

Subepithelial Dendritic B Cells in Orofacial Granulomatosis

Pritash Patel, MRCP,*^{†,§} Francesca Barone, PhD,[†] Carlo Nunes, MRCP,* Laurent Boursier, PhD,[†] Edward Odell, FRCPATH,[‡] Michael Escudier, FDSRCS,[‡] Stephen Challacombe, PhD, FDSRCS,[‡] Jonathan Brostoff, DSc (Med), FRCP,[§] Jo Spencer, PhD, FRCPATH,[†] and Jeremy Sanderson, MD, FRCP*[§]

Background: Orofacial granulomatosis (OFG) is a chronic, disfiguring, granulomatous inflammation of the lips and oral mucosa. The pathogenesis is unknown, but it has been linked previously to Crohn's disease (CD) and more recently to dietary sensitivity. The oral mucosa is an immunologically responsive site associated with the generation of protective mucosal and systemic immune responses to vaccination and also hyperresponsiveness to allergens in some individuals. Classically, immune responses in oral mucosa are considered to be mediated by mucosa-associated lymphoid tissues (MALT), secondary lymphoid follicles that are intimately associated with epithelia.

Methods: Immunohistochemistry was used to investigate the inflammatory infiltrate in OFG and control tissue samples. Polymerase chain reaction (PCR), cloning of PCR products, and sequencing were used to characterize the local immunoglobulin gene profile in OFG.

Results: We describe large, active, dendritic B cells in oral mucosa that were not associated with any organized lymphoid tissues in the local subepithelial microenvironment. They express activation induced cytidine deaminase, which is essential for immunoglobulin gene diversification by somatic hypermutation and class switch recombination. IgE is also expressed by these B cells. They do not align with any other previously described B-cell subset in secondary lymphoid tissues in terms of morphology, proliferative activity, or phenotype.

Conclusions: These subepithelial dendritic B cells may contribute to the immune responsiveness of the oral mucosa, including

IgE-mediated allergic responses. In patients with OFG, further understanding of the role these cells play in oral immunity may lead to novel therapeutic possibilities.

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Key Words: orofacial granulomatosis, Crohn's disease, immunohistochemistry, inflammation in IBD, mucosal immunology

Orofacial granulomatosis (OFG) is a chronic inflammatory disorder presenting characteristically with lip swelling but also affecting other sites in the oral cavity such as the gingivae, buccal mucosa, and the floor of mouth.¹ Most cases of OFG present as a separate clinical entity, but a proportion present in association with systemic conditions such as intestinal Crohn's disease (CD), sarcoidosis, or Melkersson–Rosenthal syndrome.^{2–4} OFG is characterized histologically by granuloma formation and lymphocytic infiltrates. However, few studies have addressed the immunological and histological features of OFG in detail, although distinct alterations in mucosal IgA have been described.^{5,6}

The internal tissues of the mouth and oral cavity are separated from the external environment by a stratified epithelium. Despite the presence of a keratinized barrier, the oral mucosa is an effective portal for administered antigens and is potentially immunologically highly active. Sublingual immunization has been shown to generate protective mucosal and systemic immune responses to pathogens and to be an effective route for administration of immunotherapeutic agents for the treatment of allergy.^{7–10}

To combat infection, saliva is rich in immunoglobulins (Igs), in particular IgA, as well as innate mediators of immunity such as mucins and defensins.¹¹ Local immune responses to oral pathogens are classically thought to be generated in the mucosa-associated lymphoid tissues (MALT). These organized lymphoid structures are intimately associated with epithelia and comprise 1 or a small number of secondary lymphoid follicles with discrete B and T cell zones, usually germinal centers containing follicular dendritic cells and lymphoid associated vascular structures. The mucosal effector IgA is actively transported across the epithelium of the salivary glands and into the

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From the *Department of Gastroenterology at Guy's & St. Thomas NHS Foundation Trust, London, UK, [†]Peter Gorer Department of Immunobiology, King's College London School of Medicine at Guy's Hospital, [‡]King's College London Dental Institute, UK, [§]Nutritional Sciences Research, Waterloo Campus, Kings College London, UK.

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Reprints: Jo Spencer, PhD, FRCPATH, Peter Gorer Department of Immunobiology, King's College London School of Medicine at Guy's Hospital, 2nd Floor Borough Wing, Guy's Hospital, Great Maze Pond, London, SE1 9RT, UK (e-mail: jo.spencer@kcl.ac.uk)

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saliva, and specific IgA to oral pathogens can be detected in saliva.¹² IgG transudes from the serum and local tissue spaces, especially the gingiva (in the so-called crevicular fluid) and can also be detected in saliva. Specific IgG has also been demonstrated, particularly to pathogens implicated in periodontitis.¹³

The lips and oral cavity are the first body surfaces to encounter food, other ingested substances, and many environmental molecules and particles, including those that are airborne. Many of these are classified as allergens that initiate hypersensitivity in the oropharyngeal mucosa and many of these responses are IgE-mediated. Despite the clear association between the induction of allergic IgE responses in the oropharyngeal/nasal mucosae, the micro-anatomical site of class switching recombination (CSR) to IgE is still debated.¹⁴ Evidence for local CSR to IgE has been observed in biopsies of nasal mucosa,¹⁵ including expression of activation-induced cytidine deaminase (AID), an absolute requirement for this process.¹⁶ However, the B-cell subset undergoing the CSR event in nasal or bronchial mucosa has not been identified.¹⁵

B-cell subsets can be defined according to several criteria. These include their cellular morphology, the anatomical microenvironment in which they are found, and the profile of molecules expressed on the cell surface and in their cytoplasm. The load of somatic mutations in the variable region genes of the Ig heavy and light chains is a reflection of exposure to the hypermutation mechanism commonly associated with germinal centers. Additionally, the use of certain gene segments can reflect selection into a particular functional repertoire. Use of the J1 segment of the heavy chain, for example, is seen in peripheral but not mucosal B-cell responses.¹⁷ The VH1-46 segment is associated with the immune response to rotavirus,¹⁸ and the VH5 family segments are associated with allergic disease.¹⁹ We have used the criteria above to study B cells in the oral mucosa.

The true nature of OFG and its relationship to CD remains uncertain. However, a role for dietary antigens in the etiology of the condition is compelling.^{20,21} Cinnamon and benzoates have been found to be common allergens on patch testing in patients with OFG²² and specific exclusion of cinnamon and benzoates from the diet resulted in objective disease improvement in up to 72% of patients.²³ How these antigens contribute specifically to the development of OFG is unclear.

In this study we investigated the lymphocytic infiltrate in OFG. We used immunohistochemistry, polymerase chain reaction (PCR), and Ig gene analysis to evaluate B cells present in the oral mucosa. We have observed active B-cell responses in oral mucosa that are independent of conventional organized lymphoid structures and that do not bear hallmarks of conventional mucosal B-cell responses. We also provide evidence for local CSR and IgE expres-

sion in inflamed oral mucosa in OFG. The B cells themselves do not align with any of the conventional classifications of B-cell subsets defined by the criteria listed above. We consider this to be a novel and previously unidentified B-cell subset that could be involved in hypersensitivity responses to food and environmental allergens and hence provide a possible link between dietary antigens and inflammation in OFG.

MATERIALS AND METHODS

Tissues

Paraffin blocks composed of oral biopsies from 70 patients attending a specialist Joint Oral Medicine and Gastroenterology service were used and included a range of characteristics from mild lymphocytic infiltrates considered within normal histological limits through to marked inflammation (Table 1). Diagnosis of OFG was based on characteristic clinical and histological findings

In addition, 8 fresh-frozen oral mucosal samples were selected, 7 from patients with lichen planus and 1 from a patient with OFG. All tissues were originally submitted for diagnostic purposes. The study was approved by the Local Research Ethics Committee (Approval No. 95/5/6; Lewisham & North Southwark REC). Specimens were graded according to the inflammatory infiltrate present to produce a semiquantitative score (nil, mild, and marked). This was undertaken blindly by 2 authors (P.P., E.O.).

Immunohistochemistry

All antibodies and reagents were purchased from DakoCytomation (Carpinteria, CA) unless otherwise stated. Paraffin-embedded tissue sections (5 μ m) were submitted to antigen retrieval by heating in Dako Target Retrieval solution at 95°C for 40 minutes. Endogenous peroxidase and alkaline phosphatase were blocked by means of a dual endogenous blocking solution. Sections were single-stained for CD20, CD21, CD23, IgD, IgG, IgA, IgM, and IgE and double-stained for AID (Cell Signaling, Beverly, MA) with CD20, Ki67 with CD20, IgE with CD20, CD68 with CD20, and CD20 with CD3 using the EnVision²⁴ single and double stain systems according to the manufacturer's instructions. After staining, sections were counterstained with hematoxylin. Sections of human tonsil were stained in parallel with and without primary antibody to provide a positive and negative control.

Microdissection, mRNA Isolation, Reverse Transcription

Microdissection and laser capture microscopy was performed on cresyl-violet (0.1% in ethanol)-stained frozen tissue sections from the biopsy obtained from a patient with OFG and a tonsil germinal center as previously

TABLE 1. Details of Paraffin-embedded Tissues Studied

Site	Histological Diagnoses	Sex F:M	Age Mean (Range)	Inflammatory Infiltrate			Numbers
				Nil	Mild	Marked	
Lip	17x orofacial granulomatosis (OFG)	5:12	27 (10–68)	0/13	12/17	5/17	28
	10x lip mucocele	5:5	32 (10–51)	6/10	2/10	2/10	
	1x normal	1:0	No data	1/1	0/1	0/1	
Gingiva	5x OFG	4:1	19 (7–72)	1/5	2/5	2/5	25
	5x inflammatory fibroepithelial hyperplasia (FEP)	3:2	62 (43–81)	0/5	1/5	4/5	
	13x gingivitis	5:8	37 (6–69)	0/13	6/13	7/13	
	1x lichen planus	1:0	70	0/1	1/1	0/1	
	1x normal	0:1	63	1/1	0/1	0/1	
Buccal	6x OFG	3:3	37 (9–68)	1/6	3/6	2/6	12
	1x inflammatory FEP	0:1	15	0/1	1/1	0/1	
	5x normal	1:4	53 (19–79)	4/5	1/5	0/5	
Palate	1x OFG	1:0	9	1/1	0/1	0/1	3
	2x inflammatory polyp	2:0	43, 83	0/2	1/2	1/2	
Tongue	2x inflammatory FEP	1:1	10, 64	0/2	2/2	0/2	2
Totals		32:38		15	32	23	70

described.²⁵ mRNA was isolated from microdissected tissue (Qiagen RNeasy, Chatsworth, CA) and reverse-transcribed using Sensiscript (Qiagen). Quantitative reverse-transcription (qRT-PCR) (Applied Biosystems, Foster City, CA) was performed as previously described for AID mRNA expression (Hs00757808_m1, AICDA; Applied Biosystems)²⁶ and quantified using GAPDH as a reference gene.

RNA was extracted from sections cut from the other 7 frozen oral samples (Qiagen RNeasy) and reverse-transcribed using a high-capacity cDNA reverse transcription kit (Applied Biosystems). PCR of V_H-C_ε transcripts and AID transcripts was then performed using standard programs and primers.¹⁵

Analysis of Ig Gene Rearrangements

Deoxyribonucleic acid was isolated from 3 sections of a lip biopsy from a patient with OFG that contained a sparse lymphocytic infiltrate and no plasma cells (confirmed by staining with CD20 and CD38). Rearranged Ig heavy chain genes were initially sampled by a multiplex nested PCR method that amplifies Ig heavy chain variable region (IGHV) genes approximately proportional to their contribution to the repertoire using standard primers.²⁷ The first round of PCR was conducted at 95°C for 10 minutes, followed by addition of 1.5 units of *Taq* DNA polymerase, followed by 7 cycles of 94°C for 1 minute, 64°C for 2 minutes, and 72°C for 45 seconds (the annealing temperature was decreased by 2°C at each cycle). This was followed by 39 cycles of amplification (30 seconds denaturation at 94°C, 30 seconds annealing at 52°C, and 1

minute 30 seconds primer extension at 72°C). A second round of PCR was performed on a 3-μL aliquot of the first-round PCR product under the same conditions, except that an annealing temperature of 66°C was used and 35 cycles of amplification with an annealing temperature of 54°C. To increase the number of sequences from this small sample, a subset of cells with rearrangements of the VH4-34 and VH5 family were sampled by VH4-34 and VH5 gene-specific PCR as described previously.²⁸ PCR products were cloned, sequenced, and analyzed using Vquest at IMGT.

Statistical Analysis

Data were analyzed in the GraphPad Prism statistical PC program (GraphPad Software, San Diego, CA) using the Mann–Whitney *U*-test or using the χ^2 test. A level of $P < 0.05$ was considered statistically significant.

RESULTS

Immunohistochemistry

Of the 70 paraffin-embedded biopsies evaluated for this study, classified according to the presence and features of the lymphocytic infiltrate, 15 showed no lymphocytic infiltrate. Of the other 55, 32 showed sparse lymphocytic infiltrates and 23 displayed marked chronic inflammation (Table 1). No acute inflammation was observed in any biopsies. Of 8 frozen biopsies evaluated, 4 samples displayed marked inflammation, 3 showed sparse lymphocytic infiltrates, and 1 showed no lymphocytic infiltrate.

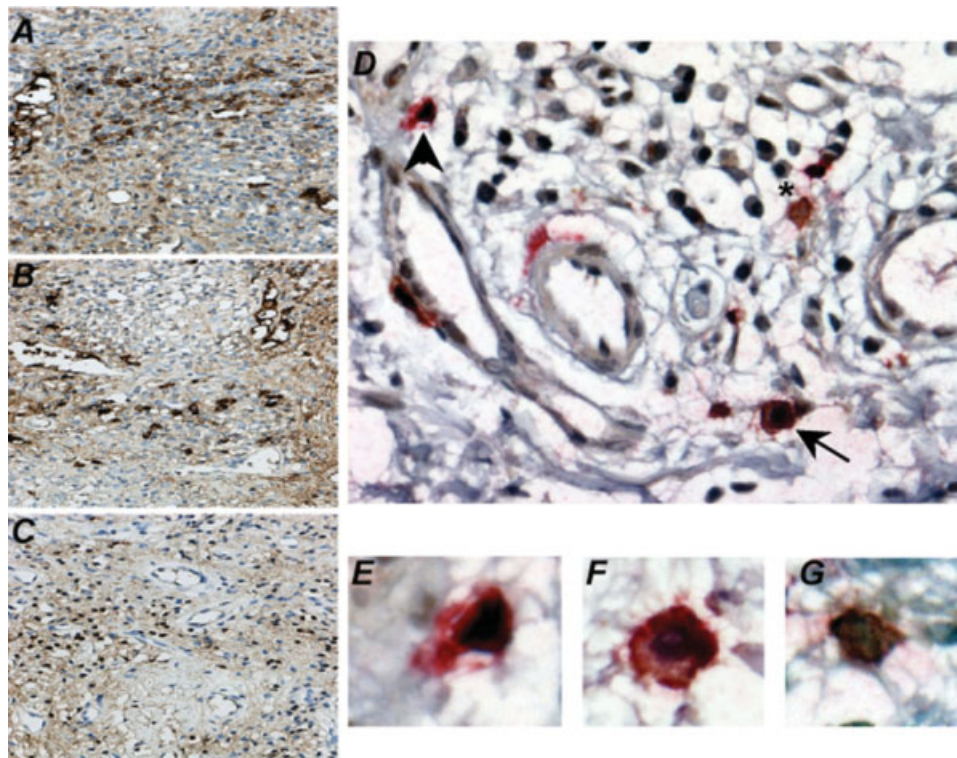


FIGURE 1. Immunoglobulin production in oral mucosa. Oral samples were single-stained for IgG (A), IgA (B), and IgM (C) (all markers in brown). (A–C) There was consistently more IgG than IgA, which in turn was present in greater amounts than IgM. Oral samples were double-stained for IgE (in brown) and CD20 (in pink) (D–G). (D) Lip biopsy demonstrating a double-positive cell (arrow), single-positive cells for CD20 (arrowhead), and a single-positive cell for IgE (asterisk) that is a mast cell. (E) Single cell positive for CD20. (F) CD20+ IgE+ B cell. (G) Single cell positive for IgE. Original magnification 20 $\times$ (A–C), 40 $\times$ (D–G).

Sections were then stained with antibodies to IgM, IgA, IgG, and IgE in order to evaluate local immunoglobulin expression (Fig. 1). It was not possible to detect surface IgG or IgA due to the background of secreted immunoglobulin, although it was clear that IgG-secreting plasma cells were consistently more numerous than IgA-secreting cells. It was also apparent that some cells expressed surface IgM and IgE. IgE+ cells included both mast cells that were CD20– and B cells that were CD20+. The CD20+ cells that included the IgE-expressing subset were predominantly large CD20+ B cells with irregular morphology. In this article we refer to these cells as subepithelial dendritic B cells.

CD20+ and IgE+ double-stained cells were counted at high power (original magnification $\times 40$) on 57 double-stained samples to determine the percentage of CD20+ B cells that had IgE on their surface (Fig. 2A). Lip biopsies from patients with OFG contained significantly more CD20+ B cells expressing IgE than any of the other oral conditions studied ($P < 0.0001$). To confirm that the IgE that was expressed on CD20+ cells was produced locally, we used PCR to identify $V_H-C\epsilon$ transcripts using cDNA from all

8 frozen oral samples. $V_H-C\epsilon$ transcripts were amplified from the only frozen OFG sample available for our study, but not from any of the other oral samples (Fig. 2B).

Phenotype and Localization of Oral Mucosal B Cells

Subepithelial dendritic B cells in oral mucosa that expressed IgE in OFG were characterized. Diffuse lymphocytic infiltrates, whether within normal histological limits or components of marked inflammation included subepithelial dendritic B cells. This finding did not vary according to site of biopsy or histological diagnosis. These cells had cytoplasmic processes that frequently made contact with adjacent cells, including both T lymphocytes and macrophages (Fig. 3). They did not express markers of naïve B cells including IgD or the low-affinity IgE receptor CD23, also confirming that the IgE staining observed was produced by the B cell and not a consequence of binding to this receptor (Fig. 4). The subepithelial dendritic B-cell population included cells that expressed the proliferation antigen Ki67 (Fig. 5). Of the 55 paraffin-embedded

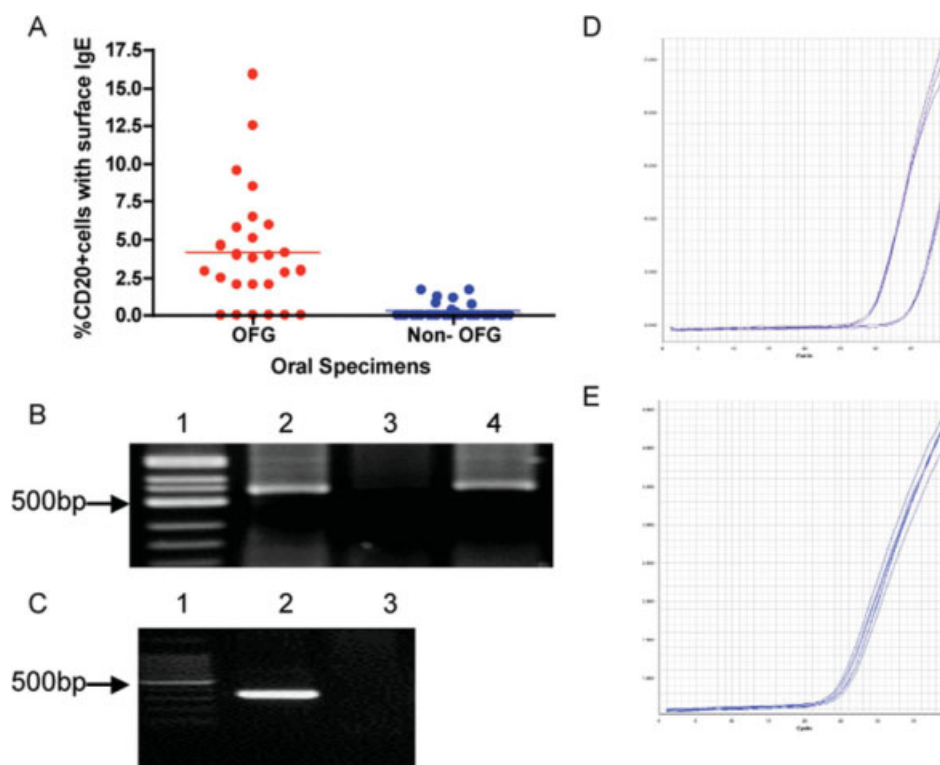


FIGURE 2. Quantification of IgE⁺ CD20⁺ cells and amplification of AID and V_H-C ϵ transcripts. (A) The percentage of CD20⁺ cells that had surface IgE were quantified from 57 oral samples. (B) Amplification of V_H-C ϵ transcripts. Products were subjected to electrophoresis on a 3% agarose gel. Lane 1, 100-bp DNA ladder (500 bp indicated); Lane 2, tonsil; Lane 3, biopsy from patient with lichen planus; Lane 4, biopsy from patient with orofacial granulomatosis. (C) Amplification of AID transcripts. Lane 1, DNA ladder; Lane 2, moderately inflamed biopsy from patient with orofacial granulomatosis; Lane 3, negative control. (D) Real-time PCR amplification plot for AID mRNA shown from patient with orofacial granulomatosis alongside tonsil germinal center control. (E) Real-time PCR amplification plot for GAPDH run in parallel with PCR from D.

biopsies with lymphocytic infiltration, this marker was found in 10% of the subepithelial dendritic B cells.

Subepithelial Dendritic B Cells Are Not Associated with Local Organized Lymphoid Tissue

Subepithelial dendritic B cells resembled the large interfollicular B cells²⁹ that are present in the T-cell zones of organized lymphoid tissues, in their morphology, their CD20⁺ and IgD[−] phenotype, and proliferation status. We therefore sought to determine whether the B cells we observed were associated with follicular structures by staining serial sections of all biopsies with CD21 that recognizes follicular dendritic cells (Fig. 4). Only 1 follicle was observed in the 70 paraffin-embedded biopsies studied, and no follicular structures were present in the frozen tissue samples. To exclude the possibility of the presence of lymphoid structures at different levels in the tissue, 2 markedly inflamed lip biopsies were stained every 5 μ m alternately for the expression of CD20 and CD21. There was no evidence of germinal centers at any level despite the presence of abundant subepithelial dendritic B cells.

Subepithelial Dendritic B Cells Express AID

Large interfollicular B cells express intense cytoplasmic AID.²⁹ Sequential sections of oral mucosa were stained to detect CD20 and AID. Subepithelial dendritic B cells did not express cytoplasmic AID, but some cells showed weak nuclear reactivity, although this was not conclusive. To examine this further, RNA was isolated from frozen sections of 7 biopsies of oral mucosa from patients with lichen planus. In each case the presence of subepithelial dendritic B cells and the lack of germinal centers were confirmed in adjacent sections. AID transcripts were PCR-amplified from the 3 samples with a marked lymphocytic infiltrate (Fig. 2C). To verify that AID expression was associated with the lymphocytic infiltrate, RNA was isolated from the lymphocytic infiltrate from an additional single frozen lip biopsy from a patient with OFG by laser capture microdissection and assessed for the expression of AID mRNA by real-time qRT-PCR. In parallel, germinal center cells from a tonsil were microdissected and used as a positive control for AID mRNA. AID mRNA relative to the expression of GAPDH was detected in the lymphocytic

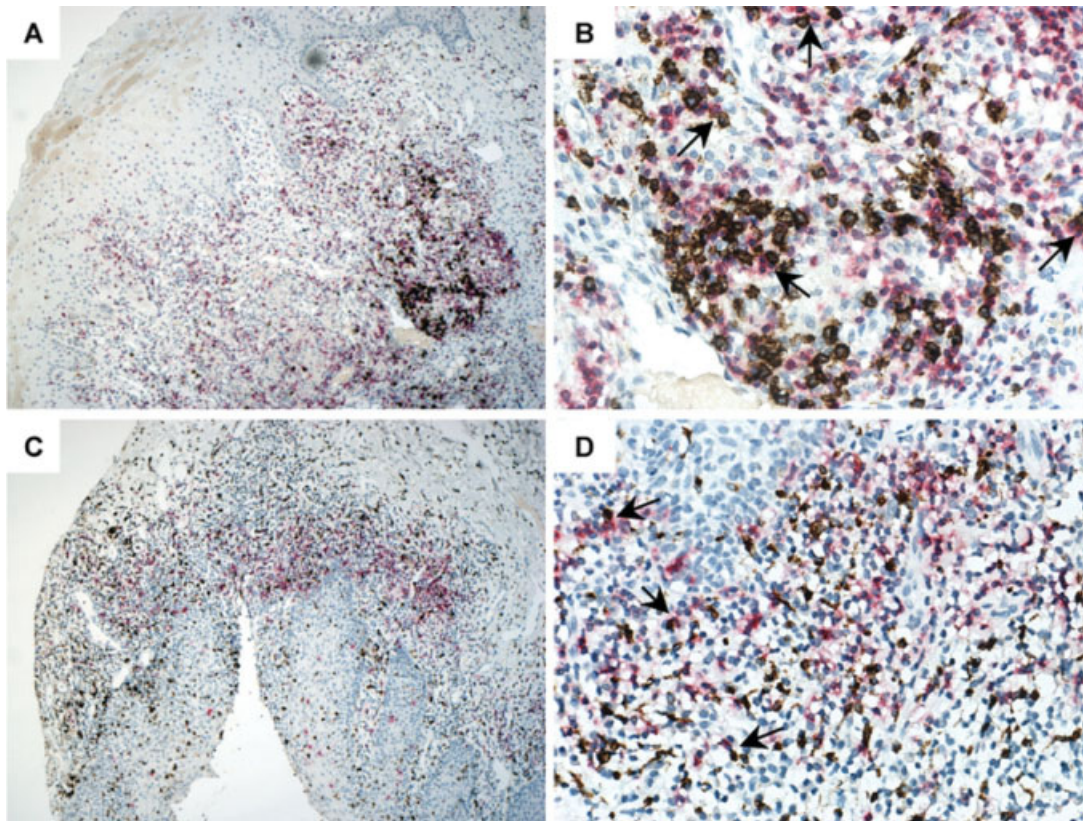


FIGURE 3. Microanatomical relationship between CD20+ cells and CD3+ cells and CD68+ cells. (A,B) Double staining of CD20 (in brown) with CD3 (in pink). (C,D) CD20 (in pink) with CD68 (in brown). These demonstrate contact between B cells and T cells and cells of the macrophage/monocyte lineage (arrows). Original magnification 10 $\times$ (A,B), 20 $\times$ (C,D).

infiltrate in OFG at a level that was 20-fold less than that detected in the germinal center from the tonsil (Fig. 2D,E).

IGHV genes sampled from subepithelial dendritic B cells are mostly mutated but are not typical of IGHV genes sampled from mucosal B cells

Overall, 27 different IGHV genes were isolated from the single biopsy of OFG available. Fifteen were from multiplex PCR of all IGHV families and a further 12 from family-specific PCR. All sequences were mutated by somatic hypermutation (mean 16 mutations, range 4–49 mutations). The JH repertoire was biased toward JH6 (10/27). In addition, 3 examples of JH1 were observed that are rare or absent in mucosal B-cell populations.³⁰

DISCUSSION

OFG is a chronic orofacial inflammatory disorder with significant associated morbidity. The condition often affects young adults and teenagers in whom facial disfigurement also results in significant psychological distress. Some cases of OFG present in association with intestinal CD but most OFG occurs as a separate entity. The etiology remains unclear but a role for dietary antigens has become

increasingly apparent, with dietary exclusion therapy a successful first-line treatment option.²³ In this study we describe a novel subepithelial dendritic B-cell population in oral biopsies from patients with OFG that are active and show evidence of class switching to IgE, providing a link between dietary antigens as a trigger and the ensuing chronic oral inflammation.

Subepithelial dendritic B cells described in this article have characteristics that distinguish them from other B-cell subsets in secondary and peripheral lymphoid compartments. Unlike naïve B cells, they are IgD– and CD23–. Although they proliferate, they are not germinal center cells because they are not associated with follicular dendritic cell networks. In contrast with large interfollicular B cells, they do not have cytoplasmic AID and are not associated with lymphoid follicles.²⁹ The plasmablasts that home to gut lamina propria before differentiation to plasma cells are CD20–, and have not been observed in cell cycle in human gut.³¹ In contrast, subepithelial dendritic B cells express CD20 and are proliferative. Unlike subepithelial dendritic B cells, plasma cells are nonproliferative. Marginal zone B cells are medium-sized cells that do not have cytoplasmic processes and so differ in morphology from

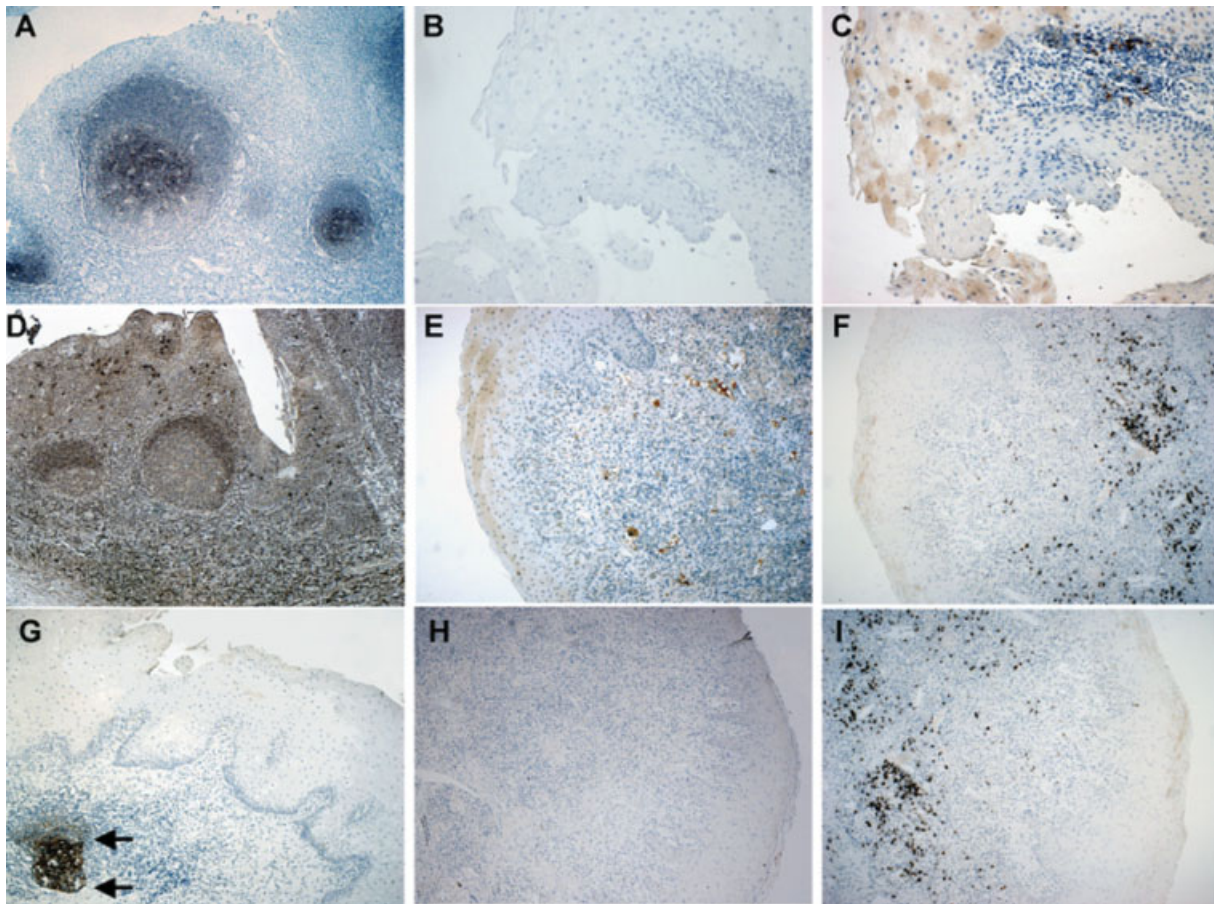


FIGURE 4. Subepithelial dendritic B cells do not express markers of mantle zone cells and are not related to follicular structures. (A) CD23 staining in the mantle zone of a tonsil control. (B) Example of lack of CD23 staining in oral samples. (D) IgD staining in the mantle zone of a tonsil control. (E) Example of lack of IgD staining in oral samples. (C,F) Serial sections to those in B and E were single-stained for CD20 to confirm the presence of B cells. Oral samples were single-stained for CD21 (G,H) and CD20 (I) (all markers in brown). (G) Staining of a follicle from a patient with orofacial granulomatosis with CD21 (arrows). Despite a thorough histological examination, only 1 follicle was seen in all samples examined. (H) The majority of oral samples were CD21-negative as this sample of OFG demonstrates with no organized lymphoid tissue. (I) Serial section to that shown in H was single-stained for CD20 to confirm the presence of B cells. Original magnification 10× (A–I).

the subepithelial dendritic B cells described here. The population that most resembles subepithelial dendritic B cells is the thymic B-cell population.³² Thymic B cells are also large, proliferative, germinal center-independent cells that have cytoplasmic processes that make contact with adjacent T cells, although clearly the microenvironment in which they exist is distinct and different from that described here. We therefore propose that subepithelial dendritic B cells are a previously unidentified B-cell subset that could play a significant role in oral immune responses.

It is not possible to know from this study whether the cells observed are resident, in transit, or recruited to the oral mucosa as part of an inflammatory response, although they were present in lymphocytic infiltrates that were considered to be within normal limits. The IgHV genes of sub-

epithelial dendritic B cells have undergone the process of somatic hypermutation and class switch recombination. This may indicate that they are the progeny of a germinal center response that would suggest that they migrate to the oral mucosa. AID expression, which would be an absolute requirement for hypermutation and class switch recombination, was detected at both the protein and mRNA level, indicating that subepithelial dendritic B cells have the potential to diversify independently from organized lymphoid tissue. Although germinal center-independent hypermutation has been proposed, the cells that undergo this process have not been identified.

It has been proposed that the diffuse B-cell infiltrate in the lamina propria of the gut may express AID and undergo class switch recombination. A study from our

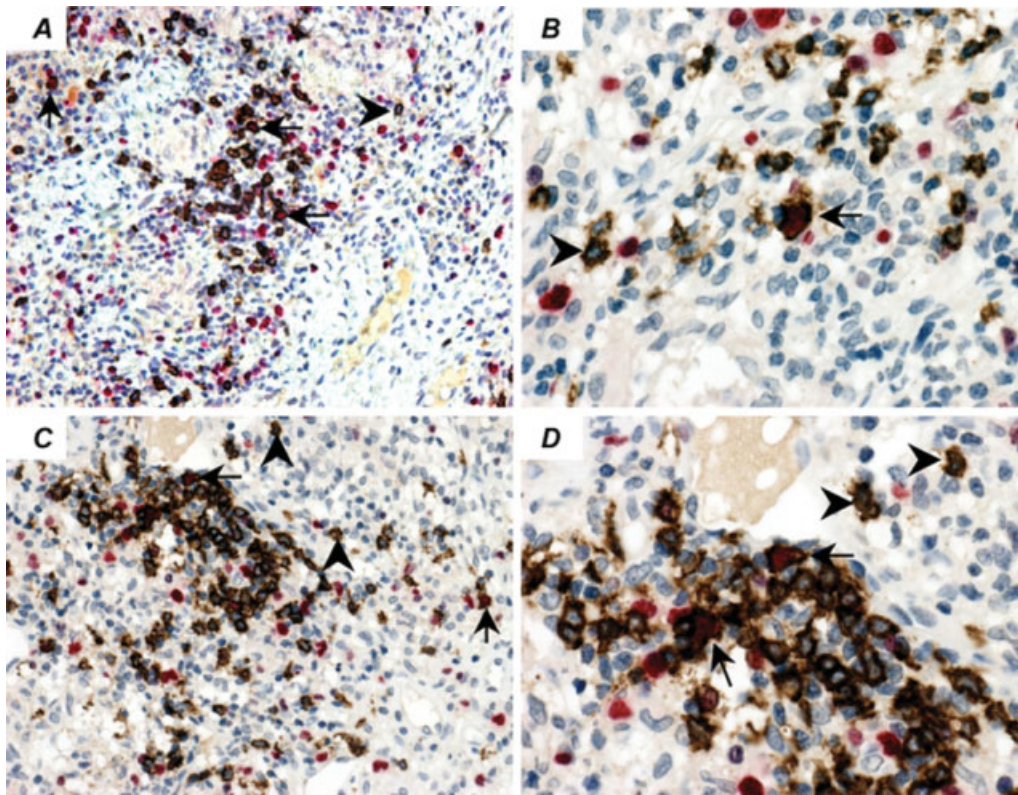


FIGURE 5. Proliferation of subepithelial dendritic B cells. Oral samples were double-stained for CD20 (in brown) and the nuclear proliferation marker Ki67 (in pink). (A,B) Lip biopsy from a patient with orofacial granulomatosis showing CD20+ and Ki67+ cells in the CD20+ B cell population (arrows). (C,D) Biopsy from a patient with gingivitis showing CD20+ and Ki67+ cells in the CD20+ B cell population (arrows). CD20+ cells that are not proliferating (Ki-67–) are shown with arrowheads in all fields. Original magnification 20 $\times$ (A,B), 40 $\times$ (C,D).

laboratory that did not observe lamina propria AID, excluded samples of lamina propria containing CD20+ cells because these may indicate adjacent lymphoid tissues in the gut.³¹ The lack of any organized lymphoid tissues in oral mucosa enabled the characterization of this follicle-independent B-cell subset, but it may ultimately be found to have a broader distribution.

Our data suggest that immune responses in the oral cavity may occur outside MALT structures. The relationship between subepithelial dendritic B cells and the gut immune system is unclear, partly due to the association between CD20+ cells and organized lymphoid tissue in the gut. Subepithelial dendritic B cells described here cannot be tied specifically to mucosal or systemic compartments. Although they are clearly situated at a mucosal interface and have highly mutated Ig genes, they show examples of IgHJ1 usage that have not been observed in the mucosal B-cell response in the gut other than in ulcerative colitis.^{31,33} In addition, subepithelial dendritic B cells exist adjacent to (predominantly) IgG secreting plasma cells. It is possible that subepithelial dendritic B cells may generate

this population, again suggesting that they may contribute to systemic responses. Sublingual immunization has been shown to generate mucosal and systemic B-cell responses.^{34,35} It is possible that subepithelial dendritic B cells that are in the oral mucosa, albeit sparsely in normal tissues, may play a role in specific oral and systemic immunity.

Subepithelial dendritic B cells were consistently present in oral biopsies, irrespective of site or degree of inflammation. IgE expression was detected on the surface of CD20+ cells in most parts of the mouth but significantly more often in biopsies from OFG (Fig. 3). Our group and others have previously demonstrated that OFG is associated with certain dietary sensitivities. Exclusion of cinnamon and benzoate from the diet provided an objective measure of disease improvement in over two-thirds of patients studied.²³ How these substances contribute to the development of OFG is as yet unclear. The subepithelial dendritic B cells, in particular the subset that are class switched to IgE identified in this study, may be relevant to the pathogenesis of this and other oral conditions by providing a bridge

between specificity and hypersensitivity responses. Allergic phenomena, particularly atopy, is a frequent observation in patients with OFG but the involvement of allergy in pathogenesis of OFG remains speculative.^{22,36} It has been shown previously that different regions within the oral cavity have varied diffusion rates to chemicals.³⁷ It has also been shown, experimentally at least, that this permeability is sufficient for allergen uptake, as allergens can reside within the oral cavity for up to 20 hours after application.^{38,39} It is thus plausible for dietary antigens to be absorbed in the oral mucosa and to cause local hypersensitivity reactions.

In conclusion, we describe for the first time a population of B cells that are present in the oral mucosa as a component of lymphocytic infiltrates. They have unique features that differentiate them from known B-cell populations. It is likely that they play a significant role in the immunological protection of the oral mucosa and possibly responses to locally administered vaccines. They are abundant in oral inflammation and may have a central role in the pathogenesis of oral diseases, including hypersensitivity responses. The role of diet in the treatment of OFG has already been demonstrated with the successful use of a cinnamon- and benzoate-free diet. The subepithelial dendritic B cells described here may play a central role in the pathogenesis of hypersensitivity reactions in OFG. Further understanding of the role these cells play may lead to more successful treatments for this difficult condition.

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