Oral lichen planus

A study of associated factors with special reference to thyroid disease

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Cover illustration: "Oral lichen planus and thyroid disease" by Pamela Uribe

To my parents,

Ana María and Jairo

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ABSTRACT

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Oral lichen planus (OLP) is one of the most common and debilitating oral mucosal lesions in the adult population. Despite large research effort over the last decades, the aetiology of OLP remains an enigma. The current series of studies aimed to identify new potential aetiological factors for OLP.

The morbidity and prevalence of oral lichenoid reactions in a non-referral adult Swedish population of 6448 subjects was determined (**Study I**). The medication profile of patients with OLP (n=956) was compared with dental patients with no oral mucosal lesions (**Study II**). Based on the results from *studies I* and *II*, the prevalence of levothyroxine supplementation and profile of thyroid disease was established in a cohort of patients with OLP (n=1611) and compared to the general population (n=1615) (**Study III**). The clinical characteristics of patients with concomitant OLP and thyroid disease (n=108) were also investigated (**Study III**). Serum levels of antithyroid antibodies and thyroid hormones were analysed in patients with OLP (n=108) and compared with different control groups (**Study IV**). Finally, the expression of thyroid proteins in OLP lesions (n=5) was determined and compared to healthy oral mucosa (n=5) (**Study IV**).

It was demonstrated that:

- Oral lichenoid reactions still represent one of the most common and debilitating oral mucosal lesions in the adult population (**Study I**).
- OLP is strongly associated with the use of levothyroxine (**Study II**).
- The prevalence of thyroid disease in patients with OLP is significantly higher compared to the general population (**Study III**).
- The clinical characteristics and the natural course of OLP lesions in patients with thyroid disease are different compared to those in patients with no thyroid disease (**Study III**).
- Patients with OLP without a previously diagnosed thyroid disease have high levels of TSH and low levels of FT_4, indicative of thyroid disease (**Study IV**).
- Elevated levels of antithyroid antibodies could not explain the high prevalence of thyroid disease in patients with OLP (**Study IV**).
- Thyroid-stimulating hormone receptor is highly expressed in basal keratinocytes of OLP lesions (**Study IV**).

In conclusion, a subgroup of patients with OLP may have an aetiological background in common with thyroid disease. The reason for this connection remains to be determined, but it is likely that some mechanisms in autoimmune thyroid disease are involved in the pathogenesis of this group of patients suffering from OLP.

Keywords: oral lichen planus, oral mucosal lesion, epidemiology, levothyroxine sodium, thyroid disease, hypothyroidism, autoimmune thyroid disease, antithyroid antibodies, thyroid hormones.

PREFACE

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. **Robledo-Sierra J**, Mattsson U, Svedensten T, Jontell M. The morbidity of oral mucosal lesions in an adult Swedish population. *Medicina Oral Patología Oral Cirugía Bucal.* 2013 Sep 1;18(5):e766-72.

II. **Robledo-Sierra J**, Mattsson U, Jontell M. Use of systemic medication in patients with oral lichen planus – a possible association with hypothyroidism. *Oral Diseases.* 2013 Apr;19(3):313-19.

III. **Robledo-Sierra J**, Landin-Wilhelmsen K, Filipsson Nyström H, Mattsson U, Jontell M. Clinical characteristics of patients with concomitant oral lichen planus and thyroid disease. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology.* In press.

IV. **Robledo-Sierra J**, Landin-Wilhelmsen K, Filipsson Nyström H, Eggertsen R, Larsson L, Dafar A, Warfvinge G, Mattsson U, Jontell M. Levels of antithyroid antibodies and thyroid hormones in patients with oral lichen planus – a connection between two autoimmune diseases? *In manuscript.*

LIST OF ABBREVIATIONS

Common abbreviations used in this thesis are listed according to their first appearance.

OLP	Oral lichen planus
OLR	Oral lichenoid reaction
OLL	Oral lichenoid lesion
WHO	World Health Organization
CD	Cluster of differentiation
MHC	Major histocompatibility complex
TNF-α	Tumor necrosis factor-alpha
HCV	Hepatitis C virus
OR	Odds ratio
CI	Confidence interval
NSAIDs	Nonsteroidal anti-inflammatory drugs
HLA	Human leukocyte antigen
MONICA	Multinational Monitoring of trends and determinants in Cardiovascular disease
ICD	International Classification for Diseases and Health Related Problems
ATC	Anatomical Therapeutic Chemical Classification System
Anti-Tg	Anti-thyroglobulin antibody
Tg	Thyroglobulin
T_3	Triiodothyronine
T_4	Thyroxine
AITD	Autoimmune thyroid disease
Anti-TPO	Anti-thyroid peroxidase antibody
TPO	Thyroid peroxidase
Anti-TSHR	Anti-thyroid-stimulating hormone receptor
TSHR	Thyroid-stimulating hormone receptor
FT_3	Free triiodothyronine
FT_4	Free thyroxine
TSH	Thyroid-stimulating hormone
qPCR	Quantitative real-time polymerase chain reaction

INTRODUCTION

Oral lichen planus (OLP) is a member of the family of oral lichenoid reactions (OLR). The concept of OLR is used to define a number of diverse immune-mediated conditions linked together by the presence of common clinical and histopathological features (Khudhur et al., 2014). Except for OLP, the other mucosal lesions that belong to the group of OLR are: oral lichenoid contact reactions, oral lichenoid drug reactions and oral lichenoid lesions of graft-versus-host disease. Although the pathological process will always result in the same reaction pattern, each of the OLR is governed by different immunological mechanisms and apparently triggered by different causative factors. For instance, oral lichenoid contact reactions are considered to be a delayed hypersensitivity reaction against dental materials, especially mercury-containing amalgam (Finne et al., 1982; Dunsche et al., 2003; Montebugnoli et al., 2012). Oral lichenoid drug reactions represent an adverse effect of systemic medications (McCartan & McCreary, 1997; Yuan & Woo, 2015), while oral lichenoid lesions of graft-versus-host disease are a common complication following allogeneic hematopoietic stem cell transplantation (Petti et al., 2013; Margaix-Munoz et al., 2015). In contrast, the aetiology or aetiologies of OLP are yet unknown.

It is well established that the pathogenesis of OLP is mediated by T cells, which presumably are targeted against the oral epithelium and trigger apoptosis of basal keratinocytes, leading to chronic inflammation (Sugerman et al., 2002). However, despite large research effort over the last decades, the mechanisms responsible for T-cell activation in OLP remain to be determined. OLP has traditionally been considered a specific disorder caused by a single antigen or factor, but the widespread use of this monocausal model has added little insight to the aetiology of this condition. Conversely, it is likely that OLP represents different forms of idiopathic OLR and, thus, should not be regarded as a specific disease but a common clinical and histopathological reaction pattern elicited by a broad range of environmental and/or self-antigens in genetically susceptible patients. Any scientific effort to identify factors that may be related to the aetiology will consequently require the investigation of an extensive number of patients that allows the identification of possible subgroups.

History

The term lichen planus is derived from the Greek word *leikhēn*, which means "what eats around itself", and the Latin word *planus*, which denotes "flat, level". The word *lichen* was probably coined by Theophrastus in the 4th century BC in order to describe a superficial growth on the bark of olive trees, but it was not until early 17th century when the term was reintroduced in the botany field. The current definition of *lichen* is "a simple slow-growing plant which typically forms a low crust-like, leaf-like, or branching growth on rocks, walls, and trees" (Oxford Dictionaries).

The first to describe and name lichen planus in a medical context was the dermatologist Sir William James Erasmus Wilson (Wilson, 1869). He characterized the disease as "an eruption of pimples remarkable for their colour, their figure, their structure, their habits of isolated and aggregated development". Although Wilson recorded the coexistence of oral lesions in some of his patients with lichen planus, it was Thibierge (1885) who published the initial clinical report on OLP. Later, Louis-Frédéric Wickham further characterized the lesions adding "*stries et punctuations grisatres*" (greyish striae and dots) to the previously described features (Wickham, 1895). This remarkable finding, which later received the name of Wickham's striae, still represents the cornerstone in the clinical diagnosis of lichen planus. Finally, Dubreuilh (1906) was the first to describe the histopathology of an OLP lesion.

Figure 1. Lichen on a rock (left; © Einar Timdal) and lichen ruber planus (right)

Epidemiology

OLP is one of the most common oral mucosal lesions in the adult population worldwide, and represents the majority of the non-infectious cases referred to oral medicine clinics (Bowers et al., 2000; Pinto et al., 2015). The mean age of the patients at the onset varies from 45 to 65 years (Carbone et al., 2009; Bermejo-Fenoll et al., 2010), although younger adults and children may also be affected (Alam & Hamburger, 2001; Laeijendecker et al., 2005). Despite the large number of studies that have been conducted in order to establish the exact prevalence of OLP in the general population, there is an apparent lack of valid epidemiological data (Kleinman et al., 1991). An improper sample design, a poorly described disease classification and the use of non-validated diagnostic criteria are the methodological issues most frequently encountered in the literature. Most of the studies have been based on samples of patients attending dental clinics or other types of medical centres, which do not provide accurate information on the prevalence in the general population (McCartan & Healy, 2008). The fact that there is no consensus for the diagnosis of OLP, and that other OLR (especially oral lichenoid drug reactions) can be easily misdiagnosed as OLP, suggests that the prevalence presented in a significant number of studies are likely to be exaggerated.

Only a few studies, in which representative random samples drawn from the general population were investigated, reveal minor methodological issues (Mehta et al., 1971; Pindborg et al., 1972; Axell, 1976; Axell & Rundquist, 1987; Ikeda et al., 1995; Kovac-Kovacic & Skaleric, 2000). The overall prevalence of OLP reported in these studies, and presented in the review by McCartan & Healy (2008) as age-standardized rates, ranges from 0.47% to 1.27%, with a markedly women predilection. The estimated overall prevalence of OLP (age-standardized rates) in the Swedish population is 1.27%, 0.96% in men and 1.57% in women (Axell & Rundquist, 1987). It is important to highlight that the clinical criteria used for the diagnosis of OLP in this study did not allow the exclusion of other forms of OLR, which may have overestimated the reported figures. The incidence of OLP has been reported in a Japanese population to be 59.7 in men and 188.0 in women per 100.000 person-years (0.06% and 0.19%, respectively) (Nagao et al., 2005).

Clinical characteristics

Lichen planus is a chronic, possibly life-long condition in which periods of remission and exacerbation are frequently observed. It affects mainly the skin and oral mucosa, but may also manifest itself on the lips, nails, scalp, glans penis and several mucosal surfaces, especially vulvar and vaginal mucosa (Al-Hashimi et al., 2007). Clinically, OLP presents with a wide range of features that vary from white papules, striae and plaques, to red and

ulcerated lesions (Andreasen, 1968a).

The most common is the *reticular form* (Fig. 2), which is characterized by white keratotic dots and lines (the so-called Wickham's striae) that coalesce to create an annular or lacy pattern. The striae are often surrounded by an erythematous area, which reflects a subepithelial inflammation. The *papular form* (Fig. 3) is considered to be an early phase of OLP (Thorn et al., 1988) and is seen as small white dots that frequently appear in combination with a reticular pattern. The *plaque-like form* (Fig. 4) shows a homogeneous and relatively well-demarcated white plaque that resembles a homogeneous oral leukoplakia. However, the presence of striae and erythema in the periphery of the lesion is not a finding compatible with oral leukoplakia. The *reticular*, *papular* and *plaque-like forms* constitute the white OLP lesions. These types of OLP may be detected in all regions of the oral mucosa, either unilaterally or bilaterally, and also on the lips, although the buccal mucosa is usually the most affected area with a bilateral distribution.

Figure 2. Reticular OLP in the buccal mucosa

The *erythematous form* (Fig. 5) appears as homogeneous red patches accompanied by white striae or papules in the periphery. When this form exclusively affects the attached gingiva, the white dots and striae are frequently absent and the lesion resembles mucous membrane pemphigoid or any other lesion associated with erythema. The *ulcerative form* (Fig. 6) is the most disabling type of OLP. It displays a fibrin-coated ulcer in the centre of the lesion surrounded by an erythematous area and white striae in the periphery.

Figure 3. Papular OLP in the buccal mucosa

Figure 4. Plaque-like OLP in the buccal mucosa

The *erythematous* and *ulcerative forms*, which constitute the red OLP lesions, are also known as *atrophic* and *erosive*, respectively. However, these terms are misleading to describe an OLP lesion from a clinical perspective, since atrophy and erosion (of the epithelium) are exclusively a histopathological finding.

Figure 5. Erythematous OLP in the gingiva

Figure 6. Ulcerative OLP in the ventral surface of the tongue

A rarely encountered type of OLP is the *bullous form* (Figure 7). It features short-lived bullae, which are often located in the buccal mucosa and concur with a surrounding reticular network. The bullae range from few millimetres to centimetres in diameter and, on rupturing, leave a painful ulcer.

Figure 7. Bullous OLP in the buccal mucosa

OLP can be a very debilitating condition with serious implications in the patients' quality of life (Lopez-Jornet & Camacho-Alonso, 2010; Karbach et al., 2014). Patients often complain from subjective symptoms, which include feeling of roughness, smarting and burning sensations, itching and pain. Although the erythematous and ulcerative forms are commonly associated with symptoms, any type of OLP may cause discomfort to the patients. In these cases, either topical or systemic immunosuppressive therapy is usually required (Lodi et al., 2005b; Thongprasom et al., 2011).

Concomitant skin lesions are present in approximately 15-20% of the patients with OLP (Silverman et al., 1985; Eisen, 1999). Lichen ruber planus manifests as violaceous papules that are flat-topped, polygonal in form and covered by Wickham's striae. It commonly affects the flexural surface of the wrists, lower legs, lumbar region and chest.

There is a longstanding debate on the potential malignant transformation of OLP (Krutchkoff et al., 1978; Krutchkoff & Eisenberg, 1986; Eisenberg & Krutchkoff, 1992; Holmstrup, 1992). At present, most experts tend to consider that patients with OLP carry an increased risk to develop oral squamous cell carcinoma, not only at the site of a pre-existing OLP lesion but also in any other location of the oral cavity (van der Waal, 2009a; Fitzpatrick et al., 2014; van der Waal, 2014). The annual incidence rate of malignant transformation has been reported to be below 1% (Murti et al., 1986; Holmstrup et al., 1988; Sigurgeirsson & Lindelof, 1991; Lo Muzio et al., 1998; Rajentheran et al., 1999; van der Meij et al., 2003; Rodstrom et al., 2004; Laeijendecker et al., 2005; Bermejo-Fenoll et al., 2009; Bombeccari et al., 2011). However, it is virtually impossible to establish an exact figure of cancer development, as there is still an

ambiguous clinical and histopathological definition of the disease. The efficacy of continuous follow-up of oral lichen planus patients to reduce oral cancer morbidity and mortality is questionable (Mattsson et al., 2002), although follow-ups at least once a year have been recommended by various authors (Mignogna et al., 2001; Mignogna et al., 2006).

Histopathological features

Histopathologically, OLP presents as a lichenoid tissue reaction (Figure 8). It is characterized by an intense band-like mononuclear cell infiltrate at the epithelium-connective tissue interface, consisting of activated T cells, macrophages and dendritic cells (Walker, 1976). This particular feature is intimately associated with a degeneration of basal keratinocytes, which has routinely described as being "liquefactive/hydropic/vacuolar" (Andreasen, 1968b; Pinkus, 1973; Bascones-Ilundain et al., 2007; Fernandez-Gonzalez et al., 2011).
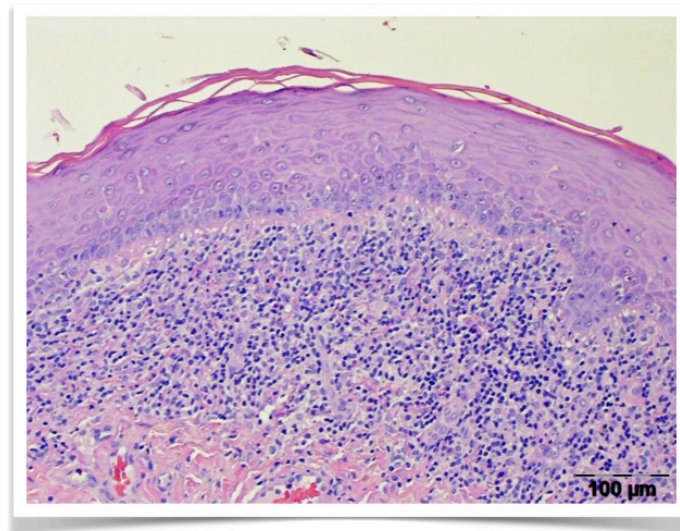

Figure 8. Lichenoid tissue reaction

For many years this cell damage has been thought to be the result of an apoptotic process, but a recent authoritative review suggest that the primary morphological changes seen in the basal layer is one of necrosis-associated cloudy swelling (Sontheimer, 2009). Areas of hyperparakeratosis or hyperorthokeratosis are frequently observed, often with a

thickening of the granular cell layer and a saw-tooth configuration of the rete pegs. Eosinophilic masses, called colloid or Civatte bodies, can also be detected in the lamina propria and basal epithelium (el-Labban, 1970), although these are more commonly associated with other conditions such as lichenoid drug reactions (Van den Haute et al., 1989). Direct immunofluorescence demonstrates the presence of fibrinogen in the basement membrane zone in most of the cases (Schiodt et al., 1981). Deposits of immunoglobulins and complement may also be found, although this is not a distinctive feature of a lichenoid tissue reaction.

Diagnostic aspects: the dilemma continues

The clinical and histopathological characteristics of OLP have been well described for decades (Andreasen, 1968a; Andreasen, 1968b). Since the first diagnostic criteria were published in 1978 (Kramer et al.) (Table 1), it has been widely accepted that in order to establish the clinical diagnosis of OLP the lesions must present with white striae and/or papules, as well as erythema, with or without ulceration. The identification of these features may be helpful to exclude a number of other oral mucosal lesions, although they are not by any means exclusive for OLP. The other members of OLR, i.e. oral lichenoid contact reactions, oral lichenoid drug reactions and oral lichenoid lesions of graft-versus-host disease, which are also known as oral lichenoid lesions (OLL), have the same clinical and histopathological features proposed for the diagnosis of OLP.

Therefore, it is crucial to include other clinical and anamnestic procedures in order to make a distinction between OLP and OLL. In the case of oral lichenoid contact reactions and oral lichenoid lesions of graft-versus-host disease, the diagnosis should not bring major problems. The former are usually located in the buccal mucosa or lateral border of the tongue in direct contact with amalgam fillings and may resolve in most cases after removal of the dental material (Lind et al., 1986; Henriksson et al., 1995; Bratel et al., 1996; Laine et al., 1997; Koch & Bahmer, 1999; Issa et al., 2004), while a history of allogeneic hematopoietic stem cell transplantation will reveal the origin of the latter.

In contrast, the diagnosis of oral lichenoid drug reactions is, at least, problematic. It can take several months for this type of lesions to develop following the start of medication. At the same time, they do not resolve immediately after medication withdrawal. This makes it extremely difficult for a clinician to establish a temporal relationship between the drug and the oral lesion. Probably the ideal scenario to confirm the role of a suspected medication in an oral lichenoid drug reaction would be to use the de-challenge-re-challenge protocol (McCartan et al., 2003), but this may be both impractical and hazardous for the patients. It has been suggested that these lesions have a tendency to be

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unilateral (Lamey et al., 1995) and ulcerative (Potts et al., 1987). Other studies have shown some particular histopathological elements different from those found in OLP, such as the presence of eosinophils and plasma cells in the subepithelial infiltrate (Van den Haute et al., 1989). However, there is currently no convincing scientific support indicating that oral lichenoid drug reactions have a different histopathological presentation than OLP (McCartan & McCreary, 1997).

Table 1. World Health Organization (WHO) diagnostic criteria of oral lichen planus (Kramer et al., 1978)

Clinical criteria
> Lesions are usually multiple and often have a symmetrical distribution
> Presence of white papules, which form reticular, annular or plaque-like patterns
> Presence of slender white lines (Wickham's striae) radiating from the papules
> Presence of a lace-like network of slightly raised gray-white lines in the reticular form
> Presence of atrophic lesions with or without erosion
> Lesions may also include bullae (rare)

Histopathologic criteria
> Presence of thickened ortho or parakeratinized layer in keratinized mucosa. In non-keratinized mucosa this layer may be very thin
> Atrophic epithelium, with variable thickness
> Presence of Civatte bodies in the basal layer, either in the epithelium or within the superficial part of the connective tissue
> Signs of "liquefaction degeneration" in the basal cell layer
> Presence of a well-defined band-like zone of cellular infiltration that is confined to the superficial part of the connective tissue (lamina propria), consisting mainly of lymphocytes

OLP is a clinicopathological diagnosis, thus a histopathological examination of a biopsy specimen is generally advised to confirm the clinical diagnosis (Eisenberg & Krutchkoff, 1992; Rad et al., 2009). However, the evidence regarding the need and value of biopsy for histological confirmation of the diagnosis is not definitive (Al-Hashimi et al., 2007). A number of studies have shown a remarkable inter- and intra-observer variability in the clinicopathological assessment of OLP using the WHO 1978 diagnostic criteria

(Onofre et al., 1997; van der Meij et al., 1999; van der Meij et al., 2002; van der Meij & van der Waal, 2003; Rad et al., 2009). With the objective of obtaining a more reproducible diagnosis of OLP that could enhance the homogeneity in the inclusion of patients in future studies, a modified set of criteria was proposed by van der Meij & van der Waal (2003). These criteria include some parameters intended to enable a distinction between OLP and what was defined as OLL. A diagnosis of OLL was proposed when the lesion(s) did not entirely fulfil clinical and histopathological criteria for OLP. But, once again, the fact that these lesions are most likely, clinically and histopathologically, indistinguishable from each other makes such recommendations insufficient for the diagnosis of OLP (Mravak-Stipetic et al., 2014; Hiremath et al., 2015). Currently, it is believed that the clinical features may be sufficient for the diagnosis, particularly when presenting with the "classical" reticular form. A biopsy, with the corresponding histopathological examination, should be taken if the lesions do not display the typical characteristics (e.g. gingival OLP) and to exclude dysplasia and malignancy (Eisen et al., 2005; Al-Hashimi et al., 2007).

There are also some OLP-like diseases that could make the diagnosis of OLP even more complex, i.e. erythema multiforme, discoid lupus erythematosus, oral lesions of systemic lupus erythematosus, lichen planus pemphigoid, paraneoplastic pemphigoid/paraneoplastic autoimmune multiorgan syndrome and chronic ulcerative stomatitis (Khudhur et al., 2014). Although infrequent, these lesions may resemble OLP both clinically and histopathologically, and some diagnostic tools may not be regularly available to reach a differential diagnosis. In the future, the implementation of new molecular technologies in the oral medicine field could contribute to a better characterization and diagnosis of oral mucosal lesions, including OLP.

Immunopathogenesis

It seems clear that a dysregulation in the cellular arm of the immune system plays a primary role in the pathogenesis of OLP (Sugerman et al., 2002). The inflammatory infiltrate consists mainly of T cells and macrophages. Activated $CD8^+$ T cells are abundant within the epithelium and are found in close contact to lesional basal keratinocytes (Zhou et al., 2002). The autocytotoxic nature of these cells has been studied (Sugerman et al., 2000). $CD8^+$ T cells isolated from lichen planus lesions were found to be more cytotoxic against autologous lesional keratinocytes than those isolated from adjacent clinically normal skin. Furthermore, their cytotoxic activity was partially inhibited by an anti-major histocompatibility complex (MHC) class I monoclonal antibody, which indicates that lesional $CD8^+$ T cells can recognize antigens associated to MHC class I on basal keratinocytes and, once activated, may trigger keratinocytes apoptosis (Sugerman et al., 2000).

Introduction

Different from the epithelium, the infiltrate in the lamina propria is dominated by CD4^{+} T cells (Ishii, 1987). These cells may be activated, in the presence of other costimulatory factors, by antigens associated to MHC class II molecules expressed on Langerhans cells and keratinocytes (Farthing et al., 1990; Walsh et al., 1990). Once activated, CD4^{+} T cells secrete the Th1 cytokines inteleukin-2 and interferon-gamma (Constant & Bottomly, 1997), which contribute to the activation of CD8^{+} T cells.

The exact mechanism of how cytotoxic CD8^{+} T cells can elicit apoptosis of basal keratinocytes is unknown, but several hypotheses have been proposed (Figure 9) (Lodi et al., 2005a): (1) Tumor necrosis factor-alpha (TNF-α) secreted by T cells binds to TNF-α receptor 1 in keratinocyte surface, (2) CD95L (FasL) in T-cell surface binds to CD95 (Fas) in keratinocyte surface, or (3) granzyme B secreted by T cells enters the keratinocyte through perforin-induced pores in surface membrane. All these mechanisms can activate caspase cascade resulting in keratinocyte apoptosis.

Although the nature of the antigen(s) that activates both CD4^{+} and CD8^{+} T cells remains an enigma, it is most likely that this process occurs in response to extrinsic antigens, altered self-peptides or superantigens (Simark-Mattsson et al., 1994; Khudhur et al., 2014). If the antigen(s) is a self-peptide, OLP would therefore be defined as an autoimmune disease (Rose & Bona, 1993). Many other features of OLP may indicate an autoimmune role in its pathogenesis, i.e. disease chronicity, adult onset, female predilection, associations with other autoimmune diseases (type 1 diabetes and autoimmune hepatitis), decreased suppressive activity in OLP patients, development of OLP-like lesions in patients with graft-versus-host disease, improvement or resolution of the lesions after immunosuppressive therapy, and presence of autocytotoxic T-cell clones (Sugerman et al., 1993; Sugerman et al., 2002; Lodi et al., 2005a).
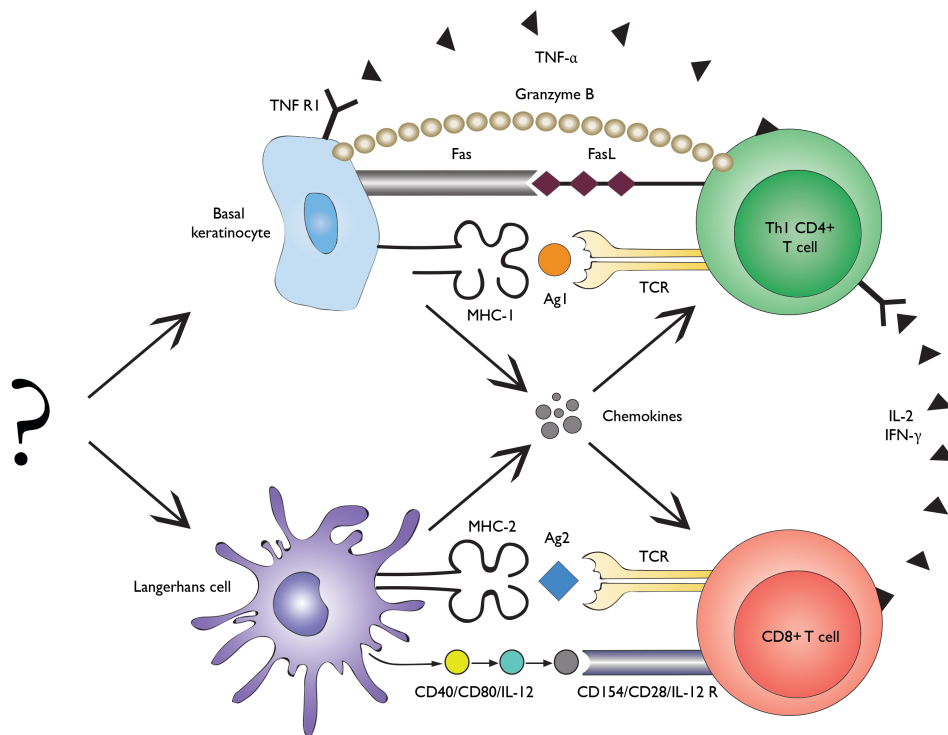
Figure 9. Hypothesis for the immunopathogenesis of OLP (Modified from Lodi et al., 2005a)

OLP: one disease or several?

Due to the relatively well-defined demarcation of OLP lesions, it is suggested that keratinocytes express the culprit antigen only at the injury site. Thus, an early event in the lichen planus lesion formation may be keratinocyte antigen expression or unmasking by some factors, e.g. medications, systemic diseases, dental materials allergens, mechanical trauma (Koebner phenomenon), viral or bacterial infection and psychological stress (Sugerman et al., 2002). Numerous studies have been conducted to investigate the role of such factors in the pathogenesis of OLP. Although most of the reported associations are weak and sustained by poor quality research, it is not unlikely that what is clinically and histopathologically seen as OLP actually represents a group of diseases which are instigated by a diverse repertoire of antigens or factors in susceptible patients. Some of the most relevant associations that have been described in the literature on the aetiology of OLP will be described below.

Hepatitis C virus and other viruses

Since an association with OLP was first reported nearly 20 years ago (Gandolfo et al., 1994), hepatitis C virus (HCV) has probably been the factor most commonly linked with the aetiology of OLP. A large number of case-control studies have been conducted all over the world, but also with somewhat conflicting results. Three recent independent meta-analyses provide strong evidence that lichen planus and HCV are associated (Shengyuan et al., 2009; Lodi et al., 2010; Petti et al., 2011). The pooled odds ratio (OR) and 95% confidence interval (CI) of HCV exposure in patients with lichen planus versus controls ranged from 2.8 (95% CI=2.4-3.2) to 5.4 (95% CI=3.5-8.3). A similar OR of having lichen planus was found in HCV-positive patients compared to controls (Shengyuan et al., 2009; Lodi et al., 2010). There is a clear geographical variability in this association, which does not always relate to the prevalence of HCV in the studied population. A higher relative risk of being HCV-positive in patients with lichen planus has been found in South America and the Middle East, while weaker figures have been reported in studies from Africa. In Europe, the relationship between HCV and lichen planus was stronger in Mediterranean countries (OR=7.0, 95% CI=4.9-9.9) than in northern countries (OR=2.1, 95% CI 0.6-7.7) (Baccaglini et al., 2013).

Despite the fact that a connection has been established by epidemiological studies, there is no conclusive biological evidence indicating that HCV could play a role in the etiopathogenesis of OLP, or that patients with OLP are more susceptible to HCV infection (Bigby, 2009). Several studies have shown the presence of both HCV in OLP lesional tissue (Arrieta et al., 2000; Nagao et al., 2000; Carrozzo et al., 2002) and HCV-specific $CD4^+$ and $CD8^+$ T-cell responses in OLP (Pilli et al., 2002). However, well-designed prospective studies are warranted to elucidate the potential causative role of HCV in OLP (Carrozzo, 2008).

The presence of other viruses, i.e. human papillomaviruses and herpes viruses, has also been detected in OLP lesions (Jontell et al., 1990; Lodi et al., 2005a; Syrjanen et al., 2011). A recent study found a high frequency of human papillomavirus 16-specific $CD8^+$ T cells in patients with OLP (Viguier et al., 2015). Nevertheless, the evidence is yet scarce and may not reflect a pathogenic connection.

Medications

As discussed previously, an OLR caused by a drug should be called oral lichenoid drug reaction (Al-Hashimi et al., 2007). Though, due to the impossibility to differentiate OLP both clinically and histopathologically from an oral lichenoid drug reaction, some authors have considered medications as the aetiological factor for OLP. Some of the earliest lichen planus-like reactions, possibly caused by a medication, were reported in World

War II military personnel who were being treated with prophylactic antimalarial drugs (Bagby, 1945; Nisbet, 1945; Schmitt et al., 1945; Bazemore et al., 1946; Wilson, 1946; Feder, 1949). Since then, an endless number of drugs have been related to lichenoid lesions in the skin and oral mucosa, including nonsteroidal anti-inflammatory drugs (NSAIDs) (Potts et al., 1987), antihypertensives (Hay & Reade, 1978; Firth & Reade, 1989; Robertson & Wray, 1992), penicillamine (Seehafer et al., 1981; Powell et al., 1983), antimicrobials (Markitziu et al., 1986), gold salts (Glenert, 1984) and hypoglycaemic agents (Dinsdale et al., 1968; Barnett & Barnett, 1984). However, most of this evidence is based on case reports or case series including a limited number of patients, which do not allow a thorough analysis of the relationship between the suspected drugs and the onset of the lesions (McCartan et al., 2003).

The exact pathogenic mechanism by which drugs may cause a lichen planus-like lesion is unknown (Scully & Bagan, 2004). An immunogenetic predisposition to drug-induced OLP might be plausible, but there are very limited data to support this notion (Femiano et al., 2008). A poor drug metabolism due to cytochrome P450-enzymes polymorphisms has been suggested as a possible explanation for the debut of OLP (Kragelund et al., 2003; Kragelund et al., 2009). However, it has not been possible to substantiate a pharmacological pathogenesis of OLP based on this theory (Kragelund et al., 2009; Paulusova et al., 2010).

Dental materials

Similarly to medications, dental materials, such as mercury-containing amalgams and composites, may induce a sensitivity response clinically manifested as a lichenoid reaction (Blomgren et al., 1996; Little et al., 2001). The question then arises as if these lesions represent OLP or oral lichenoid contact reactions, since clinically and histopathologically they may not be distinguishable from each other (Thornhill et al., 2006). It seems clear that lesions solely confined to the contact area with these materials are the result of a hypersensitivity reaction, as they improve or resolve a few weeks after the insulting agent is replaced (Henriksson et al., 1995; Bratel et al., 1996; Dunsche et al., 2003). However, "classical" OLP lesions (bilaterally symmetrical distribution with no topographical relation to dental materials) do not seem to benefit from this type of treatment (Bratel et al., 1996; Thornhill et al., 2003; Montebugnoli et al., 2012). Therefore, it is most likely that dental materials are involved in the pathogenesis of oral lichenoid contact reactions but not in the aetiology of OLP.

Stress

Patients with OLP often report an exacerbation of the lesions during periods of great stress, depression or anxiety (Vallejo et al., 2001; Ivanovski et al., 2005). The physiological response to these events has therefore been investigated, but the results are conflicting. Although some studies have found high levels of anxiety, salivary cortisol and other stress markers in patients with OLP (Rodstrom et al., 2001; Koray et al., 2003; Lopez-Jornet et al., 2015), there is no indication to date that these factors play a role in the aetiology of the lesions. The symptoms experienced by some patients with OLP can *per se* be a stressful episode and may partly explain the cases in which this association has been documented. It is more biologically plausible that psychological stress amplifies an immunological response in a previously established lesion aggravating the clinical picture and symptoms.

Genetics

A genetic predisposition in patients with OLP has also been investigated. In this context, many studies have focused on the association between human leukocyte antigens (HLA) and OLP, where high frequencies of specific alleles have been found (Watanabe et al., 1986; Jontell et al., 1987; Lin & Sun, 1990; Porter et al., 1993; Roitberg-Tambur et al., 1994; McCartan & Lamey, 1997). HLA-DR6 has been regarded as explanatory, at least partially, to the peculiar geographical heterogeneity of the association between HCV and OLP (Carrozzo et al., 2001; Carrozzo et al., 2005). Numerous gene polymorphisms have also been reported in connection with OLP susceptibility, including toll-like receptor 3 and different cytokines (Xavier et al., 2007; Fujita et al., 2009; Dan et al., 2010; Kimkong et al., 2011; Jin et al., 2012; Kimkong et al., 2012; Stanimirovic et al., 2013). A recent genetic linkage analysis identified the chromosome 3p14-3q13 as the candidate gene region for OLP in a Chinese family with erosive lesions (Wang et al., 2011). Altogether, the available data is far from being conclusive and more studies, including genome-wide association studies in several thousands of patients, are warranted in order to confirm a genetic predisposition in patients with OLP.

SCIENTIFIC QUESTIONS

The current series of studies aimed to answer the following scientific questions:

1. What is the morbidity and prevalence of OLR in a non-referral adult Swedish population? (**Study I**)

2. Is the medication profile of patients with OLP different from healthy individuals? (**Study II**)

Based on the results of *studies I* and *II*, the following scientific questions emerged:

3. What is the prevalence and profile of thyroid disease in referred patients with OLP? (**Study III**)

4. Is there any difference in the clinical characteristics between OLP patients with and without thyroid disease? (**Study III**)

5. Do patients with OLP have elevated serum levels of antithyroid antibodies and/or abnormal levels of thyroid hormones? (**Study IV**)

6. Are thyroid proteins expressed in OLP lesions? (**Study IV**)

MATERIALS & METHODS

Participants

In *study I*, a total of 6448 subjects, who visited their dentist between 2004 and 2006 for their annual examination, were invited to participate in the study. The patients were examined by one of six general dentists in six private dental clinics in Borås, a medium-sized town of 66000 inhabitants in the Southwest of Sweden. Clinical parameters of patients who presented with oral mucosal lesions (n=1031) were compared to those of patients with healthy oral mucosa (n=1029), selected from the remaining study population.

In *study II*, the medication profile of 956 patients with OLP was investigated. All patients were referred to the Clinic of Oral Medicine, Public Dental Health in Gothenburg, or to the Clinic of Oral and Maxillofacial Surgery and Hospital Dental Care, Central Hospital in Karlstad, between 1998 and 2011. The same 1029 patients with healthy oral mucosa who took part in *study I* were used as controls in *study II*.

A collaboration between the Department of Oral Medicine and Pathology and the Department of Endocrinology, at the University of Gothenburg, was established to conduct *studies III* and *IV*. All OLP patients included in these studies were referred to the Clinic of Oral Medicine, Public Dental Health in Gothenburg, between 1998 and 2013. A total of 1611 patients with OLP were included in *study III* to determine the prevalence of thyroid disease and levothyroxine supplementation in this study population. The prevalence of thyroid disease and levothyroxine supplementation was also investigated in a random population sample from Gothenburg (n=1615), who participated in the third screening of the WHO, multinational MONItoring of trends and determinants in CArdiovascular disease (MONICA) study (Wilhelmsen et al., 1997).

The clinical characteristics of patients with OLP and thyroid disease/levothyroxine supplementation (OLP+/levothyroxine+) (n=108) were compared with an age- and sex-matched group of OLP patients without thyroid disease/levothyroxine supplementation (OLP+/levothyroxine-) (n=110), selected from the remaining patients diagnosed with OLP. The thyroid disease profile of patients with OLP (n=86) was compared with the profile of subjects who participated in the MONICA study and presented with thyroid disease (n=40).

In *study IV*, the serum levels of antithyroid antibodies (anti-thyroglobulin, anti-thyroid peroxidase and anti-thyroid-stimulating hormone receptor antibodies) and thyroid hormones (free triiodothyronine, free thyroxine and thyroid-stimulating hormone) were analysed in OLP+/levothyroxine- patients (n=110), and compared with three different control groups, i.e. a random population sample without levothyroxine supplementation (MONICA study group) (n=657), patients with OLP+/levothyroxine+ (n=108), and patients with thyroid disease/levothyroxine supplementation and no history of OLP (OLP-/levothyroxine+) (n=59). In addition, tissue samples were taken from OLP lesions in 5 OLP+/levothyroxine+ patients and from healthy buccal mucosa in 5 subjects without thyroid disease/levothyroxine supplementation to analyse the expression of thyroid proteins (thyroglobulin, thyroid peroxidase and thyroid-stimulating hormone receptor) in OLP lesions.

Diagnostic criteria for OLP and other oral mucosal lesions

In *study I*, the diagnostic labels and criteria for oral mucosal lesions were in accordance with the WHO, the Application of the International Classification of Diseases to Dentistry and Stomatology and with the modifications and complementary additions suggested by Axéll (1976; 1987) and Axéll et al. (1984).

In *studies II*, *III* and *IV*, specialists in oral medicine established the diagnosis of OLP following the clinical and histopathological criteria according to the WHO (Kramer et al., 1978). However, biopsies were only taken when the disease was divergent from the typical clinical manifestations, as has been previously suggested (Al-Hashimi et al., 2007). All lesions had to present with reticular or papular features with or without plaque, erythema or ulcerations. OLP lesions were clinically classified as papular, plaque-like, reticular, erythematous or ulcerative, based on the most predominant form of the lesion. Gingival OLP with erythema but without striae or papules, which is sometimes referred to as an OLL (van der Waal, 2009), was also included. Patients who presented with other types of oral mucosal lesions, including oral lichenoid contact reactions, were excluded from these studies.

Data collection

Clinical data from patients in *studies I* and *II*, and OLP patients in *studies III* and *IV*, were collected using MedView. MedView is a computer system for the formalized registration and subsequent analysis of clinical and image-based information (Jontell et al., 2005). It operates with an input application that is focused on the collection and computerized

storage of clinical data. The resulting information was gathered in a single database and exported to MedVisualizer. This application is used for visualization and scanning of the information that is obtained from the database (Jontell et al., 2005). Finally, the data selected for evaluation were transferred to Microsoft Excel for Mac 2011 (Redmond, WA, USA) for subsequent statistical analysis.

In *studies I-IV*, systemic diseases were registered and grouped into different blocks according to the International Classification for Diseases and Health Related Problems, 10th revision (ICD-10) (World Health Organization, 2010). Medications were classified according to the Anatomical Therapeutic Chemical (ATC) Classification System (WHO Collaborating Centre for Drug Statistics Methodology, 2011a; 2011b) and the Swedish Medicines Compendium for Physicians (FASS) (Läkemedelsindustriföreningen, 2012). The ATC Classification System is a five-level index of medicaments and pharmacological substances. The system divides drugs into 14 main groups (first level), with a subsequent separation into chemical/pharmacological/therapeutic subgroups (second level–fourth level). The fifth level of the code indicates the chemical substance, e.g. ibuprofen. In cases in which a single chemical substance belonged to two or more ATC codes, the code for a systemic route of administration was selected over topical administration. A visual analogue scale was used to register symptoms from the oral mucosa. Other clinical parameters, such as smoking habits, allergies and patient's own assessment of their general health were also recorded in *studies I* and *II*.

Clinical photographs of all oral mucosal lesions (Study I) and OLP lesions (Study II) were taken on all patients. In *studies III* and *IV*, clinical photographs of all OLP lesions were taken both at the primary examination and follow-up.

In order to confirm the thyroid diagnosis of patients with OLP in *study III*, the medical records of the patients were requested from the physician who made the initial diagnosis of the thyroid disease. In case the patient was diagnosed before 2003-2004 and if the medical journal was no longer active in the physician's database, records were requested either from the archive of the corresponding institution or otherwise, from the regional archive of the Region Västra Götaland. All medical records were reviewed by an endocrinologist to establish the thyroid diagnosis.

Antithyroid antibodies, thyroid hormones and levothyroxine supplementation

Anti-thyroglobulin antibody (anti-Tg)

Thyroglobulin (Tg) is a glycoprotein produced mainly by the thyrocyte. It plays an important role in the synthesis of the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4) (Ma & Skeaff, 2014). The presence of anti-Tg is quite common in patients with autoimmune thyroid disease (AITD). Approximately 20-50% of the patients with Hashimoto's thyroiditis has high serum concentrations of anti-Tg (Pearce et al., 2003). However, it has been shown that up to 13% of the thyroid disease-free adult population and 25% of the women over 60 years of age, have circulating anti-Tg (Hollowell et al., 2002). As a result, anti-Tg is not routinely assessed in the diagnosis of AITD (Sinclair, 2006).

Anti-thyroid peroxidase antibody (anti-TPO)

Thyroid peroxidase (TPO), as Tg, is a glycoprotein synthetized by thyrocytes. It also plays a key role in the synthesis of T_3 and T_4. Anti-TPO represents the hallmark of Hashimoto's thyroiditis (McLachlan & Rapoport, 2004; Hadj-Kacem et al., 2009). High serum concentrations of anti-TPO are found in 90% of patients suffering from this condition (Pearce et al., 2003; Erdogan et al., 2009). Anti-TPO is a marker of thyroid dysfunction and is largely involved in the pathogenesis of AITD. It has been shown that this antibody has the ability to fix complement and promote antibody-dependent cell-mediated cytotoxicity against thyroid cells (Stassi & De Maria, 2002).

Anti-thyroid-stimulating hormone receptor antibody (anti-TSHR)

Thyroid-stimulating hormone receptor (TSHR), expressed on the basolateral membranes of thyroid epithelial cells, is the primary regulator of thyroid hormones synthesis and cell growth (Hadj-Kacem et al., 2009). It is a member of the G-protein-coupled receptor family. TSHR is the primary autoantigen in Graves' disease. Anti-TSHR are found in the sera of most patients with Graves's disease and 10-15% of patients with Hashimoto's thyroiditis (Michalek et al., 2009). Moreover, levels of circulating anti-TSHR correlate with the severity of the disease (degree of hyperthyroidism). Therefore, anti-TSHR is considered the hallmark of Graves' disease (Michalek et al., 2009).

Expression of TSHR is not limited to the thyroid gland. The presence of biologically active TSHR has been demonstrated in a variety of human and animal cells tissues, including adipocytes and fibroblasts, osteoblasts, osteoclasts, bone marrow cells,

cardiomyocytes, skin keratinocytes and more (Davies et al., 2002; Cianfarani et al., 2010).

Triiodothyronine (T_3)

Thyroid hormones (T_3 and T_4) are normally synthetized and secreted by the thyroid gland. T_3 constitutes approximately 20% of the total hormone production in the thyroid; the rest is produced by the conversion (deiodination) of T_4 to T_3 in peripheral tissues. T_3 and T_4 regulate a number of developmental, metabolic and neural activities throughout the body. Circulating levels of T_3 are much lower than levels of T_4, but T_3 is more biologically active (3-4 times more potent than T_4). Thyroid hormones circulate primarily bound to carrier proteins; only a small fraction (less than 1%) circulates unbound (free). Only the free forms are biologically active. Free T_3 (FT_3) is therefore a measurement of the fraction of circulatory T_3 that exists in free state in the blood and is important in evaluating the effectiveness of thyroid replacement therapy, in ruling out T_3 thyrotoxicosis, and in detecting protein-binding abnormalities. Elevated FT_3 values are indicative of T_3 toxicosis (if T_4 values are normal) or hyperthyroidism, whereas decreased FT_3 values are found in primary and secondary hypothyroidism. However, FT_3 is not a reliable marker for hypothyroidism.

Thyroxine (T_4)

T_4 is the major hormone derived from the thyroid gland. It is metabolized to T_3 peripherally by deiodination, and is therefore considered a reservoir or prohormone for T_3. Approximately 0.05% of circulating T_4 is in the free or unbound portion. Free T_4 (FT_4) may more accurately measure the physiologic amount of T_4 and is usually measured together with thyroid-stimulating hormone when thyroid disease is suspected. FT_4 testing is also used to monitor the appropriateness of thyroid replacement therapy. Elevated FT_4 values suggest hyperthyroidism or over-replacement in patients treated with levothyroxine sodium, whereas decreased levels are compatible with hypothyroidism or under-replacement.

Thyroid-stimulating hormone (TSH)

TSH, also known as thyrotropin, is synthetized and secreted by the anterior pituitary gland in response to a negative feedback mechanism involving FT_3 and FT_4. Additionally, the thyrotropin-releasing hormone (TRH), which is secreted by the hypothalamus, directly stimulates TSH production. TSH interacts with specific cell receptors on the thyroid cell surface and gives rise to 2 main actions. First, it stimulates cell reproduction and hypertrophy. Second, it stimulates the thyroid gland to synthesize and secrete

T_3 and T_4.

TSH testing is useful for detecting thyroid dysfunction (both primary hypothyroidism and hyperthyroidism) and monitoring thyroid replacement therapy. Elevated TSH levels indicate primary hypothyroidism, whereas decreased levels suggest hyperthyroidism. TSH is also important in the differential diagnosis of primary (thyroid) from secondary (pituitary) and tertiary (hypothalamus) hypothyroidism, in which TSH levels are low or normal. Elevated TSH, in the presence of normal FT_3 and FT_4 levels, is often referred to as subclinical hypothyroidism.

Levothyroxine sodium

Levothyroxine sodium is a synthetic hormone chemically identical to T_4. It is the preferred choice for T_4 replacement in patients with thyroid insufficiency. The goal of replacement therapy with levothyroxine is normalization of serum TSH values and resolution of signs and symptoms of hypothyroidism (Pearce et al., 2003). Current guidelines recommend that all patients with overt hypothyroidism and subclinical hypothyroidism with TSH>10 mIU/L should be treated with levothyroxine (Khandelwal & Tandon, 2012). Since subclinical hypothyroidism may progress to overt hypothyroidism in 2-5% of the cases annually (Vanderpump et al., 1995), most experts also recommend levothyroxine therapy in patients with subclinical hypothyroidism and TSH≤10 mIU/L, but only in the presence of symptoms, goitre, positive anti-TPO or infertility (Khandelwal & Tandon, 2012). However, despite recommendations, there is a great variability among endocrinologists and general practitioners when selecting the patients that will be prescribed with this medication.

Chronic under- and over-replacement is a common clinical practice. Data suggests that over-replacement occurs in approximately 20% of the patients treated with levothyroxine sodium (Canaris et al., 2000). Possible adverse effects of over-replacement include the typical signs and symptoms of hyperthyroidism, cardiovascular changes and bone mass and density loss (Bartalena et al., 1996).

Laboratory analyses

Biochemical analysis

Venous blood samples were taken from the antecubital vein of all patients and controls to determine the serum levels of anti-Tg, anti-TPO, anti-TSHR, FT_3, FT_4 and TSH. Analyses were performed at the Laboratory of Clinical Chemistry at Sahlgrenska

University Hospital (accredited ISO 15189:2007; ISO 22870:2006), Gothenburg, Sweden.

Concentrations of serum anti-TPO, anti-TSHR, FT_3, FT_4 and TSH were measured with the electro-chemiluminescence immunoassay (ECLIA) method using the Cobas 6000/8000 system (Roche Diagnostics Scandinavia AB, Stockholm, Sweden), and anti-Tg concentrations were determined with the Time Resolved Amplified Cryptate Emission (TRACE) technology using the B·R·A·H·M·S KRYPTOR system (B·R·A·H·M·S GmbH, Hennigsdorf, Germany). The reference level for anti-TPO was <34 kU/L, for anti-TSHR <1.22 IU/L (with a gray zone between 1.22 and 1.75 IU/L) and for anti-Tg <33 kU/L. The reference level for TSH was 0.3-4.2 mIU/L, for FT_3 3.1-6.8 pmol/L and for FT_4 12-22 pmol/L.

Immunohistochemistry

In order to investigate the expression of thyroid proteins (Tg, TPO and TSHR) in the epithelium and connective tissue of OLP lesions, 3-millimetre punch biopsies were taken from OLP lesions of 5 OLP+/levothyroxine+ patients (mean age=66.8 years; range 61-75; women n=5) and from the healthy buccal mucosa of 5 healthy controls (mean age=59.6 years; range 54-65; women n=5). Tissue specimens were fixed in formalin and subsequently embedded in paraffin for histopathological examination. The clinical diagnosis of OLP was confirmed histopathologically in all patients. Healthy thyroid tissue was retrieved from the archives of the Department of Clinical Pathology and Genetics at the Sahlgrenska University Hospital and used as a positive control as recommended by the manufacturer. The procedure was performed on 4 µm-thick paraffin sections, which were retrieved from the archives of the Department of Oral Medicine and Pathology at the University of Gothenburg. Tissue sections were baked for 1 h at 60°C, deparaffinised with X-TRA-Solv (Medite GmbH, Burgdorf, Germany) and rehydrated in decreasing isopropanol series to distilled water. For antigen retrieval, sections were boiled in citrate buffer (pH 6) at 110°C for 7 min using a pressure boiler (Decloaking chamber, Biocare Medical, Walnut Creek, CA, USA). Once the boiling was completed, the slides remained in the pressure boiler until cool down to 90°C and then rinsed with water.

Tissue sections were then washed in Tris-buffered saline (pH 7.6) and treated with peroxidase blocking solution (Dako, Glostrup, Denmark), followed by a 10% BSA wash for 1 h to reduce background. The EnVision+ System-HRP (DAB) (Dako) was used for polymer detection according to the procedure outlined by the manufacturer with TBS washes in between. The mouse monoclonal anti-Tg ([2H11] ab187378, Abcam, Cambridge, United Kingdom; 1:100 dilution with 4% BSA), mouse monoclonal anti-TPO ([MoAb47] ab12500, Abcam; 1:100 dilution with 4% BSA) and rabbit polyclonal anti-TSHR (HPA026680, Atlas Antibodies AB, Stockholm, Sweden; 1:200

dilution with 4% BSA) antibodies were applied to the sections and incubated overnight at 4°C. The mouse polyclonal IgG antibody (ab37355, Abcam; 1:100 dilution with 4% BSA) was used as a negative control for all stainings. Finally, the sections were counterstained with hematoxylin (Dako) and mounted with an aqueous mounting medium (Aquatex, Merck KGaA, Darmstadt, Germany).

Quantitative real-time PCR (qPCR)

With the objective of sustaining the results obtained from the immunohistochemical analysis, 3-millimetre punch biopsies were taken respectively from OLP lesions and healthy buccal mucosa of the same 5 OLP+/levothyroxine+ patients and 5 healthy controls included in the immunohistochemical analysis. The biopsies were kept in RNAlater (Qiagen, Hilden, Germany) for 24 h at 4°C and then stored at -80°C. Prior to RNA isolation, the biopsies were homogenized using the gentleMACS™ Dissociator (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) according to the accompanying standard protocol.

Total RNA was extracted using the RNeasy Plus Mini kit (Qiagen) according to the manufacturer's instructions and quantified using Qubit 2.0 fluorometer (Life Technologies Stockholm, Sweden) and the Qubit® RNA BR Assay Kit. A total of 0.5 µg RNA per sample was converted into cDNA using the iScript reverse transcription supermix (Bio-Rad Laboratories AB, Solna, Sweden) diluted in water to a total volume of 20 µl. The reactions were run on the MJ Mini 48-well Personal Thermal Cycler (Bio-Rad Laboratories AB) for 5 min at 25°C, 30 min at 42°C, 5 min at 85°C and put on hold at 4°C, after which the cDNA was diluted in water and stored at -20°C until further analysed. Two µl of cDNA were added to 18 µl of the reaction mix containing 1 x SsoAdvanced Universal SYBR Green Supermix (Bio-Rad Laboratories AB) and 1 µl of primers (PrimePCR™ SYBR® Green Assays for human Tg, TPO or TSHR, Bio-Rad Laboratories AB). All reactions were run in duplicates in 96-well plates. qPCR was performed on a CFX Connect Real-Time PCR Detection System (Bio-Rad Laboratories AB) using the following PCR conditions: an initial activation step, 2 min at 95°C, 40 cycles of denaturation, 5 sec at 95°C, and combined annealing/extension, 60 sec at 60°C, after which a melting curve analysis was performed for identification of nonspecific products. Stability comparisons of suitable reference genes were calculated using geNorm (Vandesompele et al., 2002), evaluating TUBB and TBP as the best suitable among five tested primers from the human endogenous control gene panel (TATAA Biocenter AB, Göteborg, Sweden). The difference between the mean CT value of TUBB and TBP and the CT value of the target gene (ΔCT) was determined and the relative gene expression was calculated using the formula $2^{\Delta CT}$. The values were adjusted so that the lowest value was set to 1 and results are presented as relative expression of the mRNA levels in

patients compared to healthy controls.

Data analysis

In *study I*, clinical variables were compared between patients with oral mucosal lesions and controls in order to identify associations between the different parameters and diagnoses. Fisher's exact test was used in the analysis and a *p*-value <0.05 was regarded as statistically significant. The statistical analysis was performed using the Statistical Analysis Software (SAS), version 9.2 (SAS Institute Inc. Cary, NC, USA).

In *study II*, the statistical analysis was performed in a stepwise procedure. The first step consisted of comparing the levels of drug usage between patients with OLP and controls at all levels of the ATC code. Moreover, differences between clinical forms of OLP, age and sex with respect to different drugs were analysed. Fisher's exact test was used in the analysis, and a *p*-value <0.05 was regarded as statistically significant. The second step was a multiple logistic regression analysis designed to determine the possible influence of confounders. This analysis was performed in two phases. The first included all significant factors found in the univariate pairwise analysis. In the second, all non-significant variables found in the first step, with the exception of levothyroxine and NSAIDs, were excluded. The statistical analysis was performed using SAS, version 9.2 (SAS Institute Inc.).

In *study III*, a Fisher's exact test was used to analyse the difference of thyroid diagnoses between patients with OLP and the general population, and also to determine differences in clinical parameters between OLP+/levothyroxine+ and OLP+/levothyroxine- patients. A McNemar's test was used to analyse differences in the clinical type of OLP and other clinical parameters between the primary examination and the re-examination both in OLP+/levothyroxine+ and OLP+/levothyroxine- patients. A paired-samples *T*-test was used to analyse the difference between the age at the time of OLP and thyroid disease diagnosis. A multivariate logistic regression analysis was performed to determine the possible influence of confounders, such as age and gender, in the difference of levothyroxine use between OLP patients and controls. Finally, an analysis of variance was used to determine the possible correlation between levothyroxine doses and the clinical characteristics and subjective symptoms of OLP lesions. All the analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 22 (IBM Corporation, Armonk, NY, USA). A *p*-value <0.05 was considered to be statistically significant.

In *study IV*, a sample size of at least 100 subjects per group was calculated (power [1β]=0.8; α=0.05) using G*Power version 3.1.9.2 (University of Düsseldorf, Germany)

for the analysis of serum levels of antithyroid antibodies and thyroid hormones based on previously published data (Chang et al., 2009; Lin et al., 2011; Robledo-Sierra et al., 2013). Data on serum levels of antithyroid antibodies and thyroid hormones followed a non-normal distribution. Thus, the Fisher's exact test and chi-square test were used to analyse differences in the number of subjects with elevated serum levels of antithyroid antibodies and elevated/decreased levels of thyroid hormones. A logistic regression analysis was used to adjust the groups for age and gender when comparing serum levels of antithyroid antibodies and thyroid hormones between patients with OLP and the general population. The results from the immunohistochemical analysis were presented in a descriptive way, while differences between groups in the qPCR analysis were determined using the Mann-Whitney U test. The threshold for statistical significance was set at a *p*-value <0.05, although weak significances (0.049-0.010) were analysed with caution due to multiple testing problem. The analyses were performed using SPSS, version 22 (IBM Corporation), and GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA).

Ethical considerations

Studies III and *IV* were approved by the Ethics Committee of the University of Gothenburg (Dnr 048-12 and Dnr 244-94) and the National Data Inspection Board. A written informed consent was obtained from all patients and controls.

MAIN FINDINGS

- OLR still represent one of the most common and debilitating oral mucosal lesions in the adult population (**Study I**).

- OLP is strongly associated with the use of levothyroxine (**Study II**).

- The prevalence of thyroid disease in patients with OLP is significantly higher compared to the general population (**Study III**).

- The clinical characteristics and the natural course of OLP lesions in patients with thyroid disease are different compared to those in patients with no thyroid disease (**Study III**).

- Patients with OLP without a previously diagnosed thyroid disease have high levels of TSH and low levels of FT_4, indicative of thyroid disease (**Study IV**).

- Elevated levels of thyroid antibodies could not explain the high prevalence of thyroid disease in patients with OLP (**Study IV**).

- TSHR is highly expressed in basal keratinocytes of OLP lesions (**Study IV**).

RESULTS

Study I

In this study, no distinction was made between OLP and OLL. Patients who presented with any of these lesions were diagnosed and denoted as OLR. The overall prevalence of OLR was 2.4% (women 53.5%). Nearly 20% of the patients reported the presence of subjective symptoms. Ninety-one (57.9%) patients with OLR were taking at least one regular medication compared to 43.9% of the controls (p=0.0011). By analysing the medications at the first level of the ATC code, it was found that patients with OLR used more cardiovascular drugs than the controls (p<0.0001). At the fifth level of ATC code, it was found that metoprolol (9.5%) and levothyroxine sodium (5.1%) were the medications more frequently used by patients with OLR. However, no significant differences were found when these specific medications were compared between patients with OLR and controls. Moreover, hypertensive diseases were more common in patients with OLR than in the controls (p=0.0061).

Although no significant differences were found in the medication between patients with OLR and controls at the fifth level of the ATC code, the fact that more patients with OLR were using cardiovascular drugs and were diagnosed with hypertensive diseases led us to further investigate the medication profile of patients with OLP.

Study II

The majority (51.2%) of patients with OLP were using at least one regular medication at the time of initial examination compared to 43.9% of the controls (p=0.0014). Regular medication was more frequent among women; 57.3% of the patients with OLP compared to 45.4% of the controls (p<0.0001). At the fifth level of the ATC code, five drugs were associated with OLP: levothyroxine sodium, diclofenac, naproxen, ketoprofen and celecoxib. This finding correlated with the results of the analysis between OLP and systemic diseases; patients with OLP reported more thyroid disorders, arthropathies and dorsopathies than the controls (p<0.0001).

When the different clinical types of OLP were analysed separately, patients with ulcerative lesions were using naproxen and ibuprofen more frequently than the

Results

controls (p=0.0010).

To further investigate the difference in regular medication between groups, patients who were only medicating with levothyroxine in the OLP (n=34) and control (n=6) group were excluded from the analysis. These results showed that 47.6% of the patients with OLP and 43.3% of the controls used regular medication (n.s.).

Since the groups were not matched for age or sex, and the OLP group comprised more women than the controls (p=0.0107), these and other variables (smoking, allergies and systemic diseases) were treated as potential confounders in a subsequent logistic regression analysis. This model demonstrated that levothyroxine sodium was associated with OLP (multivariate OR 3.39, 95% CI: 2.09–5.46, P < 0.0001), even after adjusting for confounders. In contrast, no association was found between OLP and NSAIDs using the same statistical model.

Study III

Prevalence of levothyroxine supplementation and thyroid disease in patients with OLP

The prevalence of levothyroxine supplementation among referred patients with OLP was 10.6%, compared to 2.5% in the general population. The multivariate logistic regression analysis demonstrated that levothyroxine was associated with OLP (multivariate OR 2.99, 95% CI: 2.03–4.44, p<0.0001), even after controlling for confounders. Eighty-two (76%) out of the 108 OLP+/levothyroxine+ patients reported that the diagnosis of thyroid disease and start of levothyroxine supplementation preceded the onset of OLP. Moreover, the mean age of the patients at the time of initial OLP diagnosis was 59.9 years, compared to 48.4 years when their thyroid disease was firstly diagnosed (p<0.0001). Neither the clinical presentation of the OLP lesions nor the severity of subjective symptoms was correlated with the levothyroxine dose in OLP+/levothyroxine+ patients.

The thyroid diagnosis was retrieved from the medical records in 86 (women n=77) out of 108 (80%) of the OLP+/levothyroxine+ patients and in all 40 controls (women n=37) with thyroid disease. In the OLP+/levothyroxine+ group, 58.1% of the patients were diagnosed with unspecified hypothyroidism, compared to 80.0% of the subjects from the general population with thyroid disease (p=0.0173). Hashimoto's thyroiditis was diagnosed in 22.1% of the patients with OLP compared to 2.5% of the controls (p=0.0037). However, when all cases of hypothyroidism were grouped together, including Hashimoto's thyroiditis and hypothyroidism for reasons other than surgery,

irradiation or hypopituitarism, no significant difference was found between patients with OLP and controls.

None of the individuals from the general population was diagnosed with Graves' disease, while this diagnosis was found in 10.5% of the patients with OLP (p=0.0563). Unspecified thyrotoxicosis was diagnosed in 3.5% of the patients with OLP, compared to 12.5% of general population (p=0.1084). As for hypothyroidism, when all cases of hyperthyroidism (Grave's disease, unspecified thyrotoxicosis and different forms of goitre) were merged together, it was found that 19.8% of the OLP patients and 12.5% of the controls were diagnosed with this type of condition (n.s.).

Clinical characteristics of OLP lesions

At the primary examination, the clinical characteristics of OLP lesions and the severity of the symptoms were not significantly different between OLP+/levothyroxine+ and OLP+/levothyroxine- patients. However, at the re-examination, patients in the OLP+/levothyroxine- group displayed significantly more erythematous lesions (p=0.0015) and complained of more severe symptoms (p<0.0001). Moreover, a higher number of OLP+/levothyroxine- patients were using topical steroids as treatment for the OLP lesions at the re-examination (p=0.0297). No statistically significant difference was found for the concomitant presence of extraoral lichen planus between the groups.

The mean time period between the primary examination and the re-examination did not differ between OLP+/levothyroxine+ (6.5 years, SD=4.7) and OLP+/levothyroxine- patients (6.9 years, SD=3.4). However, the clinical characteristics of OLP lesions differed between the groups at follow-up. The lesions in the OLP+/levothyroxine+ patients were more reticular (p=0.0027) and less erythematous at the re-examination (p=0.0008) compared to the primary examination. In the OLP+/levothyroxine- patients, the number of plaque-like lesions increased (p<0.0001) and the number of reticular and ulcerative lesions decreased (p=0.0269). The severity of the symptoms from the OLP lesions decreased from the primary examination to the re-examination in the OLP+/levothyroxine+ group only (p<0.0001).

Study IV

Serum levels of antithyroid antibodies and thyroid hormones

Sixteen (14.5%) patients in the OLP+/levothyroxine- group presented at least one elevated antithyroid antibody compared to 82 (16.1%) of the subjects from the general

population, 59 (54.6%) OLP+/levothyroxine+ patients and 25 (42.4%) OLP-/levothyroxine+ patients. After adjusting for age and gender, fewer OLP+/levothyroxine- patients (9.4%) presented with elevated levels of anti-TPO compared to the general population (15.0%) (p=0.0240). High levels of TSH were more frequently found in OLP+/levothyroxine- patients (2.8%) than in the general population (0.5%) (p=0.0427). Finally, more OLP+/levothyroxine- patients (9.4%) presented with low levels of FT_4 compared to the general population (2.9%) (p=0.0034).

Significant differences were found in antithyroid antibodies and thyroid hormones when OLP+/levothyroxine- patients were compared with OLP+/levothyroxine+ patients. Serum anti-Tg (p<0.0001) and anti-TPO (p<0.0001) antibodies were more frequently elevated in the OLP+/levothyroxine+ group. No differences were found in serum levels of anti-TSHR between the groups (p=0.3639). With regard to thyroid hormones, more patients in the OLP+/levothyroxine+ group presented with low levels of TSH (p<0.0001) and FT_3 (p=0.0167), and high levels of FT_4 (p<0.0001), compared to OLP+/levothyroxine- patients.

Patients with OLP+/levothyroxine+ were compared with patients with OLP-/levothyroxine+ to determine if those with thyroid disease and levothyroxine supplementation, irrespective of the presence of OLP, differed in serum levels of antithyroid antibodies and thyroid hormones. Anti-Tg (p=0.2450), anti-TPO (p=0.1366) and anti-TSHR (p=1.0000) were similar in both groups. However, more patients in the OLP+/levothyroxine+ group showed low levels of FT_3 (p=0.0387), whereas more patients in the OLP-/levothyroxine+ group showed high levels of FT_4 (p=0.0142). No differences were found for TSH (p=0.5773) between the groups.

Expression of thyroid proteins in OLP lesions

The immunohistochemical analysis showed a positive staining for TSHR in the basal layer of the epithelium in all OLP+/levothyroxine+ patients, whereas all sections from the healthy controls were negative for the same protein. All sections from both groups were negative for Tg and TPO. qPCR analysis demonstrated that there was a tendency for higher expression of TSHR in patients compared to healthy controls. Tg and TPO did not differ between the groups.

GENERAL DISCUSSION

Research on OLP has often taken a monocausal approach, which has added little to our knowledge about the aetiology of this condition. It may therefore be more reasonable to assume that OLP represents a heterogeneous disease not caused by a single aetiological factor. Any scientific effort to identify factors that may be related to the aetiology will consequently require a cohort of patients that is large enough to allow identification of possible subgroups. A systematic collection of data with the implementation of standardized registration tools and formalized diagnostic criteria are also critical aspects for the validity of retrospective research. Using this approach, the current series of studies included in this thesis enabled us to identify one possible aetiological factor for a subgroup of patients with OLP.

The use of systemic medications has historically been one of the most frequently implicated factors in the onset of OLP lesions (Hay & Reade, 1978; Hamburger & Potts, 1983; Potts et al., 1987; Firth & Reade, 1989; Bagan et al., 2004; Torpet et al., 2004; Clayton et al., 2010; Habbab et al., 2010). The support for this hypothesis comes mainly from case reports and small case series focusing on specific groups of drugs rather than from controlled scientific studies. Therefore, in *study II*, the medication profile of a large cohort of patients with OLP was compared with a control group consisting of dental patients with no oral mucosal lesions.

In accordance to the aforementioned studies, the univariate analysis showed that NSAIDs were more frequently used in the OLP group. Our results were also consistent with those published by Potts et al (1987), in which NSAIDs were associated with erosive OLP lesions. Interestingly, when a logistic regression analysis was used to control for the possible influence of confounders, we could not find any association between OLP and NSAIDs. In contrast, a strong association was found between OLP and levothyroxine. This fact emphasizes the importance of controlling for confounders with appropriate statistical methods when investigating potential aetiological factors for OLP.

The use of levothyroxine has previously been associated with OLP. A recent Finnish study (Siponen et al., 2010) showed similar results to those reported in *study II*; 10% of patients with OLP (n = 152) were using levothyroxine supplementation compared to 5% of the controls (n = 222) (OR 2.39, 95% CI: 1.05–5.61). Other studies have also reported a higher intake of thyroid drugs in patients with OLP compared to controls (Kragelund et al., 2003; Hirota et al., 2011), although specific chemical substances were not reported in these studies.

General discussion

A study design necessary to verify a connection between a drug and an oral lichenoid drug reaction has been advocated (McCartan et al, 2003). These authors claim that knowledge of the patient's medication history over the 12 months prior the onset of the symptoms is essential. They also argue that drugs should be withdrawn followed by a subsequent re-exposure. This approach is theoretically most justified, but the problem is that asymptomatic OLP may go unrecognized for several years and drug withdrawal may be impractical and hazardous for the patients. For these reasons, it is a difficult challenge to confirm the role of levothyroxine or any other medication in the development of OLP.

A question that emerged was if levothyroxine *per se* could induce a lichenoid reaction in the oral mucosa or if it was an underlying thyroid disease the responsible for this connection. Adverse effects of thyroid hormone supplementation have been widely documented. These typically result from therapeutic overdosage and mainly include the signs and symptoms of hyperthyroidism (Bartalena et al., 1996). Probably the only dermatologic side effect that has been recognized is a transient scalp hair loss that may occur during the first weeks of hormone replacement (Roberts & Ladenson, 2004). To the best of our knowledge, only one case report of lichenoid eruptions in the skin related to levothyroxine has been published to date (Kaur et al., 2003). The lesions occurred in a 10-year old girl 3 days after an accidental levothyroxine overdosage and resolved 2 weeks later. No recurrence was observed over the next 4 months of follow-up. Although levothyroxine overtreatment has been reported to occur in approximately 20% of the patients (Canaris et al., 2000), it is most unlikely that circulating levels of T_4 in these patients are comparable to those reported by Kaur et al. (2003) (9000 μg). Moreover, the half-life of levothyroxine is very short, which makes it even more improbable that all OLR in patients using levothyroxine could be explained on the basis of an oral lichenoid drug reaction. Based on this, our interpretation of the results from *study II* was that it is an underlying thyroid disease and not levothyroxine which is associated with OLP.

It could be questioned if dental patients with no oral mucosal lesions are the most appropriate controls when investigating the medication profile of patients with OLP, and if they are representative of the general population. In order to tackle this issue, we aimed to compare, in *study III*, the prevalence of levothyroxine supplementation and thyroid disease in the general population with an even larger cohort of patients with OLP. The results from this study confirmed the marked difference presented in *study II* on the prevalence of levothyroxine supplementation in patients with OLP compared to controls. Although the retrospective design of *study III* could not establish a temporal relationship between the onset of OLP lesions and the use of levothyroxine, nearly 80% of the patients reported that the start of levothyroxine supplementation preceded the diagnosis of OLP. This indicated that either levothyroxine supplementation or an underlying thyroid disease might play a role in the development of OLP lesions in a subgroup of patients. Furthermore, it was shown that nearly 80% of the patients

with OLP using levothyroxine had a diagnosis of primary hypothyroidism for reasons other than surgery or irradiation.

OLP has previously been associated with hypothyroidism (Siponen et al., 2010; Hirota et al., 2011; Lo Muzio et al., 2013). In the study by Siponen et al. (2010), it was established that all patients with OLP who were using levothyroxine (n=15) had an initial diagnosis of hypothyroidism. Hirota et al. (2011) found that 10.9% of patients with OLP (n=110) had hypothyroidism, compared to 5.3% of the controls (n=76). Lo Muzio et al. (2013) reported from a series of 105 OLP patients without a previous diagnosis of thyroid disease, that 14.3% had Hashimoto's thyroiditis. A high prevalence of hypothyroidism has also been shown in patients with lichen ruber planus (Dreiher et al., 2009). It was found that 10.0% of these patients (n=1477) had hypothyroidism compared to 5.7% of the control subjects (n=2856). Based on these studies and ours, it is evident that a subgroup of patients with lichen planus may have hypothyroidism, or eventually levothyroxine supplementation, as a common aetiological denominator.

The results from *study III* also showed that the course of OLP lesions was different between patients with and without thyroid disease. The severity of the lesions was lower and the symptoms were milder in patients with thyroid disease when comparing the oral status between the primary examination and re-examination. In patients without thyroid disease, OLP lesions and subjective symptoms remained more constant over time. OLP is a chronic condition in which periods of remission and exacerbation are frequently observed. A complete resolution of the papular and reticular form may also occur (Andreasen, 1968a). It has been shown that in the natural course of OLP the lesions tend to adopt a reticular pattern and that the initial erythematous/erosive forms are likely to decrease (Thorn et al., 1988; Carbone et al., 2009). However, in those studies, the course of OLP lesions in patients with and without thyroid disease was not examined. In *study III*, reticular forms of OLP were more prevalent and erythematous/erosive less prevalent in patients with thyroid disease than in patients without thyroid disease at the re-examination. Hence, patients with concomitant OLP and thyroid disease may represent a specific subgroup of OLP patients with a different clinical presentation of the lesions over time.

One of the hypotheses for the connection between OLP and hypothyroidism includes the presence of a common autoimmune process. Hashimoto's thyroiditis is the most common form of hypothyroidism in iodine-replete regions like northern Europe (Laurberg et al., 2006; Garber et al., 2012). Thus, it is most likely that the vast majority of the patients who were diagnosed with hypothyroidism in *study III* might suffer from Hashimoto's thyroiditis. Several investigations have shown a high prevalence of skin disorders in patients with thyroid disease (Ai et al., 2003; Burman & McKinley-Grant, 2006). Additionally, experimental studies suggest that the skin is a target of autoantibodies against thyroid-specific antigens in patients with AITD

(Slominski et al., 2002; Cianfarani et al., 2010). A similar organ-specific autoimmune response may occur in the oral mucosa influencing the development of OLP lesions in a subgroup of patients, where basal keratinocytes expressing thyroid or thyroid-like proteins become a target of cytotoxic T cells. Therefore, it may be hypothesized that an autoimmune link exists between OLP and Hashimoto's thyroiditis.

As mentioned previously, approximately 90% of the patients with Hashimoto's thyroiditis have elevated levels of circulating anti-TPO (Pearce et al., 2003; Erdogan et al., 2009). The role of anti-TPO and other antithyroid antibodies in the pathogenesis of AITD is uncertain (Stassi & De Maria, 2002; Ai et al., 2003; McLachlan & Rapoport, 2004). However, if an autoimmune connection between Hashimoto's thyroiditis and OLP actually exists, it might be reasonable to assume that patients with OLP have high serum concentrations of anti-TPO and/or other antithyroid antibodies. Two studies conducted in Taiwan did indeed find significantly higher titres of anti-TPO and anti-Tg in a group of patients with OLP in whom other autoimmune diseases were excluded (Chang et al., 2009; Lin et al., 2011). Chang et al. (2009) reported that 24.4% of the patients with OLP (otherwise healthy; no systemic medication) (n=320) had elevated levels of anti-TPO compared to 1.9% of the healthy controls ($p<0.0001$). The same study reported elevated levels of anti-Tg in 21.3% of the patients with OLP compared to 1.9% of the healthy controls ($p=0.002$). When the patients were clustered according to the clinical type of OLP, an even higher prevalence of elevated levels of anti-TPO (33.6%) and anti-Tg (31.3%) was found in patients with erosive lesions ($p<0.0001$). Lin et al. (2011) showed similar results, although no difference was found between erosive OLP and the entire OLP group. Moreover, it was shown that levamisole treatment reduced or completely abolished anti-TPO and anti-Tg levels in anti-TPO/anti-Tg-positive OLP patients. This treatment also alleviated symptoms and reduced the size and severity of ulceration in existing OLP lesions. These results support the idea that some autoimmune mechanism involved in Hashimoto's thyroiditis may also play an important pathogenic role in a subgroup of patients with OLP.

In order to further investigate the potential common autoimmune mechanism between OLP and Hashimoto's thyroiditis, we aimed, in *study IV*, to analyse serum levels of antithyroid antibodies and thyroid hormones in patients with OLP. No association was found between antithyroid antibodies and OLP. Only 10.0% of the patients in the OLP+/levothyroxine- group presented with elevated serum levels of anti-TPO and anti-Tg. Data from population-based studies show that approximately 11% and 10% of the general thyroid-disease-free population had elevated anti-TPO and anti-Tg, respectively (Hollowell et al., 2002). In the random population sample of *study IV*, 16.1% of the subjects had elevated levels of anti-TPO and corroborates the figures by Hollowell et al. (2002). Hence, patients with OLP without a previously diagnosed thyroid disease did not have elevated serum levels of anti-TPO and anti-Tg when compared to the general population.

Although we were not able to find any association between OLP and circulating antithyroid antibodies in *study IV*, their role in the pathogenesis of OLP in a subgroup of patients cannot be excluded. The cross-sectional design of *study IV* only provided a "snapshot" of the levels of antithyroid antibodies in patients with OLP at a specific time. A more comprehensive investigation of a potential aetiological mechanism in OLP involving antithyroid antibodies will therefore require the execution of large prospective studies were these antibodies are analysed at different time points and correlated with the activity of OLP lesions.

The analysis of thyroid hormones in *study IV* showed that more OLP patients without a previously diagnosed thyroid disease have high levels of TSH and low levels of FT_4 compared to the general population. This finding is indicative of a higher prevalence of hypothyroidism in patients with OLP and corroborates the results from *study III*.

Anti-TSHR antibodies have been shown to be involved in the pathogenesis of Grave's disease, as they are present in 80-100% of the patients (Michalek et al., 2009). However, in Hashimoto's thyroiditis, only 6% of the patients were seropositive for anti-TSHR (Ai et al., 2003). Expression of TSHR is not confined to the thyroid gland. The presence of biologically active TSHR has been confirmed in a variety of human and animal cells tissues, including the skin (Slominski et al., 2002; Bodo et al., 2009; Michalek et al., 2009; Cianfarani et al., 2010). Similar findings have also been reported for Tg (Cianfarani et al., 2010). No previous study has, until now, investigated the expression of thyroid proteins in OLP lesions. Although the number of patients included for this purpose was small, the results from the immunohistochemical analysis and qPCR in *study IV* showed that TSHR is highly expressed in the basal keratinocytes of OLP lesions compared to healthy oral mucosa. These results, and the fact that Grave's disease was also shown to be highly prevalent in patients with OLP (*study III*), suggest that some autoimmune mechanism involving the expression of TSHR may participate in the activation of cytotoxic T cells leading to the destruction of basal keratinocytes.

To conclude, the results presented in this thesis suggest that a subgroup of patients with OLP may have an etiological background in common with thyroid disease. The reason for the high prevalence of thyroid disease in patients with OLP remains to be determined, but it is likely that some mechanisms in AITD are involved in the pathogenesis of this group of patients suffering from OLP. One such promising factor, which will be evaluated in further research, is the high expression of TSHR in basal keratinocytes of OLP lesions.

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APPENDIX

I. **Robledo-Sierra J**, Mattsson U, Svedensten T, Jontell M. The morbidity of oral mucosal lesions in an adult Swedish population. *Medicina Oral Patología Oral Cirugía Bucal.* 2013 Sep 1;18(5):e766-72.

II. **Robledo-Sierra J**, Mattsson U, Jontell M. Use of systemic medication in patients with oral lichen planus – a possible association with hypothyroidism. *Oral Diseases.* 2013 Apr;19(3):313-19.

III. **Robledo-Sierra J**, Landin-Wilhelmsen K, Filipsson Nyström H, Mattsson U, Jontell M. Clinical characteristics of patients with concomitant oral lichen planus and thyroid disease. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology.* In press.

IV. **Robledo-Sierra J**, Landin-Wilhelmsen K, Filipsson Nyström H, Eggertsen R, Larsson L, Dafar A, Warfvinge G, Mattsson U, Jontell M. Levels of antithyroid antibodies and thyroid hormones in patients with oral lichen planus – a connection between two autoimmune diseases? *In manuscript.*