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Immune mechanisms in oral lichen planus

Asma El-Howati, Martin H. Thornhill*, Helen E. Colley, Craig Murdoch

School of Clinical Dentistry, University of Sheffield, Sheffield, United Kingdom

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Corresponding author: *Prof Martin Thornhill, School of Clinical Dentistry, University of Sheffield, Sheffield, S10 2TA, UK. Tel: +44 (0) 114 2159397

E-mail: m.thornhill@sheffield.ac.uk

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Abstract

Oral lichen planus (OLP) is a T-cell-mediated inflammatory disease of the oral mucosa that has been extensively researched over many years but as yet the mechanisms of pathogenesis are still not fully understood. Whilst the specific etiologic factors driving OLP remain ambiguous, evidence points to the development of a chronic, dysregulated immune response to OLP-mediating antigens presented by innate immune cells and oral keratinocytes leading to increased cytokine, chemokine and adhesion molecule expression. These molecules recruit T-cells and mast cells to the diseased site and orchestrate a complex interplay between cells that culminates in keratinocyte cell death, mucosal basement membrane destruction and long-term chronicity of the disease. The main lymphocytes involved are thought to be CD8+ cytotoxic and CD4+ Th1 polarised T-cells although recent evidence indicates the involvement of other Th subsets such as Th9, Th17 and Tregs, suggesting that a more complex immune cell relationship exists during the disease process. This review provides an overview of the immune mechanisms at play in OLP pathogenesis with particular emphasis on the role of the different Th subsets and how these recent discoveries may guide research toward identifying potential therapeutic targets.

Introduction

Oral lichen planus (OLP) is a relatively common mucocutaneous inflammatory disease of unknown aetiology with a global prevalence of around 1%, although there are marked geographical differences (Gonzalez-Moles et al., 2021). OLP occurs most frequently in isolation. However, it can occur with involvement of other mucosal surfaces, particularly genital (20%) or the skin (15%). In contrast, OLP is found in approximately 70% of patients with cutaneous LP (Farhi & Dupin, 2010). The condition ranges in severity from asymptomatic, mild, moderate to severe forms and commonly undergoes periods of quiescence and exacerbations that often coincide with such factors as stress, anxiety, trauma, or exposure to a low chronic irritant, for example dental plaque, tobacco use or dental filling (Kurago, 2016; Scully & Carrozzo, 2008). Classically, OLP presents with reticular white lesions that are bilaterally and symmetrically distributed on the oral mucosa, particularly the buccal mucosa and sides of the tongue. Sometimes these white patches are more plaque-like in appearance. In both cases, the thickening of the mucosa may be relatively asymptomatic apart from feeling rough. With increasing disease activity however, there is mucosal thinning to produce erosive (atrophic) lesions that are erythematous in appearance, often with surrounding reticular or striated areas. Erosive areas are often painful and particularly sensitive to strong flavours, acids, spirits etc., probably due to loss of the mucosal permeability barrier. As disease activity increases further, there is complete loss of epithelium and the development of ulcerative lesions. Like erosive lesions these may be surrounded by reticular or straited lesions and are extremely sensitive (Al-Hashimi et al., 2007; Carrozzo, Porter, Mercadante, & Fedele, 2019) (Figure 1). Hence, it is the erosive/ulcerative form that is considered the more clinically significant, causing symptoms of discomfort, burning and pain (Park, Hurwitz, & Woo, 2012; Scully & Carrozzo, 2008).

The classical histopathological features of OLP include a dense sub-epithelial band of inflammatory T-cell, along with intra-epithelial lymphocytic cell infiltration and liquefaction degeneration/apoptosis in the basal cell layer to form colloid bodies (also termed cytoid, hyaline, keratin or Civatte bodies) (Matthews, Scully, & Potts, 1984) (Figure 2). Other histological features include surface hyper-parakeratosis, irregular acanthosis with occasional areas of atrophic epithelium where the rete pegs may be pointed (saw-tooth), and the presence of a fibrinous precipitate at the epithelial-connective tissue junction (Muller, 2017).

Oral lichenoid reactions (OLR) are regarded as either a distinct variant of OLP or an OLP-like lesion and are similar in clinical and histopathological appearance to OLP. In contrast to OLP, OLR tends to present clinically as a unilateral lesion that is not accompanied by extraoral lesions (Thornhill, Pemberton, Simmons, & Theaker, 2003). Moreover, histologically, the lymphocytic band tends to be more diffuse, containing more eosinophils, plasma cells and colloid bodies than in OLP (Muller, 2017; Thornhill et al., 2006). Some cases of OLR can be difficult to distinguish from OLP. Unlike the

idiopathic nature of OLP, OLR is often associated with an identifiable factor such as a specific dental material (e.g., amalgam or nickel), chemical or medication and so is considered to occur as a result of a delayed hypersensitivity reaction (Thornhill et al., 2006). Chronic graft-versus-host disease (GvHD), whereby oral mucosal lesions present in hematopoietic stem cell transplantation recipients in response to the damaging effects of donor T cells within the transplanted tissue, can also histologically resemble OLP (Imanguli, Alevizos, Brown, Pavletic, & Atkinson, 2008). Diagnosis of these lesions can often be made on clinical presentation alone, although a correlation between both clinical and histopathological criteria are required to provide a definitive diagnosis. Therefore, there are several inflammatory mucocutaneous diseases that share characteristic clinical and histological features of OLP. These diseases probably share similar immunological events that result in pathogenesis, so understanding the immune mechanism in one disease may help to explain the immune mechanism in other similar diseases.

There is a long history concerning the proposed cellular and molecular pathogenic mechanisms in OLP, starting with Walsh and colleagues in 1990 (Walsh, Savage, Ishii, & Seymour, 1990), further developed by Sugerman et al., in 2002 (Sugerman et al., 2002) and more recently extended by others to take into account new data (DeAngelis, Cirillo, & McCullough, 2019; Khan et al., 2003; Lu, Zhang, Sun, Du, & Zhou, 2015; Lu et al., 2011). The overall consensus is that the pathogenesis of OLP is initially driven by the recognition of a non-self-antigen that evokes an intricate and complex interplay between immune and non-immune cells, cytokines and adhesion molecules, culminating in a dysregulated cell-mediated immune reaction. This leads to proteolytic-mediated basement membrane destruction and an inappropriate and harmful immune response, mainly by T-cell subsets, directed against oral keratinocytes leading to keratinocyte apoptosis. Despite much advancement in the knowledge of OLP pathophysiology, the dysregulated immune response that governs the disease is still not completely understood. This review provides an overview of the accumulated knowledge and current understanding of the specific and non-specific immune responses in OLP and reflects on areas of research that may lead to the development of new, targeted therapies.

Which antigens drive OLP pathogenesis?

The specific etiologic factors driving OLP are still unknown, although several lines of evidence point to the presentation of a foreign or altered antigen by basal keratinocytes that induce a T-cell-mediated autoimmune response against these cells as the root cause. Each T-cell expresses a specific cell surface T-cell receptor (TCR) that is composed of α and β chains which contain variable (V) regions with unique amino acid sequences that mediate binding to a specific antigenic determinant. Rearrangements in the genes that encode the α and β proteins during T-cell development create an extensive antigen-specific TCR repertoire. In response to an OLP-mediating antigen presented in association with the major

histocompatibility complex (MHC), the corresponding antigen-specific T-cell becomes activated, recruited to the target oral mucosa where it undergoes clonal expansion and directs a deleterious immune response against target oral keratinocytes resulting in keratinocyte cell death.

In order to identify the specific antigens that instigate OLP, studies have attempted to characterise the TCRs expressed by T-cells in OLP lesions. The over-representation of specific Vα and Vβ TCR families expressed by OLP lesional T-cells indicates the selective recruitment and expansion of particular T-cells at lesional sites. However, the overall heterogeneous nature of these Vα and Vβ sub-family suggests that a number of antigens rather than one distinct antigen are responsible for provoking disease progression. Moreover, since superantigens (i.e., antigens that mediate non-specific excessive T-cell activation) can bind to multiple types of Vβ regions irrespective of TCR specificity, it has been proposed that superantigens are also involved in driving OLP (Simark-Mattsson, Bergenholtz, Jontell, Tarkowski, & Dahlgren, 1994; Thomas, Stephens, Stephens, Patten, & Lim, 1997; Zhou et al., 1996).

At present no single antigenic determinant has been shown to specifically trigger OLP pathogenesis. Although still highly debated, current data points to the involvement of a number of exogenous viral or microbial antigens in driving the cell-mediated host response. Three independent systematic reviews with meta-analysis have shown an association between presence of hepatitis C virus (HCV) and OLP, although this association showed regional differences, with increased association observed in areas with higher prevalence of HCV infection (Alaizari, Al-Maweri, Al-Shamiri, Tarakji, & Shugaa-Addin, 2016; Lodi, Pellicano, & Carrozzo, 2010; Petti, Rabiei, De Luca, & Scully, 2011). Another systematic review identified that human papillomavirus (HPV) may also play a causal role in OLP, but once again the strength of this association varied across geographic populations, clinical types of OLP and HPV genotypes (Ma, Zhang, Zhang, Lv, & Liu, 2016). An association of OLP with presence of Epstein-Barr virus is less convincing (Ashraf et al., 2020). The commensal fungus, *Candida albicans* has also been studied as a potential instigator for OLP, but there is difficultly in establishing a causal relationship, even where high prevalence was reported because oral candidiasis is an opportunistic infection or can occur as a side-effect of corticosteroid and other treatments for oral diseases (Baek & Choi, 2018). Several periodontal pathogenic bacteria (*Porphyromonas gingivalis*, *Tannerella forsythia, Prevotella denticola*) have been detected in OLP lesions and direct or indirect roles of these microbes, along with superantigens derived from *Staphylococcal* and *Streptococcal* bacteria and even *Escherichia coli* have been proposed in the aetiology of OLP (Baek et al., 2020; Bornstein, Hakimi, & Persson, 2008; Ertugrul, Arslan, Dursun, & Hakki, 2013; Zhong et al., 2020) although their role in disease pathogenesis is still contentious (Villa, Sanchez-Perez, & Sieiro, 2021). The detection of intracellular bacteria within oral keratinocytes and their close association with intra-epithelial T-cells suggests a route for microbial antigen presentation by oral keratinocytes, and it has been proposed that individual bacterial species are responsible for the presentation of different OLP types (Choi et al., 2016). Sequencing of 16S rRNA

from healthy oral mucosa compared to OLP lesion shows that the OLP-associated microbiota is significantly different (Baek et al., 2020). Exogenous viral antigens have been associated with the aetiology of cutaneous LP including those linked with OLP, particularly HCV (Georgescu et al., 2019), supporting the notion that common viral/microbial antigenic factors are at play in both oral and non-oral sites. However, unlike OLP, detailed studies examining the functional microbiome at LP lesional compared to healthy skin are lacking, making it difficult to draw any comparisons (Karimova, Moyes, Ide, & Setterfield, 2021). Moreover, in all instances, it has proven difficult to establish a direct causal relationship between microbe and OLP pathogenesis.

Other predisposing factors have been related to OLP onset such as stress, hypothyroidism, nutrient deficiency and food allergy. It has been postulated that these factors as well as others such as drugs or mercury salts may cause the unmasking or alteration of endogenously expressed self-antigens that in turn stimulate a dysregulated T-cell immune response in OLP (Payeras, Cherubini, Figueiredo, & Salum, 2013; Sugerman et al., 2002). Despite this research, the precise nature of the antigen(s) that instigate OLP remain elusive and so the initial events driving OLP are still ambiguous.

Antigen presentation and initiating events in OLP pathogenesis

The initiating events in OLP pathogenesis not only revolve around acquisition of exogenous or endogenous antigens but importantly production of pro-inflammatory cytokines and response to these. Upon stimulation, oral keratinocytes release a multitude of pro-inflammatory cytokines that significantly contribute at different stages to OLP pathogenesis. Keratinocytes from OLP lesions have been reported to produce TNF-α, IL-1β, G-CSF, IL-12 and IL-6 with many at levels 10- to 20-fold greater than from healthy oral mucosa (Aragane et al., 1994; Khan et al., 2003; Yamamoto & Osaki, 1995). In addition, TNF-α, IL-1β and IL-6 were detected at high levels in the supernatant of OLP keratinocytes, tissue-infiltrated mononuclear cells and in the saliva of OLP patients (Rhodus et al., 2005; Rhodus, Cheng, & Ondrey, 2007; Yamamoto & Osaki, 1995). Gene polymorphisms in TNF-α and IL-6 have been associated with increased risk of OLP whilst no association was found for the IL-1 cluster or IL-10 (Xavier, de Sa, Guimaraes, da Silva, & Gomez, 2007). The activated keratinocytes act in concert with local dendritic cells (DC) termed Langerhans cells, which are antigen presenting cells (APCs) that reside within the oral epithelium and are found in increased numbers in OLP lesions (Rich & Reade, 1989). These DC mature by capturing, processing, and presenting OLP-mediating antigens in association with major histocompatibility complex (MHC) class II on their cell surface. The key pro-inflammatory molecules at this stage appear to be TNF-α and IFN-γ. Increased levels of IFN-γ up-regulates the expression of MHC class II by DC enhancing antigen presentation and at the same time stimulates oral keratinocytes to express human leukocyte antigen (HLA)-DR and -DQ MHC class II,

enabling them to also act as APCs (Farthing & Cruchley, 1989; J. Li, Farthing, & Thornhill, 1996; Takeuchi, Tohnai, Kaneda, & Nagura, 1988). Increased levels of TNF-α, due to both DC and keratinocyte activation, cause activation of the key intracellular pro-inflammatory transcription factor, nuclear factor kappa B (NFκB) in the surrounding cells and, in combination with the effects of IFN-γ, lead to increased expression of numerous chemokines that drives immune cell recruitment as the disease progresses (J. Li, Ireland, Farthing, & Thornhill, 1996; Marshall, Celentano, Cirillo, McCullough, & Porter, 2017; Orlando, Bragazzi, & Nicolini, 2013; Santoro et al., 2003; Zhao, Sugerman, Walsh, & Savage, 2002). Importantly, activated DC migrate to the local lymph node where they interact with antigen specific T-cells to produce a primary (often termed the specific) OLP immune response.

T-cell activation

T-cells with an OLP-mediating antigen-specific TCR can be activated either at distal lymph nodes sites or at the lesional site by APCs. The activation of naïve T-cells in the lymph nodes follows the physical interaction between T-cells and DC. The primary signal in T-cell activation is the binding of the TCR-CD3 complex and either CD4 or CD8 co-receptor with MHC class I or II, respectively (Figure 3). This interaction stimulates a cascade of intracellular signalling that results in the initial activation of T-cells. However, other co-stimulatory events are essential for T-cell proliferation and cytokine secretion. The primary co-stimulatory signal for T-cell activation is the binding of CD28 and the integrin CD18/CD11a (LFA-1) expressed on the T-cell surface with their counter-receptors, CD80 (B7-1)/CD86 (B7-2) and CD54 (ICAM-1) respectively, expressed by DC (Wingren et al., 2017) (Figure 3). Ligation of CD28 augments T-cell immune responses by inducing enhanced secretion of IL-2 that then acts in an autocrine manner to stimulate T-cell proliferation (June et al., 1989; Thompson et al., 1989). Indeed, the expression of IL-2 has been consistently found at elevated levels in OLP lesions (Orlando et al., 2013; Yamamoto & Osaki, 1995). DC constitutively express moderate levels of CD80 and CD86, however, these levels increase 100-fold upon activation. To modulate this immune response, CD80/CD86 also bind cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) but with higher affinity than CD28, blocking CD28-medaited T-cell activation and dampening intracellular signalling (Krummel & Allison, 1995). In addition to CD28, binding of CD40 with CD154 (also termed CD40-ligand), also induces the expression of CD80/CD86 as well as the secretion of pro-inflammatory cytokines such as IL-6 and IL-12, which can influence T-cell differentiation into different T-cell subsets (Mackey, Barth, & Noelle, 1998) (Figure 3).

Activated antigen-specific T-cells then enter the bloodstream and are recruited to the OLP lesional site by chemokines and adhesion molecules (see following section). Interestingly, non-activated T-cells are also recruited to lesional sites where they are activated by tissue APCs including DC, macrophages and

IFN-γ-activated oral keratinocytes that are stimulated to express MHC class II, CD40, CD54 and CD86 (Little, Griffiths, Watson, Pemberton, & Thornhill, 2003; Marshall, Celentano, Cirillo, Mirams, et al., 2017; Zhou et al., 1996). Moreover, OLP lesional mast cells can also activate T-cells through the expression of MHC class II, CD80/86 and CD11a (Kambayashi et al., 2009). Hence, both the chance encounter and the attracted migration hypothesis have been suggested for T-cell activation and recruitment in OLP.

T-cell recruitment to the OLP lesion sites

In OLP, the lesional cell population is predominantly made up of variable levels of CD4+ and CD8+ T-cell subpopulations. The crucial role of T-cells in OLP pathogenesis was first established after an oral lesion was induced following local transfer of CD4+ T-cell clones with a cytotoxic ability (Shiohara, Moriya, Tsuchiya, Nagashima, & Narimatsu, 1986). Immunostaining analysis shows that the majority of T-cells within the epithelium and adjacent to apoptotic keratinocytes are activated CD8+ cells, while most T-cells in the lamina propria are CD4+ T-cells (Jungell, Konttinen, Nortamo, & Malmstrom, 1989; Matthews et al., 1984), providing an early indication that cytotoxic CD8+ T-cells are largely responsible for driving keratinocyte apoptosis (Sugerman, Satterwhite, & Bigby, 2000).

Recruitment of T-cells from the circulation to OLP lesions is mediated by adhesion molecules that are expressed on the surface of activated endothelium with their cognate receptors on T-cells. Endothelial adhesion molecules such as CD31 (PECAM-1), CD106 (VCAM-1), CD54 (ICAM-1) and CD62E (E-selectin) are expressed at very low levels in quiescent endothelium but stimulation by pro-inflammatory cytokines such as TNF-α and IFN-γ released from OLP lesional sites dramatically up-regulates their expression on nearby blood vessels (Little et al., 2003; Meager, 1999). For example, endothelial CD31 was found to be overexpressed in OLP compared to normal mucosa with higher expression in erosive than reticular forms (Lavanya et al., 2020). Circulating T-cells, under the influence of specific chemokines, then bind to the activated vascular endothelium via multiple receptor/ligand interactions including CD18/CD11a with CD54, integrin CD49d/CD29 (VLA-4) with CD106 and CD62L (L-selectin) with CD62E (Meager, 1999).

T-cell migration from the endothelial surface into lesional tissue is directed by chemokine gradients produced by IFN-γ and TNF-α-activated immune and non-immune lesional cells and increased expression of CD54 on the surface of oral keratinocytes that facilitate leukocyte locomotion through the epithelium (Little et al., 2003). CCL5 (RANTES) is a potent T-cell chemoattractant that also causes degranulation of mast cells. *In vitro*-cultured oral keratinocytes increase gene and protein expression of CCL5 in response to IFN-γ and TNF-α (J. Li, Ireland, et al., 1996; Little et al., 2003) and high levels of CCL5 have been detected in the epithelium, lamina propria and serum of OLP patients (Hu et al., 2013;

Ichimura et al., 2006; Shan et al., 2019). Moreover, CCL5 was found to be expressed by oral keratinocytes in the majority of OLP and OLR biopsies analysed (Little et al., 2003) and CCR1 and CCR5, which are cell surface receptors for CCL5 as well as other T-cell chemokines, have also been identified in OLP lesions (Hu et al., 2013; Zhao et al., 2002). Recently, Shan *et al*., highlighted the importance of the CCL5-CCR5 axis in OLP. The authors reported that as well as acting as a potent chemoattractant, CCL5 inhibited T-cell apoptosis, increased T-cell longevity whilst simultaneously inducing expression of CCL5 and CCR5 in an autocrine manner to form a positive feedback loop to establish chronicity of OLP (Shan et al., 2019). As well as CCL5 other T-cell chemokines such as CXCL9, CXCL10, CXCL11 and CCL20 as well as their receptors have also found to be upregulated in OLP (Fang et al., 2019; Ichimura et al., 2006). It is plausible that these chemokines may mediate the recruitment of specific T-cell subsets in lesional sites or direct their migration to specific locations within the oral mucosa such as to the lamina propria or intra-epithelial sites, or be expressed in a lesion specific manner, although the precise role of each specific chemokine is yet to be determined.

The role of mast cells and macrophages in OLP

Mast cells act in concert with T-cells in the pathogenesis of OLP and are found in higher numbers in the lamina propria, near blood vessels and adjacent areas of basement membrane destruction (Sharma, Sircar, Singh, & Rastogi, 2011; Zhao, Savage, Pujic, & Walsh, 1997). Elevated degranulation of mast cells has been reported when compared to normal mucosa via detection with electron microscopy, through the loss of metachromasia following toluidine blue staining or via immunohistochemistry (Jontell, Hansson, & Nygren, 1986; Zhao, Sugerman, Zhou, Walsh, & Savage, 2001). Upon degranulation, mast cells release TNF-α, chymase, tryptase and the T-cell chemoattractants IL-16 and CCL4 as well as CCL5 (Rumsaeng et al., 1997; Zhao et al., 2002; Zhao et al., 2001). These mast cell-derived chemoattractants act in concert with the keratinocyte-derived chemokines to perpetuate T-cell recruitment to the lesional site. CCL5 and CCL3 can also act in a paracrine manner by binding to CCR1 on nearby mast cells driving their degranulation with further release of TNF-α, histamine and other pro-inflammatory molecules culminating in a positive feedback loop (Figure 4) (Miyazaki et al., 2005; Zhao et al., 2001). Mast cell-derived tryptase and chymase are potent activators of matrix metalloproteinases (MMP)-1 and -3, but not MMP-2 or -9, by proteolytic cleavage (Lees, Taylor, & Woolley, 1994). Culture supernatants from OLP lesional T-cells contained higher levels of MMP-9 than those from healthy control peripheral blood T-cells suggesting T-cell-mediated secretion of MMP-9 at lesional sites (Zhou, Sugerman, Savage, & Walsh, 2001). Moreover, increased levels of MMP-2 and the broad-spectrum protease MMP-7 have been observed in OLP tissue compared to controls (T. J. Li & Cui, 2013; Rubaci, Kazancioglu, Olgac, & Ak, 2012). This increased proteolytic activity facilitates the migration of T-cells

through the extracellular matrix of the lamina propria whilst also cleaving collagen IV and V to cause direct proteolytic destruction of the basement membrane that has two consequences; firstly, allowing CD8+ T-cells greater access to the oral epithelium and secondly detaching basal cells from the basement membrane thereby depriving these cells of basolateral attachment survival signals, driving apoptosis. Along with mast cells, inflammatory macrophages are also found in increased numbers in OLP lesions (Ferrisse et al., 2021) where they likely exacerbate inflammation by OLP antigen presentation and further secretion of TNF-α, IL-1β, MMPs and CCL5 (Figure 4).

CD8+ T-cell activation and keratinocyte apoptosis

Keratinocyte apoptosis, based on the detection of the colloid bodies in histological sections of OLP lesions, is a major outcome of the dysregulated immune response, where CD8+ T-cells are consistently reported to be the principal cell type responsible for keratinocyte death (Sugerman et al., 2002). CD8+ T-cells infiltrate the mucosal epithelium along a T-cell specific chemokine gradient, which is often enhanced by a disrupted basement membrane. These intra-epithelial T-cells are activated by two means; stimulation from IL-2, TNF-α, and IFN-γ produced by various cell types including Th1 CD4+ cells (see next section), and by direct binding to MHC class I-presented antigen on immune cells or keratinocytes (Sugerman et al., 2000). Crosstalk between CD8+ and CD4+ T-cells is essential, as CD8+ cells require an antigen-specific confirmation from CD4+ cells to enable them to proceed and eliminate target cells (Figure 4).

Current evidence suggests that CD8+ T-cells trigger keratinocyte apoptosis by several mechanisms including the interaction of CD8+ T-cell-expressed CD95L (FasL) with CD95 (FasR) on the cell surface of keratinocytes and apoptosis by caspase activation, T-cell-secreted granzyme-B entering the keratinocyte cytoplasm through perforin-induced membrane pores, the action of CD8+ T-cell-secreted TNF-α binding to TNFR1 that is up-regulated on the keratinocyte plasma membrane in OLP (Dekker et al., 1997; Khan et al., 2003; Neppelberg, Johannessen, & Jonsson, 2001; Shimizu, Higaki, Higaki, & Kawashima, 1997; Tobon-Arroyave et al., 2004). In addition, the interaction of CD40 and CD40L expressed by keratinocytes and cytotoxic T-cells, respectively, along with TNFR2 activation enhance TNFR1-induced apoptosis, suggesting other mechanisms may be at play (Grell et al., 1999). T-cell independent mechanisms also regulate apoptosis. E-cadherin and integrins are epithelial adhesion molecules involved with cell-cell contacts and contacts with the basement membrane, respectively, and both are essential for cell survival (Grossmann, 2002). Loss of keratinocyte E-cadherin expression has been reported in OLP, and this along with degradation of the basement membrane may also induce

keratinocyte apoptosis via anoikis, as well as further facilitating the migration of CD8+ cells into the epithelium (Grossmann, 2002; Neppelberg, Loro, Oijordsbakken, & Johannessen, 2007).

Th1/Th2 paradigm and other T-cell subsets

For CD8+ cells to enact their specific cell targeting and destructive behaviour in OLP they must interact and receive signals from Th-cells. It is now appreciated that upon appropriate cytokine stimulation naïve Th-cells are programmed to differentiate into several different Th-cell subsets, each with distinct receptor expression and cytokine-secretion profiles and immune modulatory functions (Figure 5). Given that many of these Th-cell phenotypes have been examined using *in vitro* cultures under defined cytokine conditions it is currently uncertain as to whether these T-cell subsets are distinct or form a continuum, with their exact phenotype dependent on the local micro-environmental cytokine milieu they experience *in vivo*.

In response to IL-12 and through the activation of transcription factors STAT4 and T-bet, naïve Th-cells are programmed to differentiate into a Th1 phenotype that secrete high levels of IL-2, IFN-γ, TNF-α, express cell surface IL-12R, and are involved in cell-mediated immunity by modulating the activity of macrophages and cytotoxic T-cells. In contrast, under the influence of IL-4, STAT6 and GATA-3 transcription factor activation, naïve T-cells polarise to a Th2 phenotype that express high levels of IL-4, IL-5, IL-10, IL-13 and tend to be involved in humoral immunity where they interact with B-cells (Crane & Forrester, 2005) (Figure 5).

These two Th subsets regulate each other's function through the antagonistic activity of their related cytokines. The secretion of IFN-γ by Th1 cells supresses Th2 cell formation by inhibiting the actions of IL-4 and silencing GATA-3 whilst increasing expression of T-bet. In contrast, IL-4 and IL-10 secretion by Th2 cells inhibit Th1 formation (Luckheeram, Zhou, Verma, & Xia, 2012). Thus, a dichotomistic relationship exists between Th1 and Th2 cells. Abundant evidence suggests that the cytokine milieu in OLP skews CD4+ T-cell differentiation toward the Th1 phenotype that then drives the activation of cytotoxic CD8+ T-cells. This was confirmed by the findings of elevated IL-12 and IFN-γ gene expression and secretion by keratinocytes and cells present in the superficial lamina propria and high levels of TNF-α adjacent to basal keratinocytes of OLP lesions (Aragane et al., 1994; Simark-Mattsson, Jontell, Bergenholtz, Heyden, & Dahlgren, 1998; Y. Wang, Zhou, Fu, Wang, & Zhou, 2015).

Several studies have recorded high IFN-γ to IL-4 ratios, inferring Th1 dominance with higher IFN-γ expression purportedly related to the presence of erythematous or ulcerated OLP lesions (Hu et al., 2015; Tao et al., 2008; Y. Wang et al., 2015; Wei et al., 2018). Moreover, Rui Lu *et al*., examined mRNA expression of T-bet and GATA-3 in peripheral blood mononuclear cells isolated from patients with OLP. Their results supported the Th1-bias pattern observed in OLP patients with significantly

higher expression of T-bet compared to GATA-3 detected. However, they indicated that different clinical forms of OLP might have different Th1/Th2 imbalanced status with different T-bet/GATA-3 ratios detected (Lu et al., 2011). TNF-α produced by Th1 synergistically works with IL-1 to increase expression of MHC classes I and II antigens, adhesion molecules and chemokines further driving OLP chronicity. In addition, IL-18 functions as a cofactor rather than as an initiator for Th1 development. IL-12 and IL-18 synergistically enhance IFN-γ mRNA transcription by activating the transcription factors STAT4 and AP-1, whilst inhibiting the synthesis of IL-10, a Th2-associated cytokine (Nakahira et al., 2002). In support of this, IL-18 serum levels are elevated in OLP patients and an IL-18 genetic polymorphism has been associated with OLP susceptibility (Negi et al., 2019), although more studies are needed to investigate the role of IL-18 in regulating Th1 differentiation in OLP.

Conversely, a lower IFN-γ to IL-4 ratio and high IL-10 levels have been reported (Pekiner, Demirel, Borahan, & Ozbayrak, 2012; Piccinni et al., 2014; Z. R. Zhang, Chen, Qi, & Sun, 2018). Elevated levels of the Th2 cytokine, IL-5 and IL-6 have been observed in the saliva and serum of OLP patients compared to healthy controls (Wei et al., 2018; Yamamoto & Osaki, 1995). IL-6 can promote IL-4 expression and render CD4+ T-cells unresponsive to IFN-γ signals leading to Th2 differentiation, suggesting that Th1 prominence in OLP may not be as straightforward as it seems. In particular, the protective role of Th2 cells and its related cytokines in controlling or even suppressing Th1 development and connection to the reticular form of OLP has been proposed (Pekiner et al., 2012; Piccinni et al., 2014; Z. R. Zhang et al., 2018).

Although instigated by host-donor antigen activation leading to activated donor T cells, the immunopathogenesis of chronic oral GvHD, in many respects, is similar to that of OLP. These include the central role of Th1 cells and their production of IFN-γ that drives CD8 cell activation. Likewise, there is evidence of Th2 cell involvement with increased levels of the Th2 cytokines IL-4 and IL-13 (Presland, 2016), indicating that common pathogenic pathways along with systemic and microenvironmental cytokine cues likely exist between these diseases once initial T cell activation occurs, leading to a similarity in pathological outcomes. This similarity may have positive aspects, opening up the way for novel therapeutic interventions that would be applicable to both OLP and GvHD.

Research into OLP has had a tendency toward investigating individual T-cell subsets and their associated cytokines to understand the pathogenic mechanism. These criteria may be a too simplistic approach to fully understand the complex pathogenic processes where it is likely that the actions of one cytokine may not be exclusive to only one T-cell phenotype. Moreover, there is a need to correlate research results with the clinical type/severity of OLP to uncover their clinical importance. Additional studies are warranted with increased sample size to cover, age, sex, genetic polymorphisms and disease severity to resolve this issue.

Th17 and the non-classical Th1 phenotype

Th17 cells have been defined through their expression of retinoic acid-related orphan receptor (RORC) transcriptional factor and secretion of IL-17A, which is involved in the recruitment, activation and migration of neutrophils by inducing the production of colony-stimulatory factors (CSF) and CXCL8 by both macrophages and tissue-resident cells (Annunziato, Cosmi, Liotta, Maggi, & Romagnani, 2012). The actions of Th17 cells were initially described in their function to clear extracellular pathogens such as fungi but recently their role in inflammatory and autoimmune diseases through secretion of potent pro-inflammatory cytokines such as IL-26, IL-22, IL-6, IL-1 and TNFα have been uncovered (H. Wang et al., 2016) (Figure 5).

Th1 and Th2 show some plasticity when they are partially differentiated but they seem to be more stable when fully differentiated and in a state of dichotomy to each other where, if one is predominant, the signalling pathway for the other is downregulated. In contrast to Th1 and Th2, Th17 cells are highly plastic throughout their entire differentiation and are able to switch into either Th1 or Th2 under the induction of IL-12 or IL-4 respectively (Figure 5) (Peck & Mellins, 2010). Th1/Th2 phenotypes derived from naïve CD4+ are often described as 'classic' whilst Th1/Th2 phenotypes derived from Th17 cells are described as 'non-classic' (Maggi et al., 2012). Factors such as IL-12, IL-23, TNF-α and IL1-β activate T-bet transcriptional factor in Th17 cells, stimulating them to produce IL-17, IFN-γ and GM-CSF and acquire features of Th1 cells but retaining the Th17-cell surface marker CD161, and as such are termed Th17/Th1 cells (Figure 5). To complicate matters further, longer induction with IL-12, IL-23, TNF-α or IL-1β may lead to the loss of IL-17, but increased GM-CSF and IFN-γ secretion along with increased expression of IL-23R whilst maintaining CD161, forming what is known as the 'non-classical Th1' phenotype (Annunziato et al., 2007; Mazzoni, Maggi, Liotta, Cosmi, & Annunziato, 2019) (Figure 5).

Th17/Th1 and the non-classical Th1 cells have been detected increasingly in different inflammatory and autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, Crohn's disease and psoriasis (Kamali et al., 2019). They have important roles in many processes that were previously thought to reflect the activities of Th1 cells. Xie *et al.*, investigated the presence of Th1 and Th17 cells through their expression signature cytokines IFN-γ and IL-17, respectively, in the tissue of 40 OLP patients and 15 healthy controls using double immunofluorescence staining of leukocytes by flow cytometry and in serum by ELISA. Their findings showed an overabundance of both Th1 and Th17 phenotypes in OLP, suggesting the presence of a mixed Th population. These authors also observed higher IL-17 secretion in the erosive compared to reticular form of OLP (Xie, Ding, Xiong, & Zhu, 2012). Indeed, elevated

secretion of IL-17 by Th-17 cells has been observed in several oral diseases where it is thought to play a key role in oral immunity (Abusleme & Moutsopoulos, 2017).

Piccinni *et al*., investigated the role of Th17, classical Th1, non-classical Th1, Th2, in patients with OLP and showed that IL-17 was specifically expressed by Th17 cells in OLP lesions with particularly high levels observed in erosive forms, while gene expression of the Th2-related molecules, GATA3 and IL-13, were more associated with the reticular form (Piccinni et al., 2014). Further immunohistochemical studies by Monteiro *et al*., also found increased IL-23 (a cytokine known to aid transcriptional expression of IL-17 by Th17 cells) and IL-17 expressing lymphocytes in OLP lesions compared to controls, with significantly higher levels of IL-23 in erosive compared to reticular forms (Monteiro et al., 2015). Similarly, Lu *et al*., also observed elevated levels of IL-17 and IL-23 in OLP lesions compared to control mucosa, but with predominance this time in the reticular form (Lu et al., 2014). These data are aligned to those from chronic GvHD where patients were found to display increased presence of Th17 cells in the circulation and at lesional sites. These Th17 cells consisted of both IL-17^{+}/ IFN-γ^{-} and IL-17^{+}/ IFN-γ^{+} phenotypes with both expressing the IL-23 receptor (Dander et al., 2009), once again highlighting similarities between OLP and GvHD disease. More studies examining the recruitment and cellular interactions of Th-17 cells in OLP are required to fully understand the importance of this Th subtype in disease pathogenesis.

Other T-cell phenotypes in OLP

In addition to Th1, Th2 and Th17, evidence is now emerging that other Th subsets have an involvement in OLP pathogenesis. For instance, CD4+ T-cells secreting IL-4, IL-5 and IFN-γ have been recognised and are referred to as Th0 cells (Firestein et al., 1989) (Figure 5). These cells synthesize multiple cytokines and are responsible for effects intermediate between those of Th1 and Th2 cells, or even a precursor of Th1 and Th2 cells. Using co-expression of IL-4, IL-5 and IFN-γ as a cytokine signature, Piccinni *et al*., observed the presence of Th0 cells in OLP lesions, where, like Th-17 cells, their increased numbers were associated with the erosive rather than reticular form (Piccinni et al., 2014).

Th9-cells are reprogrammed from Th2 following induction with TGF-b and IL-4 (Figure 5). Th9 cells produce IL-9 and, similar to Th2, can be inhibited by Th1-related cytokines (Veldhoen et al., 2008). Wang *et al*., measured intracellular IL-9 and IL-17 by flow cytometry following isolation of CD4+ lymphocytes from peripheral blood mononuclear cells of OLP patients. They reported significantly elevated numbers of Th9 cells (CD4+/IL-9+/IL-17-) in the peripheral blood from patients with reticular OLP, while elevated numbers of Th17 cells (CD4+/IL-9-/IL-17+) were observed in erosive types (H. Wang et al., 2017). In a more recent study, gene expression of IL-9 was found to be increased in OLP lesions compared to healthy controls, and interestingly was more abundant in erosive compared to

reticular OLP lesions. Co-culture of peripheral blood CD4+ cells with keratinocytes isolated form OLP patients *in vitro* increased the proportion of both Th9 and Th17 cells within the T-cell population, suggesting that OLP keratinocytes can support the differentiation of both cell types simultaneously. Moreover, culture of CD4+ cells with recombinant IL-9 increased the levels of Th17 cells in the T-cell population and increased secretion of both IL-17 and MMP9, indicating a direct effect of Th9 cytokines on Th17 activation. IL-17 was also shown to induce mRNA and protein production of MMP9 by keratinocytes isolated from OLP patients (H. Wang et al., 2018). These data suggest an interplay between Th9 and Th17 cells that may drive OLP pathogenesis to more aggressive and chronic forms of the disease via basement membrane destruction but more data is required to test this hypothesis, indeed, as yet there is no direct evidence for the presence of Th9 cells in OLP tissue.

Th22, an IL-22 cytokine-producing Th cell, have been detected in some inflammatory and autoimmune diseases but their exact role in the immune pathogenesis is not yet fully understood (Hofmann, Kiecker, & Zuberbier, 2016). Elevated levels of IL-22 have been detected in OLP lesions compared to heathy controls (Chen et al., 2013; Shen et al., 2016). There is scarcity in the literature regarding the role of Th22 in OLP, however, IL-22 has been shown to induce secretion of several pro-inflammatory molecules by keratinocytes in a STAT3-dependent mechanism and also inhibit keratinocyte differentiation whilst inducing cell migration and hyperplasia (Boniface et al., 2005). Therefore, IL-22 secretion by Th22 cells may play a role in pathogenesis in terms of hyperkeratosis or the remission phases of OLP where erosive/ulcerative lesions undergo wound healing (H. Wang et al., 2016).

Tregs roles in exacerbation and remission

Regulatory T cells (Tregs) are believed to have an important role in the regulatory control over immune responses, suppressing autoimmune disease while maintaining immune homeostasis. Tregs differentiate from naïve T-cells under the influence of IL-2, TGF-ß, and IL-10 whereupon they express transcription factor forkhead box P3 (FoxP3) and release anti-inflammatory cytokines such as TGF-ß, IL-10, and IL-35 to suppress T-cell activation (Gouirand, Habrylo, & Rosenblum, 2021) (Figure 5). Increased numbers of FoxP3+ cells in OLP lesions inversely correlated with OLP severity, with highest numbers found in reticular than erosive forms (Tao et al., 2010). The low number of Tregs in the chronic, erosive phase of OLP indicate that a high ratio of activated T-cells to Tregs as well as the high levels of pro-inflammatory cytokines may alter Tregs function, prevent T-cell resistance to apoptosis and cause T-cell accumulation in the tissue (Lei et al., 2014; Tao et al., 2010). Similar findings have been observed in chronic GvHD lesions (Dander et al., 2009). In contrast, Pereira *et al*., reported no significant difference in the numbers of FoxP3+ cells between reticular or erosive OLP lesions (Pereira, Monteiro, Nonaka, Silveira, & Miguel, 2012).

Experimental data thus far suggests a complex interplay between non-immune cells at the oral mucosal lesional site and an array of predominantly T-cell infiltrates of various phenotypes that may fluctuate in number and spatial location (sub/intra-epithelial) as the disease progresses or as lesions become more aggressive. It is highly likely that some of the identified Th-cell subtypes display plasticity and can respond to changing micro-environmental cues at lesional sites by secreting cytokines and changing cell surface receptor profile, thereby moving from one phenotype to another. The opposing role of Th1/Th17 and Tregs has led to the hypothesis that these cells play a pivotal role in regulating the immune response in OLP (Figure 6), and this may also be the case at non-oral LP sites or for OLR and GvHD where these diseases share common pathogenic pathways. Moreover, different environmental factors may cause fluctuations in systemic or lesional cytokine levels and T-cell numbers that can lead to periods of T-cell-mediated mucosal damage, which could be reflected clinically as periods of remission and exacerbation commonly observed in OLP. Current knowledge supports the likely hypothesis that the strong inflammatory process in the chronic phase of OLP is promoted by Th17/Th1, or non-classical Th1-cells, while a change in the microenvironment may alter the balance of the T-cell response promoting Tregs or even Th2-cells to suppress the actions of Th17/Th1 (Figure 6). To gain a greater understanding of the role of different Th subsets in OLP it would be invaluable to identify distinct Th subsets using standardised antibody panels for each phenotype with multichromatic immunostaining utilising WHO diagnostic criteria-defined OLP lesional tissue to examine pathogenesis more directly as opposed to examination of serum or peripheral blood leukocytes that could be subject to the influence of inflammation elsewhere in the body. This more detailed analysis may be able to tease apart subtle differences the pathogenic process of different forms of OLP and may explain the impact that various T cell subsets and mast cells have on the oral epithelium in, for example, reticular versus erosive or vesiculobullous forms, where a difference in the severity or type of tissue damage exists. Development of advanced tissue engineered oral mucosal models to include T-cell subsets would aid in delineating the molecular mechanism that drives pathological outcomes.

New molecular targets for OLP therapy

The current mainstay OLP treatment is the use of topically applied corticosteroids that are often applied as creams, gels or in mouthwashes. These application forms suffer from short mucosal contact times and inadvertent contact with the surrounding healthy mucosa. New drugs dosage forms include mucoadhesive polymer-based patches pre-loaded with corticosteroid (Edmans et al., 2020). These patches adhere for long periods to the oral mucosa and offer drug delivery directly to the lesion over a number of hours. Patch-delivered clobetasol-17-propionate has been shown to traverse the epithelium and inhibit T-cell cytokine production in a rudimentary model of OLP using tissue engineered oral

mucosa and activated T-cells and so are an improvement on current treatments (Colley et al., 2018; Said, Murdoch, Hansen, Siim Madsen, & Colley, 2021).

However, understanding the immune dysregulation in OLP will inevitably highlight molecules that can in turn be targeted by new therapeutics. One approach is through the use of specific small molecule antagonists or humanised monoclonal antibodies to inhibit the actions of cytokines such as IL-1, IFN-γ and TNF-α. For example, TNF-α inhibitors such as etanercept, infliximab and adalimumab, all of which are FDA approved for treating psoriasis and work by blocking TNF-α binding to the cell surface receptor TNFR1 (J. Zhang, Zhou, Du, Xu, & Zhou, 2011). Hence, TNF-α inhibitors constitute a promising therapeutic for OLP. However, this may not be straightforward as there is evidence of development of an OLP-like lesion in patients treated with infliximab for psoriasis and other inflammatory diseases (Asarch et al., 2009). Blockade of T-cell costimulatory molecules may also present a potential target for treatment. Inhibition of CD40-CD40L interactions using an anti-CD40L monoclonal antibody was shown to prevent secretion of IFNγ, IL2 and IL-12 by Th1 cells in favour of the Th2 cytokines IL-4 and IL-10 in a murine model of allograft rejection (Hancock et al., 1996), a similar approach for OLP may counter-balance T-cell phenotype against lesion development. Since adhesion molecules are involved in immune cell recruitment to lesional sites, antibodies targeting these molecules may unlock new treatment strategies. Likewise, inhibiting the CCL5-CCR5 axis by blocking CCR5 using Maraviroc could provide therapeutic potential in OLP treatment. Here, Shan *et al*., showed that T-cell proliferation and migration were suppressed while T-cell apoptosis was enhanced (Shan et al., 2019). Similar therapies may also work for other chemokines, such as interruption of the CXCL10/CXCL9-CXCR3 axis, although the effects of this type of therapy on leukocyte recruitment in homeostasis or infection may be problematic.

CD8+ T-cells are critical perpetrators of inflammatory diseases that have been largely overlooked as most biological therapies target pathways of CD4+ T-cell subsets. For instance, the selective expansion and targeting CD4+FOXP3+ Tregs has shown effective therapy or retro-conversion in experimental models of several inflammatory/autoimmune-related disorders (Beyersdorf, Hanke, Kerkau, & Hunig, 2006). However, emerging evidence shows that different subsets of CD8+ T-cells exist including suppressor or regulator CD8+ T-cells that are able to reset an autoimmune response and protect the host (Xu et al., 2016). There is uncertainty regarding the mechanism by which they control immunity but they constitute a promising therapeutic target that could be approached through T-cell transfer therapy or modulating their numbers and activity with novel immune modulators (Konya, Goronzy, & Weyand, 2009).

The use of recombinant cytokines (IL-4, IL-10, and TGF-ß) that down-regulate the immune response may also be useful. Enhancing IL-4 production to suppress IFN-γ secretion and Th1 development supports the notion that skewing the T-cell response towards the Th2 lineage may be a candidate for

autoimmune therapy by suppressing the excessive immune response mediated by IFN-γ in erythematous/ulcerated OLP lesions (Tao et al., 2008). On a similar note, the anti-inflammatory IL-10 is considered to play a role in controlling the progression of OLP by blocking pro-inflammatory cytokine synthesis and inhibiting both the proliferation and cytokine secretion of Th1 cells and so this cytokine may be a promising therapeutic target. Weak expression of the immune suppressive cytokine TGF-b has been reported in OLP, since TGF-b negatively modulates IFN-γ and favours phenotyping toward FoxP3+ Tregs use of exogenous recombinant TGF-b may favour disease regression. On similar notion, preventing the actions of IFN-γ and its ability to increase MHC expression on keratinocytes might be targeted through inhibition of STAT1 and Janus kinase 2 (JAK2) transcriptional factors. Indeed, results of a recent *in vitro* study support targeting downstream IFN-γ signalling through STAT1/JAK2 inhibition using JAK inhibitors as a therapeutic approach in OLP (Shao et al., 2019). Further studies are required to show the possibility of targeting different transcriptional factors involved in OLP pathogenesis. Selective and targeted biologics may provide a new therapeutic perspective in the management of OLP, but the immune regulation is very complex so blocking a specific receptor/signalling pathway or cytokine may not be enough in this multifactorial pathogenesis. Also, one cytokine may have several roles and so there may be off-target effects that may enhance disease progression rather than hinder it.

Summary

Research over many years has increased our knowledge of OLP, yet new research continues to develop our understanding, especially with regards to identification of OLP-promoting antigens and specific T-cell subsets that act in a dynamic and complex manner. Future research to define these mechanisms in more detail may lead to the repurposing of drugs or the development of new therapeutics aimed at inhibiting the disease process.

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Figure Legends:

Figure 1. Clinical presentations of some of the different forms of oral lichen planus presented on the buccal mucosa: (A) reticular, (B) plaque (C) erosive, (D) ulcerative. Images by Prof Thornhill, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, UK. (Images were taken with written, informed consent under ethical approval).

Figure 2. Histopathological features of oral lichen planus. (A) Haematoxylin and eosin-stained section of an OLP lesion showing the oral mucosa composed of a stratified squamous epithelium and underlying connective tissue containing a dense band of inflammatory T-cells in the subepithelial compartment. (B) Magnified image of area depicted in A (white hatched box) showing the presence of colloid bodies within the epithelium (yellow arrows) and infiltration of intra-epithelium T-cells (blue arrows). Scale bar in A = 200 μm and in B = 50 μm.

Figure 3. Schematic diagram illustrating the interaction of an antigen presenting dendritic cell with a naive T-cell and the binding of the primary (MHC and TCR-CD3 complex with either CD4 or CD8) and costimulatory signals responsible for T-cell activation.

Figure 4. Schematic illustration of the immune process in OLP. (1) An as yet unknown antigenic stimulus activates APCs to release pro-inflammatory cytokines such as TNF-α and IFNγ that further up-regulate the expression of cytokines, chemokines and adhesion molecules by keratinocyte and endothelial cells. (2) Activated APCs migrate to local lymph nodes and bind to antigen specific T-cells via cognate surface receptors and molecules leading to stimulation of signalling pathways that activate CD8+ cytotoxic cells and differentiate CD4+ into Th1-cells. (3) Under the action of proinflammatory cytokines, local blood vessel permeability increases and T-cells migrate to the OLP lesional site under the influence of chemokine gradients. (4) CCL5 mediates mast cell degranulation releasing molecules such as TNF-α, chymase, tryptase, histamine as well as further CCL5 to create a positive feedback loop increasing the chronicity of OLP. In addition, mast cell-derived TNF-α further up-regulates adhesion molecule and chemokine release while chymase and tryptase activate MMPs that degrade the oral mucosal basement membrane. The cytokine milieu selects for Th1/Th17 polarisation and their secreted cytokines and MMPs propagates the inflammatory response. (5) Cross talk between CD4+ and CD8+ occurs to enable CD8+ cell functions. TNF-α and other cytokines released by T-cells activate nearby macrophages that can then present OLP-antigens and release CCL5, recruiting more T-cells to the lesional site and perpetuating mast cell degranulation. (6) Under the influence of increased chemokine

and adhesion molecules expression, CD8+ T-cells infiltrate the epithelium through the disrupted basement membrane and cause keratinocytes apoptosis via a number of mechanisms.

Figure 5. Schematic illustration of CD4+ naïve Th subset differentiation and plasticity. Upon appropriate cytokine stimulation naïve Th cells are programmed to differentiate into several different T-cell subsets, each with a distinct transcriptional factor, receptor expression and cytokine secretion profile and immune modulatory function. Some subsets show different levels of plasticity in response to changes in the cytokine milieu. Th0 is a mixed phenotype but under abundance of either IFN-γ or IL-4 may switch to either Th1 or Th2 phenotypes, respectively. Th2 under influence of high levels of TGF-β or IFN-γ/ IL-12 have the ability to transform to Th0 or Th9, respectively. Th17 under high levels of IL-12 or IL-4 can switch to Th1 or Th2 subsets, and Treg cells may switch to the Th17 subset under high levels of IL-6 combined with reduced levels of TGF-β.

Figure 6. Schematic diagram illustrating the conceptualised role of Th17, Th1, non-classical Th1, Th2, and Treg subsets in the pathogenesis of erosive and reticular types of OLP. The make-up of cytokines within the local microenvironment regulates the type T-cell phenotype and their number in OLP lesional sites. Th1/Th17 favouring microenvironments tip the balance in favour of chronic inflammation and erosive lesion while cytokines that favour Tregs and possibly Th2 T-cells favour regression or less aggressive reticular forms.