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Recurrent aphthous ulcers today: a review of the growing knowledge

S. S. Natah[1,2,5], Y. T. Konttinen[2,5], N. S. Enattah[3], N. Ashammakhi[4], K. A. Sharkey[1], R. Häyrinen-Immonen[2]

[1]Gastrointestinal and Neuroscience Research Groups, Department of Physiology & Biophysics, University of Calgary, AB, Canada; [2]Oral Medicine, Institute of Dentistry, University of Helsinki, Finland; [3]Department of Molecular Genetics, Biomedicum Helsinki; [4]Institute of Biomaterial, Tampere University of Technology and Division of Plastic Surgery, Oulu University Hospital, Oulu, Finland; [5]Institute of Biomedicine/Anatomy, Biomedicum Helsinki, University of Helsinki, Helsinki, Finland

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Abstract. Recurrent aphthous ulcers represent a very common but poorly understood mucosal disorder. They occur in men and women of all ages, races and geographic regions. It is estimated that at least 1 in 5 individuals has at least once been afflicted with aphthous ulcers. The condition is classified as minor, major, and herpetiform on the basis of ulcer size and number. Attacks may be precipitated by local trauma, stress, food intake, drugs, hormonal changes and vitamin and trace element deficiencies. Local and systemic conditions, and genetic, immunological and microbial factors all may play a role in the pathogenesis of recurrent aphthous ulceration (RAU). However, to date, no principal cause has been discovered. Since the aetiology is unknown, diagnosis is entirely based on history and clinical criteria and no laboratory procedures exist to confirm the diagnosis. Although RAU may be a marker of an underlying systemic illness such as coeliac disease, or may present as one of the features of Behçet's disease, in most cases no additional body systems are affected, and patients remain otherwise fit and well. Different aetiologies and mechanisms might be operative in the aetiopathogenesis of aphthous ulceration, but pain, recurrence, self-limitation of the condition, and destruction of the epithelium seem to be the ultimate outcomes. There is no curative therapy to prevent the recurrence of ulcers, and all available treatment modalities can only reduce the frequency or severity of the lesions.

Key words: recurrent aphthous ulcers; mucosal inflammation; Behçet's disease; coeliac disease; HIV.

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Definition

Recurrent aphthous ulceration (RAU) is an inflammatory condition of unknown aetiology characterized by painful recurrent, single or multiple ulcerations of the oral mucosa[63].

Description and clinical forms of RAU

Recurrent aphthous ulceration has three different variants—minor aphthous ulcers, major aphthous ulcers and herpetiform ulcers, according to the classification described by Stanley (1972)[194].

(1) Minor RAU (MiRAU) is the common variety, affecting about 80% of RAU patients[156]. It is characterized by painful round or oval shallow ulcers, regular in outline, less than 10 mm in diameter, with a grey–white pseudomembrane surrounded by a thin erythematous halo. Minor RAU usually occurs on non-keratinized mucosa (Fig. 1) such as labial mucosa, buccal mucosa and the floor of the mouth, and it is uncommon on the keratinized gingiva, palate, or dorsum of the tongue. Minor RAU is the most common form of childhood RAU[53]. The lesions recur at varying frequencies (from every few years to almost constantly) and heal within 10–14 days without scarring[156].

(2) Major RAU (MaRAU), also known as periadenitis mucosa necrotica recurrens, occurs in approximately 10% of RAU patients[160]. The lesions are similar in appearance to those of minor RAU, but they are larger than 10 mm in

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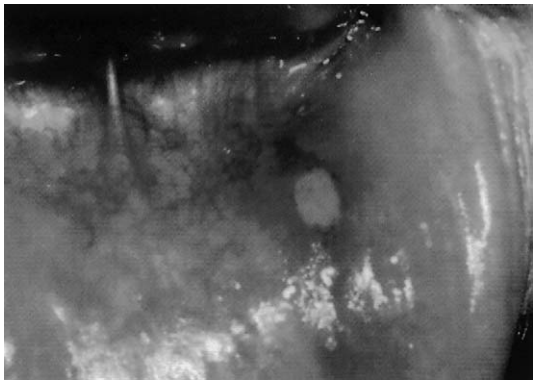

Fig. 1. Recurrent aphthous ulcer minor on the lower labial mucosa.

diameter, single or multiple and very painful (Fig. 2). Major RAU has a predilection for the lips, soft palate, and fauces, but can affect any site[176]. The ulcers of MaRAU persist for up to 6 weeks or longer and often heal with scarring. Major RAU usually has its onset after puberty[176].

(3) The third and least common variety of RAU is herpetiform (HuRAU). The name is derived from the supposed resemblance to the intraoral lesions of primary herpes simplex (HSV) infection, but HSV cannot be isolated from HuRAU lesions or from any other forms of RAU[108]. Furthermore, HuRAU lesions are not preceded by vesicular lesions, but develop—like all RAU lesions—directly as ulcers. This form is characterized by multiple recurrent crops of small, painful ulcers that are widely distributed throughout the oral cavity. As many as 100 ulcers may be present at a given time, each measuring 2–3 mm in diameter, although they tend to fuse, producing large irregular ulcers. They usually heal without scar formation, the healing time of an individual lesion being 7 to 10 days. The condition occurs more often in women and is associated with a later age of onset than other types of RAU[101,153,176].

Recurrence is the hallmark of RAU, and patients generally present with only one variant of the disease, but two forms may coexist, or a change in clinical expression may be seen with time[211].

Epidemiology of RAU

It has been estimated that 20% of the general population will suffer from RAU at some time in their lives[6,194]. In childhood, RAU is the most common form of oral ulceration[53]. It seems to be more common in children and adults of higher rather than lower socio-economic status[33,184].

In a cross-sectional study RAU lesions were found in about 2% of Swedish adults[6]. The cumulative prevalence of RAU varies from 5 to 66% of the population, depending on the group studied[48,121]. RAU seems to be infrequent in Bedouin Arabs[48] but it is relatively common in Western countries[44]. The peak age at onset is the second decade[98,192], and a high prevalence and severity of disease has been found in students of high socio-economic background[121,184,186].

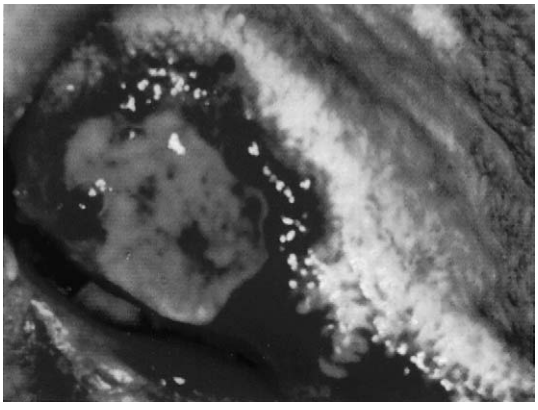

Fig. 2. Recurrent aphthous ulcer major on the left lateral border of the tongue.

Factors predisposing individuals to RAU

Age and sex

The prevalence of RAU detected in oral examination (average time point prevalence) has been found to be about 1% in children in developed countries[91], but up to 40% of children (aged 15 years or less) may have a history of RAU, with ulceration beginning before 5 years of age and the frequency of affected patients rising with age[123,147]. In the adult population, the first ulceration appears before the age of 30 years in 60–85% of cases[160]. A slight predominance has been found for females[6], and there may also be a female predisposition in affected children[53]. A decreased prevalence has been noted in males, though not in females, over the age of 50 years in a Scottish population[192], whereas Axéll (1976)[5] found a decrease in prevalence with age in both sexes in a Swedish population.

Family and heredity

In some individuals, RAU may have a familial basis. Possibly more than 40% of patients may have a familial history of RAU[192]. Patients with a positive family history of RAU develop oral ulcers at an earlier age and have more severe symptoms than individuals with no family history of RAU[121,123,183]. The probability of a sibling developing RAU is influenced by the parents' RAU status[186] with increased risk in children of two affected parents (67–90%), and there is a high correlation in the incidence of RAU in identical twins[122]. Nevertheless, there is clear variability in host susceptibility which can be explained by polygenic inheritance, with penetrance being dependent on environmental factors[183,186].

Genetic factors have been implicated in numerous studies on the association between RAU and genetically determined human leucocyte antigen (HLA) subtypes. An increased frequency of HLA2[30], B12[105,112], B51 and Cw7 in Jewish patients[189], DR2[105,136] and DR4 in Turkish patients[136], DR5 and A28 in Greek patients[2], DR7 and MT3 in Sicilian patients[59] and DRw9 in Chinese patients[196] has been reported. There may be a negative association with HLA-B5 in Sicilians[59] and DR4 in Greeks[2]. Some investigators have reported a variety of associations or absence of associations[39,59,136,150] between RAU and a particular HLA antigen. This could be explained by different ethnic backgrounds

or by multiple aetiological bases for RAU. The above-mentioned literature, however, suggests that RAU, at least in certain persons, has a genetic basis.

RAU and hormonal changes

It appears from different and sometimes conflicting studies[17,36,114,177,182] that a minor subset of women with RAU have cyclical oral ulceration related to the onset of menstruation or the luteal phase of the menstrual cycle. Complete remission during pregnancy has been reported[192,215], with exacerbation occurring in the puerperium[36]. Although 10% of women have been reported to have had their first episode of RAU between the ages of 50 and 59[192], more recent work has not uncovered an association between RAU and the menopause[114].

Food hypersensitivity

Some investigators have correlated the onset of ulcers to exposure to certain foods, such as cows' milk[203], gluten[134,221], chocolate, nuts[224], cheese[71], azo dyes, flavouring agents and preservatives[133,226]. Eversole and co-workers (1982)[46] found no significant association between RAU and three specific food items (tomatoes, strawberries and walnuts). Some investigators have noted an increased prevalence of atopy among RAU patients[206,218], whereas Wray[224] and co-workers (1982) found no significant difference in the incidence of atopy in RAU patients compared with the normal population.

Drugs

Rarely, drugs such as non-steroidal anti-inflammatory drugs (NSAIDs, e.g. proprionic acid, phenylacetic acid and diclofenac) can give rise to oral ulcers similar to those of RAU, along with genital ulceration[77] or only oral ulcers in the case of piroxicam[191]. An association between beta-blockers and aphthous ulcers has also been suggested[21]. Such ulcers usually occur as an adverse side-effect and disappear with discontinued usage of the drug. In a recent French study[22] it was found that typical clinical description of aphthous ulcers and/or clinical presentation suggesting the diagnosis of aphthous ulcers have been noted for eight drugs (Table 1), but for another group of 20 drugs the diagnosis of aphthous ulcers remains to be confirmed.

Haematinic deficiencies

Haematinic deficiencies have been found in about 20% of patients with RAU[52]. In several studies, deficiencies of iron, vitamin B12 and folate have been reported[31,49,50,151,208,220], although in many cases the deficiencies were marginally low[52]. However, Olson and colleagues (1982)[135] found that vitamin B12, folate and iron deficiencies were not significantly different between patients with RAU and controls. In a pilot study[110] on 22 HIV-infected patients with RAU it was suggested that vitamin B12 or folate deficiencies were not risk factors for HIV-associated RAU. Low serum ferritin levels have been found in 8–12% of patients with RAU, compared with 3–5% in controls, and the level did not differ in different subtypes of RAU[31]. However, in the majority of cases, there was no identifiable underlying cause for ferritin deficiency[152]. In a Scottish study, Nolan and co-workers (1991)[132] found that 28.2% of patients with RAU had deficiencies of vitamins B1, B2 and/or B6. They showed also that patients who have both RAU and a vitamin B deficiency could benefit from vitamin replacement therapy. It appears from the above-mentioned literature that the wide variations in findings may be due to differences in genetic background and dietary habits of the examined patients, or the multifactorial aetiology of RAU.

Table 1. List of drugs in association with which a complete clinical description of RAU or indicative photograph were available

Drug-induced RAU	Reference
Captopril	Corone et al. (1987)[32]
Gold salts	Kuffer et al. (1976)[92]
Nicorandil	Shotts et al. (1999)[190]
Niflumic acid	Kuffer et al. (1976)[92]
Phenindione	Kuffer et al. (1976)[92]
Phenobarbital	Kennet (1968)[89]
Piroxicam	Siegel & Balciunas (1991)[191]
Sodium hypochloride	Menni et al. (1988)[117]

Zinc deficiency

The improvement of RAU with zinc sulphate supplementation has been described in an open trial[118] and in a case report of aphthous ulcers with zinc deficiency and immunodeficiency[45], but such improvement was not been confirmed in later studies[119,223]. In a Chinese study[137] the level of serum zinc in 75 cases of RAU was found to be at a low level but within the normal range, and serum copper levels were also normal. So far no information exists on the associations between RAU and other trace elements.

Environmental factors

Stress

Earlier studies have documented an association between RAU and a variety of psychological factors including anxiety, repressed hostility, as well as job-related and other stress factors[121,181,185,192]. Conversely, other studies have failed to reveal any association between anxiety[80], depression[51], psychological life stress[140] and recurrences of RAU. In a more recent study, in which a relaxation/imagery treatment programme was used[3], a significant decrease in the frequency of ulcer recurrence among all treated subjects was noted. Although the majority of investigators have been unable to validate the concept that stress plays an important role in the development of RAU, the literature continues to indicate that stress may play a role in precipitating RAU.

Local trauma

A subset of patients with RAU is predisposed to develop aphthae at sites of trauma[165,222]. The reason why local trauma such as anaesthetic injections, sharp foods, tooth-brushing and dental treatment[93] can trigger aphthous ulceration in these patients is still unknown.

Tobacco

Several investigators have documented a negative association between smoking and the occurrence of RAU[7,178]. Such a negative association has also been documented as regards the use of smokeless tobacco (chewing tobacco and snuff)[62], as well as in patients who are smokers and seropositive for HIV[67]. Paradoxically, the majority of patients with RAU are nonsmokers[160], and in a recent study[207] only 9% of RAU patients were found to be active smokers, compared with 25% among the control subjects.

Nicotine has been reported to be beneficial in RAU[16] and in inflammatory bowel disease[97], and its effects may result from influences on nerve function, although these may also exert direct anti-inflammatory effects. However, the mechanism by which cigarette smoking protects against RAU is still unknown.

Infectious factors

Bacterial agents

In 1963, Barile and co-workers[11] isolated *S. oralis* (previously known as *S. sanguis* 2A) from an aphthous ulcer lesion. Other subsequent investigators[40–42] have found raised levels of antibodies against certain oral streptococcal strains in patients with RAU when compared with controls. Cross-reaction of antibacterial antibodies with oral mucosa has been postulated as an immunopathogenic mechanism in RAU[40]. Later studies, however, have not confirmed this[66,83,162]. In serological testing, *Helicobacter pylori* does not appear to be of aetiological significance in the development of RAU[155]. Recently, Birek et al. (1999)[15] detected *H. pylori* DNA in swabs from 23 of 32 RAU lesions, using polymerase chain reaction (PCR) assay, but in a later study[180] *H. pylori* culture was found to be negative in 12 samples from patients with RAU, suggesting that *H. pylori* does not have a direct association with the condition.

Cross-reactivity between mycobacterial 65-kDa heat shock protein (Hsp) and *Streptococcus oralis* has been demonstrated, and significantly elevated levels of serum antibodies to recombinant 65-KDa mycobacterial Hsp have been observed in RAU[106]. Lymphocytes of RAU patients have a significantly increased lymphoproliferative response to peptide epitope 91–105 of the 65-kDa mycobacterial Hsp in the ulcerative stage as opposed to the period of remission[68]. There is some cross-reactivity between the microbial 65-kDa Hsp and the 60-kDa human mitochondrial Hsp. Thus, RAU may be a T-cell-mediated response to antigens of *S. oralis* that cross-react with the mitochondrial Hsp and induce oral mucosal damage[68]. Conversely, other investigators[210] have suggested that immediate up-regulation of Hsps in any cell type, anywhere in the body, as a consequence of stress may trigger T cells with a regulatory phenotype. This would provide the immune system with an immunoregulatory mechanism which acts to monitor and control dangerous or potentially deleterious inflammatory responses. However, whether Hsps in RAU are protective or destructive or have a dual role is still unclear.

Viral agents

A viral cause behind RAU has been suggested by several investigators. Sallay and co-workers (1973)[166] isolated adenoviruses from oral aphthae, but there was no antibody response to adenovirus in RAU. Adenoviruses are ubiquitous organisms and these results need confirmation. Studd et al. (1991)[195] detected HSV-1 DNA in only two of 11 biopsies from oral aphthae in RAU patients. Other investigators have failed to detect HSV antigens in biopsies[157] and HSV cannot be cultured from RAU lesions[43,108]. Antiviral agents such as acyclovir, highly effective against HSV, appear to have only an equivocal clinical effect on RAU[141,219]. Patients with RAU have been found to have higher titres of IgM against varicella-zoster virus (VZV) and cytomegalovirus (CMV) than control subjects[142]. Further studies have revealed VZV-like DNA[143], CMV-DNA[198] and Epstein-Barr virus (EBV-DNA) in some oral ulcer biopsy specimens from some RAU and/or Behçet's disease (BD) patients[199]. However, VZV could not be cultivated from any of the oral ulcer biopsies and VZV antigen was not detected in any of the smears. A further study[60], involving PCR, revealed herpes hominis virus-6 (HHV-6-DNA) in six of 21 RAU lesions, whereas VZV-DNA and CMV-DNA were not detected in any RAU samples. The detection of human herpesvirus DNA from the oral mucosa and peripheral blood mononuclear cells of patients with RAU appears to represent normal viral shedding rather than a direct causal mechanism in this disorder[23]. Overall, the evidence for involvement of viruses such as HSV, VZV, CMV, EBV and HHV-6 in RAU is conflicting. It is possible that RAU is a non-specific response with multiple aetiologies and represents the final common pathway of mucosal inflammation. However, dormant herpesviruses may be reactivated by the immunodysregulation associated with RAU.

Serology of RAU

Increases in serum IgA, IgG, IgD and IgE have been reported in some groups of RAU patients[14,100,175], whereas in other groups of RAU patients IgG, IgM and IgA have been found to be normal or reduced[10,24,112]. In a study by Porter et al. (1992)[154] of a group of 71 RAU patients there were no significant changes in serum levels of IgG_1, IgG_2, IgG_3 or IgG_4, but in a more recent study low serum levels of IgG_2 were found during the quiescent period of the disease[214]. The presence of raised levels of anti-endothelial cell autoantibodies (AECAs) lends support to the hypothesis that a vasculitic process may underlie some cases of RAU[78]. Circulating immune complexes have been found to be present in some patients[28,105]. However, complexes have not reliably been demonstrated in MiRAU[10]. Serum levels of C9[104] and $\beta 2$ microglobulin[174] have been reported to be raised in some patients, but this may represent a non-specific acute phase response[160].

Important systemic diseases associated with RAU

Coeliac disease

Coeliac disease is characterized by inflammatory changes in the mucosa of the small intestine induced by a component of the gluten protein of wheat. Recent studies by Lähteenoja et al.[94,95] have shown inflammatory changes with increased lamina propria and intraepithelial helper-inducer T lymphocyte (CD4+) and suppressor-cytotoxic T lymphocyte (CD8+) cells in the oral mucosa of coeliac disease patients after a local challenge with gliadin. The prevalence of patients with coeliac disease who have concurrent RAU ranges from 10% to 18%[50,111,116,208], with an increase in the frequency of HLA-DRw10 and DQw1 in coeliac disease associated with RAU[111,116]. However, the aphthae usually resolve with appropriate management of the coeliac disease[50]. On the other hand, it has been found that about 5% of RAU patients suffer from coeliac disease[49,212]. Such RAU patients may particularly have IgA-class reticulin and/or gliadin antibodies[50,120]. Patients who have RAU with no detectable clinical or histological evidence of coeliac disease on jejunal biopsy may respond to gluten withdrawal[221,226]. In contrast, other investigators have failed to demonstrate any benefit from gluten withdrawal in aphthous patients, suggesting that any improvements may be due to a placebo effect[85]. So far there have been no studies involving use of the new markers for coeliac disease such as anti-tissue transglutaminase and anti-endomysium antibodies for screening patients with RAU.

Behçet's disease

Behçet's disease is a multisystem disorder that predominantly affects young men of Mediterranean, Middle Eastern and Japanese descent. Classically, it features a triad of MiRAU, genital ulcers and ocular lesions[179]. In 1990 the criteria

for the diagnosis of BD were redefined[86] to include the presence of oral ulcers plus any two of the following: genital ulcers, typical defined eye lesions (such as uveitis, hypopyon and iridocyclitis), typical defined skin lesions and a positive pathergy (cutaneous puncture hyperreactivity) test result. Aphthous ulcers are present in 99% of patients with BD and are the initial presenting symptoms in 67% of patients[101]. All three types of RAU, minor, major and herpetiform, can be found in BD and there are no features which differentiate the oral ulcers in BD from those of RAU[102]. Although the oral ulcerations in BD are both clinically and histologically identical to those seen in RAU, the exact relationship between these diseases is still unknown. Cases of complex aphthosis or bipolar aphthosis (oral and genital aphthae, but no systemic signs or symptoms) may represent an atypical form of BD[88], and follow-up of such patients may eventually disclose more complete expression of BD[88]. A high frequency of RAU has been found among relatives of patients with BD[4]. Furthermore, RAU has some, but not all of the immunological abnormalities that arise in BD. In this respect, it has been suggested that RAU and BD might represent different degrees of the same disease spectrum[103]. However, RAU is usually confined to the oral mucosa in otherwise healthy individuals, while in BD it affects the skin and oro-genital mucosa. The cause of this extension of localization to non-oral locations is unclear.

HIV-associated RAU

Severe episodes of RAU have been observed in patients infected with HIV. The ulcers are of the minor, major and herpetiform types and are often located on the soft palate, tonsils or tongue, where they hinder eating and speaking. Macphail et al. (1991)[108] showed that 66% of HIV patients affected by RAU had the usually uncommon herpetiform or major types and that patients with MaRAU were significantly more immunosuppressed than those with MiRAU or HuRAU in that they had fewer CD4 and CD8 lymphocytes. The role played by the marked neutropenia seen in most HIV patients with MaRAU is unclear, but healing of the ulcers without resolution of the neutropenia argues for the ulcers being MaRAU rather than neutropenic ulcers. About half (44%) of the patients denied or could not recall having had RAU during their childhood, which was presumably before they became infected with HIV. The rest (56%) gave a definite history of childhood RAU and described the ulcers as MiRAU[108]. Patients with HIV infection have an overall prevalence rate of recurrent aphthae ranging from 1% to 4%[126,148]. Although the lesions are mainly oral, HIV-associated aphthae have been reported in the oesophagus and more distal gastrointestinal tract[8]. HIV-associated RAU lesions tend to be more severe and longer lasting, and may cause debilitating pain with associated alteration of important oral functions such as speaking, chewing and swallowing, which ultimately lead to malnutrition and weight loss, compromise the ability to take medication and seriously interfere with the quality of life[126]. As progress in the treatment of HIV disease results in more patients living longer in a state of significant immunosuppression, managing severe RAU may increasingly become a challenge[108].

Although it has not yet been definitely accepted that RAU-like lesions found in association with HIV infection are indeed RAU, they meet the diagnostic criteria for RAU, they respond to treatment like RAU, and therefore, until proven otherwise, they must be considered to be RAU[109]. Although HIV DNA has been identified in buccal mucosal scrapings from apparently healthy mucosa of (18/45) HIV-seropositive subjects[158], there are no studies that have demonstrated the presence of HIV in oral ulcers. It is unknown whether such lesions represent a localized autoimmune reaction, developing in response to an undefined antigen which triggers a normal immunological response, or whether they represent overactive HIV in the mucosa of T cell-deficient hosts.

Important effector cells participating in the inflammatory events of RAU

Neutrophils

Although the chemotactic function of neutrophils is normal in RAU[1,34], their marked concentration at the ulcer area in the ulcerative phase of the lesion suggests that they may play an active role in the pathogenesis and/or healing of RAU. Indeed, the production of oxygen radicals by neutrophils in RAU has been found to be similar to that in controls[225], and their phagocytic function does not seem to be defective[209]. Oral aphthae are a prominent feature of cyclic neutropenia[173], and major aphthae in HIV-infected patients have been associated with a depressed absolute neutrophil count[108]. The rapid healing of aphthae on a regimen of granulocyte-colony stimulating factor (G-CSF)[113] and the clinical response to similar regimens in patients with cyclic neutropenia[54] suggest that neutrophils are important in the healing of recurrent aphthae. On the other hand, human neutrophil-type matrix metalloproteinase-8 (MMP-8) has been found intracellularly in the ulcer area, and extracellularly in the area of basement membrane lateral to the ulcer[75], suggesting that neutrophils containing MMP-8 are likely to be involved in the tissue destruction seen in aphthae. However, the exact role of neutrophils in the pathogenesis or healing of recurrent aphthae is still not known and remains to be identified.

Macrophages

In spite of the fact that macrophages are likely to participate in every stage of the inflammatory process, they have not yet been adequately studied to definitively establish their role in RAU pathogenesis. In a histopathological study of RAU, Schröeder et al. (1984)[172] found the presence of numerous macrophages loaded with phagolysosomes containing debris of neutrophilic granulocytes, implying that macrophages mainly function to clear the tissue of neutrophil remnants. Results described by Häyrinen-Immonen et al. (1991)[72] indicated that CD11b- and nonspecific esterase-positive mature tissue macrophages formed about 14% of all inflammatory cells in RAU lesions, with increased frequency around the periphery of the lymphoid cell infiltrates.

Mast cells

Mast cells (MCs) have the ability to provide numerous mediators[25] and have long been regarded as potentially important in the inflammatory events in RAU. In a histopathological study involving Alcian blue/Safranin staining of MiRAU lesions, Dolby & Allison (1969)[38] found that the MC count in the first 2 days did not differ from that of the normal buccal mucosa, but there was an approximately 50% reduction in MC count in lesions of more than 48 h duration. In contrast, increased numbers of MCs were noted by Lehner (1969)[99] in all three types of RAU (minor, major

and herpetiform) and in oral aphthae associated with BD, particularly when using toluidine blue stain. Such an increase in MC numbers has also been found in skin lesions and oral aphthae in patients with active BD[107]. Using more sensitive markers for MCs, we recently showed that MCs were 63% more numerous in RAU than in healthy mucosa and that local MCs showed signs of activation/degranulation, suggesting an active role in RAU pathogenesis[128].

Gamma/delta T-lymphocytes

Gamma/delta T-cells have the ability to recognize non-peptide molecules commonly associated with micro-organisms and stressed cells[19]. In general, recognition of these antigens by γ/δ T-cells involves the antigen receptor but does not require antigen processing, presenting cells or MHC gene products. In rodents, γ/δ cells are preferentially localized in epithelial tissues such as skin, intestine and lung[35], but they have been found to be rare in normal human oral epithelia[130,138,146]. Although the γ/δ T-cell population constitutes only about 5% of circulating T-cells, they are much more common in the peripheral blood of patients affected by RAU or Behçet's disease, especially during the active phase of the disease[145,201]. Furthermore, Hasan and colleagues (1996)[69] showed that γ/δ T-cells from patients with mucocutaneous BD have a specific, proliferative response to four peptides derived from the 65-kDa microbial heat shock protein.

Keratinocyte growth factors, such as the epithelial cell-specific fibroblast growth factor (FGF)-7, produced by γ/δ T-cells, may play a role in healing epithelia which have been damaged by infection or inflammation by promoting cell growth there and hence reinstalling tissue integrity[18,20,70]. However, the role of γ/δ T-cells in mucosal immunity might not be restricted to nursing the epithelial injury. A study involving a murine model[87] showed that γ/δ T-cells lining the orogastric tract can stimulate the production of nitric oxide (NO) by neighbouring epithelial cells. This finding may be important because human oral epithelial cells might also be stimulated by neighbouring γ/δ T-cell-derived IFN-γ[57] to produce NO via upregulation of inducible NO synthase (iNOS) expression. As a gaseous free radical, NO could serve as a physiological cytoprotective agent for the mucosa. However, large amounts of NO alone, or after reaction with superoxide released from activated phagocytes[13,90], could lead to epithelial autotoxicity[55] and possibly ulceration. Interestingly, in a recent study[130] we have shown that, as in the peripheral blood of patients with RAU and Behçet's disease, γ/δ T-lymphocytes were also increased locally at the sites of RAU lesions. Such an increase was concomitant with the RAU inflammatory process and did not extend to mucosal areas other than the inflammatory sites. Although the study provided no answers regarding the function of intraepithelial γ/δ T-lymphocytes in RAU lesions, it suggested that they might have pathogenic and/or regulatory roles in aphthous ulceration. Future studies are needed to elucidate the exact role of γ/δ T-cells in RAU pathogenesis.

Cytokines and recurrent aphthous ulceration

High plasma levels of IL-2 and a significant increase in IL-2 receptor expression by activated peripheral lymphocytes have been found in patients with RAU during the exacerbation stage[200]. Gamma/delta T-cells from RAU patients have also been reported to produce IFN-γ when induced by mitogenic stimulation[57]. The relevance of TNF-α to the pathogenesis of RAU has stemmed from the observations that thalidomide, which reduces the activity of TNF-α by accelerating the degradation of its messenger RNA[125], and pentoxifylline, which inhibits TNF-α production[229], have been found to be effective in the treatment of RAU in HIV-infected patients and in otherwise healthy persons with RAU[149,161,205]. Enhanced release of TNF-α by peripheral blood monocytes of patients with RAU has also been demonstrated[202]. Furthermore, a recent study has shown low resting levels of interleukin-10 (IL-10) mRNA in non-lesional mucosa of RAU patients and high levels of the mRNAs of the pro-inflammatory cytokines IL-2, IFN-γ and TNF-α in lesional and non-lesional mucosa of patients with RAU compared with controls[26]. These findings partially parallel the results of our recent study[129] in which we demonstrated that RAU lesions are characterized by high expression of TNF-α. Such expression occurred in the mononuclear inflammatory cells, mast cells and vascular endothelial cells. However, the relative importance of the role of cytotoxicity versus cytokine release in mediating epithelial cell death and aphthous ulceration is still unknown.

Diagnosis

There is no known laboratory procedure available to establish a definite diagnosis, and histopathological examination of biopsies does not provide a definitive diagnosis. Detailed virological investigations of lesional tissue or serum are usually not warranted unless to exclude atypical herpetic infection[156].

Diagnostic criteria

Due to the absence of a definitive aetiology or diagnostic test for RAU, the identification of RAU in clinical practice usually relies on the combination of history, clinical features and histopathology. We propose in this review a set of diagnostic criteria for MiRAU which are meant to distinguish the condition from other diseases, and to be practical, all based on working knowledge of aphthous ulcers and clinical experience. They have not been tested for sensitivity and specificity and further studies might be required before widespread use of these criteria. Further refinement of the diagnostic criteria described here (Tables 2 and 3) will depend on properly conducted studies to validate them.

The diagnosis of primary RAU minor (idiopathic) or secondary RAU minor (that occurs in association with systemic diseases) can be made if the condition fulfils the four major criteria (which are necessary to establish the diagnosis of MiRAU) plus at least one of the minor (supportive) criteria (Tables 2 and 3).

Histopathology of recurrent aphthous ulcers

The ulcer area

Superficial tissue necrosis with fibrinopurulent exudate consisting of clotted fibrin, and numerous red blood cells forming haemorrhagic foci. Neutrophils and cellular debris cover the necrotic area. The epithelium is infiltrated with variable numbers of intraepithelial lymphocytes and some neutrophils[194]. Neutrophils predominate in the immediate ulcerated area, although peripheral areas surrounding the ulcer remain mononuclear in nature[72,99,124,171].

Table 2. Major criteria for recognizing and diagnosing the condition as RAU minor

Major criteria	Description
1. External appearance	Single or multiple round/oval shaped ulcers, never preceded by vesicles. The ulcers are shallow and have regular margins and a yellow–grey base surrounded by thin erythematous halos. Variable in size, but less than 1 cm in diameter.
2. Recurrence	At least three attacks of RAU within the past 3 years and the recurrences do not affect the same focal site.
3. Mechanical hyperalgesia	The lesion is painful and the pain is exacerbated by movement of the area affected by the ulcer.
4. Self-limitation of the condition	The ulcer heals spontaneously without sequelae either with or without treatment.

The area lateral to the ulcer

Defined as the epithelium-covered area extending from the edge of the ulcer and sideways to the periphery of the biopsy. There is intense leucocytic infiltration with predominance of lymphocytes in non-ulcer regions, where they outnumber neutrophils[72,124]. Monocytes/macrophages are also numerous in the tissue adjacent and lateral to the ulcer. The density of MCs is increased in the lamina propria[99,128,172]. The lymphocytes in RAU lesions are primarily T cells, and only 5–12% of all cells in the lesion are B cells[72]. A small proportion of plasma cells and eosinophils can be found, more often in older lesions[99]. Dilatation of blood vessels is a constant and prominent feature of RAU lesions, as are foci of perivascular mononuclear cell infiltrates[99,172].

Immunohistopathology of RAU

Immunological aberrations involving both cell-mediated and humoral immunity have been reported in previous studies of RAU[156]. Both class I and II MHC antigens have been found to be expressed on the epithelial basal cells in preulcerative RAU lesions and more diffusely within the epithelium at the ulcer stage, consistent with active cell-mediated inflammation[157,169]. Studies carried out *in vitro* have shown that peripheral blood lymphocytes from patients suffering from RAU are cytotoxic against oral epithelial cells[37,164], which, however, has not been confirmed by others[27,58,139]. Patients with RAU have significantly increased antibody-dependent cellular cytotoxic (ADCC) activity in the early stage of the disease[64]. However, ADCC values such as those assessing effector function of polymorphonuclear neutrophils have been found to be higher during acute RAU when compared with those of controls[193].

Immunofluorescence studies have demonstrated deposits of IgG, IgM, IgA and C3 in and along mucosal blood vessels and in the cytoplasm of stratum spinosum cells in aphthous ulcer lesions in patients with RAU and Behçet's disease[99,112,211]. Some researchers have reported a decrease in the number of circulating CD4+ cells, but normal or reduced numbers of CD8+ cells and a normal or slightly reduced CD4/CD8 ratio in RAU patients[96,144,170,193]. Comparison of major and minor types of RAU suggests that CD8+ cells are more common in the major type than in the minor type. The CD4+/CD8+ ratio in the major type was lower than in the minor type[9]. In oral mucosa, the percentage of CD4+ lymphocytes has been shown to be increased in ulcerative lesions[72] and the proportion of CD8+ cells has also been shown to be significantly increased in the lesion sites[168].

Previous studies on peripheral NK-cells in patients with RAU have been contradictory, as their percentages have been reported to be either increased[65] or similar to that of controls[144,170]. Furthermore, Thomas et al. (1990)[204] found that depletion of CD-16-positive NK-cells produced no change in cytotoxicity towards the oral epithelial target cells. Another report demonstrated that among patients with major RAU, NK-cell activity is increased when active oral lesions are seen, depressed during periods of resolution and becomes normal in patients in remission[197]. In contrast, a significant depression in NK-cell activity has been observed in patients with acute RAU as well as in the remission period, when compared with controls[193].

Formation of perivascular lymphocyte infiltrates is probably in part mediated by endothelial intercellular adhesion molecule-1 (ICAM-1) and lymphocyte function-antigen-3 (LFA-3)-binding to their counterpart ligands lymphocyte function-antigen-1 (LFA-1) and CD-2 on lymphocytes, respectively[74,213]. ICAM-1 is expressed on the epithelium and submucosal capillaries and venules, suggesting that it may support T-cell adhesion and control the trafficking of leucocytes into the submucosa and epithelium[47,74,79,169], while LFA-3 and its counterpart ligand CD-2 are likely to be involved in T-cell activation in RAU[74]. Increased numbers of CD1+ Langerhans cells have been found in the epithelium and lamina propria in BD and RAU[73,157]. Moreover, factor XIIIa+ dendrocytes, dendritic cells which seem to be involved in regulatory functions[82] and/or antigen handling in the subepithelial connective

Table 3. Minor criteria for recognizing and diagnosing RAU minor

Minor criteria	Description
1. Family history of RAU	A positive family history of RAU is present.
2. Age at onset	The first RAU attack started before the age of 40 years.
3. Location of ulcers	Occur on non-keratinized oral mucosa.
4. Duration of the lesion	Each bout of ulceration lasts from a few days to two weeks.
5. Pattern of recurrence	Irregular
6. Histological examination	Shows non-specific inflammation.
7. Presence of a precipitating factor	The attacks are triggered by hormonal changes, exposure to certain foods or drugs, intercurrent infections, stress and local trauma.
8. Presence of haematinic deficiencies	Laboratory investigations reveal an accompanying haematinic deficiency. In particular, ferritin, folate, iron, vitamin B and zinc.
9. Negative association with smoking	RAU patient is a non-smoker or develops the ulcer after stopping smoking.
10. Therapeutic trial with gluco-corticosteroids	Positive response to treatment with local or systemic steroids.

tissue[131,228], have been found to be enlarged[127,159] and increased in numbers[127] in the subepithelial compartment of RAU lesions. It is thus evident that there is no unifying theory of the immunopathogenesis of RAU.

Pain and quality of life

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage[217]. RAU is a painful phenomenon, characterized by necrotizing ulcers of the oral mucosa that persist, remit, and recur for variable periods of time. Despite the self-limitation of the disease, pain and ulceration may disable patients and prevent them from performing their daily activities. Painful RAU lesions usually affect the movable mucosa, and the frequency of attacks ranges from only once or twice a year to more or less continuous batches of lesions that cause considerable discomfort, often with pain and difficulty in eating and speaking, and a decreased quality of life (QOL)[187].

Quality of life is a relatively new concept in the measurement of health. It broadens the assessment of the impact of disease to include physical, psychological and social functioning[56]. Indeed, the impact of RAU on QOL has not yet been studied, but the clinical impression is that RAU causes much suffering in affected patients, due to repeated painful attacks[163]. However, future work is needed to determine QOL in RAU and the exact factors affecting it.

Management of RAU

Most patients with RAU need no treatment because of the mild nature of the disease. Some manage with maintenance of good oral hygiene, the right kind of toothpaste (without irritating sodium lauryl sulfate, e.g. Biotene)[29,81] and occasional palliative therapy for pain. Patients who experience multiple episodes of RAU each month and/or present with symptoms of severe pain and difficulty in eating should, however, be considered for drug therapy. Since the exact nature of RAU remains unclear, no curative therapy is available at the present time. Major goals of current treatments are: to ensure adequate food intake by palliation of pain symptoms, reduction of lesion duration, and in cases where ulcers are constant or frequent, minimization of recurrence[29,167]. Before initiating medications for RAU one should determine possible nutritional deficiencies or allergies causing the onset of the disease. Haematologic work-up should be done including complete blood count, serum ferritin, folate and B-vitamin levels. In the case of deficiencies practitioners should start replacement therapies and/or make appropriate referrals[29,188]. Practitioners should understand that sudden severe attack of RAU in an older patient can be an initial sign of some other systemic disease.

Glucocorticoids and antimicrobial therapy constitute the traditional treatments for RAU. These medications have been administered as topical pastes, mouthrinses, intralesional injections and systemically by oral route[61]. In addition patients are advised to maintain good daily oral hygiene. Patients should avoid irritating agents, such as acid, crusty/hard, spicy and salty foods and alcoholic beverages. Topical anesthetics such as 2% viscous lidocain hydrochloride (Xylocain, Astra) are widely used to palliate the pain[188].

Topical agents

Topical agents are the first choice of treatment for RAU. They are cheap, effective and safe. The problem with topical agents is obtaining effective drug delivery, because substances applied to mucosal surfaces are inevitably rubbed or rinsed away. To ensure maximum effect patients are instructed to dab the area of ulcer dry, apply a small amount of gel or cream after rinsing, and avoid eating or drinking for 30 min. This is repeated three or four times daily while ulcers persist[12,29].

Mouthwashes

Anti-microbial mouthwash use in RAU is intended to control microbial contamination and secondary infection. Tetracycline, an antibiotic mouthwash reduces ulcer size, duration and pain because of its ability to reduce not only secondary infection but also to inhibit collagenase activity[76,188]. There is no effect on incidence or recurrence[76]. Adverse effects such as dysgeusia, skin reactions, thrush, angular cheilosis and burning and soreness of throat has been reported if tetracycline was used for more than five days[12].

Chlorhexidine gluconate is an antibacterial agent available as a mouthrinse. Its efficacy has been evaluated in the treatment of RAU. When used three times a day it decreased the number of ulcer days in some studies[29,76], but had no effect at all in other studies[12]. Chlorhexidine has a bitter taste and causes brown staining of the teeth and tongue. Listerine (Pfizer Inc., New York, NY; 800/223-0182) was evaluated in a 6-month double-blind study and found to reduce the duration and severity of oral aphthae when used twice a day[115].

Steroids are used as rinses only if the patient is unable to apply topical agents directly to ulcers or if lesions cover a larger area. An aqueous preparation of 0.1% or 0.2% triamcinolone, 0.3% hydrocortisone mouthrinse and dexamethasone elixir 0.5/5.0 ml were all effective when used three or four times per day[188].

Topical gels, creams and ointments

Topical medications are easily washed away from the target area. This problem can be addressed by using different kinds of adhesive vehicles[227] in combination with the drug (e.g. Orabase, Bristol-Myers Squibb; isobutyl-cyanoacrylate or Iso-Dent, Ellman International). For example, strong topical corticosteroids when compounded with mucosal adherents are effective despite limited contact time[29]. Topical corticosteroid use in patients with RAU is intended to limit the inflammatory process associated with the formation of aphthae. Corticosteroids may act directly on T lymphocytes and alter the response of effector cells to precipitants of immunopathogenesis (e.g. food allergies, trauma, mircroorganisms).

Topical glucocorticoids that have demostrated efficacy for RAU are fluocinonide, triamcinolone and clobetasol[29,215]. Triamcinonide acetonide with Orabase, however, may not be as effective as stronger glucocorticoids, such as fluocinonide and clobetasol. Therefore, fluocinonide or clobetasol used alone or mixed with Orabase, may be preferable for the treatment of recurrent RAU[188]. Topical use of glucocorticoids may cause oral pseudomembranous candidiasis, however it is safer for the hypothalamic–pituitary–adrenal axis system than systemic glucocorticoids[12].

Amlexanox (5%) is another topical paste used in the treatment of RAU. It has anti-inflammatory and antiallergic properties. Given topically, amlexanox facilitates the healing of aphthous ulcers but does not reduce the frequency of RAU episodes[12]. It has proved to be clinically safe and efficient in several vehicle-controlled multicentre clinical studies. Amlexanox is a potent inhibitor

of the formation and release of inflammatory mediators from mast cells, neutrophils and mononuclear cells[12,188]. In a review of clinical trials and premarketing studies involving 991 subjects, only 2.1% of those using 5% amlexanox paste reported adverse events. These included stinging, dryness, bumps on the lips and mucositis[12].

Systemic medications

For the severe and constantly recurring ulcerations, topical management of RAU may not be enough. In these cases, systemic medications are employed. Though a great number of medications have been tried over the years, oral prednisone is still most commonly used[188]. It can be used in combination with topical gels and rinses. Systemic prednisone therapy should be started at 1.0 mg/kg a day as a single dose in patients with severe RAU and should be tapered after 1 to 2 weeks[188]. Because long term prednisone use carries the risk of adverse effects including depression, hyperglycemia, lipodystrophy, moon faces and hypothalamic-pituitary-adrenal axis suppression, it should be used only for a short time[29]. Prednisone can be combined with another immunosuppressive agent, azathioprine, to reduce the dosage of prednisone required to provide effective treatment. Azathioprine can be used in combination with systemic or topical steroid therapy.

The potential serious side effects of azathioprine include thrombocytopenia, leukopenia, secondary infections, anemia, nausea, vomiting, anorexia, diarrhea, and lymphoreticular and other malignancies after long-term therapy[29,188].

Levamisole[12] is an immunopotentiating agent and has been demonstrated to improve symptoms in RAU patients. It can significantly reduce pain intensity and ulcer number, duration, and frequency in many studies. Levamisole was well tolerated in a majority of the patients. Among 128 patients receiving levamisole, 2 withdrew as result of adverse effects (nausea and flu-like symptoms). The most frequent adverse effects were dysgeusia (21%) and nausea (16%). The other adverse effects occurred in fewer than 10% of the patients and included dysosmia, headaches, diarrhea, influenza-like symptoms and rash.

Thalidomide[12] was first introduced into the European market in 1957 as a sedative and soon became infamous for its teratogenicity. An interest of thalidomide was renewed in 1980 after it was used successfully to treat erythema nodosum leprosum. Thalidomide inhibits the production of various cytokines (e.g. tumor necrosis factor-alpha). Thalidomide therapy in the treatment of RAU has been studied more thoroughly with HIV-positive patients. Significant improvements are noted, including diminished pain and an increased ability to eat, after a 4-week course of 200 mg of thalidomide daily. However, 20% of the patients in the study had to reduce the dose or terminate the treatment because of toxicity (rash, somnolence, or peripheral sensory neuropathy).

Several other immunomodulating and anti-inflammatory drugs, including colchicine, cyclosporine, pentoxyfylline, azelastine and dapsone have shown some effectiveness for treatment of RAU[188]. However, most of these drugs require further research of their suitability in the management of RAU.

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Address:
Dr Sirajedin S. Natah
Gastrointestinal and Neuroscience Research Groups
Department of Physiology & Biophysics
University of Calgary
3330 Hospital Drive N.W.
Calgary
AB T2N 4N1
Canada
Tel: 1-403-220 4491
Fax: 1-403-283 2700
E-mail: snatah@ucalgary.ca