IMMUNOHISTOCHEMICAL STUDY OF S-100 POSITIVE LANGERHANS CELLS IN ORAL LICHEN PLANUS

SUB.LT SUPASAWAT SIRIPIPATTHANAKUL

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE (ORAL MEDICINE)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2004

ISBN 974-04-4666-3

COPYRIGHT OF MAHIDOL UNIVERSITY

Copyright by Mahidol University

IMMUNOHISTOCHEMICAL STUDY OF S-100 POSITIVE LANGERHANS CELLS IN ORAL LICHEN PLANUS

Supasawat Siripipatthanakul

Sub. Lt. Supasawat Siripipatthanakul
Candidate

Mullika Sirirat

Assoc. Prof. Mullika Sirirat,
D.D.S.,Grad.Dip in Clin.Sc.
(Periodontics), Dip.Thai Board of
Periodontology
Co-advisor

Waranun Buajeeb

Assoc. Prof. Waranun Buajeeb,
B.Sc.,D.D.S., M.Sc.(Oral Medicine)
Ph.D. (Oral Biology), Dip.Thai Board
of Oral Diagnostic Sciences
Major advisor

Jirawan Thumpipit

Assist. Prof. Jirawan Thumpipit
D.D.S., M.Sc.(Oral Pathology),
Dip.Thai Board of Oral Diagnostic
Sciences
Co-advisor

Jirapa Ptinyasingh

Assoc. Prof. Jirapa Ptinyasingh,
D.D.S., M.Sc.(Oral Pathology),
Dip.Thai Board of Oral Diagnostic
Sciences
Co-advisor

Rassmidara Hoonsawat

Assoc. Prof. Rassmidara Hoonsawat,
Ph.D.
Dean
Faculty of Graduate Studies

Mullika Sirirat

Assoc. Prof. Mullika Sirirat,
D.D.S., Grad Dip in Clin Sc.
(Periodontology), Dip.Thai Board of
Periodontology
Chair
Master of Science Programme in
Oral Medicine
Faculty of Dentistry

Copyright by Mahidol University

IMMUNOHISTOCHEMICAL STUDY OF S-100 POSITIVE LANGERHANS CELLS IN ORAL LICHEN PLANUS

was submitted to the Faculty of Graduate Studies, Mahidol University

For the degree of Master of Science (Oral Medicine)

on

9 April, 2004.

Supasawal Siripipatthanakul

Sub. Lt. Supasawal Siripipatthanakul
Candidate

Assoc. Prof. Woranut Weerapradist,
D.D.S., M.Sc. (Pathobiology),
Dip.Thai Board of
Oral Diagnostic Sciences
Member

Waranun Buajeeb

Assoc. Prof. Waranun Buajeeb,
B.Sc., D.D.S., M.Sc.(Oral Medicine)
Ph.D.(Oral Biology), Dip. Thai Board
of Oral Diagnostic Sciences
Chair

Jirawan Thumpipit

Assist. Prof. Jirawan Thumpipit,
D.D.S., M.Sc. (Oral Pathology)
Dip.Thai Board of Oral Diagnostic
Sciences
Member

Mullika Sirirat

Assoc. Prof. Mullika Sirirat,
D.D.S., Grad.Dip in Clin.Sc.
(Periodontics), Dip.Thai Board of
Periodontology
Member

Assoc. Prof. Rassmidara Hoonsawat,
Ph.D.
Dean
Faculty of Graduate Studies
Mahidol University

Assist. Prof. Jurai Nakaparksin,
D.D.S., M.S.(N.Y)
Dean
Faculty of Dentistry
Mahidol University

Copyright by Mahidol University

ACKNOWLEDGEMENT

I would like to express my sincere gratitude and my deepest appreciation to my major advisor, Assoc.Prof.Waranun Buajeeb for her excellent supervision, guidance, encouragement, and constructive critism throughout this research.

I am very grateful to my co-advisor, Assist.Prof.Jirawan Thammapipit, Assoc.Prof.Jirapa Boonyasingha for their advice and help in this work.

I would like to thank Assoc.Prof Mullika Sirirat, my co-advisor and Chirman of the M.Sc.course: Major in Oral Medicine for her advice in this work.

I am deeply like to thankful to Assoc.Prof.Woranut Werapradist, for his kind in providing suggestions for this research, and who was the external examiner of the thesis defense.

I would like to express my special thanks to all staffs in Department of Oral Pathology, Faculty of Dentistry, Mahidol University and in Division of Surgical Pathology, Ratchavithi Hospital for their valuable support and suggestion during the course of this study, especially Dr.Cheepsumon Suthipintawong and Miss. Supathip Tujinda, their support are much appreciated.

I am deeply appreciation to all the lecturers and staffs of the Oral Medicine Department for their kind advice and hospitality.

Finally, my greatest debt to family for their financial support, encourangement, understanding, entirely care and love.

Sub.Lt.Supasawat Siripipatthanakul

Copyright by Mahidol University

IMMUNOHISTOCHEMCICAL STUDY OF S-100 POSITIVE LANGERHANS CELLS IN ORAL LICHEN PLANUS

SUB.LT.SUPASAWAT SIRIPIPATTHANAKUL 4237121 DTOM/M

M.Sc.(ORAL MEDICINE)

THESIS ADVISORS : WARANUN BUAJEEB, Ph.D.(ORAL BIOLOGY), DIP.THAI BOARD OF ORAL DIAGNOSTIC SCIENCES., JIRAWAN THUMPIPIT, M.Sc.(ORAL PATHOLOGY), DIP.THAI BOARD OF ORAL DIAGNOSTIC SCIENCES., JIRAPA PUNYASINGH, M.Sc.(ORAL PATHOLOGY), DIP.THAI BOARD OF ORAL DIAGNOSTIC SCIENCES., MULLIKA SIRIRAT, DIP.THAI BOARD OF PERIODONTOLOGY.

ABSTRACT

Oral lichen planus (OLP) is a chronic inflammatory mucosal disease of unknown cause, however many studies have supported the immunopathogenesis of OLP. Because Langerhans cells (LCs) is an essential cell in the processing of antigen and promulgation of the immune response in skin and mucosa, they may be important in OLP. The purpose of this study was to determine number of S-100 positive LCs in OLP.

Polyclonal antibody reacting with LCs was used to characterize the expression of S-100 proteins in OLP compared with the adjacent tissues, fibroma and normal epithelium by using immunohistochemical technique. The specimens were fixed in formalin and embedded in paraffin. S-100 positive LCs should be considered as LCs when fulfilling 2 criterias : 1) the whole cell body being visible ; 2) with at least one dendritic process. Graticular outlining an area of 0.064 mm^2 was used at magnification of 400x to assist in counting cells in stained sections. The grid was positioned parallel to the epithelial-connective tissue interface. Counts from 3 adjacent high power fields in a single section were averaged. Differences in cell counts between OLP and unaffected adjacent tissues were statistically evaluated using Wilcoxon Matched-Pairs Signed-Ranks test. Whereas the differences in cell counts between OLP and fibroma ; OLP and normal epithelium, were statistically evaluated using Mann Whitney U-Wilcoxon Rank Sum W test.

The results demonstrated that the number of stained LCs in OLP were significantly much more prominent than in unaffected-adjacent tissues, fibroma and normal epithelium ($p<0.05$), and the number of stained LCs in unaffected adjacent tissues were significantly more than in normal epithelium ($p<0.05$). It suggested that significantly increased LCs might play a role in pathogenesis of OLP or represent an event secondary to the pathologic changes in the disease.

KEY WORD : ORAL LICHEN PLANUS / S-100 PROTEIN / LANGERHANS CELLS / IMMUNOHITOCHEMICAL STUDY

76 pp. ISBN 974-04-4666-3

Copyright by Mahidol University

การศึกษาทางอิมมูโนฮิสโตเคมิสตรีของลังเกอร์ฮาร์นเซลล์ที่ให้ผลบวกต่อ เอส-100 โปรตีนในรอยโรคไลเคนพลานัสในช่องปาก (IMMUNOHISTOCHEMICAL STUDY OF S-100 POSITITVE LANGERHANS CELLS IN ORAL LICHEN PLANUS)

ร.ต.ศุภสวัสดิ์ ศิริพิพัฒนกุล ร.น. 4237121 DTOM/M

วท.ม. (เวชศาสตร์ช่องปาก)

คณะกรรมการควบคุมวิทยานิพนธ์ : วรานันท์ บัวจีบ, ปร.ด., อ.ท. (วิทยาการวินิจฉัยโรคช่องปาก) ., จิระวรรณ ธรรมพิพิธ, อ.ท.(วิทยาการวินิจฉัยโรคช่องปาก) ., จีราภา บุณยสิงห์, อ.ท. (วิทยาการวินิจฉัยโรค-ช่องปาก) ., มัลลิกา ศิริรัตน์ อ.ท. (ปริทันตวิทยา)

บทคัดย่อ

รอยโรคไลเคนพลานัสในช่องปากเป็นรอยโรคที่เกิดแบบเรื้อรัง สาเหตุยังไม่ทราบแน่ชัด เชื่อว่าอาจเกี่ยวข้องกับระบบภูมิต้านทาน โดยที่ลังเกอร์ฮาร์นเซลล์ เป็นเซลล์ชนิดหนึ่งที่มีบทบาทในการตอบสนองต่อระบบดังกล่าวของผิวหนังและเนื้อเยื่อในช่องปาก การศึกษาครั้งนี้จึงใช้ เอส-100 โปรตีนสำหรับการย้อมลังเกอร์ฮาร์นเซลล์ โดยมีวัตถุประสงค์เพื่อตรวจหาปริมาณของลังเกอร์ฮาร์นเซลล์ในรอยโรคไลเคนพลานัสในช่องปาก

ทำการศึกษาโดยการย้อมลังเกอร์ฮาร์นเซลล์ ด้วย เอส-100 โปรตีนโพลีโคลนัลแอนติบอดี้ โดยขบวนการทางอิมมูโนฮิสโตเคมิสตรี โดยใช้เนื้อเยื่อที่ถูกแช่ในฟอร์มาลีน และฝังในพาราฟฟินแล้ว เปรียบเทียบปริมาณของเซลล์ในบริเวณที่เป็นรอยโรคไลเคนพลานัสในช่องปากกับบริเวณใกล้เคียงกับรอยโรคในสไลด์เดียวกัน และทำการเปรียบเทียบปริมาณของเซลล์บริเวณรอยโรคกับบริเวณไฟโบรมาและเนื้อเยื่อปกติในช่องปาก ลังเกอร์-ฮาร์นเซลล์ที่ให้ผลบวกต่อ เอส-100 จะต้อง 1) เห็นตัวเซลล์ชัดเจน 2) มีแขนงอย่างน้อย 1 แขนง ทำการนับจำนวนเซลล์โดยใช้ตารางนับขนาดพื้นที่ 0.064 ตารางมิลลิเมตร กำลังขยาย 400 เท่า ศึกษา 3 บริเวณและใช้ค่าเฉลี่ย เปรียบเทียบความแตกต่างของจำนวนเซลล์โดยใช้สถิติ สำหรับรอยโรคกับบริเวณใกล้เคียงรอยโรค ใช้ Wilcoxon Matched-Pairs Signed-Rank test และระหว่างรอยโรคกับไฟโบรมา และรอยโรคกับเนื้อเยื่อปกติใช้ Mann Whitney U-Wilcoxon Rank Sum W test.

ผลการศึกษาพบว่า จำนวนลังเกอร์ฮาร์นเซล์ที่ให้ผลบวกต่อ เอส-100 โปรตีน ในรอยโรคไลเคนพลานัสในช่องปากมากกว่าในบริเวณใกล้เคียงรอยโรคในสไลด์เดียวกัน และมากกว่าที่พบในบริเวณไฟโบรมา และเนื้อเยื่อปกติอย่างมีนัยสำคัญ ($p<0.05$) นอกจากนั้นจำนวนลังเกอร์ฮาร์นเซลล์ที่ให้ผลบวกต่อ เอส-100 โปรตีนในบริเวณที่ใกล้เคียงรอยโรคยังมีจำนวนมากกว่าที่พบในเนื้อเยื่อปกติอย่างมีนัยสำคัญ ($p<0.05$) จากข้อมูลบ่งชี้ว่าลังเกอร์ฮาร์นเซลล์ที่ให้ผลบวกต่อ เอส-100 โปรตีนอาจมีส่วนเกี่ยวข้องกับการเกิดรอยโรคไลเคนพลานัสในช่องปาก แต่ทั้งนี้ การเจอเซลล์ดังกล่าวอาจเป็นผลตามมาหลังจากการเกิดรอยโรคก็เป็นไปได้

76 หน้า ISBN 974-04-4666-3

Copyright by Mahidol University

CONTENTS

Page

ACKNOWLEDGEMENT iii
ABSTRACT iv
LIST OF TABLES viii
LIST OF FIGURES ix
LIST OF ABBREVIATIONS xii
CHAPTER

I INTRODUCTION 1
II LITERATURE REVIEW 4
III MATERIALS AND METHODS 34
IV RESULTS 47
V DISCUSSION 54
VI CONCLUSION 56
REFERENCES 57
BIOGRAPHY 64

Copyright by Mahidol University

LIST OF TABLES

Table **Page**

1 The number of S-100 positive LCs in OLP lesions and adjacent tissue ……..... 48
2 The number of S-100 positive LCs in fibroma ..... ……... 49
3 The number of S-100 positive LCs in normal epithelium…… 50
4 Statistically analysis of S-100 positive LCs…….. 50

Copyright by Mahidol University

LIST OF FIGURES

Fig **Page**

1 Reticular type of OLP 5
2 Papular type of OLP 6
3 Plaque type of OLP 6
4 Atrophic type of OLP 7
5 Erosive type of OLP 7
6 Bullous type of OLP 8
7 Histopathology of OLP showing keratosis and inflammatory cells infiltrate 9
8 Histopathology of OLP showing interface change 9
9 Immunofluorescence shows basement membrane deposition of fibrinogen 11
10 Diagram shows complement deficiencies and their association with auto-immunity 12
11 Distribution of mast cells in a lesion of OLP 13
12 HLA-DR positive cells and keratinocyte in OLP 16
13 Distribution of mast cells in a lesion of OLP 17
14 CDw29 expression on basal and parabasal keratinocytes and dendritic cells in normal mucosa 18
15 Immunohistochemical staining of T-cell marker CD4 reveals infiltration into the submucosa 19
16 Immunofluorescence staining of Helper T cells double labeled for CD3 20
17 Immunofluorescence staining of Helper T cells double labeled for CD4 20
18 Immunofluorescence staining of Helper T cells double labeled for CD45RA 21
19 ICAM-1, VLA-1 expression in OLP 22
20 Immunoperoxidase staining of collagen along BMZ 22

Copyright by Mahidol University

LIST OF FIGURES (Cont.)

Fig **Page**

21 Immunoperoxidase staining of collagen along BMZ ……23
22 CK 1/10 immunostaining of OLP ……..24
23 Diagram shows pathogenetic mechanism in LP ……....26
24 Diagram shows modulation of the T helper immune response ……....27
25 HSP60 immunostaining of OLP …….....28
26 HSP70 immunostaining of OLP …….....28
27 Positive S-100 protein staining in OLP ……...31
28 Odontogenic myxoma cells showing strong immunoreactivity with anti-vimentin antibody immunoperoxidase ……32
29 The specimens were cut by using Microtome ……...39
30 After cutting, the sections were warmed in water bath ……...39
31 Automatic pipettes were used for dilution the antibodies ……..40
32 Antigen retrieval treatment by Microwave ……40
33 Hot air oven for drying the sections ……..41
34 Ultrasonic cleaner for cleaning the instruments ……...41.
35 Red rotor for rotoring the sections in PBS ……....42
36 Antibodies and the solutions were kept in the refrigerature ……..42
37 Preparating the primary, secondary and tertiary antibodies ……..43
38 The antibody was dropped on the sections ……...44
39 Put the sections in PBS and turned on red rotor ……...44
40 After rinsing in PBS, washed the sections with running water ……....45
41 The sections were developed in DAB ……..45
42 The sections were stained with Mayer haematoxylin …….....45
43 Interpretation the sections by using light microscope ……...46
44 Numerous S-100 postive LCs in OLP ……...51
45 S-100 positive LCs in adjacent tissue ……...51
46 Numerous S-100 postive LCs in another sample of OLP ……....52

Copyright by Mahidol University

LIST OF FIGURES (Cont.)

Fig **Page**

47 S-100 positive LCs in adjacent tissue in the same case ……..52
48 Few S-100 postive LCs in fibroma ……...53
49 A few S-100 positive LCs in normal epithelium ……..43

Copyright by Mahidol University

LIST OF ABBREVIATIONS

OLP	=	oral lichen planus
Ig	=	immunoglobulin
LCs	=	Langerhans cells
C	=	complement
EBZ	=	epithelial basement membrane zone
BMZ	=	basement membrane zone
HLA	=	human leukocyte antigen
Ia-like antigen	=	immune-associated like antigen
AET	=	2-aminoethylisothiouronium bromide
T cell	=	Thymus dependent lymphocyte
ICAM-1	=	intercellular adhesion molecule-1
LFA-1	=	lymphocyte function-associated antigen
APC	=	antigen presenting cell
IL	=	interleukin
IFN	=	interferon
HSP	=	heat shock protein
H_20_2	=	hydrogen peroxide
PBS	=	phosphate buffer saline
NSS	=	normal swine serum
SWAR	=	Swine anti-rabbit
PAP	=	peroxidase antiperoxidase complex
DAB	=	3,3 Diaminobenzidine tetrahydrochloride
Tris	=	trisma
SRP	=	streptavidin peroxidase
^0C	=	degree Celsius

Copyright by Mahidol University

LIST OF ABBREVIATIONS (Cont.)

mm^2	=	square millimeter
g	=	gram
N	=	normal
NHS	=	normal horse serum
VLA	=	ß1-integrin subfamily
α	=	alpha
ß	=	beta
NaCl	=	sodium chloride
NaH_2PO_4	=	sodium hydrogen phosphate
NaOH	=	sodium hydroxide
HCl	=	hydrogen chloride
CK	=	cytokeratin
RAS	=	recurrent apthous stomatitis
HGS	=	herpetic gingivostomatitis
SCC	=	squamous cell carcinorma
GM-CSF	=	granulocyte-macrophage colony stimulating factor
NSOU	=	non-specific oral ulceration

Copyright by Mahidol University

CHAPTER I
INTRODUCTION

Background and rationale

Oral lichen planus (OLP) is a chronic inflammatory mucosal disease first described in 1869 by Wilson. (1) These lesions may be active or in remission at any given time, the severity and discomfort may vary . The average patient with OLP is of middle age, with affected in slightly higher female population. (2) OLP has multiple-site involvement with the buccal mucosa being the most prevalent. (3) Andreasen classified OLP into six types according to their clinical appearance : reticular, papular, plaque, atrophic, ulcerative and bullous. The reticular type of lesion is the most common type and whereas atrophic and ulcerative types were accompanied by severe pain. (4) There has been considerable controversy as to whether OLP has a premalignant potential. An association between OLP and malignant development has been reported. (5-7) In the last decade, p53 protein, an indicator for potential malignant development in benign lesions of the oral mucosa, was studied in OLP because of this hypothesis. (8-16) Some studies suggested that p53 overexpression in OLP may be a physiological response to the hyper-proliferative state, so that the presence or absence of p53 staining could not be used to predict the outcome of potentially malignant oral mucosal lesions. This may usefully serve to protect against mutagenesis, and so be a factor in the low incidence of carcinoma associated with OLP. (8,10)

In spite of extensive researches , the etiology of OLP is still unknown. Psychologic factors have been mentioned as possible factors related to the development of OLP. One report showed the patient with OLP likewise exhibited greater depression than controls.(17) But others showed no statistically significant differences. (18 -19) Both humoral and cell mediated immunity are thought to be important in the pathogenesis of OLP. Significantly increased levels

Copyright by Mahidol University

of serum Immunoglobulin (Ig)G ($p<0.05$) and a significant reduction of serum IgA concentration ($p<0.05$) in the experiment group as compared with normal controls were observed. (20) Immunofluorescence studies have shown that dermal LP and OLP showed deposits of Ig and complement at the basement membrane zone (21-24). Many studies supported OLP is the lesion that has the nature of the subepithelial infiltratrate resembles that described for delayed type hypersensitivity reactions in that the predominant cell type was the T-lymphocyte (25-34). Immunohistochemical technique was used to identified keratinocytes, macrophage, Langerhans cells (LCs) and mast cells because of a unifying hypothesis which attempted to integrate cellular signals in the local immune respose in OLP (35-41). Some studies supported that expressions of cytokine , integrins , cytokeratin, adhesion molecules and heat shock proteins involving in pathogenesis of OLP (42-50). As the pathogenesis of OLP was related to immune response, topical and systemic corticosteroids have been used to treat OLP because of immunosuppressive and anti-inflammatory properties. (51-53). The clinical evaluation in treatment of OLP with topical vitamin A acid showed that lesion elimination via topical vitamin A was often very rapid and very rapid recurrence after the medication was discontinued.(54) Because LCs are essential cells in the processing of antigen and promulgation of the immune response in skin and mucosa. S-100 proteins were used to demonstrate LCs. The number of positive LCs were determinded in OLP specimens in the study. The findings might reveal the role of LCs in the pathogenesis of OLP.

Objective

To determine the number of S-100 positive LCs in OLP lesions.

Research Hypothesis

The number of S-100 positive LCs would be increased in OLP lesions than unaffected adjacent tissue, fibroma lesions and normal epithelium.

Copyright by Mahidol University

Scope of the study

This study is an experimental research by using immunohistochemical technique. Polyclonal antibody reacting with LCs were used to characterized the expression of S-100 protein. The samples included paraffin embedded tissues of 42 OLP specimens, 12 fibroma specimens, 1 normal epithelium from normal oral mucosa and 4 normal epithelium near fibroma lesions. The tissues were collected from the Department of Oral Pathology, Faculty of Dentistry, Mahidol University. And the procedure was setted and processed in the Division of Surgical Pathology, Ratchavithi Hospital.

Significance of the study

This study might reveal the role of LCs in the pathogenesis of OLP.

Copyright by Mahidol University

CHAPTER II
LITERATURE REVIEW

The literature review is presented in sequence as follows :

1. Clinical forms and classification of OLP.
2. Histopathology of OLP.
3. Immunological study of OLP.
   - 3.1 Humoral immunity.
   - 3.2 Complement and fibrinogen.
   - 3.3 Cell mediated immunity.
     - 3.3.1 Mast cell.
     - 3.3.2 Granular cell.
     - 3.3.3 LCs and keratinocyte.
     - 3.3.4 Thymus-dependent lymphocyte (T cell).
   - 3.4 Miscellanous.
     - 3.4.1 Adhesion molecules.
     - 3.4.2 Extracellular matrix proteins.
     - 3.4.3 Chemoattractants.
     - 3.4.4 Heat shock proteins.
4. S-100 protein.
   - 4.1 Biology of S-100 protein.
   - 4.2 S-100 protein expression in normal epithelium and oral lesions.
   - 4.3 Immunohistochemical technique for S-100 staining.

Copyright by Mahidol University

1. Clinical forms and classification of OLP :

Andreasen,(55) classified OLP into six types according to their clinical appearance.

1. Reticular is the most common type. It consists of slightly raised fine whitish lines in and interlocking lace-like pattern or Wickham's striae. (Fig. 1)

2. Papular presents as small white raised papules.(Fig. 2)

3. Plaque is white plaque closely resembling leukoplakia.(Fig.3)

4. Atrophic presents red atrophic areas surrounded by reticular elements. (Fig. 4)

5. Erosive presents as the typical ulcerative pattern. (Fig. 5)

6. Bullous presents as bulla formation. (Fig. 6)

Fig 1. Reticular type OLP. (56)

Copyright by Mahidol University

Fig 2. Papular type of OLP. (56)

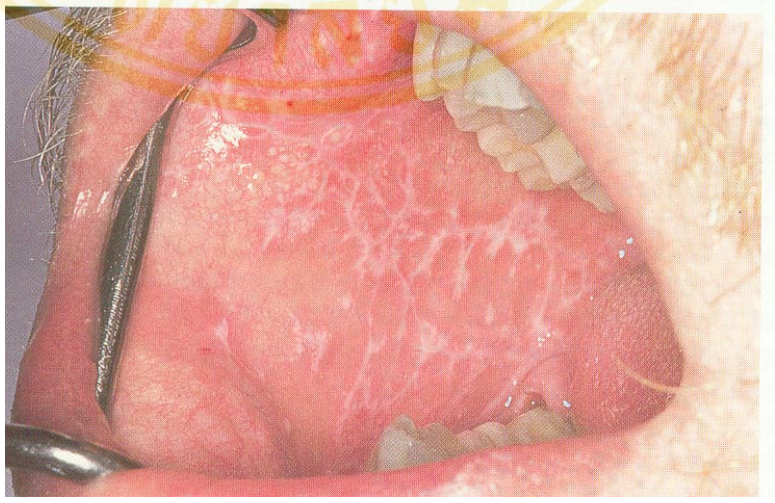

Fig 3. Plaque type of OLP. (56)

Copyright by Mahidol University

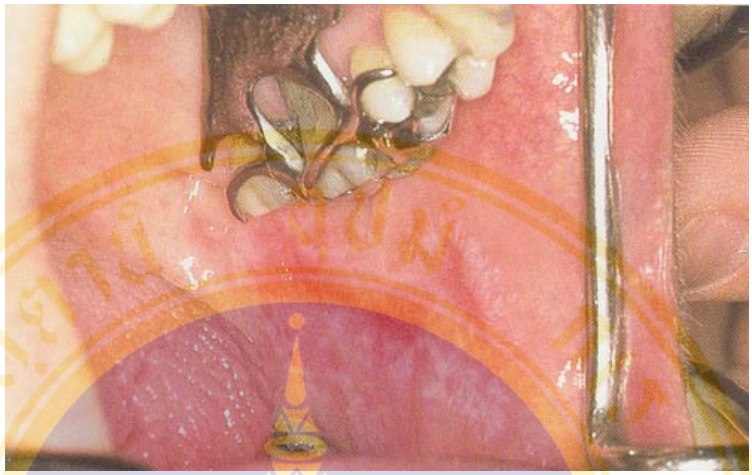

Fig 4. Atrophic type of OLP. (56)

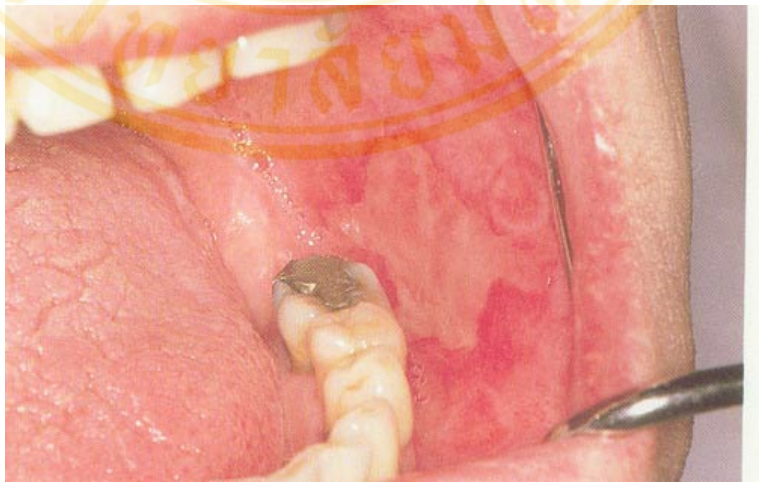

Fig 5. Erosive type of OLP. (56)

Copyright by Mahidol University

Fig 6. Bullous type of OLP. (56)

2. Histopathology of OLP

Microscopically, the white raised lesions is composed of the main feature : (1, 57-58) (Fig 7,8)

1. Hyperorthokeratosis or hyperparakeratosis.
2. Acanthosis.
3. Thickening of the granular layer.
4. Basal cell degeneration.
5. “ Saw tooth “ configuration of the rete pegs.
6. Band-like dense inflammatory cellular infiltrate in the upper lamina propria.

Copyright by Mahidol University

Fig 7 : Histopathology of OLP showing keratosis and inflammatory cells infiltrate. (59)

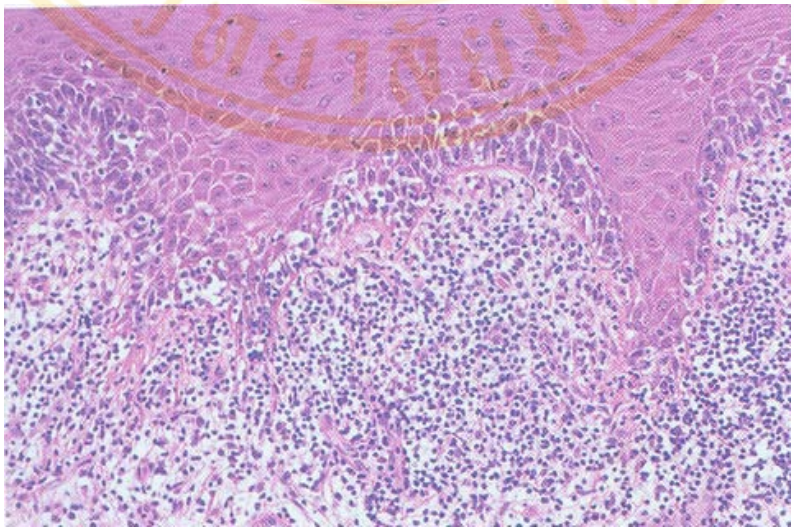

Fig 8 : Histopathology of OLP showing interface change. (59)

Copyright by Mahidol University

3. Immunological study of OLP

3.1 Humoral immunity

Few studies suggested that humoral immunity is related to the pathogenesis of OLP. The deposition of Immunoglobulin (Ig)G , IgM and IgA in the epidermal basement membrane zone (EBZ) has been studied. It was studied in 40 samples of dermal LP by using immunofluorescence technique. IgM was found in all lesions, IgA was never detected, whereas linear deposition of IgG was encountered in small areas of the EBZ. (21) In the same year , Lai *et.al*., (24) had studied about Ig-positive cells (IgM, IgA, IgG, IgD, IgE) in OLP by the same technique. It was found that in all 2 OLP specimens showed IgG-positive cells but IgA-positve cells was found in only 1 OLP specimens and no showed of other types of Ig-positive cells. However, results were found by Schiodt *et al*, (23) only 4% of OLP specimens found IgM at the basement membrane zone (BMZ) but no deposition of IgG and IgA .

Serum Ig in OLP patients has been studied by single radial immunodiffusion. It was found that a significantly increased levels of serum IgG ($p<0.05$) and a significant reduction of serum IgA concentration ($p<0.05$) in the experimental group as compared with normal controls were observed , but mean serum IgM levels was similar in patients and controls. It suggested that patient with OLP may have a generalized immunologic disorder in which humoral immunity is disturbed. Whether humoral immunity is of etiologic significance, contributes to the disease process, or , finally, represent an event secondary to the pathologic changes seen in the disease remains to be determined. (20)

3.2 Complement, Fibrinogen

Complement deposititon in dermal LP by immunofluorescence technique has been studied . It was found that C3 and C4 stained in all 2 samples of OLP specimens. It suggested that classical pathway and several other pathways exist by which complement and fibrinogen may be activated in LP lesions. These include enzymes generated during the interactions of coagulation, fibrinolysis, kinin systems and other proteolytic enzyemes. (21) Schiodt *et al.*, (23) also studied about the deposition of complement (C3) and fibrinogen in OLP by immunofluorescence technique. The C3

Copyright by Mahidol University

was found in 33 % , and the fibrinogen was found in 91 % of OLP. However, Lai *et al.*, (24) had studied about the deposition of complement in dermal LP but the complement-postitive cells could not be demonstated with the polyvalent antiserum used.

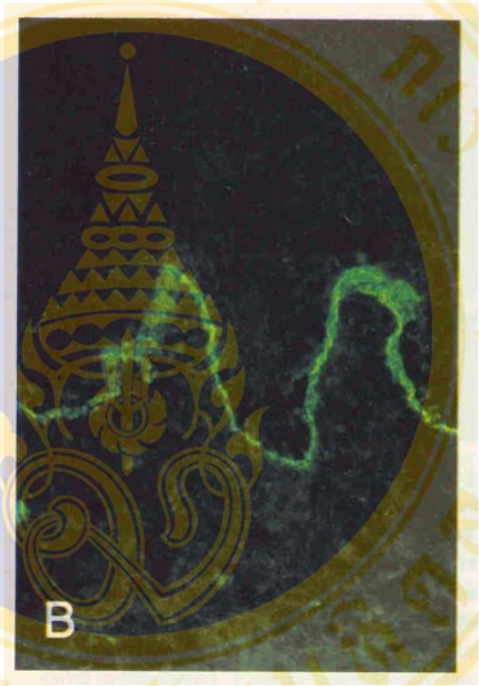

Fig 9. Immunofluorescence shows BMZ deposition of fibrinogen (60)

Sleasman, (61) reviewed about the complement deficiencies and their association with infection and autoimmunity. The classical pathway is activated by binding of C1 to immunoglobulin Fc receptor and alterative pathway, initiated by microbial proteins binding to C3 and the membrane attack complex ; and the final common pathway ; consisting of C5 through C9, leading to cytosis. (Fig 10)

Copyright by Mahidol University

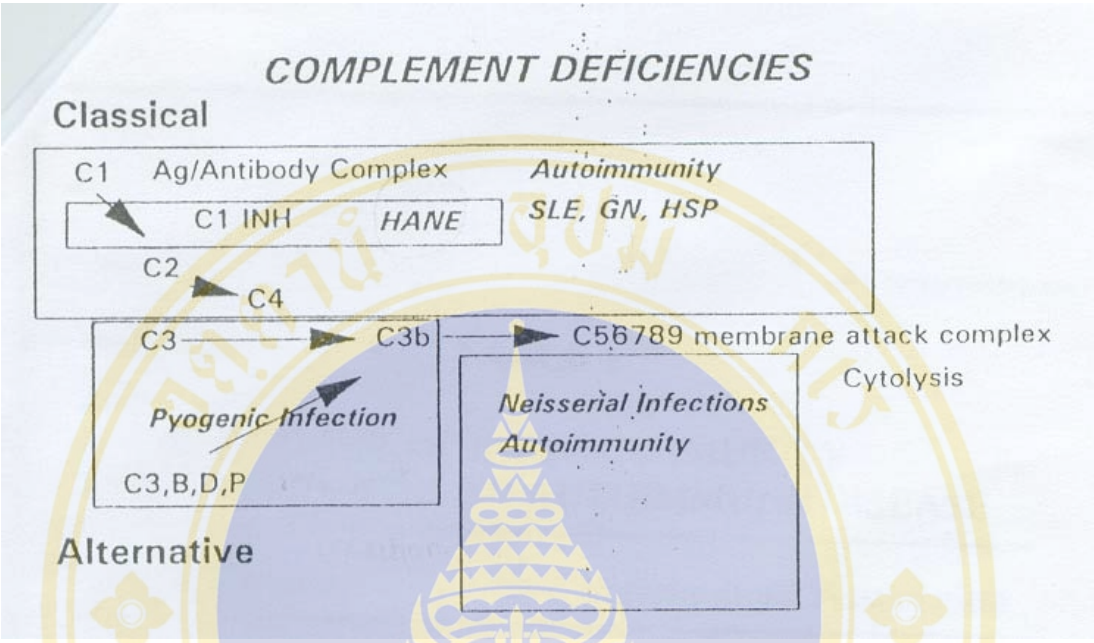

Fig10. Diagram shows complement deficiencies and their association with autoimmunity (61)

3.3 Cell mediated immunity

3.3.1 Mast cell

Mast cells distribution in bullous OLP by using EACNAS-GBC (Epsilon amino caproate ester of napthol AS-Garnet Borden C dye) technique has been studied. It was found that significantly more mast cells were found at BMZ in OLP than in normal gingiva. (37) Jontell *et al*., (38) also studied about the mast cells distribution in OLP. In comparison with the normal oral mucosa and increased number of mast cells was found below the subepithelial infiltrate. This difference was statistically highly significant ($p<0.01$). They suggested that mast cells participate in the recruitment of macrophage and lymphocytes to the subepithelial infiltrate.

Copyright by Mahidol University

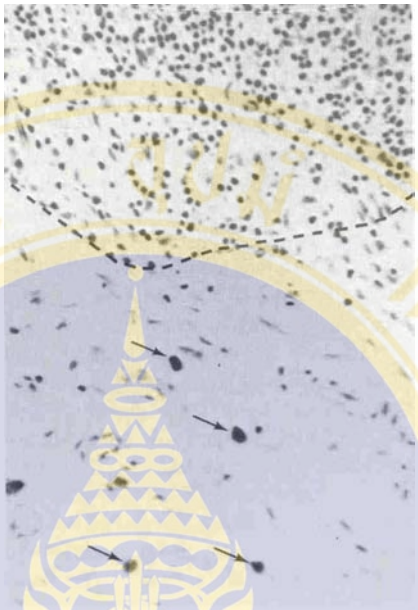

Fig 11. Distribution of mast cells (arrow) in the reticular layer of lamina propria in a lesion of OLP. The infiltrated-noninfiltrated interface of the reticular layer is indicated by a dashed line (x20) (38)

3.3.2 Granular cell

Cytological and cytochemical investigations on granular cells in OLP by staining with the labeled steptavidin biotin technique for lysozyme has been studied. It was indicated that the granular cells might be macrophages because of their lysozyme immunoreactivity. (39)

3.3.3 LCs and keratinocyte

The distribution, density and activation of LCs were established in biopsies of normal human ; buccal mucosa, hard palate, lateral border and dorsum of the tongue, floor of the mouth and lip taken from sudden death post mortem. LCs were identified

Copyright by Mahidol University

with monoclonal antibodies against CD1, HLA-DR, HLA-DQ and HLA-DP by using immunoalkaline phosphatase technique. It was found that in floor of the mouth, lip, lateral border of the tongue; the cells were found along with the epithelium. In buccal mucosa, a tendency to cluster around the connective tissue papillae was also noted. In hard palate LCs were found parallel to the surface in the mid zone of the epithelium. Dorsum of tongue had the highest density of LCs per millimeter epithelial surface length which significantly greater ($p<0.05$) than buccal which in turn had significantly more cells ($p<0.05$) than lip. The lowest density of LCs was found in lateral border of tongue, hard palate and floor of the mouth. It indicated that although there were regional difference in LCs distribution and density, normal LCs may be capable of acting as fully functional and efficient antigen presenting cells. (62)

Immunohistochemical study of HLA-DR positive LCs in OLP has been studied. The tissue were fixed in formalin and embedded in paraffin. HLA-DR positive cells were located in the middle to lower epithelium and in the submucosa in relative proportion to the severity of disease.(63) The expression of HLA-DR on keratinocytes in OLP by immunofluorescence technique has been determined. It was found that the expression of HLA-DR by keratinocytes does not appear to be a specific marker for OLP. This was demonstrated by the reactivity of the epithelium in a variety of lesions of the oral mucosa, then keratinocyte reactivity would not expected to occur in only OLP. (41) However, Ishii (64) had also studied about immunohistochemical demonstration of HLA-DR , HLA-DQ positive monocyte/macrophage and LCs. It suggested that monocytes/macrophages and LCs played a role in antigen presentation, and also that keratinocytes may progress a similar function.

LCs expressing HLA-DR, and T6 antigens in OLP has been studied. Acetone-fixed frozen sections were studied using indirect immunoperoxidase technique and monoclonal antibodies to T6 and HLA-DR antigens. It was found that significantly more T6 positive and HLA-DR positive LCs were present in OLP than normal controls. (65) But the result was different from that of Chou *et al.* (36), studied in the same year by the same technique. It was found that comparing with controls, OLP contained significantly more HLA-DQ-positive LC ($p=0.04$) and fewer HLA-DR-positive LC ($p=0.05$), but there was no such difference in T6-positive LC. In OLP

Copyright by Mahidol University

specimens, there were significantly more LCs expressing HLA-DQ and T6 than HLA-DR ($p=0.0001$ and 0.02 respectively). No such differences were found in normal mucosa. (36) Farthing *et al*., (66) also studied about CD1,HLA-DR, HLA-DP and HLA-DQ positive LCs distribution in OLP compared with normal oral mucosa by the immunoalkaline phosphatase technique. It was found that in normal mucosa , there were equal numbers of CD1 and HLA-DR positive cells but significantly fewer cells were positive for HLA-DP ($p<0.01$) and HLA-DQ ($p<0.05$). In OLP, the cells appeared more dendritic and equal numbers of CD1, HLA-DR, HLA-DP and HLA-DQ ($p<0.05$) positive cells than in normal mucosa.

The distribution of CD1 and CD4-positive LCs in OLP related to keratinocyte HLA-DR has been studied. The number of CD1- and CD4-positive LCs were compared in area of keratinocyte HLA-DR and areas showing no expression in OLP and with normal mucosa. Cells were identified using an immunoalkaline phosphatase technique. In OLP, an increase both in number of LCs and in the number expressing CD4 were found in areas of keratinocyte HLA-DR expression compared with HLA-DR negative areas and with normal oral mucosa. There was no difference in the number of LCs or their expression of CD4 between HLA-DR negative areas in OLP and normal oral mucosa. These results show that the distribution of LCs entry may be enhanced in these areas. (67) McCartan *et al*., (68) also studied expression of T6 and HLA-DR by LCs in oral lichenoid drug eruptions and idiopathic OLP. The immunoperoxidase technique was used to demonstrate binding of T6 and HLA-DR antibodies to identify dendritic intraepithelial cells as LCs and activated LCs, respectively. In lichenoid drug eruptions, the number of HLA-DR positive LCs was significantly lower than the number of T6 positive LCs ($p<0.05$), whereas in idiopathic OLP the number of T6 positive LCs and HLA-DR positive LCs did not differ significantly ($p=0.2$). The results provide evidence for differences in the routes of antigen presentation in lichenoid drug eruptions and idiopathic OLP.

Copyright by Mahidol University

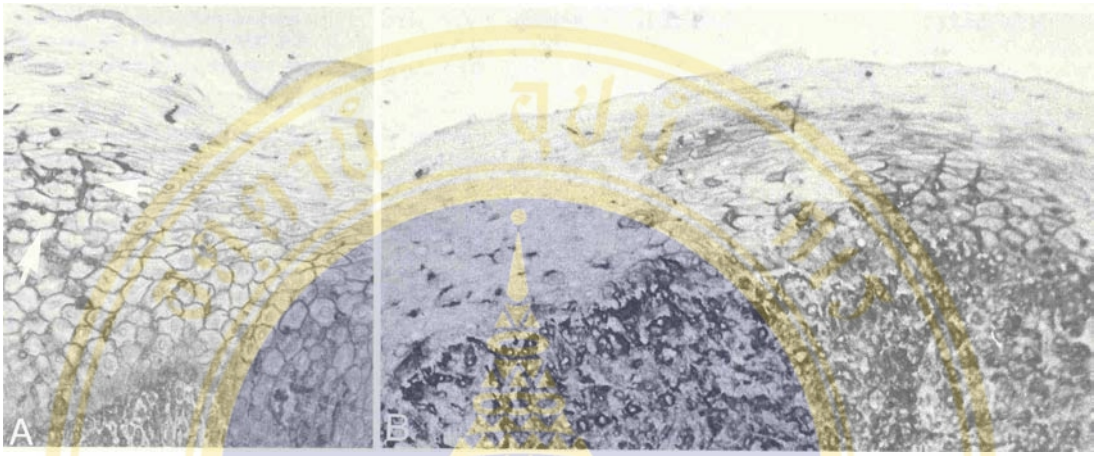

Fig 12. HLA-DR positive LCs and keratinocytes in OLP (67)

Distribution of immune-associated response (Ia)-like antigen positive LCs in OLP by using immunohistochemical technique has been studied. It was found that LCs more frequently expressed Ia-like antigens in lesions of OLP than in non-diseased oral mucosa. It was further demonstrated that cells in the sub-epithelial inflammatory infiltrate expressed Ia-like antigens of the same identity as Ia-like antigens found on LCs. In addition, treatment of OLP with topically applied tretinoin resulted in a decrease of the Ia-like antigen expression. (35)

Copyright by Mahidol University

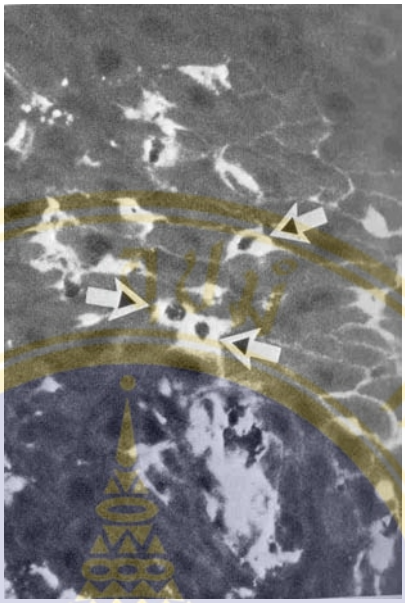

Fig 13. Distribution of Ia-like antigen in epithelium of OLP. Suprabasal cells are connected by dendritic network. The nuclei of LCs are visible as a negative-stained area in centre of the cell (arrow x340).(35)

3.3.4 T cell

T cell and B cell identification of the submucosal infiltrate in dermal LP has been studied. In frozen tissue sections , sheep erythrocytes treated with 2-aminoethyliso thiouronium bromide (AET) was used to form immunologic rosettes with the lymphocytes in the infiltrate, designating them as T cells. Rosette assays with reagent erythrocytes, IgGEA, IgMEA, IgMEAC, and E resulted in nonadherence, indicating a lack of B cells and macrophages, and indirectly implicating them as T cells. Scanning electron microscopy of the cellular infiltrate, in situ, showed that the cells had smooth, non villous surfaces. These observations were consistent with a T cell origin. The humoral immune system (B cells) apparently plays only a minor role in the development of LP. (25) Bhan *et al*., (40) also identified T cell subsets distributions in dermal LP. It was found that the majority of infiltrating cells stained with anti-T4 antibodies, which react with helper/inducer cells and the minority of cells stained with anti-T8 antibodies, which reacts with cytotoxic/suppressor cells. This result was supported by the study of Matthews *et al.*, (27). Leu 2a antibody

Copyright by Mahidol University

reacted with suppressor/cytotoxic T cell and Leu 3a antibody reacted with helper/suppressor T cells were used to study. The cell counting demonstrated mean Leu 3a : Leu 2a positive cell ratios of 2.1 : 1 and 2.5 : 1 in the epithelium and lamina propria respectively. (34) Leu 2a, Leu 3a, Leu 4 (Leu 4 reacted with pan T cell) and IL-2 receptor (IL-2 receptor reacted with activated T cell) antibodies were used in the study of T cell distribution by using immunohistochemical technique. It was found that abundant lymphocyte were present between the keratinocytes and in the lamina propia. These cells were mostly Leu 4-positive and other were Leu 3a- or Leu 2a-positive cells and some of them expressed IL-2 receptors, showing a significant difference from the control group.

CD45R and CDw29 positive cells distributions in OLP by immunohistochemical study has been studied. CDw29 was expressed by LCs and lymphocytic cells whereas keratinocyte reactivity varied from negative through to full thickness staining. Expression of CD45R was confined to intraepithelial cells with either lymphocytic or dendritic morphology. A relatively constant ratio of CD1a positive to CD45R positive cells (2:1) was seen. These result demonstrated the existence of intraepithelial cells expressing antigens which are functionally important in T cell responses and which may provide local immunoregulatory influences. (28)

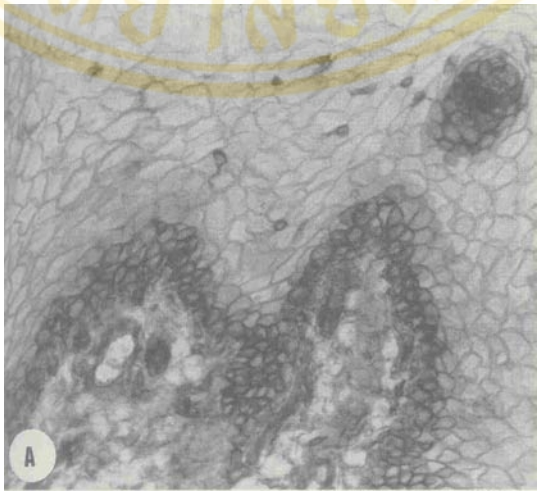

Fig 14. CDw29 expression on basal and parabasal keratinocytes and dendritic cells in normal bucall mucosa (28).

Copyright by Mahidol University

Distribution of lymphocyte subsets quantitatively using OLP specimens compared to the oral keratoses by the same technique has been studied . The results showed T helper: T suppressor/cytotoxic cell ratio was the same (1:2) for both OLP and leukoplakia. These data indicated that T cell subset analysis is of no value in distinguishing OLP from other oral keratoses. (33)

Peripheral T cell distribution in OLP patients by double color flow cytometry method has been studied. It was found that CD8CD11b (suppressor T) and CD3HLA-DR positive cell populations increased significantly in OLP when compared with controls, and CD4/CD8 cell ratio decreased in OLP. These suggested that OLP patients lymphocyte and neutrophil functions are impaired, and that cellular immunosuppression is a pathologic characteristic of OLP. (31) Walton *et al*., (69) also studied about peripheral T cell and LCs subpopulations distribution in OLP patients by using flow cytometry and double immunofluorescence technique. The circulating 'memory' subset (CD45RO positive) of T-helper cells (CD4 positive) were increased from 49.1% in controls to 65.7 % in patients (p=0.005), while the 'naive' subset (CD45RA positive), which was absent from control epithelium, compromised 24% of helper cells in OLP (p=0.016). Fewer LCs expressed CD45RO in OLP than in controls (p=0.037).

Fig 15. Immunohsitochemical staining of T-cell marker CD4 reveals infiltration into the submucosa (60)

Copyright by Mahidol University

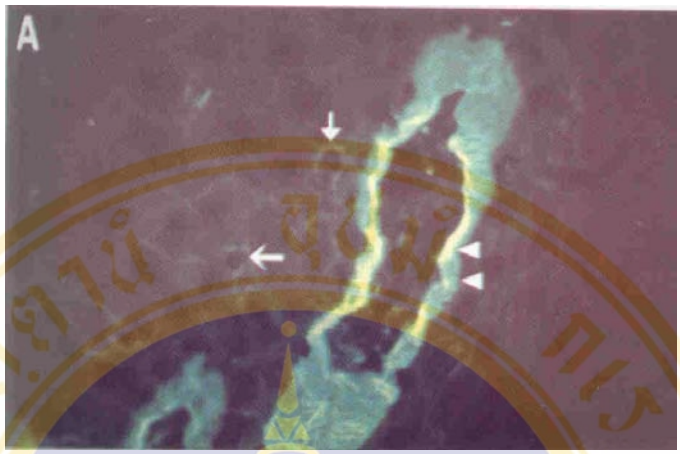

Fig 16.Immunofluorescence staining of Helper T cells double labeled for CD3 (69)

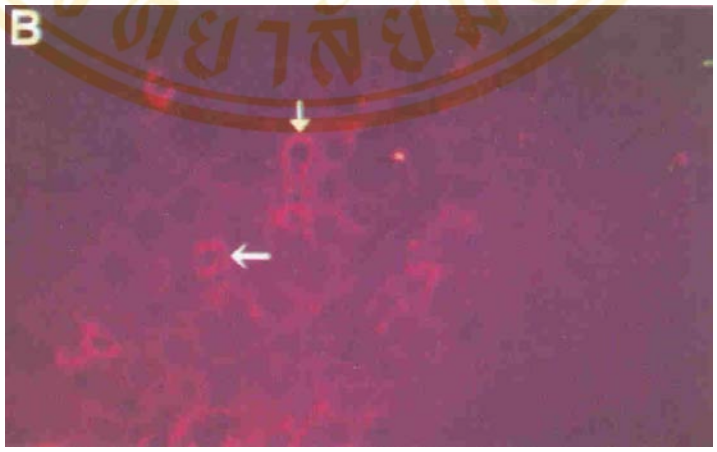

Fig 17. Immunofluorescence staining of Helper T cells double labeled for CD4 (69)

Copyright by Mahidol University

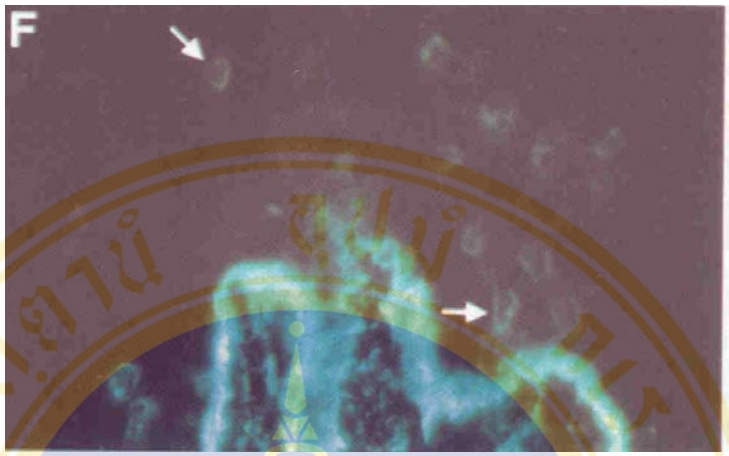

Fig 18. Immunofluorescence staining of Naive helper cells labeled for CD45RA (69)

3.4 Miscellanous

3.4.1 Adhesion molecules

ß1-integrin subfamily (VLA) and intercellular adhesion molecule-1 (ICAM-1) upregulation in OLP and dermal LP compared to normal uninvolved human oral mucosa by using an indirect immunoperoxidase technique has been studied. In both OLP and dermal LP, an induction of the VLA-1 and VLA-3 and an upregulation of VLA-6 was found on T cells infiltrating the BMZ. These cell surface molecules function as receptors for collagen, fibronectin and laminin. A focal induction of ICAM-1 on basal keratinocytes could be detected at sites of intramucosal T cells. These results suggested that investigated adhesion receptors are crucially involved in the aggregation of T cells in both conditions. (42) These results were different from that of Verdick *et al*., (44). They had studied in OLP, recurrent apthous stomatitis, secondary Sjogren syndrome and traumatic ulceration specimens. It was found that no expression of keratinocyte ICAM-1 antigen in OLP and other oral mucosal inflammation. They also studied about lymphocyte function-associated antigen (LFA-1) expression in these lesions. It was found that the number of LFA-1 positive cells were present in 33 of 56 lesions. They suggested that ICAM-1/LFA-1 adhesion interactions may not be critical in the initiation of oral mucosal inflammation.

Copyright by Mahidol University

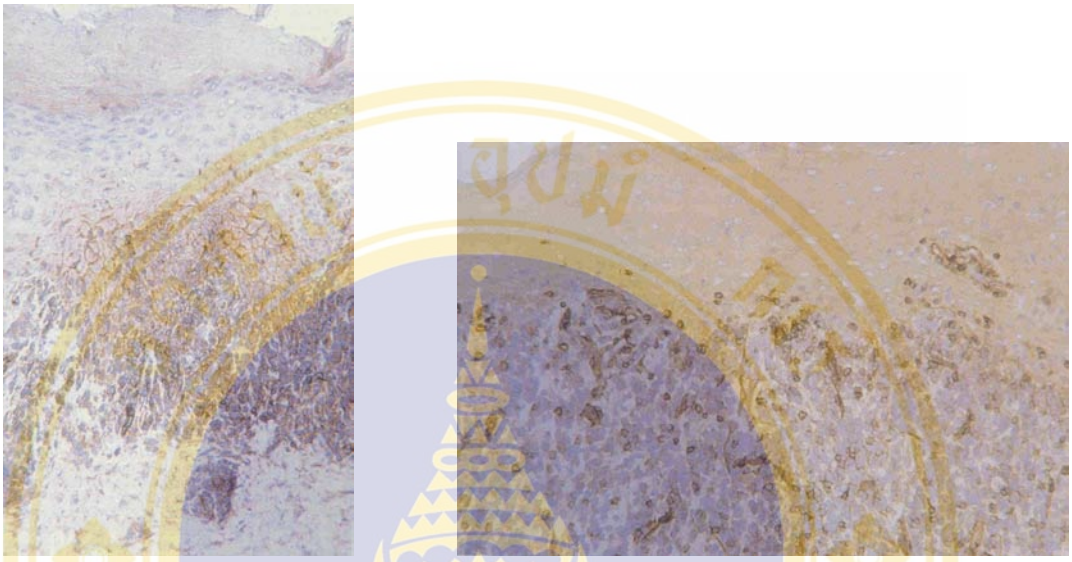

Fig 19.Left ICAM-1 expression in dermal LP, Right :VLA-1 expression in OLP.(42)

Fig 20. Left : VLA-6 expression in dermal LP x160
Right :VLA-6 expression in OLP x 100 respectively. (42)

Copyright by Mahidol University

3.4.2 Extracellular matrix proteins

Extracellular matrix proteins expression in OLP by using immunohistochemical technique has been studied. It was found that the localization of extracellular matrix proteins showed altered expression in OLP when compared to normal oral mucosa, with two principal patterns corresponding to the reticular or atrophic type. Whereas in the reticular type a focal loss of immunoreactiveity for collagen type I, III, V, VI and undulin was noted in areas with a cellular infiltrate, in the atrophic variant almost complete loss of immunoreactivity of the subepithelial extracellular matrix was found. The results suggested that the autoimmune reaction in OLP might not be primarily targeted to oral keratinocytes but to an unknown antigen in the connective tissue stroma. (48)

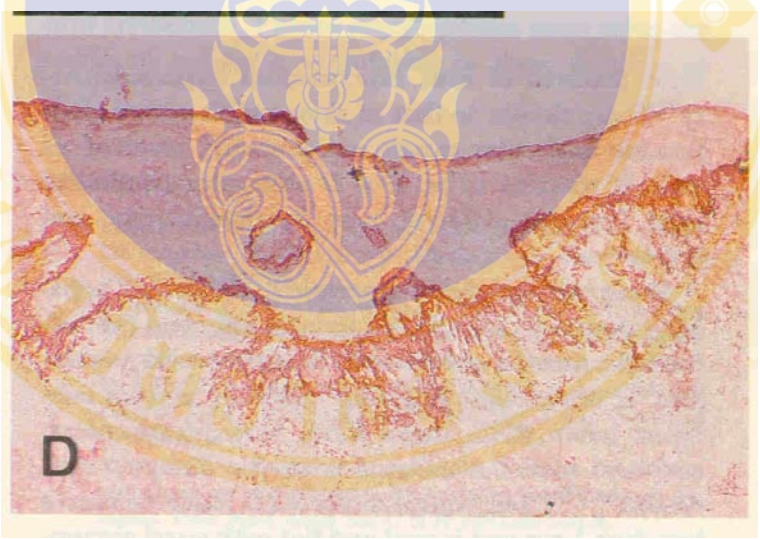

Fig 21. Immunoperoxidase staining of collagen along BMZ (60)

Cytokeratin and filaggrin expression in OLP by using immunohistochemical technique has been studied. It was found that in buccal mucosa, which is not keratinized, cytokeratin (CK) 4 and 13 and filaggrin are expressed in the majority. In buccal OLP, the appearance of CK 1,2,10 and 11 coincides with a decrease in CK 4 and 13 and a moderate increase in CK 6,16,17 and 19. In normal gingival, which is

Copyright by Mahidol University

normally keratinized, the main CK are 1,2,10 and 11 and filaggrin . In gingival OLP, a slight decrease in these CK and in CK 13 expression was noted. They suggested that CK 1,2,10,11 and filaggrin are sensitive tools that may help detecting early relapse before clinical exacerbation. (47) The study was supported by the study of Chiyarit *et al.,* (49) . It was found that the expression of CK 1, 10 in the epithelial basal and suprabasal layers was significantly higher in OLP than in fibroma. They suggested that CK may involve in a unifying hypothesis of pathogenesis of OLP.

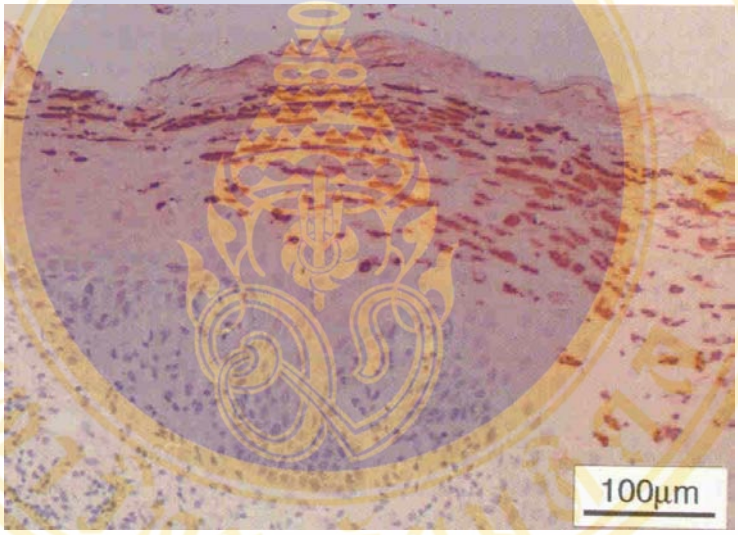

Fig 22. CK1/10 immunostaining of OLP (49)

3.4.3 Chemoattractants

Modified hybridoma growth assay to detect interleukin-6 (IL-6) and the conventional bio-assay to detect tumor necrotic factor (TNF) in OLP patients has been studied. From all cytokines studied, TNFα and IL-6 activity could be measured in OLP sera and both values were significantly higher than those in sera from healthy controls. The results indicated that an alteration in the immune condition of OLP patients and an impairment in T lymphocyte function. (50) The results supported by the study of Yamamoto *et al*., (45) . They also studied the cytokine production by

Copyright by Mahidol University

keratinocytes in OLP by using chemotaxis assay. It was found that keratinocyte in OLP patients produced much more IL-6, TNFα and granulocyte-macrophage colony stimulating factor (GM-CSF) when compared to normal keratinocytes, chronic inflamed gingival keratinocytes. They indicated that keratinocytes play a critical role in OLP, producing cytokines including monocyte-chemotactic factors and that the cytokine produced by mononuclear cells and OLP-keratinocytes through autocrine and paracrine processes enhance the local inflammatory respose. (45)

The serum cytokine levels in patients with OLP , recurrent apthous stomatitis (RAS) , herpetic gingivostomatitis (HGS) , squamous cell carcinoma (SCC) and psudomembranous candidiasis by using a solid phase enzyme linked immunosorbent assay (ELISA) has been studied . It was found that IL-6 activity was elevated with improvement of HGS lesions. TNFα increased in some OLP specimens, but the level were below than that found in RAS, HGS and candidiasis. Most SCC possesd higher GM-CSF activity than the controls. Increase of the cytokine corresponding with improvement of the oral lesion was seen in HGS but not in OLP. From these results, each serum cytokine seems to reflect a characteristic pathophysiology of individual oral disorder. (46)

Eversole, (70) reviewed the pathogenesis mechanism in LP. A putative antigen stimulus upregulates adhesion molecules in the epithelium and the BMZ, all of which bind lymphocyte integrins. Keratinocyte chemoattractants create a diffusion gradiant for trafficking of T cells to the epithelial : connective tissue interface where release of lymphocyte lytic proteins results in basal cell necrosis. (Fig.23)

Copyright by Mahidol University

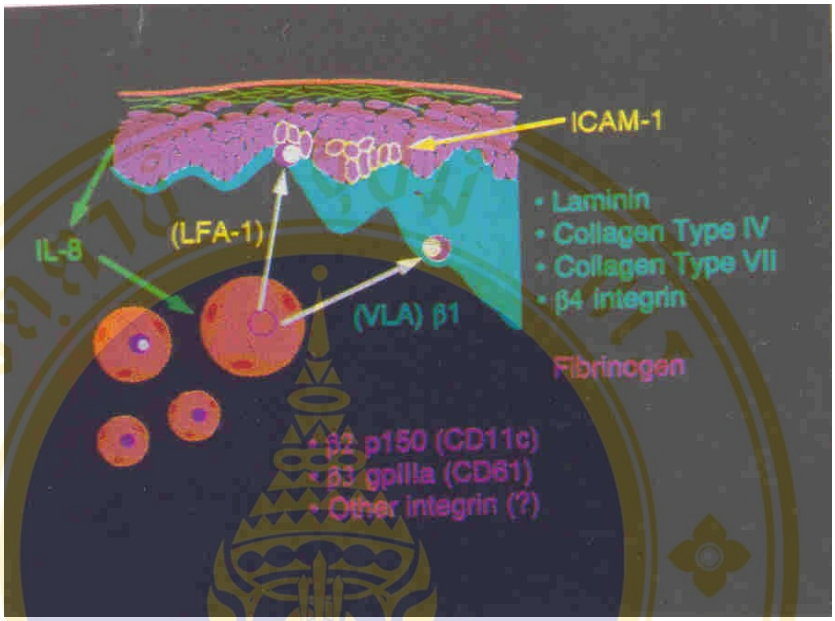

Fig 23. Diagram shows pathogenetic mechanism in LP.(70)

Sleasman, (71) reviewed about modulation of the T helper immune response. Intracellular bacteria stimulate antigen-presenting cells (APC) to elabolate IL-1, interferon, and IL-12. These cytokines activate ThO T lymphocytes to commit to a Th1-activated T-cell clone which produces IL-2 and gamma interferon. These T-cells induce a local inflammatory response and have been associated with autoimmunity. Parastitic infections stimulate APC to produce IL-4 , IL-5, and IL-6. ThO T helper cells activated by these cytokines become Th2 T-cell clones and secrete IL-4, IL-5 and IL-10. These cytokines evoke a humoral immune response chacterized by the production of IgA, IgE and eosinophilia. This immune response has been associated with allergy. (Fig.24)

Copyright by Mahidol University

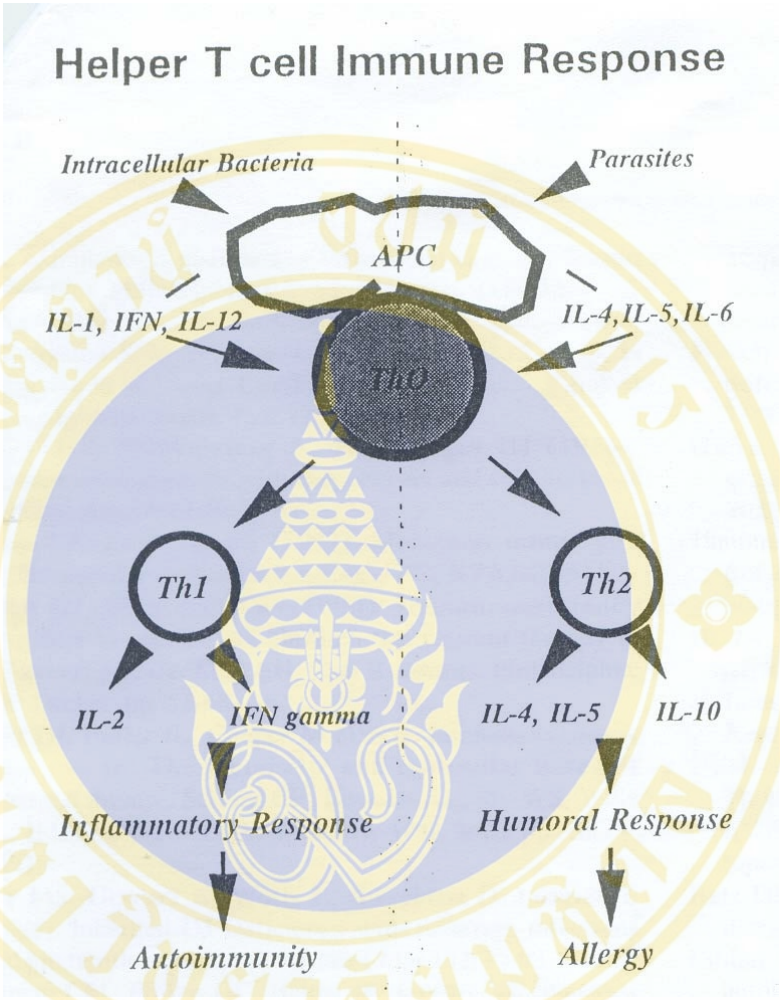

Fig 24. Diagram shows modulation of the T helper immune response. (71)

3.3.4 Heat shock protein

Heat shock protein-70 (HSP-70) expression in OLP, normal oral mucosa, non-specific oral ulceration (NSOU) and dysplastic OLP by using immunohistochemical technique has been studied. It was found that there were statistically significant differences in both the vertical and horizontal staining distribution when other groups compared with the OLP group ($p<0.01$). The mean staining intensity in OLP, dysplastic OLP and NSOU was elevated in comparison with normal oral mucosa

Copyright by Mahidol University

($p<0.01$). The results suggested that expression of HSP-70 by oral keratinocytes may cause a reaction of cytotoxic T lymphocytes may then result in tissue destruction wich is characteristic of OLP lesions. (43) The study was different from that of Chiyarit *et al*., (49). They also studied about HSP-60 and HSP-70 expression in OLP by using immunohistochemical technique. It was found that the expression of HSP-60 in the basal layer was significantly higher in OLP than in fibroma but no difference in HSP-70. The finding supported a role of HSP-60 in pathogenesis of OLP.

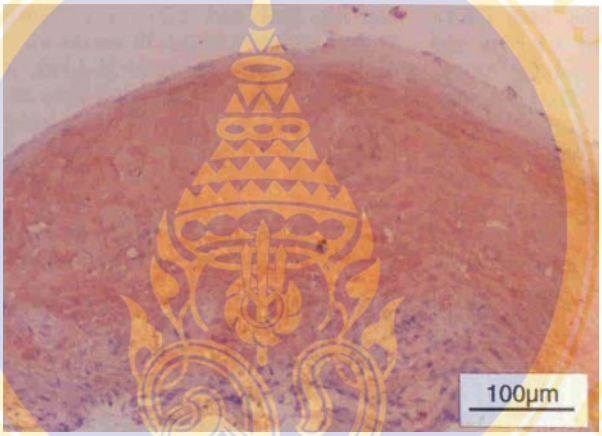

Fig 25. HSP60 immunostaining of OLP (49)

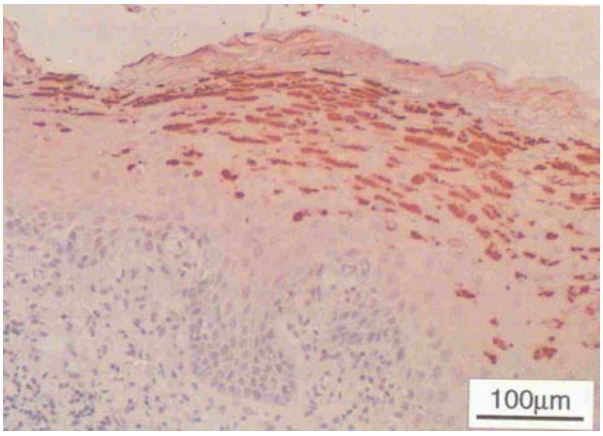

Fig 26. HSP70 immunostaining of OLP (49)

Copyright by Mahidol University

4. S-100 protein

4.1 Biology of S-100 protein

Barrett *et al.,* (72) reviewed about S-100 protein in oral biology and pathology aspects. S-100 is a protein first isolated from bovine brain and so called because of its soloubility in a 100% sateruated solution of ammonium sulphate at neutral pH. The alpha or beta subunits are each composed to two “hands”. The dimmer of two alpha subunits is termed S-100a_0 , that of two beta subunits is S100b and the heterodimer of one alpha and one beta subunits S-100a. Monoclonal antibodies to alpha and beta subunits of S-100 can distinguish melanocytes from LCs. Melanocytes are positive both for S-100 alpha and beta subunits, whereas LCs express only beta subunits. S-100 diffusely distributed throughout the cytoplasm and was also presented in some cell oraganelles. It can generally be detected on the plasma membrane and the outer membranes of the Golgi body, endoplasmic reticulum and mitochodria. The role of S-100 is not fully understood, but its function is probably attribute to calcium-binding properties, regulate enzyme involved intracellular calcium signals , involve in cell cycle control, stimulates expression of growth-associated proto-oncogenes, may important in immunity and regulation of pH , water and electrolyte balance. S-100 protein was demonstrated by these cells for example ; salivary gland cell, melanocyte , LCs , Schwann cells and chondrocytes. Expression of S-100 protein can still confirm a difficult diagnosis which is suggested by routine histological stains and the clinical history , for example in ; salivary gland tumor, pigmented lesions, nervous tissue lesions and other oral lesions (odontogenic myxoma, granular cell myoblastoma, Langerhans histiocytosis, chondroma).

4.2 S100 protein expression in oral epithelium and oral lesions

S-100 imumunolabelling of LCs in gingiva and alveolar mucosa from fibroma, fibromatosis and healthy palatal epithelium autopsy specimens fixed in Bouin’s solution by using immunohistochemical technique has been studied. A method for staining LCs, in routine fixed and paraffin-embedded material, based on the use of antiserum against S-100 proteins was presented. Positive staining cells were recognized as dark brown granular reaction. They displayed distribution located within the suprabasal layer of the epithelium between basal keratinocyte, more

Copyright by Mahidol University

frequently scattered suprabaslly in the spinous layers between suprabasal keratinocytes. Dendrites were numerous and more prominent in the positive cells located suprabasally. The problem arising from the simultaneous staining or melanocytes was dicussed. S-100 positive dendritic cells should be considered as LCs when fulfilling in 3 criteria : 1) located in the suprabasal layer of the epithelium ; 2) the whole cell body being visible ; 3) with at least one dendritic process. (73)

Development of human fetal labial salivary glands and their S-100 protein reactivity by using immunohistochemical technique has been studied. It was found that S-100 protein reactivity was demonstrated in the cytoplasm of cells of the labial gland primordial from their origin. As gland differentiation progressed, the S-100 reactivity became localized in basophil acinar cells and in proximal (intercalted and intralobular), but not in distal, duct cells. (74)

S-100 protein expression in dermal and oral blue nevi has been studied. Three common blue nevi from skin of the trunk and upper extremities and 5 from oral mucosae were studied using immunoperoxidase stain for S-100 protein. The skin lesions were uniformly negative whereas all of the oral mucosal lesions contained numerous positively stained dendritic cells. This difference may be explained by the different embryonic origins of the connective tissue stromas. The connective tissues of the head and neck are thought to be of neural crest origin whereas the connective tissue in the rest of the body are of mesodermal origin. These finding strengthen and refine the association between S-100 protein content and neural crest derivation. (75)

Immunohistochemical localization of S-100 protein and its subunits in melanotic lesions in the oral mucosa and skin. S-100 α and ß subunits were examined in 23 pigmented nevi, 20 malignant melanomas and 8 metastaic melanomas ; originated from the oral mucosa and skin. Primary oral mucosal melanomas demonstrated no ß subunit immunoreactivity while one metastatic lesion from oral melanoma that showed only a small number of melanoma cells reacted with the ß subunit. In contrast, most cutaneous melanomas and their metastatic lesions showed immunoreaction with the S-100 ß subunit. As for pigmented nevi, immunoreactions with the three antibodies were frequently detected regardless or the site. The difference in the expression of S-100 protein ß subunit between cutaneous and

Copyright by Mahidol University

mucosal melanomas may be related to differences in inductive interactions between the melanoma cells and the connective tissues in skin and mucosa. (76)

Immunohistochemical staining for S-100 protein in two jaw myxoma has been studied. It was found that the antibody against S-100 protein showed a strong positive reaction in the cytoplasm of the tumour cells examined. It indicated that a mesenchymal derivation for odontogenic myxoma, and is the first demonstration of S-100 protein within the cells of this tumour type. (77)

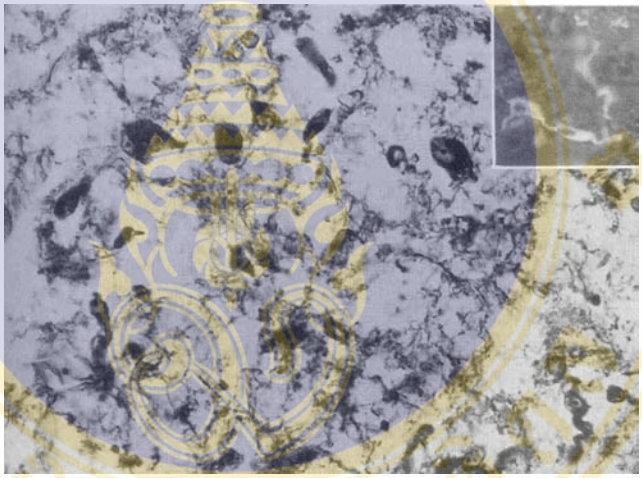

Fig 27. Odontogenic myxoma cells showing strong immunoreactivity with anti-vimentin antibody immunoperoxidase (x250) (77)

Immunohistochemical stainig of LCs and macrophages in OLP has been studied. It was found that S-100 positive cells were found predominantly in the middle and lower levels of epithelium. Stained cells were greater in number where lymphophagocytic infiltrates were intense. Frequently lymphocytes were seen adjacent to stained dendritic cells. Fewer positive dendritic cells were found in the epithelium that had separated from connective tissue in areas of severe disease expression. Normal epithelium adjacent to diseased areas meeting the histopathologic criteria of OLP always had fewer S-100 positive cells. Within the affected submucosa,

Copyright by Mahidol University

positive stellate and polygonal cells were also noted. These cells ranged from few to numerous and were proportional to the density of the lymphophagocytic infiltrate. It can be concluded that these cells are processing antigens for presentation to T-lymphocytes. (78) This study was supported the study of Regazi *et al.,* (79). It was found that S-100 positive connective tissue were also significantly increased in number in the submucosal of the diseased tissue as compared to the unaffected adjacent tussue ($p<0.00001$). Cell morphology was typically oval or polygonal. Dendritic processes were often seen extend from the cell body.

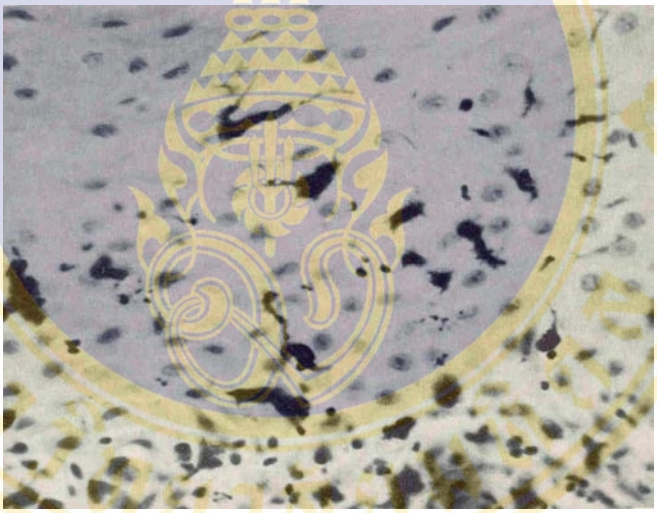

Fig 28. Positive S-100 protein staining in OLP (Magnification, x400) (79)

4.3 Immunohistochemical technique for S-100 staining

Charbit *e t al*., (73) had studied about S-100 satining by using modified immunoperoxidase technique. The sections were stained according to the procedure describe as follows :

Copyright by Mahidol University

Antisrum against S-100 protein-PAP procedure

Step N^0	Procedure
1	H_2O_2 in methanol for 30 min
2	Section washed in tap water and distilled water
3	Run PBS (a) -Bath PBS
4	NSS (b) diluted 1:10 in PBS for 30 min
5	Anti S-100 antiserum (c) diluted 1:160 in PBS for 30 min
6	Run PBS-Bath PBS
7	SWAR (d) diluted 1:20 in PBS for 30 min
8	Run PBS-Bath PBS
9	PAP (e) diluted 1:200 in PBS for 30 min
10	Run PBS-Bath PBS
11	DAB (f) n Tris (g) H_2O_2 0.03% fpr 2-5 min
12	Section washed in tap water and in distilled water
13	Section counterstained with Mason's hematoxylin

(a) Phosphate buffer saline (PBS) ph 7.4

(b) Normal swine serum (NSS) (DAKO)

(c) Antiserum against S-100 protein (HSC-Reseach Development Corporation)

(d) Swine anti-rabbit (SWAR) (DAKO)

(e) Peroxidase antiperoxidase complex (PAP) (DAKO)

(f) 3,3' Diaminobenzidine tetrachydrochloride

(g) Tris : add 0.1 g Tris in 500 ml distilled water, adjust to pH 7.6

Copyright by Mahidol University

CHAPTER III
MATERIALS AND METHODS

MATERIALS

1. Acetone
2. 3% 3-aminopropyltriethoxy silane (Sigma)
3. Distilled water
4. Xylane
5. Absolute alcohol
6. 0.5% hydrogen peroxide
7. Methanol
8. Citrate buffer
9. Phosphate buffer solution
10. Pap-pen
11. 3% normal horse serum (NHS)
12. Anti-S100 protein (Dako, Z 3111)
13. Secondary antibody ; swine anti-rabit (Dako, E 0431)
14. Tertiary antibody ; SRP (Dako, P 0397)
15. 3,3' Diaminobenzidine tetrahydrochloride (DAB)
16. Mayer's haematoxylin
17. Tris (hydroxymethyl) aminomethane

INSTRUMENTS

1. Slide and cover slide
2. Water bath with thermostat (Electrothermal ®)
3. Microtome
4. Jar
5. Red roter (Hoefer ®)
6. Ultrasonic cleaner (Metter ®)
7. Microwave (National ®)

Copyright by Mahidol University

8. Vortex mixer (Scientific industries ®)
9. auto-pipette (Socorex ®)
10. hot air oven (Memmert ®)

METHOD

1. Preparation :

1.1 Preparation of the specimens

The specimens were from the oral pathology biopsy service, formalin-fixed paraffin-embedded tissue specimens of 42 OLP sample (34 females ; mean age = 46 ; range 23-72) and 12 fibroma lesions (10 females ; mean age 45 : range 14-68) and 5 normal epithelium (1 from normal mucosa and 4 from fibroma specimens ; 4 females ; mean age 47 ; range 37-68) . The specimens were taken from patients with the clinical and histological diagnosis of OLP and fibroma. The specimen of 1 normal epithelium from the histological diagnosis of normal epithelium and others from the normal epithelium adjacent to the fibroma lesion. The tissues were used from oral mucosal biopsy (buccal, palate, attached gingiva, the tongue and the lower lip). The OLP histologic criteria that had to be met for inclusion in the study were hyperkeratosis, basal layer degeneration and lymphocytes infiltration locate at the epithelial connective tissue . Anti S-100 proteins antiserum 1:30000 (DAKO) was used to stain the mucosal biopsies.

1.2. preparation of the solution

1.2.1 10 mM sodium citrate buffer pH 6

- Citric acid (Anala R) = 5.25 g in distilled water 2 litres
- Buffering pH 6 with 5 N NaOH (NaOH 40 g + distilled water 200 ml)
- Add the distilled water until the volume =2.51

1.2.2 Phosphate buffer solution pH 7.4 (PBS)

Stock solution

- Distilled water 3,250 ml + NaCl 1,052 g + NaH_2PO_4 165.6 g
- Buffering pH 6.2 with 5 N NaOH 190 ml
- Add distilled water until the volume = 4 liter

Copyright by Mahidol University

Working solution

- Stock solution 166.6 ml + distilled water 5 liter
- Buffering pH 7.4 with 5 N NaOH (NaOH 40g + distilled water 200 ml)

1.2.3 Tris HCL buffer pH 7.4

- 0.2 M tris (6g trisma base + 250 ml distilled water)
- 0.2 M HCl (1.7 ml. conc.HCL + 100 ml distilled water)
- 0.2 M tris 12.5 ml/0.2 HCL 13.6 ml
- Add the distilled water until the volume=50 ml
- Buffering pH 7.4 with 5 N NaOH (NaOH 40 g +distilled water 200 ml)

1.2.4 DAB (diaminobenzidine tetrahydrochloride)

- DAB 0.1 g +tris HCL buffer pH 7.4 10 ml

1.2.5 Mayer's haematoxylin

- Boiled distilled water + potassium alum
- Add haematoxylin
- Add sodium iodate
- Put the jar at the room termperature for 1 day
- Add chloral hydrate + citric acid in the boiled solution for 5 minutes

1.2.6 S-100 polyclonal antibody dilution 1 : 30,000

- S-100 polyclonal antibody 1 microliter
- Dilute with 3 % normal horse serum 30 mililiter

1.2.7 Secondary antibody ; swine anti-rabbit (SAR)

- SAR 40 microliter
- Dilute with 3% normal horse serum 20 mililiter

1.2.8 tertiary antibody ; streptavidin peroxidase (SRP)

- SRP 20 microliter
- Dilute with 3% normal horse serum 20 mililiter

2. Research method :

Steps of tissue staining with indirect immunohistochemical technique

2.1 the specimen (4 microns) was coated with silane by

- Wash the slide in acetone for 5 minutes

Copyright by Mahidol University

- Fix slide in 3% 3-aminopropyltriethoxy silane in acetone for 5 minutes (3% 3- aminopropyltriethoxy silane = 7.5 ml + acetone 250 ml)
- Wash the slide in acetone for 2 dips
- Wash the slide in distilled water for 2 dips
- Incubate slide in incubator

2.2 Dewaxed the specimen by rinsing in xylane x 3 jars (5-10 minutes) and then in absolute alcoholx3 jars (1 min)

2.3 Blocking endogenous peroxidase by 0.5 % hydrogen peroxide in methanol about 30 minutes (0.5% hydrogen peroxide in methanol=3.32 ml+ methanol 200 ml)

2.4 Antigen retrieval treatment by microwave

- Put the specimens in 10 mM citrate buffer jar, pH 6.0
- Put the citrate buffer jar in the microwave at the highest power level until the solution was boiled and then reduce the power level at the optimal level for 20 minutes

2.5 Bring the jar out off the microwave for 25 minutes (rooms temperature)

2.6 Running water about 1 minute, and wash with distilled water 2-3 dips

2.7 Put the slide in PBS (phosphate buffer solution), pH 7.4

2.8 Limit the outline with pap-pen and blocking nonspecific reaction with 3% NHS (3%NHS=NHS 1 ml + PBS 29 ml), incubate 20 min

2.9 Slide was incubated overnight at room temperature with anti S100 protein as the primary antibody

2.10 After a PBS wash, the sections were incubated for 30 minutes with a secondary antibody

2.11 Followed by a PBS wash

2.12 Incubation for 30 minutes with tertiary antibody

2.13 After a PBS wash, the section were developed in diaminobenzidine (DAB) for 15-20 minutes, brown color is the positive result

2.14 Rinsing with PBS x2 (3min) and running water 3-5 minutes

2.15 Counter stain with Mayer's haematoxylin

- Fix tissue paraffin section in microwave (high power level : 2 mins)
- Dewax in xylene 2 jars x 3 mins
- Clear in absolute alcohol 2 jars x 1 min (or 20 dips) and 95 % alcohol 2

Copyright by Mahidol University

jars x 1 min (or 20 dips), respectively

- Running water 1 min
- Stain in haematoxylin 3 mins
- Running water 1 min
- Dip in ammonium solution 1-2 dips
- Running water 20 dips
- Stain in Eosin 2 mins
- Dehydrate in 95 % alcohol 2 jars x 20 dips and absolute alcohol 2 jars x 20 dips, respectively
- Clear in xylene 2 jars x 1 min (or 20 dips)
- Mount

2.16 Interpretation the slide

3. The counting stained cell and interpretation the slide :

S-100 positive cells should be considered as LCs when fulfilling 2 criteria 1) the whole cell body being visible ; 2) with at least one dendritic process. A 10x10 ocular graticule outlining an area of 0.064 mm^2 was used at magnification of 400x to assist in counting cells in sections stained for S-100 protein. The grid was positioned parallel to the epithelial-connective tissue interface. Counts from 3 ajacent high-power fields in a single section were averaged. Histologically unaffected tissue adjacent to the diseased zone ; showing no hyperkeratosis, basal cell vacuolation, or significant lymphocytic infiltration were used for comparison. Differences in cell counts in OLP and unaffected (adjacent tissue) “control” sites were statistically evaluated using Wilcoxon Matched-Pairs Signed-Ranks Test . The differences in cell counts in OLP and fibroma lesions were statistically evaluated using Mann-Whitney U-Wilcoxon Rank Sum W Test. The differences in cell counts in OLP and normal epithelium were statistically evaluated using Mann-Whitney U-Wilcoxon Rank Sum W Test.

Copyright by Mahidol University

Fig 29. The specimens were cut by using Microtome.

Fig 30. After cutting, the sections were warmed in water bath.

Copyright by Mahidol University

Fig 31. Automatic pipettes were used for dilution the antibodies and preparating the solutions.

Fig 32. Antigen retrieval treatment by Microwave

Copyright by Mahidol University

Fig 33. Hot air oven for drying the sections.

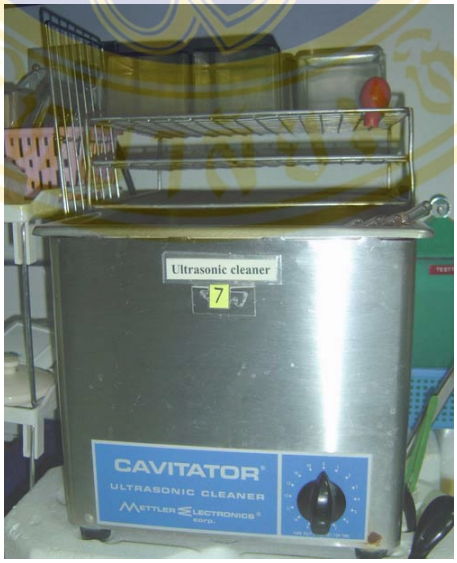

Fig 34. Ultrasonic cleaner for cleaning the instruments.

Copyright by Mahidol University

Fig 35. Red rotor for rotoring the sections in PBS.

Fig 36. Antibody and the solutions were kept in the refrigerature.

Copyright by Mahidol University

Fig 37. Preparating the primary, secondary and tertiary antibodies.

Fig 38. The antibody was dropped on the sections.

Copyright by Mahidol University

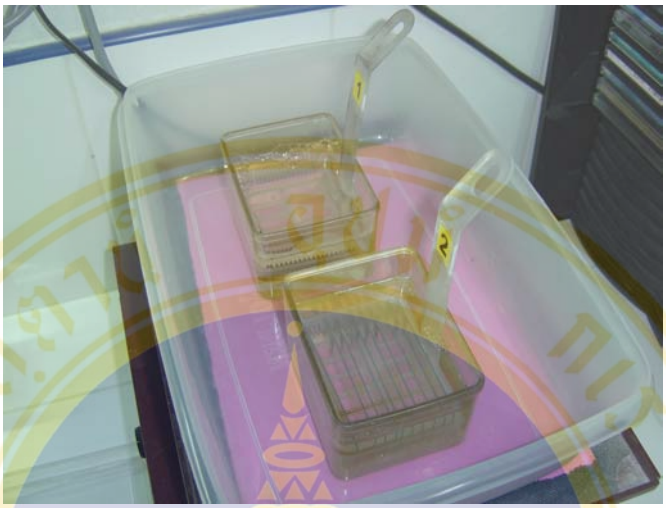

Fig 39. Put the sections in PBS and turned on the Red rotor.

Fig 40. After rinsing in PBS, washed the sections with running water.

Copyright by Mahidol University

Fig 41. The sections were developed in DAB.

Fig 42. The sections were stained with Mayer haematoxylin.

Copyright by Mahidol University

Fig 43. Interpretation the slides by using light microscope.

Copyright by Mahidol University

CHAPTER IV
RESULTS

Forty two OLP, 12 fibroma and 5 normal epithelium specimens were selected for this study. Positive results for indirect immunohistochemical staining with specific polyclonal antibody against S-100 proteins were recognized as dark brownish granular reaction. These cells exhibited dendritic process. S-100 positive LCs in OLP are shown in Fig. 44 and 46, in adjacent tissue are shown in Fig 45 and 47, in fibroma are shown in Fig.48 and in normal epithelium are shown in Fig.49.

It was found that the number of S-100 positive LCs in OLP specimens ranged from 7 to 96 (mean value 27.10) (Table 1) , in adjacent tissue near OLP specimens ranged from 2 to 45 (mean value 8.31) (Table 1), in fibroma specimens ranged from 1 to 14 (mean value 4.08) (Table 2), and in normal epithelium specimens ranged from 2 to 7 (mean value 4.20) (Table 3).

Differences in cell counts between OLP and adjacent tissue were statistically evaluated using Wilcoxon Matched-Pairs Signed-Ranks Test. Differences in cell counts between OLP and fibroma lesions ; OLP and normal epithelium were statistically evaluated using Mann-Whitney U-Wilcoxon Rank Sum W Test. It was found that S-100 positive LCs were significantly increased in number in OLP specimens as compared to unaffected adjacent tissue, fibroma and normal epithelium($p<0.05$) (Table4), and S-100 positive LCs were also significantly increased in number in unaffected adjacent tissue as compared to normal epithelium ($p<0.05$). (Table 4)

Copyright by Mahidol University

Table 1. The number of S-100 positive LCs in OLP and adjacent tissue specimens.

Pathologic no.	Age	Sex	Location	No. of positive cells	
				OLP	Adjacent tissue
41-151	46	F	Buccal	57	9
41-208	50	F	Buccal	38	11
42-062	59	F	Buccal	12	4
42-014	49	M	Buccal	52	12
42-125	34	M	Attached gingiva	18	6
42-126	46	F	Buccal	11	5
42-147	33	F	Buccal	12	4
42-168	70	F	Buccal	39	12
42-201	63	F	Buccal	31	8
42-215	65	F	Buccal	12	5
43-042	46	F	Buccal	20	10
43-080	49	F	Buccal	33	15
43-097	47	M	Attached gingiva	22	8
43-103	34	F	Buccal	18	11
43-195	35	F	Buccal	96	45
43-196	23	M	Buccal	47	6
43-223	43	F	Attached gingiva	19	7
43-232	46	M	Buccal	15	3
43-237	49	M	Attached gingiva	27	9
43-284	38	F	Buccal	62	5
43-305	48	F	Buccal	43	11
43-316	43	F	Buccal	13	6
43-352	46	F	Buccal	31	9
44-029	46	F	Buccal	26	7
44-034	40	F	Attached gingiva	32	4
44-226	43	F	Attached gingiva	10	6
44-317	40	F	Buccal	34	9
44-371	50	F	Buccal	24	6
45-034	24	F	Buccal	24	8
45-214	53	F	Buccal	26	7
45-341	52	M	Buccal	8	2
45-381	42	F	Buccal	10	7
45-374	24	F	Attached gingiva	14	8
46-033	37	F	Attached gingiva	7	2

Cont. next page.

Copyright by Mahidol University

Table 1. The number of S-100 positive LCs in OLP and adjacent tissue specimens. (Cont.)

Pathologic no.	Age	Sex	Location	No. of positive cells	
				OLP	Adjacent tissue
46-058	46	F	Buccal	41	19
46-065	46	F	Buccal	8	2
46-154	66	F	Buccal	40	9
46-159	42	M	Buccal	18	5
46-244	37	F	Buccal	22	6
46-251	72	F	Buccal	22	4
46-308	72	F	Buccal	17	5
46-319	46	F	Buccal	27	12
Mean				27.10	8.31

Table 2. The number of S-100 positive LCs in fibroma specimens.

Pathologic no.	Age	Sex	Location	No.of positive cells
43-086	51	M	Lower lip	14
43-152	50	F	Buccal	2
43-173	41	F	Buccal	2
43-201	58	F	Attached gingiva	10
43-212	68	F	Palate	3
43-221	14	F	Attached gingiva	1
43-265	46	F	Buccal	1
44-008	45	F	Tongue	1
44-016	35	F	Tongue	2
44-039	37	F	Attached gingiva	7
44-088	61	F	Palate	5
45-027	39	M	Palate	1
Mean				4.08

Copyright by Mahidol University

Table 3. The number of S-100 positive LCs in normal epithelium specimens.

Pathologic no.	Age	Sex	Location	No.of positive cells
44-010	42	F	Buccal	4
43-152	50	F	Buccal	5
43-212	68	F	Palate	3
44-039	37	F	Attached gingiva	7
45-027	39	M	Palate	2
Mean				4.20

Table 4. Statistically analysis of S-100 postive LCs.

Specimens		Mean No.of S-100 positive LCs	Significance
OLP	Affected	27.10	*
	Unaffected	8.31	*
Fibroma		4.08	*
Normal epithelium		4.20	*

* Significant differences ($p<0.05$)

Copyright by Mahidol University

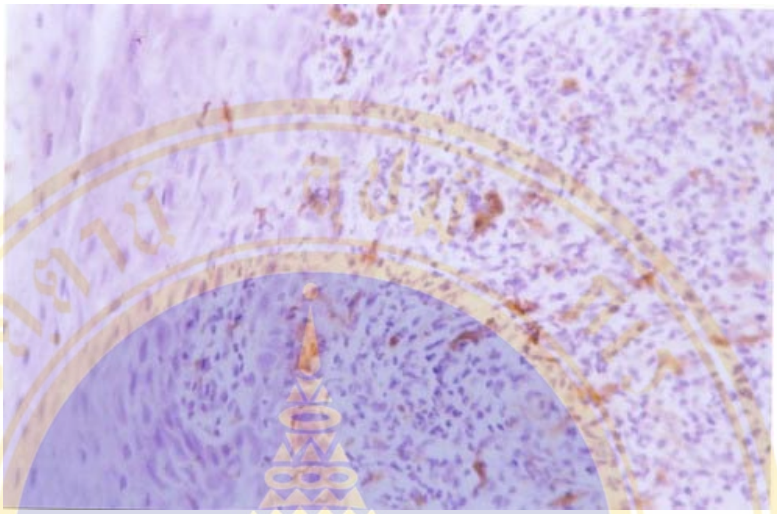

Fig 44. Numerous S-100 positive LCs in OLP.
(Original Magnification, x 400)

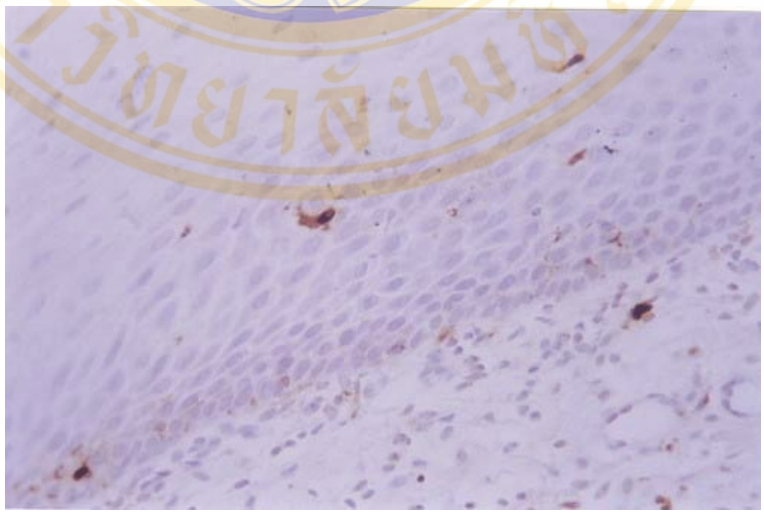

Fig 45.S-100 positive LCs in unaffected adjacent tissue of OLP.
(Original Magnification, x 400)

Copyright by Mahidol University

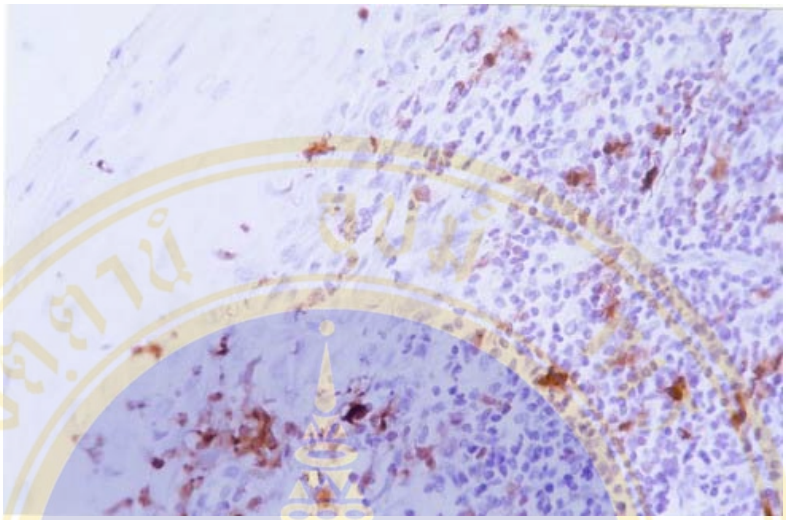

Fig 46. Numerous S-100 positive LCs in another sample of OLP.
(Original Magnification, x 400)

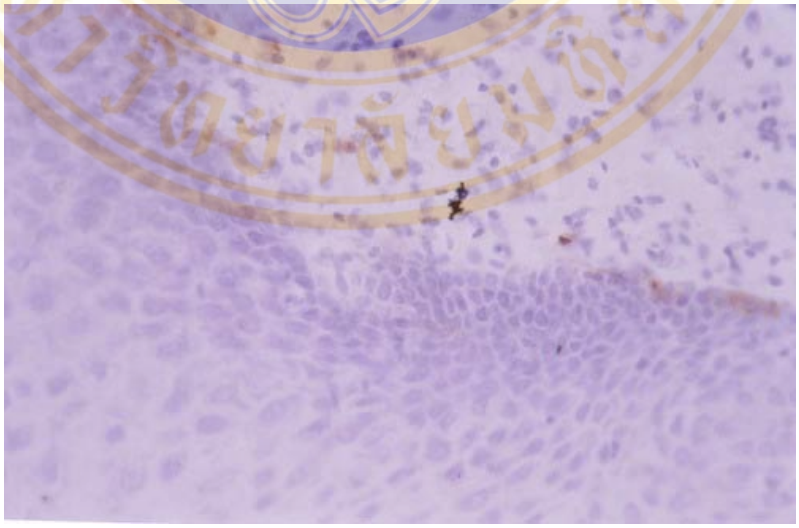

Fig 47. S-100 positive LCs in unaffected adjacent tissue in the same case.
(Original Magnification, x 400)

Copyright by Mahidol University

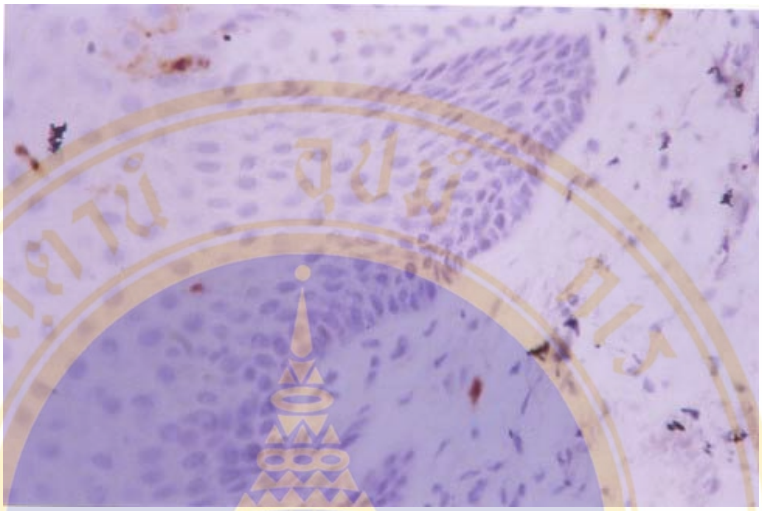

Fig 48. Few S-100 positive LCs in fibroma.
(Original Magnification, x400)

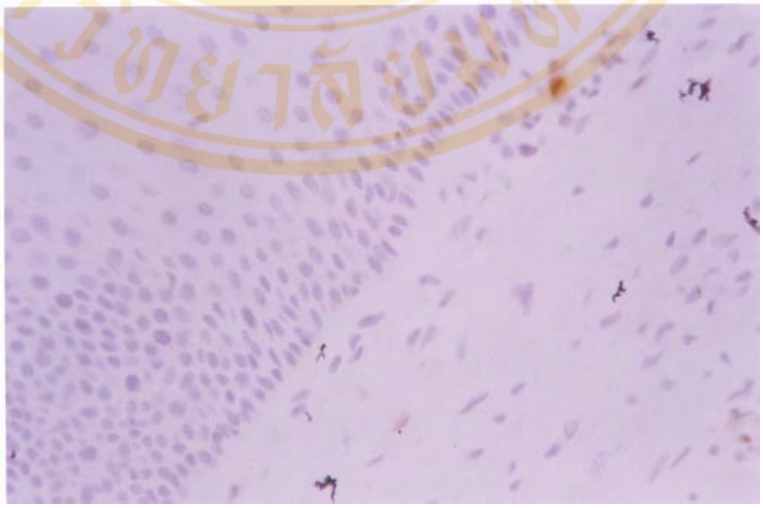

Fig 49. A few S-100 positive LCs in normal epithelium.
(Original magnification, x400)

Copyright by Mahidol University

CHAPTER V
DISCUSSION

In this study, the finding of positive LCs in OLP , supports some previous studies that these cells are the one which may play the immunological reaction in the pathogenesis of OLP. (35,36,40,41) The number of positive LCs were in proportion to the lymphocyte infiltration and were significantly increased in the OLP lesion as compared to the unaffected adjacent tissue, the fibroma lesions and the normal epithelium ($p<0.05$). The number of S-100 positive LCs were significantly much more in OLP than in unaffected adjacent tissue. Increasing antigen-processing activity in the zones of active disease supports the study of Josep *et al,* (78) However, the absolute numbers of LCs are difficult to establish in this type of study, as numbers of LCs may vary from one patient to another. The sex, age, location of the lesions and the time of the specimens were embedded in the paraffin are probably the result of variation in S-100 proteins expression.

Other immunological lesions including pemphigus, pemphigoid or lupus erythematosus may be interesting lesions in addition to times series. An increase in number of S-100 positive LCs has been reported in oral SCC. (80) If there are differences in the number or distribution of LCs in these lesions, S-100 positive LCs may be a new tool for differential diagnosis between oral lesions.

Although clinical evaluation is a method for comparison between effects of difference corticosteroid medication (51,53) , expression of antigen is a tool useful in evaluating the effects of two experimental groups after medication. (35) Therefore S-100 positive LCs may be used in evaluation of the effects of different medicines between the experimental groups.

Nowadays, OLP occupied one of the most common oral mucosal lesions. Many cells and molecules have been widely studied because we would like to know the exact cause of this lesion. Although corticosteroid is the first choice of drug

Copyright by Mahidol University

for treatment of OLP, the recurrence of this lesion is very often. Perhaps we may require the new way to treat. Molecular biology, cell biology and genetic knowledges may concern in the future to seek for the exact cause of OLP. If we know which one of molecule or cell that is the cause, so we can use the drug or medication specific to that molecule or cell instead of corticosteroid. For this reason, futher study should be continued.

The result suggested that patient with OLP may have the immunologic disorder in which significantly increased LCs might play a role of pathogenesis of OLP or represent an event secondary to the pathologic changes in the disease.

Copyright by Mahidol University

CHAPTER IV
CONCLUSION

This study determined the number of S-100 positive LCs in OLP compared with unaffected adjacent tissue, fibroma lesions and the normal epithelium. The following are conclusions :

1. The number of S-100 positive LCs were significantly increased in OLP as compared to unaffected adjacent tissue ($p<0.05$).

2. The number of S-100 positive LCs were significantly increased in OLP as compared to the fibroma specimens ($p<0.05$)

3. The number of S-100 positive LCs were significantly increased in OLP as compared to the normal epithelium ($p<0.05$).

4. The number of S-100 postive LCs were also significantly increased in unaffected adjacent tissue as compared to the normal epithelium ($p<0.05$).

Copyright by Mahidol University

REFERENCES

1. Scully C , El-Kom M. Lichen planus : Review and update on pathogenesis. Journal of Oral Pathology 1985 ;14 : 431-458.
2. Nancy W, Eileen J, Jefferson E, Laurie W. Assessing the characteristics of patients with oral lichen planus. JADA 1996 ; 127 : 648-662.
3. Meir G, Mili R, David M, Michal L, Lipa B, Tel A, Beer S. Clinical characteristics and treatment of patients with oral lichen planus in Israel. Oral Surg Oral Med Pathol Oral Pathol Oral Radiol Endod 1996 ; 82 : 644-649.
4. Andreasen JO. Oral lichen planus, a clinical evaluation of 115 cases. Oral Surg Oral Med Oral Pathol 1968 ; 31-42.
5. Murti PR, Daftary DK, Bhonsle RB, Gupta PC, Mehta FS, Pindborg JJ. Malignant potential of oral lichen planus : observations in 722 patients from India. J Oral Pathol 1986 ; 71-77.
6. Bernard NS, Scully C, Eveson JW, Cunningham S, Porter SR. Oral cancer development in patients with oral lichen planus. J Oral Pathol Med 1993 ; 22 : 421-424.
7. Voute AB, Jong WF, Schulten EA, Snow GB, Waal VD. Possible premalignant character of oral lichen planus : the Amsterdam experience. J Oral Pathol Med 1992 ; 21 : 326-329.
8. Girod SC, Krueger G, Pape HD. p53 and Ki 67 expression in preneoplastic and neoplastic lesions of the oral mucosa. Int. J. Oral Maxillofac. Surg. 1993 ; 22 : 285-288.
9. Dekker NP, Lozada NF, Lagenaur LA, Macphail LA, Bloom CY, Regezi JA. Apoptosis-associated markers in oral lichen planus. J Oral Pathol Med 1997 ; 26 : 170-175.
10. Schifter M, Jones AM , Walker DM . Epithelial p53 gene expression and mutational analysis, combined with growth fraction assessment, in oral liche planus. J Oral Pathol Med 1998 ; 318-324.

Copyright by Mahidol University

11. James TC , Massimo C , James K , Susan L , Abbondanzo, Harvey K, Paul LA Andrew Y. p53 and cyclin D1 staining patterns of malignant and pre-malignant oral lesions in age-dependent population. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999 ; 88 : 326-332.

12. Alison MR. p53 expression in oral precancer and cancer. Australian Dental Journal 1999 ; 44 : 103-105.

13. Cox SC , Walker DM . Epithelial growth fraction and expression of p 53 tumour suppressor gene in oral submucous fibrosis. Australian Dental Journal 1996 ; 41(2) : 91-96.

14. Saito T , Nakajima T , Mogi K . Immunohistochemical analysis of cell cycle associated proteins p16, pRb , p53, p27 and Ki-67 in oral cancer and precancer with special reference to verrucous carcinoma . J Oral Pathol Med 1999 ; 28 : 226-232.

15. Konstantinos IT, Elina KA, Nikiforos K. Immunohistochemical study of bcl-2 protein, Ki-67 antigen and p53 protein in epithelium of glandular odontogenic cysts and dentigerous cysts. J Oral Pathol Med 2000 ; 29 : 139-144.

16. Souza PE, Mesquita RA, Gomez RS. Evaluation of p53, PCNA, Ki-67, MDM2 and AgNOR in oral peripheral and central giant cell lesions. Oral Diseases 2000 ; 6 : 35-39.

17. Jose LR, Josse VB, Juan RM, Javier SD, Maria AM, Yolanda J. Psychologic factors and oral lichen planus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998 ; 86 : 687-691.

18. McCartan BE. Psychological factors associated with oral lichen planus. J Oral Pathol Med 1995 ; 24 : 273-275.

19. Carl MA, Frank MB, Karen MR, Theodore JK. Relation of stress and anxiety to oral lichen planus. Oral Surg Oral Med Oral Pathol 1986; 61 : 44-46.

20. Shuttleworth D, Graham B, Campbell AC. The autioimmune background in lichen planus : British Journal of Dermatology 1986 ; 115 : 199-203.

21. Baart EH, Baart H. An immunofluorescence study of lichen planus. Bristish journal of Dermatology 1974 ; 90 : 365-371.

22. Alaxandra DS , George L , Angelos P. Serum immunoglobulins and complement

Copyright by Mahidol University

(C3) in oral lichen Oral Surg 1983 ; 55(1) : 47-51.

23. Charlses ET. Deposits of immunoglobulins, complement, and fibrinogen in oral lupus erythematosus, lichen planus, and leukoplakia. Oral pathology 1981 ; 51(6) ; 603-608.

24. Lai AF , Cormane RH , Van RF . An immunohistopathological study on the synthesis of immnoblobulins and complement in normal and pathological skin and the adjacent mucous membranes. British Journal of Dermatology 1974 ; 90 : 123-136.

25. Joseph AR, Michael JD and James RH, Ann AM. Lichen planus, Immunologic and morphologic identification of the submucosal infiltrate . Oral Surg 1978 ; 46(1) : 44-52.

26. Sugarman PB, Savage NW, Seymour JG. Phenotype and suppressor activity of T-lymphocyte clones extracted from lesions of oral lichen planus. British Journal of Dermatology 1994 ; 131 : 319-324.

27. Takeuchi Y , Tohnai I, Kaneda T , Negura H . Immunohistochemical analysis of cells in mucosal lesions of oral lichen planus. J Oral Pathol 1988 ; 17 : 367-373.

28. Walsh LJ , Tsen PW , Savage NW , Seymour GJ . Expression of CDw29 and CD45R antigens on epithelial cells in oral lichen planus. J Oral Pathol Med 1989 ; 18 : 360-365.

29. Walton LJ , Thornhill MH , Macey MG , Farthing PM . Cutaneous lymphocyte-associated antigen and integrin are expressed by mononuclear cells in skin and oral lichen planus. J Oral Pathol Med 1997 ; 26 : 402-407.

30. Sugarman PB, Voltz MJ, Savage NW, Basford KE, Seymour GJ. Phenotypic and functional analysis of peripheral blood lymphocytes in oral lichen planus. J Oral Pathol Med 1992 ; 21 : 445-450.

31. Yamamoto T, Yoneda K, Ueta E, Osaki T. Cellular immunosuppression in oral lichen planus. J Oral Pathol Med 1990 ; 19 : 464-470.

32. Alaibac M, Morris J, Yu R, Chu AC. T lymphocytes bearing the gamma-delta T cell receptor : a study in normal human skin and pathological skin and pathological skin conditions. British Journal of Dermatology 1992 ; 127 : 458-462.

Copyright by Mahidol University

33. Robertson WD , Wray D. Immunohistochemical study of oral keratoses including lichen planus. J Oral Pathol Med 1993 ; 22 : 180-182.

34. Matthews JB, Scully CM, Potts AJ. Oral lichen planus : an immunoperoxidase study using monoclonal antibodies to lymphocyte subsets. British Journal of Dermatology 1984 ; 111 : 587-595.

35. Sloberg K, Jonsson R, Jontell M. Assesment of Langerhans cells in oral lichen planus using monoclonal antibodies. Journal of Oral Pathology 1984 ; 516-524.

36. Chou MJ, Daniels TE. Langerhans cells expressing HLA-DQ, HLA-DR and T6 antigens in normal oral mucosa and lichen planus. J Oral Pathol Med 1989 ; 18 : 573-576.

37. Walter BH. Mast cells in desquamative gingivitis lichen planus, and pemphigoid. Oral Surg Oral Med Oral Pathol 1969 ; 28(5) : 646-659.

38. Jontell M, Hansson HA, Nygren H. Mast cells in oral lichen planus. J Oral Pathol 1986 ; 15 : 273-275.

39. Triantafyllou A. Cytological and cytochemical investigations on granular cells in oral lichen planus. J Oral Pathol Med 1996 ; 25 : 151-156.

40. Bhan AK, Harrist TJ, Murphy GF, Mihm MC. T cell subsets and Langerhans cells in lichen planus : in situ characterization using monoclonal antibodies. Journal of Dermatology 1981 ; 105 : 617-622.

41. Hedberg NM , Hunter N. The expression of HLA-DR on keratinocytes in oral lichen planus. J Oral Pathol 1987 ; 16 : 31-35.

42. Konter U , Kellner K , Hoffmeister B, Sterry W. Induction an upregulation of adhesion receptors in oral and dermal lichen planus. J Oral Pathol Med 1990 ; 19 : 459-463.

43. Sugarman PB , Savage NW , Xu LJ, Walsh LJ, Seymour GJ. Heat shock protein expression in oral lichen planus. J Oral Pathol Med 1995 ; 24 : 1-8.

44. Verdickt GM , Savage NW , Dodd NM , Walsh LJ . Expression of the CD54 (ICAM-1) and CD11a (LFA-1) adhesion molecules in oral mucosal inflammation. J Oral Pathol Med 1992 ; 21 : 65-69.

45. Yamamoto T, Osaki T, Yoneda K, Ueta E. Cytokine production by keratinocytes and mononuclear infiltrates in oral lichen planus. J Oral Pathol Med

Copyright by Mahidol University

1994 ; 23 : 309-315.

46. Yamamoto T, Yoneda K, Ueta E, Hirota J, Osaki T. Serum cytokine levels in patients with oral mucous membrane disorder. J Oral Pathol Med 1991 ; 20 : 275-279.

47. Sylvie B , Jean PO , Branchet MC , Frances C , Beranger JY , Charpentier Y. Alteration of cytokeratin expression in oral lichen planus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1995 ; 79 : 207-215.

48. Becker J, Schuppan D. Altered expression of extracellular matrix proteins and integrins in oral lichen planus (OLP). J Oral Pathol Med 1995 ; 24 : 159-164.

49. Chaiyarit P, Kafrawy AH, Miles DA, Zunt, SL, Van ML, Gregory RL. Oral lichen planus ; an immunohistochemical study of heat shock proteins (HSPs) and cytokeratins (CKs) and a unifying hypothesis of pathogenesis. J Oral Pathol Med 1999 ; 28 : 210-215.

50. Karagouni EE , Dotsika EN , Sklavounou A . Alteration in peripheral blood mononuclear cell function and serum cytokines in oral lichen planus. J Oral Pathol Med 1994 ; 23 : 28-35.

51. Buajeeb W , Pobrurksa C, Kraivaphan P. Efficacy of fluocinolone acetonide gel in the treatment of oral lichen planus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000 ; 89(1) : 42-45.

52. Vincent SD, Fotos PG , Baker KA and Williams TP . Oral lichen planus ; the clinical, historical and therapeutic features of 100 cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1990 ; 70 : 165-171.

53. Thongprasom K , Luengvisut P , Wongwatanakij A , Boonjatturus C. Clinical evaluation in treatment of oral lichen planus with topical fluocinolone acetonide : a 2-year follow-up. J Oral Pathol Med 2003 ; 32(6) : 315-322.

54. David JZ. Treatment of oral lichen planus with topical vitamin A acid. Journal of Oral Medicine 1984 ;39(3) : 186-191.

55. Andreasen JO. Oral lichen planus II. A histologic evaluation of ninety-seven cases. Oral Surg Oral Med Oral Pathol ; 25(2) : 158-170.

56. Manfred S , Gerdt K . Diseases of the oral mucosa a color atlas. 2 ed ed. Illinois : Quintessence ; 1994.

Copyright by Mahidol University

57. Kenneth D, Mc Clatchey, Sol S , Louis SH. Studies on oral lichen planus. III. Clinical and histologic correlation in 213 patients. Oral Surg 1975 ; 39(1) : 122- 129.

58. Andreasen JO. Oral lichen planus II. A histologic evaluation of ninety-seven cases. Oral Surg Oral Med Oral Pathol ; 25(2) : 158-170.

59. Joseph AR , James JS , Anthony AP . Oral and maxillofacial pathology. 1 ed . Philadephpia : W.B.Sauder ; 2000.

60. Eversole LR . Immunopathology of oral mucocal ulcerative, desquamative, and bullous diseases. Oral Surg Oral Med Oral patho 1994 ; 77 : 555-571.

61. Sleasman JW. The association between immunodifficiency and the development of autoimmune disease. Adv Dent Res ; 1996 ; 10(1) :57-61.

62. Cruchley AT , Williams DM , Farthing PM , Lesh CA , Squire CA . Regional variation in Langerhans cells distribution and density in normal human oral mucosa determined using monoclonal antibodies against CD1, HLA-DR, HLA-DQ and HLA-DP. J Oral Pathol Med 1989; 18:510-516.

63. Regazi JA, Stewart CB, Lloyd RV. Immunohistochemical staining of Langerhans cells and macrophages in oral lichen planus. Oral Surg Oral Med Oral Pathol. 1985 ; 60 :396-402.

64. Ishii T. Immunohistochemical demonstration of T cell subsets and accessory cells in oral lichen planus. J Oral Pathol 1987 ; 16: 356-361.

65. Pitigala AA , Crane IJ , Scully C , Prime SS . Epithelial dendritic cells in pathological human oral tissues. J Oral Pathol Med 1989 ; 18 : 11-16.

66. Farthing PM , Matear P , Cruchley AT. The activation of Langerhans cells in oral lichen planus. J Oral Pathol Med 1990 ; 19 : 81-85.

67. Farthing PM, Matear P, Cruchley AT. Langerhans cell distribution and keratinocyte expression of HLADR in oral lichen planus. J. Oral Pathol Med 1992 ; 21 : 451-455.

68. McCartan BE, Lamey PJ. Expression of CD1 and HLA-DR by Langerhans cells (LC) in oral lichenoid drug eruptions (LDE) and idiopathic oral lichen planus (LP). J. Oral Pathol Med 1997 ; 26 : 176-180.

69. Walton LJ, Macey MG,Thornhill MH, Farting PM. Intra-epithelial subpopulations of T lymphocytes and Langerhans cells in oral lichen planus. J Oral

Copyright by Mahidol University

Pathol Med 1998 ; 27 : 116-123.

70. Eversole LR . Immunopathology of oral mucocal ulcerative, desquamative, and bullous diseases. Oral Surg Oral Med Oral patho 1994 ; 77 : 555-571.
71. Sleasman JW. The association between immunodifficiency and the development of autoimmune disease. Adv Dent Res ; 1996 ; 10(1) :57-61.
72. Barrett AW, Scully C. S-100 protein in oral biology and pathology. J Oral Pathol Med 1994 ; 23 : 433-440.
73. Charbit Y , Monteil RA , Hitzig C , Sauget P , Benaiche N, Jasmin JR. S-100 immununolabelling of Langerhans cells in oral epithelium. J Oral Pathol 1986 ; 15 : 419-422.
74. Adi MM , Chisholm DM , Waterhouse JP. Stereological and immunohisto-chemical study of development of human fetal labial salivary glands and their S-100 protein reactivity. J Oral Pathol Med 1994 ; 23 : 36-40.
75. Lovas G, Wysocki G, Daley T, Rowden G. S-100 protein content of dermal and oral mucosal blue nevi. J Oral Pathol 1986 ; 363-365.
76. Fujita S, Takahashi H , Tsuda N, Okabe H. Immunohistochemical localization of S-100 protein and its subunits in melanotic lesions in the oral mucosa and skin. J Oral Pathol Med 1991 ; 20 : 429-432.
77. Lombordi T, Kuffer R, Bernard JP, Fiore DG, Samson J. : Immunohistochemical staining for vimentin filaments and S-100 protein in myxoma of the jaws. J Oral Pathol 1988 ; 17 : 175-177.
78. Joseph AR , Jeffery CB , Ricardo VL, John TH, Ann AM. Immunohistochemical Staining of Langerhans cells and macrophages in oral lichen planus. Oral Surg, Oral Med Oral Pathol 1985; 60: 396-402.
79. Regazi JA, Daniels TE, Saeb F, Nickoloff BJ. Increased submucosal factor XIIIa positive dendrocytes in oral lichen planus . J Oral Pathol Med 1994 ; 23 : 114-118.
80. Kurihara K, Hashimoto N. The pathological significance of Langerhans cells in oral cancer. J Oral Pathol 1985 ; 14 : 289-98.

Copyright by Mahidol University

BIOGRAPHY

NAME	Sub.Lt.Supasawat Siripipatthanakul
DATE OF BIRTH	10 January 1973
PLACE OF BIRTH	Chonburi, Thailand
INSTRITUTIONS ATTENED	Chiang-Mai University 1997 : Bachelor of Doctor of Dental Surgery Mahidol University, 2003 : Master of Science (Oral Medicine)
POSITION & OFFICE	1997-1998 , Department of Medicine, Directorate of Joint Communication, Bangkok, Thailand. Position : Dentist 2000-present, Department of SCOPD Faculty of Dentistry, Mahidol Univ., Thailand Position : Dentist Tel. (66) 0-1665-8452
HOME ADDRESS	327/21 moo 5 , Chonburi, Thailand Tel. (66) 0-3827-6240

Copyright by Mahidol University