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REVIEW ARTICLE

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RECURRENT APHTHOUS STOMATITIS: CLINICAL CHARACTERISTICS AND EVIDENCE FOR AN IMMUNOPATHOGENESIS*

ROY S. ROGERS III, M.D.

Department of Dermatology, Mayo Clinic and Mayo Foundation, Rochester, Minnesota, U.S.A.

Recurrent aphthous stomatitis is one of the most common diseases affecting the oral mucosa, and one of its variants is the most painful affliction of the oral mucosa. The lesions of recurrent aphthous stomatitis can be manifested as part of a broad spectrum of clinical disease ranging from the common minor aphthous ulcers to Behçet's syndrome. Differential diagnosis, although not often difficult, must include many conditions capable of producing erosive and ulcerative oral mucosal lesions. The salient features of recurrent aphthous stomatitis suggest that it is a heterogeneous entity. Recent immunologic investigations have focused attention on a possible immunopathogenesis and the evidence for this is reviewed.

Recurrent aphthous stomatitis (RAS) is one of the most common diseases affecting the oral mucosa, and one of its variants is the most painful affliction of the oral mucosa [1]. In the writings of William Shakespeare, some characters averred that they would be cursed with blisters of the tongue for speaking an untruth: "If I prove honey-mouth'd, let my tongue blister" (Paulina, *The Winter's Tale* 2.2.32). Juliet was not above cursing her nurse for shaming Romeo: "Blister'd be thy tongue for such a wish!" (Juliet, *Romeo and Juliet* 3.2.90). These references to "blisters of the tongue" might well refer to RAS, as the tongue is commonly involved (Fig 1).

Hippocrates (460 to 370 BC) is credited with the first use of the word "aphthai" in regard to oral diseases, although probably he was referring to thrush [2]. The first valid clinical description of RAS was published by Mikulicz and Kümmel [3] in 1888. Sibley [4] provided the first description of RAS in the English language in the *British Medical Journal* in 1899. He described what is now recognized as minor aphthous ulcers and attributed their cause to psychic stress, naming them "neurotic ulcers." Sibley's index case was a dowager woman whose husband had died and left her a meager pension. She attempted to maintain the style of life to which she had been accustomed and found this very difficult. Because of the psychologic stress of this situation and the coincidental RAS that she suffered, Sibley [4] hypothesized that the latter was a psychosomatic disease. Sutton [5] described the first case of major aphthous ulcers in 1911 and coined the phrase "periadenitis mucosa necrotica recurrens," although careful reading of his article reveals little mention of the glandular structures; indeed, he stated that the "deeper glands" were hardly affected. Both Cooke [6] and Lehner [7] pointed out the semantic confusion surrounding Sutton's designation, and for this reason the term "major aphthous ulcers" has supplanted "periadenitis mucosa necrotica recurrens" in the recent literature. The third type, herpetiform ulcers, was described by Cooke [1] in 1960. This author detailed the inability to discover a viral pathogen by cytologic, serologic, cultural, or histopathologic methods but advocated the adjective "herpetiform" because it describes the clinical appearance of the lesions.

In the past several years, RAS has prompted considerable interest and research. The purpose of this review is to describe the clinical features of RAS, to discuss the differential diagnosis, and to

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Reprint requests to: Dr. R. S. Rogers III, Mayo Clinic, Rochester, Minnesota 55901.

Abbreviations:
ADCC: antibody-dependent cellular cytotoxicity
BS: Behçet's syndrome
GSE: gluten-sensitive enteropathy
HU: herpetiform ulcers
IBD: inflammatory bowel disease
MiAU: minor aphthous ulcers
MjAU: major aphthous ulcers
RAS: recurrent aphthous ulcers

review recent investigations that suggest an immunopathogenesis for RAS.

CLINICAL DESCRIPTION AND CLASSIFICATION OF RAS AND ITS VARIANTS

Cooke [8] classified RAS into minor aphthous ulcers, major aphthous ulcers, and herpetiform ulcers (Table I). Minor aphthous ulcers (MiAU) are recurrent crops of 1 to 5 punched-out ulcers usually affecting the "movable" or nonkeratinized oral mucosa (lips, buccal mucosa, mucobuccal and mucolabial sulci, and tongue) (Fig 2). Major aphthous ulcers (MjAU) are recurrent, large, chronic, and usually solitary ulcers that begin as nodules, destroy deep tissue, and heal with scarring. MjAU affect the "movable" oral mucosa also as well as posterior mucosal surfaces (Fig 3). Herpetiform ulcers (HU) are recurrent, multiple, shallow, pinpoint ulcers that may affect any part of the oral mucosa (Fig 4). MjAU and HU are distinctly less common than MiAU.

Lehner [7] characterized these types from a study of 210 patients with recurrent oral ulcerations. Table II describes the characteristics of these three variants and is partially derived from Lehner's study.

Several studies of patients alleged to have RAS [2,9,10] have included from 8 to 13% of the patients with aphthous lesions of both the oral mucosa and genitalia. These patients had 1 of 3 types of recurrent oral and genital aphthous lesions: ulcus vulvae acutum, an incomplete Behçet's syndrome, or aphthous stomatitis et vulvitis.

Ulcus vulvae acutum was described by Lipschütz [11] in 1913 in association with recurrent aphthous stomatitis. The entity was established by Berlin [12], who accepted two variants. The first variant was an acute, gangrenous, self-limited, nonrelapsing, ulcerative disorder of the vulva accompanied by an infectious disease, frequently enteric fever. This condition occurred in young females with a sudden onset, fever, and gangrenous ulcers of the labia minora with sharp borders, a dirty, firmly adherent membrane, and an erythematous halo. These lesions healed spontaneously with scarring in 2 to 3 weeks.

The second variant that Berlin [12] accepted was a less acute, relapsing ulcerative disorder that he believed probably represented a forme fruste of Behçet's syndrome (BS). These lesions were associated with RAS. Recurrent oral and genital aphthous ulcerations in men, although less often encountered, also represent the incomplete BS.

Kierland (RR Kierland, personal communication) accepts a third variant called "aphthous stomatitis et vulvitis." This disorder is characterized by recurrent oral and genital aphthous ulcers and persists for long periods without evolving into the complete BS. The vulvar or genital ulcers are not always associated with the recurrent oral aphthous ulcers but are present with enough frequency to distinguish this group of patients from those with RAS.

Recurrent oral aphthae, alone or in association with genital aphthae, can antedate the complete picture of BS by months to years. The oral aphthae that occur in BS conform to any 1 of the 3 types described above; however, the incidence of HU and MjAU is increased in patients with this disease when compared with patients who have RAS

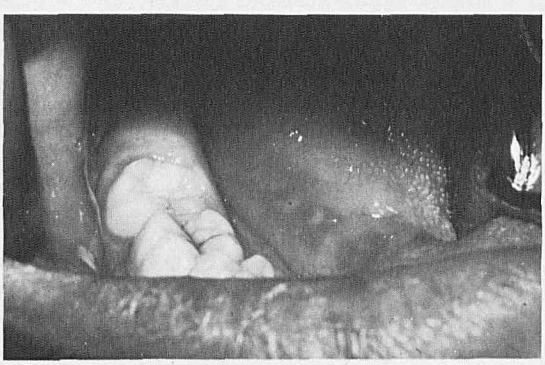

FIG 1. Aphthous lesions occurring on lateral border of tongue. Lesions such as these may have prompted Shakespeare to write about "blisters of the tongue." These minor aphthous ulcers are characterized by a central gray or yellow membrane and peripheral erythematous halo.

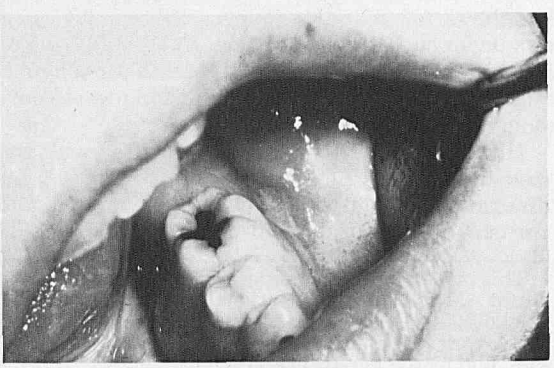

FIG 2. Typical lesion of minor aphthous ulcer. Note occurrence of lesion in mucobuccal sulcus and characteristic long oval conformation of lesions occurring in sulcus.

TABLE I. *Classification of recurrent aphthous stomatitis*

Type	Author	Year	% of cases[a]
Minor aphthous ulcers	Mikulicz & Kümmel [3]	1888	80
Major aphthous ulcers	Sutton [5]	1911	8
Herpetiform ulcers	Cooke [1]	1960	8

[a] The remaining 4% of patients with recurrent aphthous stomatitis fall into the other portion of the spectrum of the aphthosis diathesis (see text).

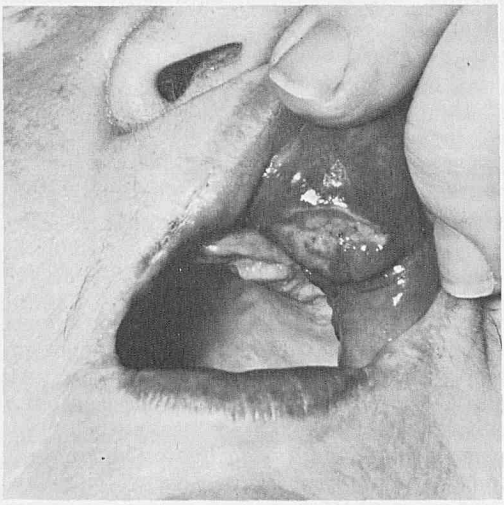

FIG 3. Large, nodular quality of Sutton's aphthae (major aphthous ulcers).

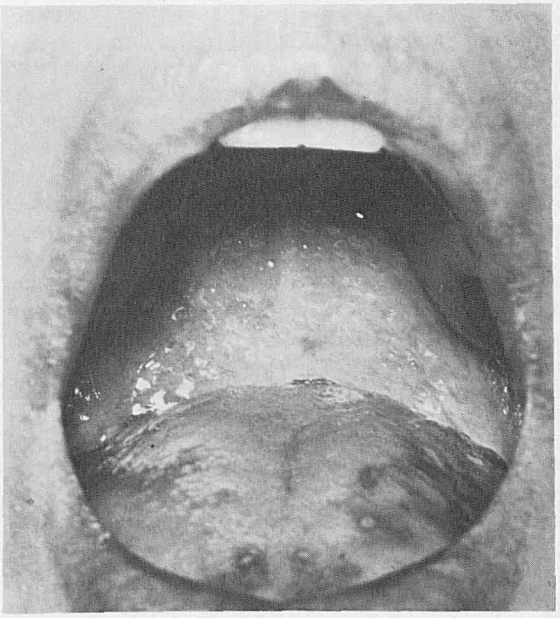

FIG 4. Diffuse distribution on oral mucosa of numerous small aphthae that characterize herpetiform variant of recurrent aphthous stomatitis.

TABLE II. *Characteristics of recurrent aphthous stomatitis*[a]

Characteristic	MiAU	MjAU	HU
Female-male ratio (% females)	1.3:1 (56)	0.8:1 (44)	2.6:1 (73)
Age at onset (yr)	10–19	10–19	20–29
Lesions			
Size (mm)	<10	>10	1–2
Site	"Moveable oral mucosa"		Entire oral mucosa
	Lips, cheeks, tongue	Lips, cheeks, tongue, palate, pharynx	
Number	1–5	1–10	10–100
Healing with scarring (%)	8	64	32

[a] Derived, in part, from Lehner's [7] study. MiAU = minor aphthous ulcers; MjAU = major aphthous ulcers; HU = herpetiform ulcers.

only [7]. A careful history and physical examination will often reveal the other characteristics of BS. The classic triad includes oral aphthous ulcers, ocular inflammation, and genital ulceration. With only 2 elements of the classic triad present, the addition of either synovitis or cutaneous vasculitis is sufficient for the diagnosis [13]. O'Duffy and Goldstein [14] would add meningoencephalitis to the latter group. As with other multisystem diseases, nonspecific symptoms such as fever, malaise, arthralgias, and myalgias may be noted. Other reported manifestations of BS include superficial and deep thrombophlebitis, epididymitis, colitis, pancreatitis, peripheral neuropathy, subungual infarctions, and malignant lymphoma [15]. The differential diagnosis should include erythema multiforme, systemic lupus erythematosus, Reiter's disease, Crohn's disease, and chronic ulcerative colitis.

SALIENT FEATURES OF RAS

In order to attempt to understand RAS and its variants with respect to pathogenesis, review of some salient features and associations may be helpful. The lesions of RAS may represent one disease, but they also may well represent mucosal manifestations of varying diseases.

The results of several epidemiologic studies [2,7,10,16–18] reflect both the common occurrence of RAS in the population at large and the inability to determine a single etiologic agent. However, no random population studies have been conducted to determine the true prevalence of this disease. The prevalence of RAS varies greatly with the demographic characteristics of the population studied. Higher rates occur among middle-class and upper-class professional students, intermediate rates among outpatients of general medi-

cal and dental clinics, and lower rates among indigent inpatients [16]. The range varies from 5 to 60%. A reasonable estimate of prevalence would be 20% [16]. Several authors [10] report a 2:1 female-to-male ratio of incidence, and Lehner [7] reported minor differences in sex incidence among the types of RAS. Sex incidences in his study are shown in Table II. RAS is typically a disease of young adults.

The stages of natural evolution of lesions of RAS have been synthesized by Stanley [19], who divides the natural history into the following 4 stages: premonitory, preulcerative, ulcerative, and healing. Stage 1, the premonitory stage, lasts for up to 24 hours. This stage is characterized by tingling, tense, burning, painful, raw, or hyperesthetic sensations in the absence of any clinical changes. Some patients do not report a premonitory stage. Stage 2, the preulcerative stage, lasts for 18 hours to 3 days. The painful sensation varies in intensity but is usually moderately severe. Clinically, the aphthae begin as erythematous macules or papules with slight induration. They are single or multiple, or circular or oval, depending on their location. The aphthae are surrounded by an erythematous halo and range from 2 to 20 or 40 mm in diameter. On the cheeks or lips, lesions are circular, whereas in the buccal or labial sulci or vestibule, oval lesions occur. Lesions overlying fibromuscular bands such as the frenum are exceptionally painful.

Stage 3, the ulcerative stage, lasts from 1 to 16 days. Early, these lesions are usually severely painful. Clinically, the papule or macule, which had begun to erode in the second stage, enlarges and ulcerates but remains a discrete lesion. The maximum size is usually attained 4 to 6 days after the onset; following this an indolent period sets in which persists until stage 4. The aphthae are gradually covered by a gray or yellow membrane and are surrounded by a dusky, red halo. Two or 3 days later, there is an abrupt cessation of pain, leaving residual discomfort that correlates clinically with the appearance of the covering fibromembranous slough. It should be noted that this clinical characteristic must be considered in any study purporting to demonstrate therapeutic efficacy which uses reduction of pain as a criterion. Stage 4, the healing stage, lasts from 4 to 35 days. The lesions usually heal without scarring in 10 to 21 days. Scarring occurs most commonly with MjAU and correlates with the depth of necrosis.

The natural history of RAS is one of eventual remission. Sircus, Church, and Kelleher [2] found no significant differences with respect to sex in the length of time that the disease persisted. These authors stated that approximately 2 of 3 patients with RAS will undergo remission within 15 years, whereas one of three patients will continue to have RAS lesions for periods up to 40 years. Lehner [7] found that most patients with MiAU had remissions by 15 years and that more than half of MiAU patients had remissions in less than 5 years. The duration of MjAU lesions was almost the opposite of that of MiAU lesions; almost two of three patients with MjAU (especially males) had activity for more than 15 years. Forty percent of patients with HU had activity for less than 5 years, 30% had activity for 6 to 15 years, and the remaining 30% had activity for more than 15 years.

Other salient features include clinical observations about RAS such as association with the menstrual cycle and induction of lesions by trauma.

The association of new RAS lesions with the premenstrual phase of the menstrual cycle has been reported [9,17]. The striking occurrence of remissions during the third trimester of pregnancy with prompt exacerbation after parturition lends credence to an endocrine association [9]. Dolby [20] demonstrated a consistently increased incidence of new ulcer formation during the 7 days after ovulation. This was associated with increasing progesterone and decreasing estrogen levels. Bishop, Harris, and Trafford [21] reported considerable improvement in 30 of 33 women with premenstrual aphthae treated with systemic estrogens in doses sufficient to suppress ovulation. Carruthers [22] was able to demonstrate a beneficial response with the use of oral anovulatory medications. This therapy was most successful with medications containing a high concentration of estrogen. Main and Ritchie [23] have studied the cornification index of the oral mucosa in relation to menstrual hormonal changes. The degree of cornification is low during the premenstrual phase (low estrogen), and this perhaps renders the oral mucosa more susceptible to trauma. The association of the hormonal changes and keratinization lends credence to the role of endocrine factors in some patients.

Trauma appears to play an important role in the induction of lesions of RAS. Clinically, self-inflicted, incidental dental trauma is by far the most common aggravating factor [24]. Lesions occur in areas of the mouth most often subjected to trauma and least well keratinized. Lesions allegedly have been produced by dental manipulation, but Ross et al [25] were unable consistently to induce lesions in susceptible patients by brushing, pinching, needle puncture, and scalpel incision. Some edentulous sufferers of RAS have remissions while awaiting completion of their dentures and have exacerbations once they are fitted.

The association of smoking and a decrease in the frequency and severity of RAS lesions in Dorsey's [26] patients lends credence to the concept of protection from trauma by oral hyperkeratosis. Bánóczy and Sallay [27] studied the oral cytology of a nonsmoking population, some with RAS, others with leukoplakia, and still others with no oral disease. They found significantly diminished keratinization in the patients with RAS when compared with those who had no oral disease and suggested that decreased keratinization was con-

ducive to the occurrence of ulceration in patients with RAS.

The most frequent sites of the lesions are the lips, cheeks, tongue, and sulci, where keratinization is less well developed than in other areas of the oral mucosa. The studies of Main and Ritchie [23] on keratinization and female hormones and of Bánóczy and Sallay [27] on the relative lack of hyperkeratosis in patients with RAS are pertinent to the role of trauma. One has little difficulty in consigning at least a permissive role to trauma in the induction of lesions of RAS.

Several studies [2,18,28,29] have indicated a familial tendency in RAS. It is not unusual to find that 1 or more first-degree relatives of a patient with RAS also have RAS, but there is no definite evidence that this is genetic in origin. The overall prevalence is so high that a chance familial association would not be unreasonable; however, there may be an immunogenetic basis for these observations, as has been described in psoriasis [30,31], pemphigus vulgaris [32,33], and dermatitis herpetiformis [34].

The association of RAS with gastrointestinal disease has been recognized for years [35]. According to DuBois and van den Berghe [36], the term "sprue" is derived from the Dutch word for aphthous disease, "spruw." Truelove and Morris-Owen [37] have emphasized the association of RAS with both idiopathic steatorrhea (celiac disease, nontropical sprue) and chronic ulcerative colitis. Sircus, Church, and Kelleher [2] believed that the incidence of RAS among the general population was so great that the association of RAS with gastrointestinal disease was probably of minor significance.

Ferguson et al [38] performed jejunal biopsies in 33 patients with RAS and found 8 with flat mucosal architecture consistent with gluten-sensitive enteropathy (GSE, celiac disease). All 8 responded to a gluten-free diet with clinical remission of their aphthous lesions. This response was not dependent on concurrent administration of hematinics. Unfortunately, there was no difference in the RAS/GSE patients when compared with RAS patients with respect to gut-related symptoms. The authors stated that, as a group, the RAS/GSE patients had lower mean hemoglobin and serum folate concentrations.

Recently, Wray et al [39] screened 130 consecutive outpatients with RAS for deficiencies of iron, vitamin B_{12}, and folate. Twenty-three of the 130 patients (17.7%) were deficient in 1 or more factors, whereas 11 of 130 (8.5%) age and sex-matched controls were deficient. The deficiencies were replaced, and all patients were followed for 1 year. Of the 23 RAS patients with deficiencies, 15 showed complete remission and 8 showed definite improvement. Of the remaining 107, 33 had a remission or improved. Underlying diseases included pernicious anemia, GSE, other malabsorption states, and other gastrointestinal diseases.

RAS is associated with chronic ulcerative colitis and Crohn's disease, collectively termed inflammatory bowel disease (IBD) [40]. Indeed, at times differentiation of IBD from BS can be difficult (RS Rogers III and JD O'Duffy, personal communications).

The heterogeneous character of RAS can also be documented by the evidence gleaned from claims of success of various therapeutic modalities. Often these successes [41,42] are not confirmed in other studies [43,44]. The recent enthusiasm for levamisole as a treatment for RAS provides evidence for the heterogeneity of the RAS population. Several small, uncontrolled studies [45,46] have reported the efficacy of levamisole in some patients with RAS. A larger, open trial [47] of 50 patients resulted in 6% with complete remission, 56% with improvement, and 38% with no response. A double-blind, crossover trial has been carried out in 47 patients by Lehner, Wilton, and Ivanyi [48], who reported 64% positive response and 36% nonresponders, of which 23% had negative responses (worsening). Agranulocytosis has developed during treatment with levamisole [49–51]. Whether this will limit the usefulness of the drug is not known at this time.

Evidence gleaned from therapeutic trials suffers from several shortcomings. First is the variable history of RAS lesions—specifically, their variable duration and frequency of recurrence. This variability casts doubt on results in individual patients and requires very large numbers of patients for confident conclusions. Second is the natural reduction of pain that occurs during the ulcerative stage when the lesion is covered by a fibromembranous slough. Utilization of pain reduction as a parameter of efficacy is therefore highly suspect. Third is the paucity of adequately controlled studies. At a minimum, 33 of 107 patients will report a remission or definite improvement with local treatment, as has been demonstrated by the study of Wray et al. [39]. Long-term studies on large populations with double-blind, crossover provisions are necessary. Finally, it appears from reviewing the literature on therapeutic intervention in RAS that almost anything rationally conceived will yield some benefit in some patients, including placebos.

Clearly, the population of sufferers of RAS is not a homogeneous one. Some patients with RAS may show evolution into BS and some may have, or may acquire, gastrointestinal diseases. The differentially responsive populations among the levamisole-treated RAS patients may demonstrate different etiologic factors related to the RAS. With these considerations in mind, a review of the evidence that supports an immunopathologic concept of the cause of RAS can be better appreciated.

EVIDENCE FOR AN IMMUNOPATHOGENESIS FOR RAS

Observations to support an immunopathogenesis for RAS can be gleaned from several sources. The association of RAS with certain diseases, the response of RAS to therapy, light and electron

microscopic observations, and in vitro immunologic studies relative to streptococcal and oral mucosal antigen hypersensitivity are observations that lend credence to an immunopathogenesis for RAS.

An immunopathogenesis has been proposed for IBD. This thesis is based on the demonstration of immunologic aberrations in both cellular and humoral immunity [52] and also an antibody-dependent cellular cytotoxicity mechanism demonstrated in vitro [53]. RAS lesions occur in IBD and often correlate with clinical activity of the disease [37,40]. BS is another disease for which an immunopathogenesis is suspected [15,16,54,55]. RAS lesions are a hallmark of BS and commonly reflect the clinical activity of the syndrome.

The dramatic resolution of typical RAS lesions in those RAS patients with GSE who were treated with a gluten-free diet [38] is another example of the correlation of RAS activity with the clinical activity of an underlying disease. GSE has been strongly associated with the HLA haplotype HLA-B8 [56], and this possibly implicates an immunogenetic basis for GSE. Other diseases with strong HLA-B8 associations include Graves' disease, myasthenia gravis, dermatitis herpetiformis, and chronic active hepatitis [57], diseases for which an immunopathogenesis has been proposed. RAS is not notably associated with these diseases. In light of the familial tendency of RAS and its association with GSE, study of HLA patterns, the Ir region, and the major mixed lymphocyte culture determinants may provide insight into the strong family history of RAS and may provide immunogenetic information with respect to the pathogenesis of RAS.

The demonstration of a small group of RAS patients with iron deficiency and the dramatic response to iron therapy [39] is another association that might be pertinent to immunopathogenic mechanisms. Chandra [58] has shown that the number of T lymphocytes, the phytohemagglutinin-induced transformation response, and cutaneous delayed hypersensitivity are reduced in iron-deficient subjects. These immunologic alterations relate to serum iron and transferrin saturation rather than to hemoglobin concentration. Furthermore, neutrophil function is impaired in iron deficiency.

Finally, the response of some subjects with RAS to levamisole [45–48], a drug thought to possess immunopotentiating activity, lends further circumstantial evidence to an immunopathogenesis for RAS.

Pathologic observations of early RAS lesions also provide circumstantial evidence for this thesis. A cell-mediated immunopathogenesis has been suggested by the histopathologic appearance of early lesions of RAS [10,19,59] which fulfills the histologic criteria of Coe, Feldman, and Lee [60] for a delayed hypersensitivity reaction. These criteria are (1) at least 3 clusters of mononuclear cells per cross section perivascularly or perineurally, (2) dispersed mononuclear cells in the dermis without obvious pattern, (3) polymorphonuclear leukocytes constituting less than 5% of the inflammatory infiltrate, and (4) absence of necrosis or smudging of the wall of venules.

Recent investigations into self-tolerance and autoimmunity, reviewed with respect to thyroid disease by Allison [61], imply that such mononuclear infiltrates as described above may not necessarily imply T cell-mediated immunopathologic damage. The present evidence also implies that a B lymphocyte-mediated immunopathologic mechanism with antibodies, immune complexes, antibody-dependent cellular cytotoxicity (ADCC), and K (killer) cells possibly play key roles.

Additional support for a delayed hypersensitivity reaction is the electron microscopic demonstration of the presence of cytoplasmic phagosomelike bodies in mononuclear cells that invaded the epithelium of early lesions of RAS [62]. This "invasive-destructive" reaction pattern is considered by Waksman [63] as the forerunner of delayed hypersensitivity damage to antigen-bearing epithelial cells. Electron microscopic studies by Honma [64] have demonstrated mononuclear cells in the prickle cell layer of early RAS and BS lesions that resemble activated T or B lymphocytes as well as phagocytic mononuclear cells resembling macrophages. Other pathologic studies have not provided evidence for vasculitis in the submucosal vessels or immune complex-type damage to the epithelial cells or vessel walls in early RAS lesions, although Lehner (personal communication) has reported the presence of immune complexes in the sera of patients with RAS and BS.

Studies of serum immunoglobulins revealed mildly elevated levels of IgA and IgG in all 3 types of RAS, with significant elevations in patients with MjAU. Serum complement levels (C3) have been normal. The incidence of autoantibodies to thyroid, gastric parietal cells, and nuclei did not differ from that in control populations. Cross-reactions between oral mucosa and a variety of oral bacterial sonicates were negative except for the *Lactobacillus acidophilus*, the significance of which has not been investigated [65].

Lehner [65] hypothesized that oral epithelial antigens or some microbial cross-reacting antigens act as the stimulus for the immunologic responses that have been noted for RAS. Lehner [65] favored a cell-mediated immune response on the basis of the available data at the time of his hypothesis. A review of recent immunologic investigations focusing attention on these two antigenic sources will permit an assessment of most of the evidence for an immunopathogenesis for RAS.

Historically, the discovery of new microorganisms has been reflected in the list of microbial agents evoked as etiologic culprits for RAS. Among these microorganisms studied and discarded are *Bacillus crassus, Mycobacterium tuberculosis, Candida albicans, Toxoplasma, Mycoplasma,* and the herpes simplex virus.

The pleomorphic L-form of the alpha streptococcus, *Streptococcus sanguis* ($2A_{2+3}$ HOT), became the thrust of the National Institute of Dental Research of the National Institutes of Health in the early 1960's, culminating with the Graykowski et al [10] provocative *JAMA* article in 1966. Barile et al [66] isolated pure cultures of an L-form from the base of lesions of RAS in three patients, and an identical organism was cultured from the blood of 1 of these patients during a severe exacerbation. In addition, cultures of biopsy specimens were also positive for the same organism. These authors [66] invoked the "L-form theory" to explain RAS. This theory presumes that the stable L-form is the dormant carrier state for recurrent infections and that it may live symbiotically in an intracellular location. Under appropriate conditions, the stable L-form may revert to the pathogenic form or a bacterium. They hypothesized that antibiotics given to a patient for another disease could stimulate conversion to the stable L-form and that trauma could cause the reversion to a pathogenic state. Stanley, Graykowski, and Barile [67] reported that 93% of lesions of RAS contained pleomorphic bacterial microorganisms as demonstrated by histochemical stains. These same forms were found in 47% of autopsy specimens and 40% of nonaphthous lesions. However, the pleomorphic forms are difficult to differentiate from parakeratin granules or mast cell granules by Giemsa stain. Tissue identification of these pleomorphic forms has not been established, although the authors favored the alpha streptococcus as a source. These investigators [67] suggested that their data supported their "L-form theory."

Graykowski, Barile, and Stanley [68] then injected 10^6 live transitional L-form organisms into rabbit skin and this produced a nodule in 12 hours. Clinically and histopathologically the nodule resembled a MjAU. The microbes were histochemically visible in the lesions, and the organisms were cultured from the lesion. This was reproduced in guinea pig oral mucosa.

Graykowski et al [10] then used heat-killed *S. sanguis* 2A organism in skin tests. They reported that a positive tuberculin-like response developed in 30 of 30 patients with RAS, whereas 1 of 4 patients with lichen planus and 1 of 6 normal patients had positive reactions.

Francis and Oppenheim [69] performed lymphocyte transformation tests using phytohemagglutinin, smallpox vaccine, and heat-killed streptococci, including the *S. sanguis* prototype used by Graykowski et al [10] in their skin test studies. These authors found a diminished response of lymphocytes from RAS patients when compared with controls when the streptococcal 2A antigen was used. The lymphocytes of RAS patients were not hyporesponsive to the control antigens or the other heat-killed streptococcal antigens. No suppressive plasma factors were found when the authors removed autologous plasma and studied the lymphocytes in normal plasma.

Donatsky and Bendixen [70] studied streptococcal hypersensitivity by the leukocyte migration technique [71], which is an in vitro parameter of cellular hypersensitivity. These authors found that 8 of 17 patients with RAS but none of 13 normal controls demonstrated cellular hypersensitivity to the streptococcal 2A antigen. Donatsky [72] has recently extended his studies of in vitro cell-mediated immunity by the leukocyte migration technique. The migration index of patients with RAS differed significantly from that of normal controls and controls with other oral diseases when the lymphocytes were exposed to antigenic extracts of *S. sanguis* 2A, *Streptococcus pyogenes* M5, and adult human oral mucosa. In a further study, Donatsky [73] demonstrated that the leukocyte migration index correlated with clinical activity of RAS when streptococcal 2A and adult human oral mucosa antigenic extracts were utilized. However, other antigenic extracts, including *S. pyogenes* M5, did not show clinical correlation.

Donatsky and Dabelsteen [74] examined sera of RAS patients and controls for the presence of humoral antibodies to the same streptococcal 2A antigen by means of a double-layered indirect immunofluorescent technique. Antibodies to the 2A antigen were demonstrable in both populations, but the mean end-point titers were significantly higher among the RAS patients when compared with controls. However, Donatsky [73] was unable to demonstrate differences in end-point titers in antibodies to streptococcal 2A, streptococcal M5, and adult human oral mucosa antigens with respect to clinical activity of the RAS.

Thus, there is strong evidence that a hypersensitivity response to the streptococcal 2A antigen exists. The positive delayed hypersensitivity skin tests, the positive in vitro leukocyte tests correlated with RAS disease activity, the hyporesponsive lymphocyte transformation tests, and the evidence of humoral antibody to the streptococcal 2A antigen may indicate that a specific subset of lymphomononuclear cells is implicated in the hypersensitivity response and that mechanisms such as ADCC may be pertinent.

The studies of Donatsky [72,73] indicate an in vitro immunologic response to an adult human oral mucosa antigen extract as well as to the streptococcal 2A antigen. Oral mucosal antigens have been studied by other investigators as a possible stimulus for the putative autoimmune pathogenesis of RAS. Oshima et al [54] demonstrated antibody directed against an oral mucosal antigen by the tanned-cell hemagglutination technique in 17 of 40 patients with BS. A similar antibody was found in the sera of 4 of 25 control patients and in none of 18 healthy controls. Twelve of the 17 patients with antimucosal antibodies had oral lesions. The authors stated that the titer was higher before attacks of BS and that it declined with remissions.

Humoral immune responses also have been im-

plicated by the demonstration of antimucosal antibodies to human fetal oral mucosa by the tanned-cell hemagglutination technique by Lehner [55,75]. This author found that the sera of 75% of 237 patients with MiAU and MjAU, 20% of patients with HU, and 86% of 21 patients with BS contained the antimucosal antibodies. Of controls, 10% possessed the antimucosal antibodies. These antibodies were found predominantly in the IgM class and to a lesser extent in the IgG class of antibodies. However, immune absorption studies demonstrated a lack of species or tissue specificity, and the antibody titers did not correlate with the course or the severity of the disease [76]. Lehner's study differs from that of Oshima et al [54], who noted correlation of titers with disease activity. Donatsky [73] and Donatsky and Dabelsteen [77] confirmed Lehner's work [55,75].

Using homologous adult human buccal mucosal tissue from an RAS patient and from a normal control subject as substrates for the indirect immunofluorescent test, Donatsky and Dabelsteen [77] studied the sera of 24 patients with RAS and 24 normal controls.The mean value of the end-point titer of the controls (1:2) was lower than that of the RAS group (1:8). A positive reaction was the apple-green fluorescence of the cytoplasm of the stratum spinosum cells. Rogers, Movius, and Jordon [78] have been unable to demonstrate an immunofluorescent pattern with the use of sera in a 1:10 dilution from RAS patients and monkey esophageal or human gingival mucosal substrates for the indirect immunofluorescent test.

Lehner [59] has demonstrated binding of fluorescein-conjugated immunoglobulins to oral mucosal tissue of 10 of 19 patients with MiAU, MjAU, and BS by the direct immunofluorescent technique. The third component of complement was bound in 5 of 19. This author also demonstrated binding of patients' sera to autologous oral mucosal tissue specimens in 10 of 19 patients by a modified indirect immunofluorescent technique. The use of normal control oral mucosal substrate yielded negative results. In the tests performed by Lehner [59] and by Donatsky and Dabelsteen [77], specific immunofluorescent localization was limited to the intracytoplasmic portion of the stratum spinosum cells. It is noteworthy that this is the site of epithelial damage in histopathologic specimens of early lesions of RAS.

The study of Donatsky and Dabelsteen [77] is reminiscent of that of Bystryn, Abel, and Weidman [79], in which sera of several groups of patients, diluted 1:5, was reacted with human skin and a cytoplasmic fluorescence was obtained. These anticytoplasmic antibodies are fairly common; they occur in 1 of 6 normal persons, 1 of 5 patients with various dermatoses, and 1 of 3 patients with cancer. The pathogenic significance of these antibodies is poorly understood, but they may represent a nonspecific immunologic reaction to chronic or recurrent epithelial tissue damage.

In vitro studies of lymphocytes harvested from patients with RAS add suggestive evidence to the thesis that cell-mediated immune responses to oral mucosal antigens are involved in the pathogenesis of RAS.

The lymphocyte transformation test is a means of detecting lymphocyte sensitization to a particular antigen [80]. Lymphocyte transformation tests [81] using a 1:50 dilution of a sterile saline homogenate of human fetal oral mucosa as antigen were positive in 10 of 19 patients with MiAu and MjAU and in 2 of 9 patients with HU. Lymphoblast transformation was negative when the disease was inactive but became positive with recrudescence of the aphthae in 1 patient who was followed sequentially. The antigen used in this study was crude, but 12 normal controls and 13 controls with miscellaneous oral ulcerative disorders showed negative results. In 5 of 9 patients with BS, the lymphoblast transformation tests also were positive [81]. Donatsky [72,73] has demonstrated a similar cellular response to adult human oral mucosal antigen and its correlation with disease activity.

Lymphocyte cytotoxicity for target cells is another method of evaluating specific lymphocyte sensitization [82]. The presence of lymphocyte cytotoxicity in vitro for colonic target cells in IBD [83,84] and muscle target cells in polymyositis [85–87], and the lymphocyte transformation studies in RAS and BS [81], led Dolby [88] to evaluate the lymphocytes of patients with RAS by cytotoxicity assays on gingival epithelial target cells. This author demonstrated that the peripheral blood lymphocytes of 7 patients with MiAU reduced the survival in vitro of gingival target cells when compared with controls. This lymphocytotoxicity was blocked by preincubation of the lymphocytes of patients with RAS with rabbit antihuman lymphocytic serum [89]. Recently, Dolby [90] demonstrated that lymphocytes of patients with RAS are not cytotoxic for colonic epithelial target cells when incubated for 24 hours at a ratio of 5 lymphocytes to 1 target cell and that hydrocortisone succinate sodium (100 μg/ml) blocked the cytotoxic activity of the aggressor lymphocytes when this agent was added to the in vitro short-term culture medium [91].

Rogers, Sams, and Shorter [92] have previously described lymphocytotoxicity for gingival epithelial target cells as measured by exclusion of the supravital dye trypan blue. This property of peripheral blood lymphocytes harvested from patients with RAS and BS was not noted when studying 14 normal subjects, 11 disease controls without oral lesions, and 10 disease controls with oral lesions. Movius, Rogers, and Reeve [93] also have demonstrated an increased lymphocytotoxicity of peripheral blood lymphocytes of patients with destructive periodontal disease for gingival epithelial target cells when compared with periodontal disease-free patients' lymphocytes. Recently, Rogers, Movius, and Pierre [94] confirmed

and extended these observations with a second method to demonstrate target cell death.

In vitro cell-mediated cytotoxicity mechanisms can be separated by the character of the effector cell and the dependence of the reaction on antibody [95,96]. For example, immunization of a laboratory animal with nucleated allogeneic cells induces a population of lymphomononuclear cells capable of in vitro cytolysis of the immunogen, the nucleated allogeneic cell. The effector cells apparently are T lymphocytes, and the cytotoxicity is antibody-independent [95]. Another cytotoxicity system, the ADCC system [96,97], utilizes effector cells that may not have been specifically sensitized to attack target cells coated with antibody directed against their own cellular surface determinants. Cells bearing Fc receptors are capable of effecting the damage in this system (killer or K cells).

These observations of immunologic investigations of RAS may represent secondary phenomena. The pathogenic relationship of these in vitro studies to the in vivo situation remains to be demonstrated. The elegant studies of self-tolerance and autoimmunity with respect to thyroid disease as reviewed by Allison [61] and the investigations of Stobo et al [53] and Shorter et al [84] with respect to IBD are examples of pathways of investigation which may be fruitful with respect to an immunopathogenesis for RAS, as would the development of an animal model for RAS.

SUMMARY AND CONCLUSIONS

The heterogeneity of RAS is beginning to be appreciated, and subpopulations are being defined which may allow a more efficacious therapeutic approach to RAS. Clinical studies purporting to demonstrate therapeutic efficacy will be difficult to conduct because of the variable duration and frequency of lesions and the pain reduction that naturally attends the covering of the ulcer by the fibromembranous slough.

The evidence for an immunopathogenesis for RAS is based on clinical and pathologic observations that lend credence to the thesis. These observations are becoming better founded as the associated diseases are studied more intensively and the subpopulations of RAS sufferers are better defined.

Evidence for a lymphocyte-epithelial cell interaction that may have pathogenetic significance can be gleaned from the pathologic features of early lesions, the evidence by lymphocyte transformation tests, lymphocytotoxicity tests, and leukocyte migration tests for sensitization of the lymphocyte (or the target cell), and the presence of antimucosal antibodies as an indication of immunologic reaction to epithelial tissue damage.

Antigens such as the *S. sanguis* 2A microorganism and the oral mucosal saline homogenates and epithelial cells require purification. Sequential studies utilizing these antigens are necessary to correlate the immunologic reactivity with the disease activity. Cross-reactivity between the oral mucosal and streptococcal 2A antigens requires study.

Specific immunopathologic mechanisms can be better defined by utilizing the sophisticated immunologic techniques that have been developed in recent years. Further elucidation of these mechanisms and their role in the pathogenesis of RAS may provide a more rational approach to therapy for this common disorder.

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