Chronic ulcerative stomatitis (CUS) is a mucocutaneous disease primarily involving mucosal surfaces, but occasionally may involve the skin. Clinically, CUS patients exhibit erosive or ulcerative lesions of the oral mucosa that resemble erosive oral lichen planus. Direct immunofluorescence (DIF) studies of mucosal or skin biopsies reveal a unique pattern of IgG immunoglobulin bound to nuclei of keratinocytes of the basal and lower one third cell layers, the stratified epithelial specific (SES) antinuclear antibody (ANA) pattern. Patient sera also exhibit circulating SES-ANA reactions on indirect immunofluorescence (IIF) using an esophagus substrate. We report the clinical and immunopathologic findings of 3 cases of CUS and demonstrate autoantibody recognition of the CUS antigen on Western blot. An important reason to distinguish CUS from other oral ulcerative conditions is that it may be refractory to standard treatments with topical corticosteroids, and favorable clinical responses may be achieved with hydroxychloroquine pharmacotherapy. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;96:718-26)
bination of small doses of steroids and hydroxychloroquine may be needed to induce remission.\(^4\)\(^-\)\(^6\)\(^,\)\(^8\)\(^,\)\(^9\) Only 31 cases of CUS have been reported in the literature.\(^3\)\(^-\)\(^5\) In this article, we report 3 additional cases of CUS with emphasis on the immunologic features.

**CASE DESCRIPTIONS**

**Case #1**

A 54-year-old Caucasian woman was referred to a periodontist with a chief complaint of “gum soreness.” Her past medical history was significant for 2 previous episodes of bullous skin lesions at ages 38 and 51 that were diagnosed clinically as LP by her primary physician and a dermatologist. The skin lesions were treated with systemic and topical corticosteroids, although she did not recall their names. She correlated the onset of her oral symptoms with an increase in psychological stress that occurred in the previous 2 months. The only medication she was taking at that time was a conjugated estrogen, for hormone replacement.

On oral examination, a prominent finding was the bilateral presence of deeply erosive lesions on the buccal mucosa adjacent to the molar areas (Fig 1). A clinical diagnosis of erosive oral LP was made, and lesional tissue biopsies were submitted for hematoxylin and eosin (HE) and DIF. Histopathologic examination showed a specimen that was devoid of epithelium and covered by a fibrinopurulent exudate with a mixed inflammatory infiltrate in the underlying connective tissue. The diagnosis was nonspecific ulceration of the buccal mucosa.

DIF revealed an SES-ANA reaction in the lower levels of the stratified squamous epithelium (Fig 2, A) characteristic of CUS. Serum studies were requested for IIF, and positive findings confirmed the diagnosis of CUS (Table I).

The initial treatment consisted of scaling and prophylaxis of the teeth and prescriptions for chlorhexidine and 0.05% fluocinonide ointment. When the patient returned for a 3-month follow-up, she had not filled the prescriptions but reported that she currently was comfortable despite refractory areas of mucosal erosion. At this visit, the patient reported feeling less stress, which seemed to correlate with the symptomatic improvement of her condition.

**Case #2**

A 71-year-old Caucasian woman with a several-year history of the chief complaint of “sore mouth and tongue and red gums” was referred to a periodontist. The patient complained of tongue and gingival hypersensitivity to spicy foods as well as xerostomia. Past medical history was significant for hypertension and “heartburn.” Her systemic medications included atenolol, omeprazole, and hydrochlorothiazide with triamterene. Clinically the patient presented with multifocal inflammation and prominent areas of desquamation of the gingiva (Fig 3), buccal mucosa, and palate. In addition, focal areas of the attached gingiva displayed
white lichenoid striae. No history of skin lesions was obtained nor evidence of cutaneous involvement was seen at the time of examination.

A clinical diagnosis of oral LP was made and gingival biopsies were taken for H&E and immunofluorescent examination. Histopathologic examination showed features typical of oral LP. The parakeratinized, stratified squamous epithelium was atrophic and a “band-like” lymphocytic inflammatory infiltrate, containing occasional plasma cells, was present in the papillary lamina propria. In addition, basal cell hydropic degeneration and cytoid bodies were observed (Fig 4). Focal areas of degeneration created splitting of the epithelial connective tissue interface. The microscopic diagnosis rendered was LP of maxillary gingiva.

DIF showed SES-ANA type deposits of IgG (Fig 2, B) and IgA in the nuclei of the basal and parabasal layers of the stratified squamous epithelium. Serum studies were requested for IIF, and positive findings confirmed the diagnosis of CUS (Table I).

The initial treatment consisted of topical applications of 0.05% clobetasol propionate to areas of soreness every 12 hours. This was discontinued after 10 days because it did not provide relief and the patient experienced side effects (burning tongue and throat and candidiasis). The patient’s primary care physician instituted therapy with hydroxychloroquine (200 mg/day). After 7 months of treatment the patient reported a decrease in gingival soreness, although she still experienced sensitivity to spicy foods and the gingiva continued to be erythematous. The patient discontinued the medication on her physician’s advice 2 weeks before a periodontal surgical procedure and the mucosa reverted to its appearance at the initial presentation. One month after

### Table 1. Indirect immunofluorescence serum studies

<table>
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<td>SES-ANA pattern</td>
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*Ab*, antibody; *IgG*, immunoglobulin G; *ME*, monkey esophagus; *SES-ANA*, stratified epithelial specific-antinuclear antibody; *GPE*, guinea pig esophagus; *MK*, mouse kidney; *HEp2*, human epithelial cell line.

Fig 2. Direct immunofluorescence staining of nuclei of basal and epithelial cells in the lower third of the epithelium (A, Case 1; B, Case 2) (fluorescent stain; original magnification ×200 and ×400 respectively).
resuming hydroxychloroquine therapy, the patient reported an increase in oral comfort and the tissues appeared less inflamed and erythematous.

Case #3

A 39-year-old Caucasian female was referred to a periodontist for evaluation of gingival lesions. She presented with a chief complaint of 2 months of progressively worsening “sore gums.” Her medical history was significant for Type I diabetes, hypertension, ehrlichioses, and glaucoma. Her medications consisted of insulin, lisinopril, atorvastatin calcium, and timolol. Clinically, the patient presented with erythematous and flat to slightly raised white lesions primarily localized to

Fig 3. Erosion and ulceration of buccal attached and interproximal gingiva (Case 2).

Fig 4. Histologic findings in biopsy from lesional attached gingiva. Insert shows a “band-like,” subepithelial inflammatory cell infiltrate and parakeratosis. The square in the insert indicates the enlarged area that shows basal cell hydropic degeneration and numerous intraepithelial cytoid bodies (Case 2) (hematoxylin-eosin; original magnification ×20 and ×100, respectively).
the maxillary and mandibular buccal attached gingiva (Fig 5). The left buccal mucosa had a lichenoid pattern of white striae, erythema, and erosion adjacent to the molar and premolar area. No history of skin lesions was retrieved, nor evidence of cutaneous involvement was seen at the time of examination.

A clinical diagnosis of oral LP was made and lesional and perilesional biopsies were taken for immunological studies and H&E examination. Histopathologic examination showed a specimen surfaced by stratified squamous epithelium with hyperorthokeratosis and irregularly spaced rete ridges extending a short distance into the subjacent stroma. In discrete areas there was a focal loss of the basal cell layer and formation of “sawtooth” rete ridges, although these areas were not associated with a prominent inflammatory cell infiltrate. A patchy, predominantly lymphocytic, inflammatory cell infiltrate with scattered plasma cells was seen in the deeper lamina propria. At one edge of the specimen the inflammatory cell infiltrate was more dense and included occasional eosinophils and neutrophils. The diagnosis provided was chronic inflammation of the gingiva.

DIF revealed the SES-ANA pattern of IgG deposits that were most prominent in the nuclei of basal and parabasal epithelial cells. Serum studies were requested for IIF, and positive findings confirmed the diagnosis of CUS (Table I). The patient has not returned to the office for follow-up or treatment.

SEROLOGIC STUDIES

Serum samples were obtained from all 3 patients for IIF and immunoblotting.

MATERIALS AND METHODS

Indirect immunofluorescence

HEp2 cells and 4-μm frozen sections of monkey esophagus (ME), guinea pig esophagus (GPE), and mouse kidney (MK) were obtained from IMMCO Diagnostics, Buffalo, NY. IIF studies were performed as described by Beutner et al.10

CUS protein preparation

The cDNA for the human cus, in pThioHis A (Invitrogen, Carlsbad, Calif) was kindly provided by Wendy C. Weinberg, PhD, Center for Biologics Evaluation and Research, Food and Drug Administration. Plasmid DNA was prepared by transforming Select96™ Competent Cells (cat. #L3300, Promega, Madison, Wisc) as per manufacturers instructions, recovered, then purified with the Wizard® Plus Midiprep DNA purification System (cat. #A7640, Promega). Primers were designed for PCR amplification using the published sequence.7 The primer sequences were as follows:

**Forward**

GGATCCTAATACGACTCACTATAGGGAAACAGCTAACATGTTGTACCTGGAA

**Reverse**

TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCACCCCCTCCTCCTTTGATGC.

Gel purified primers were purchased from Integrated DNA Technologies (Coralville, IA). Amplification using the AccuPrime polymerase chain reaction (PCR) system (cat. # 12342-010, Invitrogen), used as per manufacturer’s instructions, gave a 2-kb PCR product as expected (data not shown). The TNT® T7 Quick for PCR System (cat. #L5540, Promega) was used as per manufacturer’s instructions, with the addition of 2 μL of the Transcend™ tRNA Translation Detection Sys-
tem (cat. #L5070, Promega), to express CUS protein directly from the PCR product as a biotinylated in vitro translated product (IVTP). A negative control was prepared by performing the in vitro translation, without addition of the PCR product.

**Western blotting**

For Western blotting, CUS IVTP protein was prepared as described above and 2 μL of the biotinylated CUS protein or the negative control reaction was loaded onto each lane of a NuPAGE™ NOVEX 4-12% Bis-Tris precast gel (cat. #NP0322, Invitrogen) for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by immunoblotting. Direct detection of the biotinylated CUS protein was accomplished with Western Blue® Stabilized Substrate for Alkaline Phosphatase (cat. #S384C, Promega). For immunodetection of CUS autoantibodies, serum from all 3 cases was used at a 1:50 dilution. Alkaline phosphatase-conjugated goat antihuman IgG (cat. #A3312, Sigma, St. Louis, Mo) was used as the secondary antibody at a 1:100 dilution. Mouse antihuman p63 monoclonal antibody 4A4, specific for CUSP (the ΔN isoform of p63) was purchased (cat. #OP132, Oncogene Research Products, San Diego, Calif) and used at a dilution of 1:500. Alkaline phosphatase-conjugated goat antimouse IgG (cat. #115-055-062, Jackson ImmunoResearch Laboratories, Inc, West Grove, Pa) was used as the secondary antibody at a 1:100 dilution. BCIP/NBT substrate was used to develop the blots.

**RESULTS**

**Indirect immunofluorescence**

All 3 cases showed positive SES-ANA staining in the basal epithelial layer of GPE, (Fig 6, A) and ME (Fig 6, B). The antibody titers were generally higher on the GPE (Table I), which is in agreement with previous cases reported in the literature.5,8 MK and HEP2 cells usually yield negative or low antibody titers, findings that were reflected in our series. Table 1 summarizes these findings.

**DISCUSSION**

CUS is typically a disease of middle-aged women (average age at diagnosis of 58.9 years) characterized by the presence of atrophic or erosive lesions of the oral mucosa that heal without scarring and display alternated periods of exacerbation and remission. CUS may be indistinguishable clinically from oral LP, lichenoid stomatitis, mucous membrane pemphigoid, dermatitis herpetiformis, linear IgA disease, pemphigus vulgaris, erythema multiforme, pyostomatitis vegetans, and epidermolysis bullosa acquisita. About 14% of CUS cases exhibited concurrent, biopsy-proven, cutaneous LP. None of our cases were accompanied by synchronous skin lesions and all were diagnosed clinically as oral LP.

The light microscopic features (H&E) of CUS are
reported to be reminiscent and in some cases, indistinguishable from oral LP. In our series, 1 case met the microscopic criteria of oral LP while the other 2 cases displayed nonspecific findings. The histopathological variability of CUS and oral LP warrants the use of DIF.

Both circulating and tissue-bound IgG antibodies to a keratinocyte nuclear antigen are required for the diagnosis of CUS. Only one case of a false negative result on DIF of the 31 cases in the literature has been reported. Because of the persistent nature of the disease and recalcitrance to treatment in this case, a serum sample was later examined and the diagnosis of CUS was made.

Preliminary diagnosis of CUS is nearly always made when there are characteristic positive findings on DIF. However, in vivo ANAs may be also be detected on DIF of biopsies from patients with idiopathic connective tissue diseases, eg lupus erythematosus, systemic sclerosis, and mixed connective tissue disease. The DIF pattern of ANAs in idiopathic connective tissue diseases is positive throughout the thickness of the epithelium, not limited to the basal and parabasal layers as in CUS. Because of the possibility of tangential sectioning or atrophic epithelium, it may not be possible to evaluate the location of the SES-ANAs with certainty. Thus, a false positive diagnosis for CUS cannot be ruled out without a serum sample for IIF.

The IIF panel for SES-ANAs consists of HEp2 cells, mouse kidney, and monkey and guinea pig esophagus sections. SES-ANA, limited to basal and parabasal epithelial cells on IIF using monkey and guinea pig esophagus, is considered diagnostic for CUS; IIF is negative or very low titer on HEp2 cells and mouse kidney sections. A positive SES-ANA on DIF, with a negative IIF, is indicative of a false positive DIF for CUS. There are no reports in the literature regarding the incidence of false positive cases of CUS on DIF.

In idiopathic connective tissue diseases IIF may be positive for ANAs on HEp2 cells and mouse kidney sections. In such cases, a positive SES-ANA on DIF with positive IIF on HEp2 cells and mouse kidney sections is inconsistent with a diagnosis of CUS (if the IIF is negative on monkey and guinea pig esophagus). In this situation, clinical correlations should be sought and the patient’s physician notified of the results.

Conflicting evidence has been reported in the literature regarding the correlation of antibody titers with disease activity in CUS. However, more recently, Chorzelski et al concluded that, although the titers show fluctuations and are frequently lower on improvement, their levels do not correlate with clinical activity.

Several studies have reported clinical remission of CUS, and even reduction in SES-ANA titers, with hydroxychloroquine (Plaquenil), a potent antimalarial drug. It has been suggested that the mechanism of action of hydroxychloroquine is an interference with the “antigen processing” mechanisms of macrophages and other antigen-presenting cells, resulting in down regulation of the immune response against autoantigenic peptides. In initial data from human clinical trials, it has been shown to inhibit the development of graft-versus-host disease in bone marrow transplant patients.

Caution should be exercised with this off-label use of hydroxychloroquine because significant side effects such as retinopathy, toxic psychosis, neutromyopathy, agranulocytosis, and aplastic anemia may arise, necessitating discontinuation of therapy. The neuromuscular and hematologic complications usually are reversible whereas the retinopathy is not. Therefore, close follow-up of patients who take hydroxychloroquine is warranted. Doses as low as 200 mg/day may induce
improvement (42% of patients) and in some cases (23%) complete clearing of oral lesions.5,6,8,9

A variety of topical steroids such as fluorocinide, betamethasone, clobetasol, dexamethasone elixir, and dapsone also have been used to treat CUS. All of them seem to improve symptoms. However, severe gastrointestinal side effects are commonly produced by dapsone.3,4,8,9,18

The antigenic target of CUS autoantibodies, the CUS protein, has been identified as a 70-kd nontransactivating isoform of p63 (CUSP, ΔNp63α, p73L, p68β15) and its gene has been cloned.7,19,20 Several groups have used immunoblotting to demonstrate the recognition of a 70-kd protein by CUS patient sera, using extracts from calf esophagus, cultured human keratinocytes, and epidermis.7,13,17,21,22 COS cells, which normally do not express CUS protein, were transfected with the CUS cDNA and nuclear protein expression was confirmed using CUS patient sera.7 On Western blots a mouse antihuman p63 monoclonal antibody, and sera from all 3 of our cases, recognized the 70-kd CUS protein that was translated in an in vitro system using the CUS cDNA. At this time the diagnostic protocol for CUS includes DIF and IIF, however these are in situ techniques. Our results indicate that in the future, the availability of immunoblotting may provide a more specific test to detect CUSP autoantibodies.

The CUS protein appears to be essential for epithelial development and regeneration, and the presence of autoantibodies in CUS raises the issue of whether they may interfere with normal CUS protein function, potentially leading to poor wound healing and chronic ulcerations.23 Clinically diagnosed oral erosive and ulcerative lesions and biopsied lesions with nonspecific histopathology that do not respond to standard therapies should be biopsied for DIF in an effort to establish a definitive diagnosis. A diagnosis of CUS on DIF should be followed up with IIF for confirmation. This diagnostic approach eliminates the need for numerous appointments with clinicians due to failure of treatment to resolve the condition. In addition, the expense derived from purchase of ineffective medications is eliminated.

The more widespread use of immunofluorescence studies for the evaluation of refractory atrophic and erosive oral lesions may ascertain the real prevalence of CUS and reduce morbidity from putative oral LP cases that are actually undiagnosed CUS.

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REFERENCES


Reprint requests:
Lynn Solomon, DDS
Department of Oral Diagnostic Sciences
School of Dental Medicine
State University of New York at Buffalo
3435 Main Street
Buffalo, New York 14214
lsolomon@buffalo.edu