation between the frequency of cells with cytoplasmic IgD⁺ and the degree of inflammation (grades 0–3; $R = 0.531$, $P = .023$) using Pearson correlation analysis.

Discussion

We have shown that cells expressing intracellular IgD represent a dominant isotype of antibody-secreting cells in the sinus tissue from healthy control subjects and from patients with 2 forms of CRS compared with IgA or IgM. Although IgD also can be present on the surface of naive and other B-cell subsets; such surface IgD⁺ cells were not identified in sinus tissue. The identification of IgD⁺ cells as being consistent with plasma cells in sinus tissues was confirmed

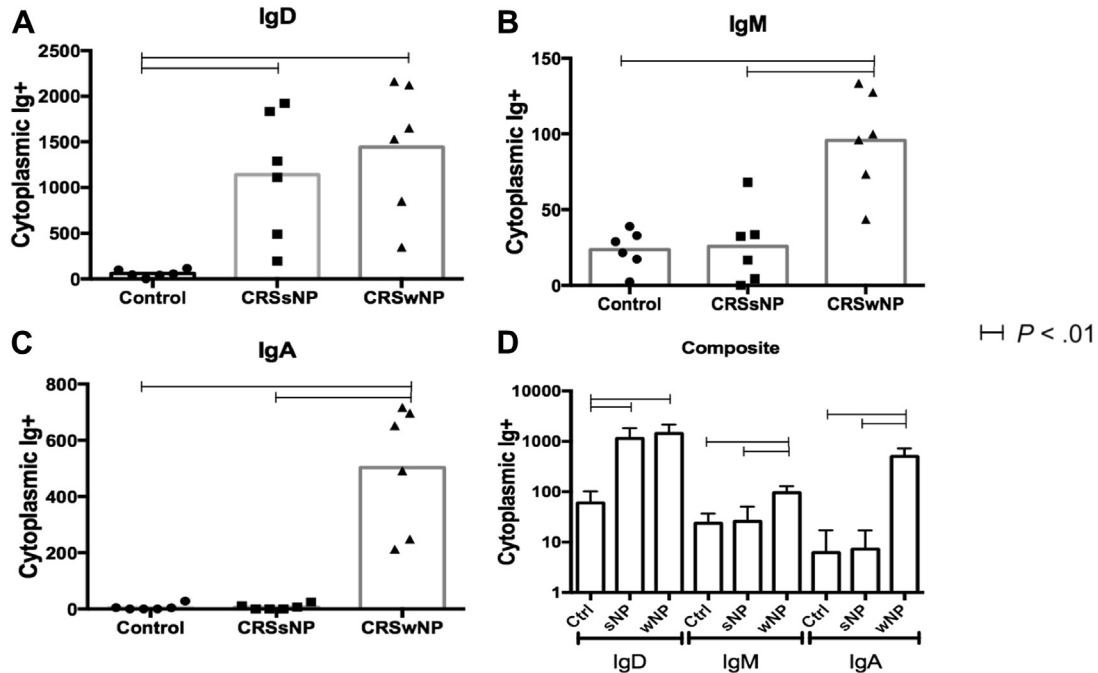


Figure 2. Frequencies of cytoplasmic immunoglobulin-positive (Ig⁺) cells in sinus tissues. Cells expressing cytoplasmic IgD (A), IgA (B), and IgM (C) were detected by 4-color immunofluorescence in sinus tissues from healthy control subjects (circles), patients with chronic rhinosinusitis without nasal polyps (CRSsNP; squares) and patients with chronic rhinosinusitis with nasal polyps (CRSwNP; triangles). Dots represent mean cell counts from 10 regions of interest per tissue sample and the box represents median values. Horizontal bars represent statistical significance between groups by analysis of variance ($P < .01$). (D) Composite graph of IgD, IgM, and IgA in a log₁₀ scale showing relative counts of antibodies in each disease state. Horizontal bars represent statistical significance between 2 points. Error bars represent SD. Statistical significance calculated by analysis of variance.

by the absence of IgD and IgM costaining, cytoplasmic localization of the antibody, and coexpression of CD138 (syndecan-1; Fig 1B). This cytoplasmic localization is distinct from the surface staining of IgD⁺ naive cells in tonsil controls (eFig 2). In the context of sinus pathology, increased frequencies of IgD⁺ plasma cells in the sinus

tissue most consistently distinguished patients with CRS (with or without polyps) from control subjects.

In addition to their presence in the sinus lamina propria, only cells with cytoplasmic IgD⁺ were identified in the epithelial layer. However, we cannot exclude the presence of cells expressing

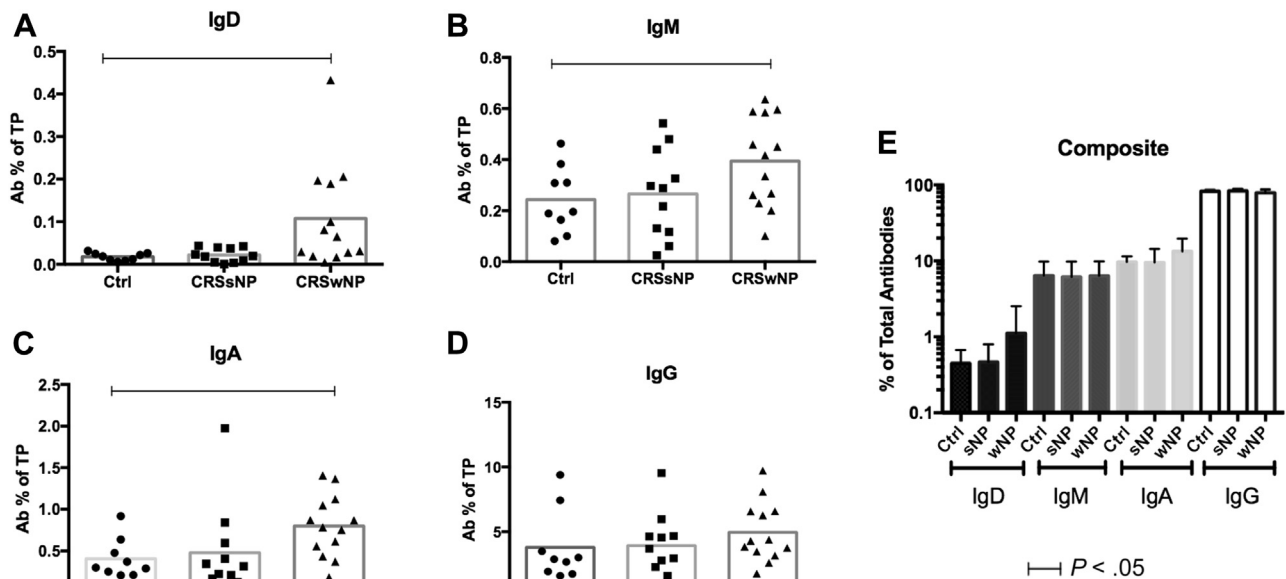


Figure 3. Levels of antibodies in sinus tissue secretions eluted from swabs and measured by enzyme-linked immunosorbent assay. Values for immunoglobulin (Ig) D (A), IgA (B), IgM (C), and IgG (D) from each subject (1 dot per subject) are normalized and expressed as antibody (Ab) percentage of total protein (TP). Horizontal bars represent significance differences between 2 points calculated by analysis of variance ($P < .05$). (E) Composite graph showing relative antibody values as percentage of total antibodies on a log₁₀ scale.

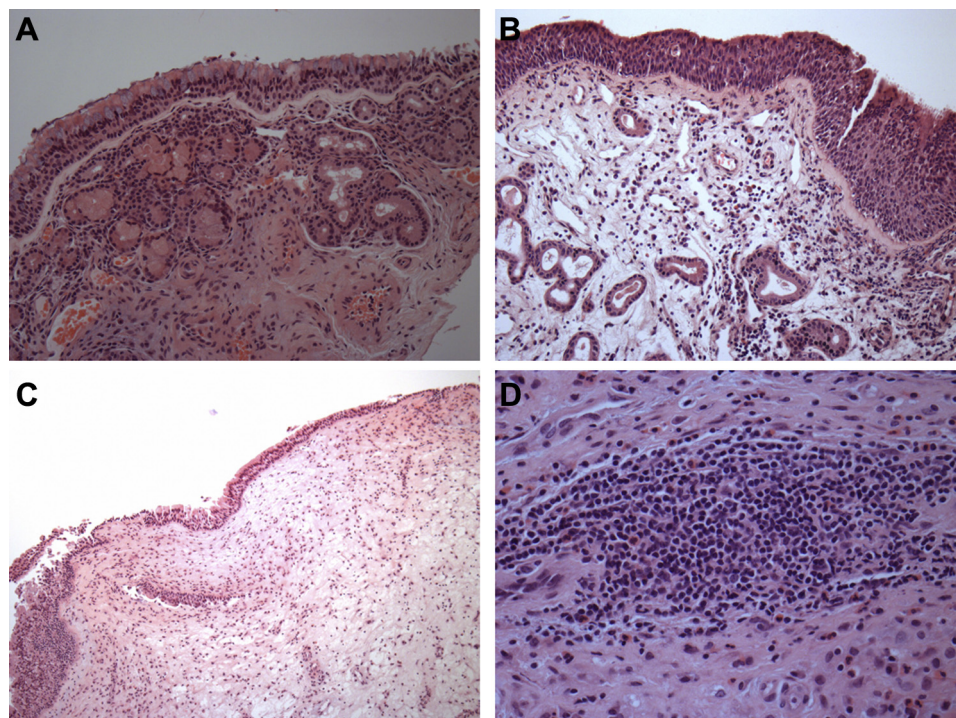


Figure 4. Hematoxylin and eosin staining of sinus tissue. (A) Normal control: Normal sinonasal mucosa is lined by ciliated, pseudostratified, columnar epithelium with identifiable mucocytes (goblet cells) with underlying seromucous glands scattered throughout the submucosa (magnification 200×). (B) Chronic rhinosinusitis without nasal polyps: Submucosal edema with mixed inflammatory cells, including mature lymphocytes with variable plasma cells, eosinophils, histiocytes, and neutrophils (magnification 200×). (C) Chronic rhinosinusitis with nasal polyps: The surface epithelium is composed of intact respiratory epithelium. The underlying stroma is markedly edematous and is noteworthy for the absence of seromucous glands. A mixed chronic inflammatory infiltrate is present and predominantly composed of eosinophils, plasma cells, and lymphocytes (magnification 100×). (D) Lymphoid follicle: Dense and sizable (>50 lymphoid cells) aggregate with predominantly lymphoid cells (ie, other chronic inflammatory including plasma cells, eosinophils, histiocytes, and neutrophils cells constitute only a minor subset within the aggregate). All other lymphoid patterns are considered part of the chronic inflammatory infiltrate (magnification 400×).

cytoplasmic IgG and IgE that were not examined. Despite the high frequency of cells with cytoplasmic IgD in the lamina propria and epithelium, levels of IgD protein were quite low in secretions.

Consistent with the results of Hulse et al,²⁶ IgA and IgM were present at increased concentrations in patients with CRSwNP. These polymeric antibodies engage the polymeric immunoglobulin receptor on the basolateral surface of mucosal epithelial cells and undergo active receptor-mediated transport from the lamina propria to the lumen as secretory IgA or IgM. Polymeric immunoglobulin receptor expression and transport is upregulated in the presence of inflammation.^{27,28} IgG also can be actively transported across the epithelium by the neonatal Fc receptor,²⁹ which is prominently expressed in the upper respiratory mucosa. In contrast, no specific or active mechanisms by which IgD could be transported to the lumen have been identified, which could explain its presence within the epithelial layer in the absence of other antibodies. Thus, the function of IgD might be most active within the sinus tissue rather than sinus secretions.

We identified high frequencies of IgD⁺ plasma cells in the lamina propria of sinus tissues from healthy adults. Increased frequencies of these cells in CRS suggest that pathologic stimulatory or regulatory mechanisms might enhance and drive their development. In contrast to our results, Chen et al⁹ found that virtually none of the IgD⁺IgM[−] cells isolated in tonsils were positive for CD138. Unlike the study by Chen et al, cells that we localized were consistent with mature plasma cells, capable of secreting IgD antibodies into the lamina propria. One likely explanation for this is that the IgD⁺ CD138[−] plasmablasts identified by Chen et al in the tonsil could represent an earlier stage of differentiation. Given that most of the IgD⁺ cells that we identified also were CD138⁺, IgD[−] could switch cells differentiated elsewhere (eg, tonsils, local lymphoid tissue) and enter the sinus as a terminally differentiated

population. Subjects with CRSwNP but not CRSsNP also showed larger numbers of IgA⁺ and IgM⁺ plasma cells, indicating that the pathogenesis of these 2 syndromes differ to some extent in the same target tissue.

Pathogen-specific functions of IgD are poorly characterized but could depend largely on the antigen-binding Fab variable region of the molecule. Binding of bacterial surface antigens of upper respiratory pathogens by the exposed Fab fragments of IgD or IgM on naive B cells could initiate B-cell activation and drive antigen-specific antibody responses, which could support agglutination or, potentially, phagocytosis. In organized germinal centers in secondary lymphoid tissues, such as tonsils (eFig 2), T-cell–dependent mechanisms could contribute prominently to this evolution. However, selective cross-linking of the constant region Fc of IgD by selective IgD-binding surface proteins on respiratory mucosal bacteria such as *H influenzae* and *M catarrhalis*^{11–13} could activate and initiate non–antigen-specific differentiation of these IgD⁺ cells into IgD⁺ plasma cells in the local environment. In the sinus, T-cell–independent mechanisms might be sufficient to drive this process to generate IgD⁺ plasma cells in the sinus lamina propria. Extrafollicular T-cell–independent mechanisms of B-cell differentiation into IgD plasma cells could include internalization of *H influenzae* into B cells to stimulate toll-like receptor-9, soluble B-cell activating factor, and A proliferation-inducing ligand derived from IgD-activated basophils, each of which also can facilitate B-cell activation alone or in combination.¹¹ The presence of lymphoid aggregates, rather than follicles, in sinus tissue serves as evidence for local lymphocyte proliferation in the sinus tissue. We found such lymphoid aggregates in 46.3% of patients with CRSwNP (Fig 4D), consistent with a recent publication highlighting the presence of tertiary lymphoid organ-like structures in eosinophilic nasal polyps.³⁰ A study by Tsurumaru²⁰ identified immunologically

activated lymphoid follicles in sinus tissue that produce plasma cells, as evidenced by the patterns of T- and B-lymphocyte subpopulations and follicular dendritic cells.

Another isotype, IgE, also has been shown to be more prevalent in the mucosa of subjects with CRSwNP and has been implicated in its pathogenesis.³¹ Baba et al³² reported a local increase in total IgE and class-switch recombination to IgE in nasal polyps in CRS. The T-helper cell type 2 inflammatory pathway and increased concentrations of interleukin-5 and total IgE are proposed to play a role in the pathogenesis of CRSwNP. That increased IgE levels are reported in CRSwNP, coupled with the present findings that cells with cytoplasmic IgG are elevated in this population, suggests mature B cells might migrate and localize to polyp tissue. Factors that govern class-switch recombination and further tissue-specific differentiation of plasma cells warrant further investigation. From a clinical context, further work is required to compare the relative prevalence of IgE⁺ and IgD⁺ plasma cells in control subjects and subjects with different forms of CRS.

Potential limitations of this study include the inclusion of different study cohorts for immunohistochemistry and sinus fluid analyses. However, the study design limits potential bias by performing a second test (enzyme-linked immunosorbent assay) on a different cohort to build on the findings of the initial immunostaining. Although automated cell counting software for enumeration of isotype-specific plasma cells in immunohistochemistry (Cellprofiler) could generate inaccuracies, we verified the cell counts using a manual cell counting software (ImageJ), which showed 98% correlation of image counting. Despite the descriptive nature of this study with a somewhat small sample (n = 6–13 per group), we have shown consistent, statistically significant, and biologically relevant differences between patients with CRS and control subjects. We did not stain for IgG or IgE, because previous studies also found that IgG plasma cells are prominent in sinus tissues.³³

In summary, CRS is associated with increased expression of IgD plasma cells in tissue and secreted IgD. Current work in our laboratory is directed to determine whether sinus tissue-derived IgD⁺ plasma cells do or do not show evidence of antigen specificity. Secreted antibodies and those that on the B-cell surface could bind to local microbiota and/or mucosal pathogens based on specific binding of the Fab fragments to discrete bacterial antigens. Antibodies also might bind to IgD-binding proteins on the surface of, for example, *H influenzae* or *M catarrhalis* through the constant regions. Such binding by secreted IgD could serve as a “decoy” to sterically block more functional bacteria-specific antibodies.^{13,34,35} Binding by the constant regions on the surface of B cells also could activate B cells to secrete irrelevant antibodies, thereby diverting more effective specific antibody production.³⁶ Determining the specificity and functions of IgD in sinus tissue is an intriguing and relevant target of our ongoing work to consider the mechanisms of IgD induction and the activity of these antibodies in health and disease.

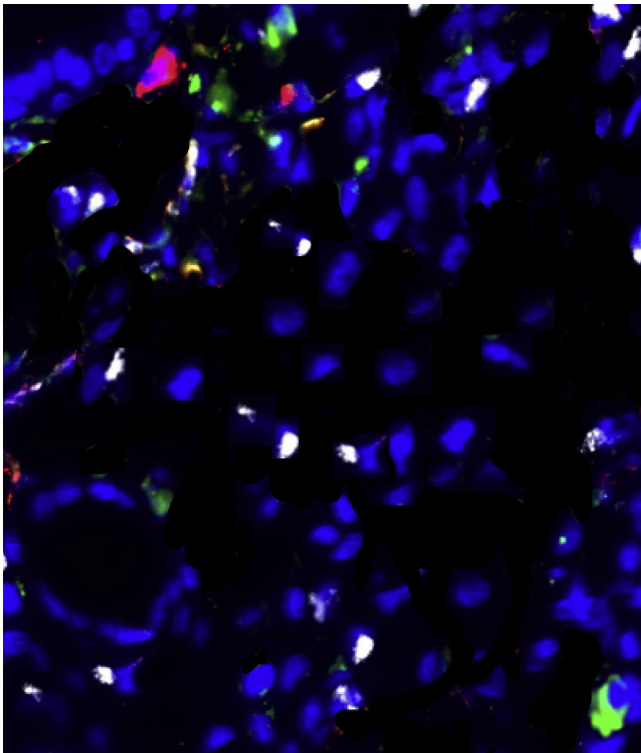
Supplementary Data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.anai.2017.07.024>.

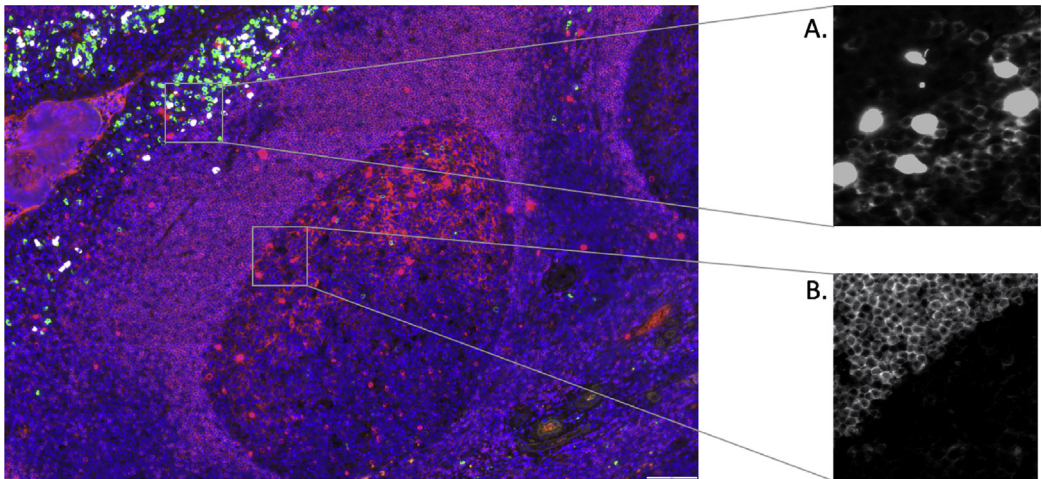
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Supplementary Data



eFigure 1. Low-power image showing distribution of immunoglobulins D, A, and M in sinus tissue (magnification 100×).



eFigure 2. Immunofluorescent triple staining for cells expressing cytoplasmic and surface immunoglobulins D (white), M (red), and A (green) in tonsil germinal centers. Nuclei are stained with 4',6-diamidino-2-phenylindole (blue). (A) High-power field showing cytoplasmic immunoglobulin D in the germinal center mantle zone. (B) High-power field showing surface immunoglobulin D in the germinal center mantle zone.