PROFESSOR YOUNGNIM CHOI (Orcid ID : 0000-0002-6496-5560)

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The microbiology of oral lichen planus: Is microbial infection the cause of oral lichen planus?

Keumjin Baek and Youngnim Choi

Department of Immunology and Molecular Microbiology, School of Dentistry and Dental Research Institute, Seoul National University, Korea

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Correspondence to

Youngnim Choi, D.D.S., Ph.D.

Department of Immunology and Molecular Microbiology,

School of Dentistry and Dental Research Institute, Seoul National University

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101 Daehak-ro, Jongno-gu, Seoul 03080, Republic of Korea

Tel: 82-2-740-8643, Fax: 82-2-743-0311, e-mail: youngnim@snu.ac.kr

Summary

Oral lichen planus (OLP) is a variant of LP, a common chronic mucocutaneous inflammatory disease. While cutaneous lesions of LP are self-limiting, OLP lesions are non-remissive, alternating periods of exacerbation and quiescence, and only symptomatic treatments exist for OLP. The precise etiology and pathogenesis of OLP are hardly understood, which is a major obstacle to the development of new therapeutics for this disease. OLP is considered a T cell-mediated inflammatory disease. Although various antigens have been considered, what actually triggers the inflammatory response of T cells is unknown. Suggested predisposing factors include genetic factors, stress, trauma, and infection. The aim of this review was to determine whether microbial infection can cause OLP. We first reviewed the association between OLP and microbial factors, including viral, fungal, and bacterial infections. In addition, each microbial factor associated with OLP was assessed by modified guidelines of Fredricks and Relman to determine whether it establishes a causal relationship. In conclusion, no microbial factor yet fulfills the guidelines to establish the causality of OLP. By focusing on the unclarified issues, however, the potential roles of microbial factors in the pathogenesis of OLP will be soon elucidated.

Introduction

Lichen planus (LP), first described by Erasmus Wilson in 1869, is a common chronic mucocutaneous inflammatory disease affecting the skin, nails, eyes, urogenital tract, and oral mucosa.[1] The prevalence of LP in the general population is 0.22% to 5%.[2,3] As a variant of LP, oral lichen planus (OLP) most frequently affects the buccal mucosa but also the gingiva, tongue, lip, and palate. The estimated prevalence of OLP in adult is 0.5% to 2%. The age of onset is generally between 30 and 60 years, and it appears to be slightly more common in females.[2,4,5] Lesions are characterized by a bilateral symmetric distribution of a lacelike network of white lines (reticular pattern). Papular, plaque-like, atrophic, erosive/ulcerative, and bullous lesions can also appear in the presence of reticular lesions. According to World Health Organization diagnostic criteria for OLP, in addition to the abovementioned clinical criteria, histopathologic criteria, including i) band-like lymphocytic infiltration confined to the superficial part of connective tissue, ii) liquefaction degeneration in the basal cell layer, and iii) absence of epithelial dysplasia, should be fulfilled.[6]

While cutaneous lesions of LP are self-limiting, OLP lesions are non-remissive, alternating periods of exacerbation and quiescence, and only symptomatic treatments exist for OLP.[5] The precise etiology and pathogenesis of OLP are hardly understood, which is a major obstacle to the development of new therapeutics for this disease. OLP is considered a T cell-mediated inflammatory disease, because the infiltrated lymphocytes are mainly T cells. Both intrinsic and extrinsic antigens have been speculated as targets of the infiltrated T cells. Although OLP is sometimes regarded as an autoimmune disorder, it is not classified as an autoimmune disease because no autoantigens have been identified. Suggested predisposing factors include genetic factors, stress, trauma, and infection.[5,6] In this article, microbial factors associated with OLP are reviewed, and the possibility that microbial infection causes OLP is evaluated.

Association with viral infection

The T cell mediated nature of OLP histopathology suggests that viral infection could be involved in the pathogenesis of the disease. Therefore, the potential association between OLP and several viruses has been widely studied.

Human papilloma virus (HPV) infects oral epithelia and is an established causal factor for oropharyngeal squamous cell carcinoma.[7,8] Since the first report of detection of HPV DNA in OLP biopsies[9], many groups have investigated the presence of HPV in oral tissues or exfoliated cells from OPL patients and control subjects by PCR, immunohistochemistry, or in situ hybridization, but conflicting results have been reported. A recent meta-analysis of 22 case-control studies concluded that OLP patients have a significantly higher risk of HPV infection than healthy controls (35.09% vs. 7.77%, odds ratio [OR]: 6.83, 95% confidence interval [CI]: 4.15 – 11.27), with the variation depending on geography, clinical types of OLP, and HPV genotype.[10]

Hepatitis C virus (HCV) infection causes diverse extra-hepatic as well as hepatic disorders, and LP has been suspected as one of the extra-hepatic manifestations. The sero-positivity of anti-HCV antibodies in OLP and control groups has been widely studied without yielding consistent results. However, three papers that performed meta-analyses of 11 studies[11], 44 studies[12], and 19 studies[13] uniformly reported a significantly higher HCV sero-positivity in OLP patients than in controls (OR: 5.56, 2.8, and 6.07, respectively). Two of the three studies found significant variation in the OLP-HCV association by geographical distributions attributable to differences in the prevalence of HCV and HLA-DR genetics.[11,13] Campisi *et al.* reported that the association between OLP and HCV infection in HCV-endemic cohorts disappeared after correcting for age.[14] In the three meta-analyses, age was not properly corrected for; therefore, the age factor must be further considered in future studies. LP is currently classified as an extra-hepatic disorder of HCV infection with a

significant but weak association.[15] As the underlying mechanism, a direct role of HCV-specific T cells in epithelial damage at OLP lesions has been suggested.[16] Although the presence of both genomic and replicative forms of HCV RNA in the epithelial cells of normal oral mucosa or OLP lesions has been reported[17-19], evidence to support the epithelial tropism of HCV is insufficient.[20]

Several groups have investigated the potential association of OLP with Epstein-Barr virus but have found no association.[21-24] Recently, distinct expression profiles of human cytomegalovirus-encoded miRNAs in the plasma of OLP patients have been reported without known roles of these miRNAs in the etiology of OLP.[25]

Association with fungal infection

The relationship between *Candida* and OLP has been suggested since 1980.[26] The *Candida* species are a common oral flora in healthy individuals without any symptoms or clinical signs of *Candida* infection. Among the *Candida* species, *Candida albicans* is the most prevalent in both healthy and diseased oral cavities, and shows carriage rates ranging from 60 to 80%.[27] Certain systemic or local factors, including systemic antibiotics therapy, diabetes mellitus, immunodeficiency, poor oral hygiene, and systemic or local steroid therapy, promote susceptibility to *Candida* infection.[28]

Currently, the high prevalence of *Candida* in OLP patients is controversial. Two groups have reported no significant differences in the prevalence of *Candida* species between healthy individuals and patients with erosive OLP. [26,29] Artico *et al.* presented a significantly higher prevalence of *Candida* species in healthy individuals than in OLP patients.[30] By contrast, several studies have demonstrated a higher prevalence of *Candida* culture in OLP patients than in control subjects.[28,31-33] Among OLP patients, no significant difference has been observed in the colonization of *Candida* species between erosive and non-erosive

OLP.[31] Non-*C. albicans* species including *C. glabrata, C. tropicalis, C. fukuyamaensis, C. parapsilosis,* and *C. dubliniensis* were isolated in a small percentage of total isolates.[28,33] Notably, topical corticosteroid therapy, the most commonly employed treatment of OLP, often increases the incidence of fungal infection and secondary candidiasis.[28,34,35]

Whereas most studies have focused on the carriage rate of *Candida* species, Zeng *et al.* studied the genotypes and virulence factors of *C. albicans* in OLP patients. Type A and type C were predominantly found in erosive OLP, while type A and type D were mainly identified in non-erosive OLP. Among the four genotypes, the isolates with type A had the highest adherence ability, and the phospholipase activity of the isolates with types A and C was higher than that of the isolates with types D and B that were prevalent in healthy individuals.[36]

Collectively, it is difficult to conclude that fungal infections are associated with OLP. However, antifungal treatment of OLP patients with *Candida* has been shown to improve clinical symptoms of OLP.[37,38] Therefore, superinfection with *Candida* may aggravate the symptoms of OLP. All previous studies have investigated the prevalence of *Candida* using a culture-based method involving mucosal swabs, saliva, or oral rinses but not in OLP tissues. Fungal hyphae are sometimes observed in histopathologic examinations of OLP biopsies, while mucosal swabs result in negative *Candida* culture (Personal communication with Dr. Hee Kyung Park at the Department of Oral Medicine, Seoul National University Dental Hospital). A PCR-based method would identify low levels of *Candida* that fail to grow. In addition, the application of high-throughput sequencing technology would provide an overall view of the fungal community. Therefore, further studies are requested to characterize fungal communities associated with OLP not only on the mucosal surface but also within tissues.

Association with bacterial infection

After the detection of *Helicobacter pylori*, a well characterized pathogen for gastric and duodenal ulcer, in saliva[39], several groups studied the association of OLP with *H. pylori*. Although two groups reported the presence of *H. pylori* DNA detected by nested PCR in 38%-45% of OLP biopsies, most studies reported no association.[40-44] In particular, the *H. pylori* organisms were not detected in OLP lesions by either H&E staining or immunohistochemistry.[40,44]

Bornstein *et al.* compared the colonization of 74 bacterial species on mucosa with asymptomatic OLP lesions to that on healthy sites of OLP patients and heathy subjects using the checkerboard DNA-DNA hybridization method. They reported increased colonization of several species, including *Capnocytophaga sputigena*, *Eikenella corrodens*, *Lactobacillus crispatus*, *Mobiluncus curtisii*, *Neisseria mucosa*, *Prevotella bivia*, *P. intermedia*, and *Streptococcus agalactiae*, on OLP lesions. However, it was unclear whether the bacterial speceies increased on the mucosal surface invaded the diseased mucosa.[45] Ertugrul *et al.* investigated the prevalence of five periodontopathogenic bacterial species in subgingival plaque samples obtained from OLP patients and healthy subjects by a PCR-based method. They reported increased prevalence of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *P. intermedia*, and *Treponema denticola* in OLP patients and proposed direct or indirect roles of the periodontopathogenic bacteria as local irritant factors in the etiology of OLP.[46] However, the relationship between the subgingival plaque microbiome and bacterial communities on OLP lesions is not known.

The advent of high-throughput sequencing technology has allowed researchers to study the complex profiles of the microbiome associated with OLP. To date, three groups have investigated oral microbiota associated with OLP by high-throughput sequencing of the 16S rRNA gene. Wang and colleagues analyzed salivary microbiota of healthy, reticular

OLP, and erosive OLP groups.[47] In contrast, the other two groups including our group analyzed buccal mucosal microbiota of healthy subjects and OLP patients.[48,49] All our patients carried both reticular and erosive lesions, and our sampling method using a 4 cm^2 membrane often covered both lesions. He and colleagues sampled from erosive and non-erosive OLP patients separately but reported few difference between the two groups.[49]

Despite the differences in ethnic populations, sampled sites, the targeted variable region of the 16S rRNA gene, and sequencing methods among the three studies, several common findings were revealed. First, the OLP-associated microbiota was characterized by increased diversity (Figure S1).[47,49] The periodontitis-associated subgingival microbiome was also characterized by an increase in diversity.[50,51] OLP and periodontitis are quite unique examples because most other mucocutaneous diseases, such as atopic dermatitis, psoriasis, Crohn's disease, and ulcerative colitis, are associated with a microbiome exhibiting decreased diversity.[52-54] Second, OLP patients are colonized with dysbiotic oral flora. When we performed a principal coordinates analysis of our data together with the data of Wang and colleagues obtained from the SRP database, segregation of bacterial communities not only by anatomical site (*i.e.*, saliva *vs*. buccal mucosa) but also by OLP status was evident. In particular, the salivary bacterial communities of erosive OLP patients were completely segregated from those of healthy controls (Figure 1). In terms of bacterial composition, a decrease in the most prevalent genus *Streptococci* and an increase in *Leptotrichia* in OLP patients were common in the three studies. He *et al.* reported the low abundance of *Helicobacter* genus both in healthy and OLP groups with no significant difference.[49] In our analysis of the data reported by the other two groups, *H. pylori* was not detected in any sample. Therefore, *H. pylori* is unlikely to be associated with OLP.

Considering the altered characteristics of mucosal surfaces observed at OLP lesions, the dysbiosis of the oral microbiota is no surprise and could be the result of disease. We

previously showed the presence of intracellular bacteria within epithelial cells and infiltrated T cells by *in situ* hybridization using a eubacterial probe in the all OLP biopsies observed. Furthermore, the presence of immunologic synapse-like structures at the contact sites between the infected epithelial cells and T cells suggested that the intracellular bacteria may provide target antigens to the infiltrated T cells.[48] In our previous study, specific species responsible for the intracellular infection were not clarified. Recently, Mizuki *et al.* reported that *Mycoplasma salivarium* was detected in 58.5% of OLP tissues by immunohistochemistry using an antibody to *M. salivarium*. The bacteria were localized intracellularly in epithelial cells although intra- or extracellular localization in the lamina propria was not clear.[55] Several *Mycoplasma* species, including *M. salivarium*, were detected among the buccal mucosal microbiota analyzed by our group, although there was no difference in relative abundance between control and OLP groups. Whether *M. salivarium* triggers a specific T cell response in OLP lesions must be verified.

Evaluation of microbial factors as the cause of OLP based on the modified guidelines of Fredricks and Relman

Collectively, microbial factors associated with OLP include HPV, HCV, and dysbiotic oral bacterial communities. To verify a causal relationship, the association observed in repeated cross-sectional studies should be confirmed by prospective and intervention studies[56], which are few in number or lacking among OLP studies. According to Koch's postulates, the best way to prove a causal relationship between microorganisms and OLP would be to reproduce OLP in animal models using the suspected microorganisms isolated from OLP lesions. However, HPV does not infect other animals because of species restriction, and less than half of oral bacteria are currently cultivable. Fredricks and Relman proposed sequence-based guidelines to establish causal relationships between microbes and diseases in the absence of a

cultivated or purified microorganism.[57] While the principles underlying Koch's postulates were upheld, relative numbers of pathogen-derived sequences were applied rather than their absence or presence to overcome several limitations of Koch's postulates. The authors also emphasized coherence and plausibility rather than strict adherence to every one of the proposed guidelines.

We modified the guidelines of Fredricks and Relman to evaluate HPV, HCV, or bacteria as the cause of OLP (Table 1). First, a putative pathogen or a nucleic acid of the pathogen should be present in most cases of OLP. The prevalence of HPV in OLP patients ranged from 2.7% to 100% depending on the study, and the pooled prevalence was 35.09%.[10] The prevalence of HCV in OLP patients ranged from 0% to 67.8%, resulting in a pooled prevalence of 16.3%.[58] Petti *et al.* estimated the fraction of global OLP cases associated with HCV to be only 2.1%.[12] Although a eubacterial 16S rRNA sequence was detected in 100% of OLP tissues[48], whether it belongs to a single bacterial species is not clear. *M. salivarium* was detected in 58.5% of OLP cases.[55] These findings indicate that a single microbial factor responsible for all cases of OLP has not yet been identified. Of course, as observed in the example of pneumonia, a single disease condition can be caused by different microorganisms.

Second, fewer or no pathogen or pathogen-associated nucleic acid sequences should occur in control subjects or the healthy oral mucosa of OLP patients. The pooled prevalences of HPV and HCV in control subjects were 7.77% and 2.9%, respectively.[10,58] It is not known whether those control subjects with HPV or HCV have lower levels of viral titer than OLP patients with the respective virus. Little or no signal of a eubacterial 16S rRNA sequence or *M. salivarium* was detected in the oral tissues of control subjects, but the size of the control group was small in both studies.[48,55]

Third, the number of pathogen or pathogen-associated nucleic acid sequences should increase during periods of exacerbation but decrease during quiescent periods of OLP. There are no published data on the levels of suspected microbial factors during the clinical course of OLP. However, obtaining a biopsy again after alleviation of OLP lesions is neither practical nor ethical.

Fourth, pathogen/sequence detection should predate OLP development, the number of pathogens/sequences should correlate with the clinical score or pathology of OLP, or clearance of pathogens/sequences should be followed by remission of OLP. Whether HPV infection precedes OLP development is not known. HPV infects only the basal cells of stratified epithelium through micro-abrasions or other epithelial trauma.[7] A significantly higher prevalence of HPV in erosive/atrophic-OLP (OR 9.34) than in non- erosive/atrophic-OLP (OR: 4.32)[10] may indicate that HPV infects following the development of OLP. There are few well-designed prospective studies that followed subjects with or without HCV infection with respect to the development of OLP. Several groups have reported that IFN and ribavirin therapy cleared serum HCV RNA but not OLP or LP.[59-61] Even the exacerbation or development of OLP lesion by IFN therapy has been reported, although the sample size was small.[59] Very recently, the disappearance or improvement of HCV-associated OLP lesions by IFN-free therapy with direct-acting antivirals has been reported in a small number of patients.[62] These results indicate that HCV may contribute to the pathogenesis of OLP through a host immune response rather than acting as the direct causal agent of OLP. In contrast, several lines of evidence indicate a causal role of bacteria. The levels of bacteria detected within the lamina propria correlated positively with those of T cell infiltration in OLP lesions, suggesting that intracellular bacteria may drive T cell infiltration.[48] The levels of salivary *Porphyromonas* correlated with disease scores and salivary levels of IL-17 and IL-23.[47]

Fifth, the nature of the microorganisms inferred from the available sequence should be consistent with the known biological characteristics of that group of organisms. This guideline is important to prove causality by unidentified taxa.

Sixth, a pathogen or pathogen-associated nucleic acid sequences should be localized to OLP tissues, particularly to the basal cells of the epithelium, by specific immunohistochemistry or in situ hybridization. Localization of HPV or bacteria to almost all basal epithelial cells in OLP tissues has been demonstrated by immunohistochemistry or in situ hybridization.[48,55,63,64] In contrast, HCV RNA has been detected in a small portion of suprabasal epithelial cells.[17]

Seventh, efforts should be made to reproduce the known pathology of OLP using a pathogen or that inferred from available sequences *in vitro*. HPV-induced modification of the cell cycle of epithelial cells[65] may be responsible for hyperkeratosis or hyperplasia often observed in OLP lesions. We previously showed that oral bacteria can induce the production of T cell-recruiting chemokines from monocytes and CD4 T cells, regardless of the bacterial species.[48]

Eighth, these evidence for microbial causation of OLP should be reproducible.

Conclusion and perspective

In conclusion, no microbial factor yet fulfills the guidelines to establish the causality of OLP. We believe that our previously proposed model for the pathogenesis of OLP is still valuable by including virus and fungus. Epithelial barrier dysfunction may precede intracellular infection of basal epithelial cells with bacteria, virus, or possibly fungus. The species increased as the result of dysbiosis of the oral microbiome may be responsible for epithelial barrier dysfunction and/or intracellular infection. Intracellular infection, given its nature, would predominantly recruit T cells. Recognition of microbial antigens presented on epithelial cells by the recruited T cells would lead to liquefaction of the basal cell layer,

which would aggravate barrier dysfunction, completing a vicious cycle. Finally, continuous or persistent infection is responsible for the chronic and non-remissive nature of disease (Figure 2). The most likely role of HCV in the pathogenesis of OLP appears to be increased serum proinflammatory cytokines that can contribute to epithelial barrier dysfunction.[48,66] HPV can infect the basal cells of epithelial cells and may provide antigens to T cells. However, HPV infection cannot be responsible for all OLP cases, and different consequences of HPV infection from clearance or OLP development to carcinogenesis must be clarified. The distribution patterns of bacteria in the epithelia and infiltrated T cells suggest bacteria as a promising cause of OLP. As *M. salivarium* was detected in only 58.5% of OLP cases, several different species could be responsible for different OLP cases. Alternatively, OLP may be a polymicrobial disease such as periodontitis. Although the relationship between the bacteria detected in OLP tissues and those colonized on the mucosal surface is not known, bacteria infecting OLP tissues are likely to be indigenous, such as *M. salivarium*, rather than exogenous. Identification of the bacteria detected in OLP tissues to the species level would clarify these speculations. The examples of intracellular pathogenic bacteria are fewer than those of extracellular pathogenic bacteria. How bacteria in the complex oral mucosal microbiome establish only intracellular infections and how neutrophils are not recruited if there are also extracellularly located bacteria must be addressed. By focusing on the unclarified issues, the potential roles of microbial factors in the pathogenesis of OLP will be soon elucidated.

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Conflict of Interest

Authors declare that there is no conflict of interest.

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Table 1. Guidelines to evaluate a causal relationship between microbial factors and OLP

Guidelines[a]
1. A putative pathogen or a nucleic acid of the pathogen should be present in most cases of OLP.
2. Fewer or no pathogen or pathogen-associated nucleic acid sequences should occur in control subjects or the healthy oral mucosa of OLP patients.
3. The number of pathogen or pathogen-associated nucleic acid sequences should increase during periods of exacerbation but decrease during quiescent periods of OLP.
4. Pathogen/sequence detection should predate OLP development, the number of pathogen/sequence should correlate with clinical score or pathology of OLP, or clearance of pathogen/sequence should be followed by remission of OLP.
5. The nature of the microorganisms inferred from the available sequence should be consistent with the known biological characteristics of that group of organisms.
6. A pathogen or pathogen-associated nucleic acid sequences should be localized to OLP tissues, particularly to the basal cells of epithelialium, by specific immunohistochemistry or in situ hybridization.
7. Efforts should be made to reproduce the known pathology of OLP using a pathogen or that inferred from available sequences *in vitro*.
8. These evidence for microbial causation of OLP should be reproducible.

[a]Modifed from those of Fredricks and Relman[57]

Figure Legends

Figure 1. Principal coordinates analysis plot generated using weighted UniFrac metric. The data pertaining to the salivary microbiota[47] were obtained from the SRP database and analyzed. The data for the buccal mucosal microbiota include our previously published data[48] and new samples from five healthy individuals and three OLP patients.

Figure 2. Proposed pathogenesis model for OLP. The diagram is adapted from a previously published figure.[48]