

Oral epithelial barrier function and the role of nuclear factor kappa- β pathway in the pathogenesis of aphthous ulceration

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Background/aims: The normal oral mucosa is usually tolerant to its special microenvironment. Epithelial integrity and a well-managed immune system are important in sustaining harmony. A close look at the role played by adaptive immunity during recurrent aphthous ulcerations may throw some light into the pathogenesis. **Materials and Methods:** In this report, we provide a concise review of oral epithelial barrier function and present data on the possible pathogenetic mechanism of aphthous ulceration using immunohistochemical signs of nuclear factor kappa beta pathway activation on fourteen cases of mucosal aphthous ulcerations. **Results:** We strongly support the hypothesis that oral aphthous ulcerations develop as a result of loss of epithelial barrier function and that nuclear factor kappa beta signaling pathway seems to be involved in this type of injury. **Conclusion:** Interventions that strengthen the mucosal barrier function or modulate inappropriate activation of nuclear factor kappa beta signaling pathway can be considered in the treatment of oral aphthous ulcerations.

Key words: Mucosa, barrier function, immunity, nuclear factor kappa beta, aphthous ulceration

Ağız epitelinin bariyer fonksiyonu ve aftöz ülserasyon patogeneğinde nükleer faktör kappa beta'nın rolü

Giriş ve Amaç: Normal ağız mukozası çok yönlü mikroçevresine karşı genellikle tolerans gösterir. Epitelin devamlılığı ve iyi organize immün sistem dengenin devamlılığı için önemlidir. Kazanılmış immünitenin aktivasyonu rekürrent aftöz ülserasyonlar için esas mekanizmayı oluşturabilir. **Gereç ve Yöntem:** Bu yazıda, ağız epiteli bariyer fonksiyonu kısaca gözden geçirilmiş ve on dört olguluk mukozal aftöz ülserasyon serisi üzerinde, nükleer faktör kappa beta yolağının aktivasyonunun immünohistokimyasal belirteçleri kullanılarak, aftöz ülserasyonun muhtemel patogenetik mekanizması ile ilişkili veriler sunulmuştur. **Bulgular:** Oral aftöz ülserasyonun, epitelyal bariyer fonksiyon kaybı sonucu ve nükleer faktör kappa beta sinyal yolağının dahil olduğu bir yaralanma ile oluştuğu hipotezini kuvvetle desteklemekteyiz. **Sonuç:** Mukozal bariyer fonksiyonunu güçlendiren girişimler veya nükleer faktör kappa beta sinyal yolağının uygun olmayan aktivasyonunu düzenlemeler oral aftöz ülserasyon tedavilerinde göz önünde bulundurulabilir.

Anahtar kelimeler: Mukoza, bariyer fonksiyonu, immünite, nükleer faktör kappa beta, aftöz ülserasyon

INTRODUCTION

Oral mucosa is exposed to a special microenvironment which contains commensal flora, pathogenic microorganisms, foods, and various chemicals.

Normally, the mucosa is tolerant to these highly antigenic microenvironment and does not mount an unnecessary defensive response. This tolerance

to the microenvironment depends on the integrity of the mucosal epithelium and its barrier functions. Saliva and antimicrobial proteins secreted by oral epithelia contribute to the protection of epithelial surface and then actively participate in the maintenance of tolerance (1). In addition to the mechanical barrier function, oral epithelium plays important roles both in the innate and the adaptive immune systems of oral mucosa. When necessary, the oral epithelium upregulates the expression of major histocompatibility class II antigens as an active component of adaptive immunity (2).

The functions of the mucosal epithelium as a part of local immune system of the oral cavity is closely related with the thickness, stratification and maturation of epithelium. A variety of genetic, nutritional and hormonal factors affect the organization, flexibility, and integrity of the epithelium.

The normal, "friendly" oral flora contains numerous different species of bacteria and fungi. Although the most predominant microorganisms are streptococci, the relative proportions may show great individual and regional differences. Normally, there is a harmonious relationship between oral flora and oral mucosa. When the epithelial barrier functions are lost or deranged, the commensal flora may invade the oral epithelium and activate various pattern recognition receptors. Such derangements can also expose the immune system to a variety of antigens. Aberrant stimulation of some of these receptors may initiate immuno-inflammatory reactions and can result in tissue injury. Changes in the composition of the "friendly" flora (dysbiosis) may disrupt the normal barrier function by increasing the permeability of the epithelium and facilitate activation of receptors. Pathogenic bacteria and soluble antigens may also activate the deeply located receptors and can directly initiate adaptive immunity. Pattern recognition receptors are found both on the surface and the inner part of the mucosal epithelial layer and Langerhans cells. The type and location of the activated pattern recognition receptors may determine the severity of the ensuing immuno-inflammatory reaction (1,3).

Recurrent aphthous ulceration is a frequent oral mucosal disorder with a multifactorial etiology (4-6). The presence of ulceration and the relatively long period of regeneration suggest involvement of adaptive immunity. Mucosal injury due to a similar barrier function deficiency has been shown in other parts of the body such as the eyes, skin, air-

ways, and intestines (7-10). Inflammatory bowel diseases are prototypes of this type injury, and they have been better studied (8,11,12). The loss of barrier function and the resulting activation of nuclear factor-kappa B (NF- κ B) pathway, which is the main activator of adaptive immunity, cause rapid tissue destruction in the pathogenesis of inflammatory bowel diseases (8,13,14). NF- κ B is a master transcription factor and a central regulator of immuno-inflammatory reactions and apoptosis (13-15). It is involved in the pathogenesis of some autoimmune diseases and neoplasia (13-15).

In this study, we have reviewed the oral epithelial barrier function briefly and focused on a series of clinically diagnosed cases of mucosal aphthous ulcerations. Tissue samples were evaluated histologically and immunohistochemically to clarify the possible pathogenetic mechanism of aphthous ulceration.

MATERIALS and METHODS

This retrospective study was performed in Gülhane Military Medical Academy (GMMA), Military Medical School, Department of Pathology. Histologic slides and paraffin blocks of biopsies from twelve cases of clinically diagnosed oral mucosal aphthous ulcerations were retrieved from the files. Two normal biopsies were also taken as control. The patients were 8 females and 4 males with an age range of 19-70 years. Samples were from the cheek (3 cases), lip (2 cases), tongue (2 cases), gingival mucosa (2 cases), and other areas (3 cases). Medical records of all patients were reviewed. Most of the biopsy specimens were from patients with clinically recurrent aphthous mucosal ulcerations. One of the oral mucosa specimens was from a patient with Behçet disease. No other patient had a systemic disease. Two oral mucosa specimens containing normal epithelium were used as controls. Some of the samples had previously been used for a different study (16). Hematoxylin and eosin stained archival sections were reexamined and verified for the presence of ulceration. Histohchemically, periodic acid-Schiff (PAS) staining was applied. All these slides were evaluated by one of the authors (ÖG).

New sections were immunohistochemically stained with CD3, CD4, CD8 (Neomarkers, Fremont, CA, USA) and CD 20 (Dako, Denmark) to determine the nature of inflammatory cells.

NF- κ B antibody [Neomarkers, p65 (Rel A) Ab-1,

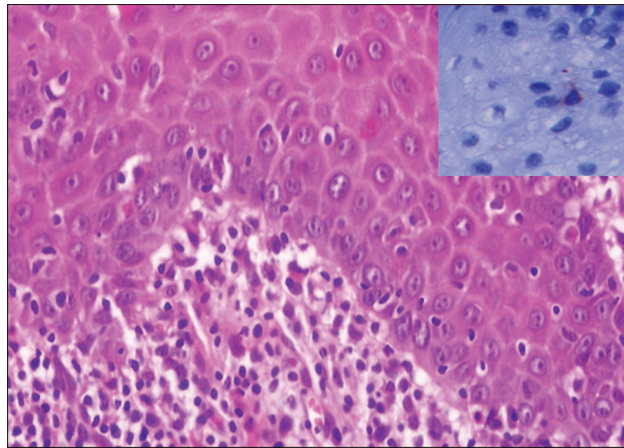


Figure 1. Intraepithelial lymphocytes in the preulcerative stage of aphthous ulceration. Lymphocytes were CD 3 positive (inset) (X400).

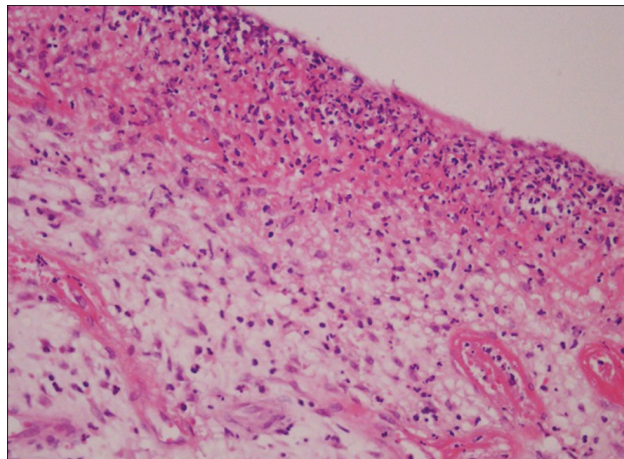


Figure 2. Ulceration covered by a thin inflammatory exudate. Note the edematous connective tissue and PMN leukocyte-rich inflammatory cell infiltration (HEX 200).

transactivating subunit, 1/200 dilution, Fremont, Ca, USA] was selected to evaluate adaptive immunity within the lesions. Streptavidin-peroxidase technique was used for immunohistochemical procedure.

RESULTS

Histologic examination showed that ulceration was present in all of the cases (Figure 1). No specific inflammatory reaction or finding suggestive of vasculitis were found. Inflammatory infiltrations were rich in neutrophil leukocytes and lymphocytes. The overlying squamous epithelium of the oral mucosa at the edges of ulceration contained intraepithelial neutrophil leukocytes and lymphocytes. Both the intraepithelial and submu-

cosal lymphocytes were rich in CD3, CD4 and CD8 positive T lymphocytes (Figure 2). CD 20 positive B cells were numerous at the base of ulceration and within the subepithelial connective tissue. Ulceration was superficial, and there was regenerative activity at the edges of the epithelium. No excessive connective tissue reaction was seen except for some edematous changes, capillary proliferation and nonspecific inflammatory cell infiltration (Figure 1). In occasional areas, there was PAS-positive basal membrane overlying the subepithelial connective tissue suggesting that the injury involved mainly the epithelium. Strong NF- κ B immunostaining was found in all cases. NF- κ B expression was prominent within the squamous epithelium adjacent to areas of ulceration, the endothelial cells of subepithelial vascular structures and the accompanying inflammatory cells (Figure 3). The mucosa epithelium far from areas of ulceration showed only some basal layer NF- κ B positivity. The histologic appearance and the results of immunohistochemical staining were not noticeably different in the case with Behçet disease. The main histopathologic findings of our study were shown in Table 1.

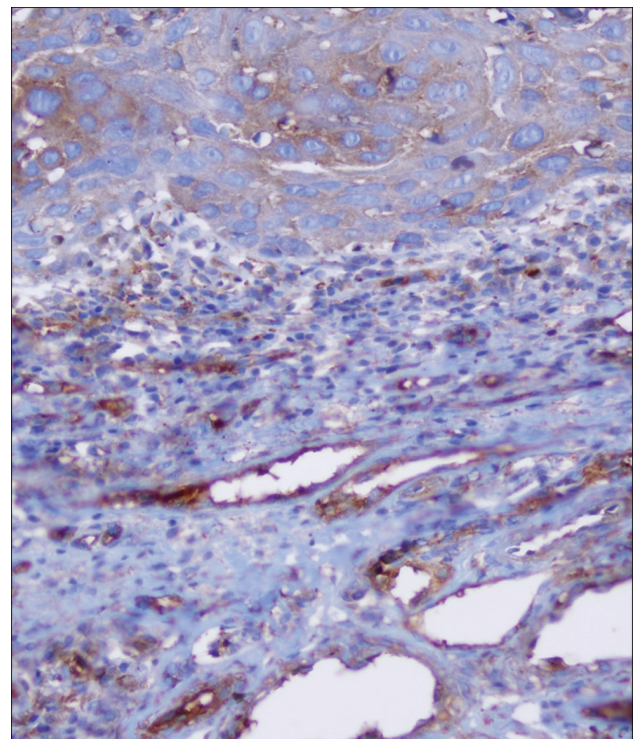


Figure 3. Mucosal epithelium adjacent to areas of ulceration. Epithelial, endothelial and inflammatory cells express NF- κ B immunoreactivity (Immunostaining X200).

Table 1. The main histopathologic findings of the cases

Location (number)	Ulceration	Vasculitis	IEIC*	SEIC*	NF- κ B activation
Cheek (3)	+	–	+	+	+
Lip (2)	+	–	+	+	+
Tongue (2)	+	–	+	+	+
Gingiva (2)	+	–	+	+	+
Others (3)	+	–	+	+	+
Control (2)	–	–	±	–	–

*: IEIC: Intraepithelial inflammatory cells. SEIC: Subepithelial inflammatory cells.

DISCUSSION

Maintenance of the oral mucosal integrity is a complex process. Numerous local, genetic, nutritional and metabolic factors contribute to the defense mechanisms. These factors can affect the integrity of the epithelium by modulating receptors and postreceptor signal activation in different ways.

Genetic factors are important determinants of disease susceptibility, and they regulate the type and intensity of immune reactions. For example, major histocompatibility complexes are closely related with T lymphocyte reactions (4). Similarly, the frequency of aphthous ulcerations, as an aberrant immuno-inflammatory reaction, shows marked individual differences. The mucosal surface of the oral cavity exhibits a complex homeostatic balance between immune activation and tolerance. The physiologic interactions between the commensal flora and the epithelium result in diminished sensitivity and set a basal immune threshold (17). Once believed to be a passive barrier, the epithelial layer is now regarded as an information relating device. Oral epithelial cells are involved in the initiation and regulation of local immune responses. The increased number of intraepithelial T lymphocytes in our cases may be a result of keratinocyte activation as a part of adaptive immunity.

Epithelial integrity is the primary factor in sustaining tolerance. The stratified squamous epithelium covering the oral mucosa forms a dynamic layer and maturation is essential for its proper function. Unlike the gastrointestinal mucosa, it lacks the protective mucus secreting cells and the thickness of the epithelium is a physically important protective feature. Factors such as menopause, malabsorption, avitaminoses, some drugs, and metabolic stress impair the maturation of the epithelium and increase mucosal vulnerability. Inte-

restingly, smoking may improve some mechanical properties of the oral epithelium by increasing its thickness and promoting keratosis. That is probably why aphthous ulceration is relatively rare in smokers.

The oral epithelium is constantly renewed by proliferation of the basal cells. During maturation of the basal cells, there are continuous posttranslational protein modifications within the keratinocytes, which are important for generation of new proteins with various functions related to the integrity of the oral epithelium. Keratins, loricrin, involucrin, and small proline-rich proteins are among these proteins, and they constitute the oral mucosal epithelial “cornified cell envelope” located just beneath the keratinocyte plasma membrane in the most superficial cells (18-21). These are insoluble proteins contributing to the flexibility, integrity, strength and regular aging of the epithelium. Any genetic or metabolic derangement involving these proteins effects the stability and may impair the barrier function of the epithelium (18). Epithelial cells are joined together by tight junctions, macula adherens, and desmosomes forming a protective layer against bacterial invasion. Adhesion molecules such as occludin, claudin, desmosomal proteins, and intermediate filament keratins are important for this function. Epithelial layers contain a variety of keratin proteins depending on the cell type, location, function, and stage of differentiation (19,22). Certain acantholytic disorders are characterized by defect of some adhesion molecules (19).

Normally, antigens in the oral cavity cannot easily travel between the epithelial cells due to the presence of tight junctions between oral keratinocytes. The immune system ignores noninvasive commensal flora and their antigens. When the surface layers of the epithelium are damaged, the perme-

ability increases. The permeability of the epithelium is also related to the solubility and the amount of the antigens. This may explain the observation that some foods can cause aphthous ulceration relatively easy.

Saliva, IgA, and antimicrobial peptides form part of the first line of host defense and they prevent luminal bacteria from crossing the epithelial barrier and making a contact with pattern recognition receptors. For this reason, they prevent unnecessary and deleterious inflammation. The major antimicrobial peptides are defensins and cathelicidins (LL-37) (1). These may inactivate the pathogenic flora, particularly the aerobic microorganisms, by interacting with bacterial wall lipopolysaccharides (1). It is suggested that defensin application may be utilized as a possible therapeutic measure in aphthous ulcerations (23).

The oral microenvironment and flora influence the physiology of oral mucosa. Oral flora is rich in streptococci (7). It can metabolize various carbohydrates, remnants of squamous cells, and salivary mucin. Healthy oral epithelium does not respond to normal oral flora and does not mount an inflammatory response. The relations between the oral mucosa and flora can be more properly described as mutualistic rather than commensal. Clinically, noninflamed oral epithelium is in a partially stimulated state and this may be due to the effects of commensal microorganisms (24). The commensal flora protects the overgrowth of pathogens and stimulates the innate system mildly to keep it on a low level of alert. This state of low level alert helps protecting the epithelium against bacterial and other antigenic penetration, particularly around the sites that accumulation of bacteria is easy and the mucosa is relatively more vulnerable. For example, there is continuous neutrophil migration within the thin layer of pocket epithelium and a high concentration of antimicrobial protein secretions. Many factors, including the unnecessary use of antibiotics can change this harmony. Shinnick *et al.* (25) suggested that a high load of *Streptococcus sanguis*-like microorganisms may initiate a local immune response stimulating Langerhans cells and activating a cross-reacting autoimmune response to the homologous peptides within the epithelial heat shock proteins. This process can initiate the immunopathological changes that lead to recurrent aphthous ulceration (25).

The response of oral mucosa against changing microenvironment starts with epithelial damage

and subsequent antigen penetration leading to activation of pattern recognition receptors such as Toll-like receptors (TLR) expressed on the squamous epithelium, Langerhans cells, and neutrophil leukocytes (26). TLR activation results in proinflammatory cytokine secretions such as interleukin-1, tumor necrosis factor- α , interleukin-8, interleukin-10, and interferons (27,28). Different types of TLRs recognize different antigenic substances, and the specific location of TLR specify the form of the ensuing immuno-inflammatory reaction. Abnormal expression of pattern recognition receptors may also play a role in the pathogenesis of aphthous ulcerations. The mucosal reaction to pathogenic bacteria or permeated antigens also activates the postreceptor NF- κ B pathway which is the main pathway of adaptive immunity (13,27). A growing number of studies demonstrate that aberrant NF- κ B signaling is critical in the development of various inflammatory diseases (13,14,28,29). NF- κ B pathway may be activated by Toll-like receptors/pathogen signals, and proinflammatory cytokines (29). Proinflammatory cytokines increase mucosal permeability, facilitate and stimulate adaptive immunity (3). Nonpathogenic flora may delay the activation of NF- κ B pathway. Antigenic substances entering the cell cytoplasm can easily activate adaptive immunity. It is suggested that modulation of NF- κ B signaling is also important in homeostasis and this pathway may be involved in the pathogenesis of inflammatory bowel diseases (14). The role of NF- κ B pathway has not been studied previously on the pathogenesis of aphthous ulceration. In our cases, NF- κ B expression was observed within the squamous epithelium adjacent to areas of ulceration, the endothelial cells of subepithelial vascular structures, and on some inflammatory cells. We have also observed that mucosal epithelium far from the ulceration showed only some basal layer NF- κ B positivity. These findings suggest that blocking the NF- κ B pathway can be a conceivable alternative in the treatment of a variety of diseases (13).

Cytokines secreted from T helper lymphocytes mediate apoptosis, secretion of matrix metalloproteinases, and sudden tissue breakdown (30,31). The preulceration phase of aphthous ulceration is characterized by increased number of intraepithelial T helper and T cytotoxic lymphocytes (2). Intraepithelial CD4 and CD8 positive lymphocytes have been observed within the epithelium at the edge of ulceration in our biopsy specimens.

The clearance of destructive enzymes secreted as a result of activation of the NF- κ B pathway and T lymphocyte stimulation, and the removal of apoptotic debris can take a longer period than ordinary tissue healing. Apoptotic degeneration of the oral mucosa was suggested as a possible mechanism in recurrent aphthous ulceration (31,32). This may explain the rapid tissue destruction and the relatively long healing period of aphthous ulcerations.

Maintaining the barrier function of the epithelium is an energy-dependent process (33). Metabolic stress-related reduction of epithelial ATP synthesis can result in widening of the tight junctions (33). Later, deranged epithelial mitochondrial function can lead to increased epithelial permeability and may result in loss of epithelial integrity (33).

The interaction of the commensal flora and the covering layer of epithelium is quite similar in oral mucosa and intestine. Barrier function deficiency and early activation of NF- κ B pathway in the pathogenesis of aphthous ulceration shows striking similarities to the pathogenesis of inflammatory bowel diseases. The thinner and more easily vulnerable single layer of columnar surface epithelium of the intestine is prone to severe destruction in inflammatory bowel diseases. Mdinaridze et al.

suggested that patients suffering from inflammatory intestinal diseases also had an increased incidence of aphthous ulceration (34). Infectious diseases and antibiotic usage may change the flora and initiate mucosal ulcerations. On the other hand, some antibiotics, through their inhibitory effects on the commensal flora which may act like a pathogen, can induce remission of the lesions for a certain period. This is especially true for Behçet disease, inflammatory bowel disease, and oral aphthous ulcerations (6,35,36). Preshaw et al. suggested that subantimicrobial doses of doxycycline has a promising effect in the treatment of recurrent oral aphthous ulceration (36). However, it is unlikely to be a primary treatment procedure in this type of diseases since the main cause of the tissue breakdown is the deficiency of the barrier function and not of the flora. Additionally, antigens other than those supplied by the flora may also incite the mucosal injury. Anti-inflammatory drugs inhibiting this aberrant hyperinflammatory reactions such as thalidomid and colchicum were suggested in the treatment of this type of diseases (6,37). Interventions that either strengthen the mucosal barrier function or modulate inappropriate activation of NF- κ B signaling pathway may offer better strategies in the treatment of oral aphthous ulcerations.

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