IMMUNOHISTOCHEMICAL STUDY OF INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1) IN ORAL LICHEN PLANUS, PREMALIGNANT AND MALIGNANT LESIONS

NIS OKUMA

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NIS OKUMA

Mr. Nis Okuma
Candidate

Waranun Buajeeb

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

Sopee Poomsawat

Assist. Prof. Sopee Poomsawat,
D.D.S., M.Sc. (Oral Pathology),
Ph.D. (Oral Pathology)
Co-Advisor

Prof. M.R. Jisnuson Svasti, Ph.D.
Dean
Faculty of Graduate Studies

Mullika Sirirat

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

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on 31 January, 2006

.....NIS OKUMA.....
Mr. Nis Okuma
Candidate

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

Sopee Poomsawat
.....
Assist. Prof. Sopee Poomsawat
D.D.S., M.Sc. (Oral Pathology)
Ph.D. (Oral pathology)
Member

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

.....
Prof. M.R. Jisnusob Svasti, Ph.D,
Dean
Faculty of Graduate Studies
Mahidol University

Theeralaksna Suddhasthira
.....
Assist. Prof. Theeralaksna Suddhasthira,
B.Sc., D.D.S., Grad. Dip. in Clin. Sc.
(Oral surgery), Dip. Amer. Board of Oral
and Maxillofacial Surgery,
Ph.D. (Biomedical Science)
Dean
Faculty of Dentistry
Mahidol University

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Nis Okuma

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NIS OKUMA 4636127 DTOM/M

M.Sc. (ORAL MEDICINE)

THESIS ADVISORS : WARANUN BUAJEEB, Ph.D.(ORAL BIOLOGY), Dip.Thai Board of Oral Diagnosis Sciences., SOPEE POOMSAWAT, Ph.D.(ORAL PATHOLOGY).

ABSTRACT

Intercellular adhesion molecule-1 (ICAM-1) is an adhesion protein which predominantly interacts with leukocytes. It is accepted as an important adhesion molecule in the inflammatory process in many diseases, including oral lichen planus (OLP). Furthermore, correlation of this molecule and premalignant lesions, as well as malignant lesions is reported, but not in oral premalignant lesions.

The purpose of this study was to compare the expression of ICAM-1 in normal oral mucosa with its expression in OLP, oral premalignant lesions, including OLP with dysplasia, oral leukoplakia with and without dysplasia, as well as oral squamous cell carcinoma. This study was performed using an immunohistochemical technique in paraffin-embedded tissue with monoclonal anti-ICAM-1 antibody. The number of specimens in groups of normal mucosa, OLP without dysplasia, OLP with dysplasia, leukoplakia without dysplasia, leukoplakia with dysplasia, and squamous cell carcinoma were 10, 20, 4, 10, 7, and 10 specimens, respectively. The positive staining cells were counted and calculated into percentage of positive cells in total cells, then analyzed by ANOVA.

The results exhibited constitutive expression of ICAM-1 in normal mucosa with a mean percentage of positive cells as 8.92±1.04 in normal mucosa, 15.05±5.01 in OLP, 17.01±1.06 in OLP with dysplasia, 17.88±2.16 in leukoplakia, 16.71±1.06 in leukoplakia with dysplasia, and 23.41±3.23 in squamous cell carcinoma. The expression of ICAM-1 was statistically increased in OLP, OLP with dysplasia, leukoplakia, leukoplakia with dysplasia, and squamous cell carcinoma, compared with normal mucosa ($p<0.05$). However, there were no significant differences between groups of lesions ($p>0.05$).

The expression of ICAM-1 was increased in OLP, as well as in oral premalignant and malignant lesions. This probably indicates the role of ICAM-1 in inflammatory and immunoregulating processes involved in the pathogenesis of disease.

KEY WORDS : INTERCELLULAR ADHESION MOLECULE-1 / ORAL LICHEN PLANUS / ORAL LEUKOPLAKIA / DYSPLASIA / ORAL SQUAMOUS CELL CARCINOMA

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การศึกษาทางอิมมูโนฮีสโตเคมีสตรีของโมเลกุลยึดเกาะระหว่างเซลล์ในรอยโรคไลเคนพลา นัสช่องปาก, รอยโรคก่อนมะเร็งและรอยโรคมะเร็ง

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นิษณ์ โอกูม่า 4636127 DTOM/M

วท.ม.(เวชศาสตร์ช่องปาก)

คณะกรรมการควบคุมวิทยานิพนธ์ : วรานันท์ บัวจีบ, ปร.ด., อ.ท. (วิทยาการวินิจฉัยโรคช่องปาก), โสภี ภูมิสวัสดิ์, Ph.D. (Oral Pathology)

บทคัดย่อ

โมเลกุลยึดเกาะระหว่างเซลล์เป็นโปรตีนยึดเกาะที่มีหน้าที่หลักในการยึดเกาะกับเม็ดเลือดขาว และเป็นโมเลกุลที่สำคัญในขบวนการอักเสบในหลายๆโรค ซึ่งรวมไปถึงรอยโรคไลเคนพลานัสในช่องปาก นอกจากนี้ยังมีการรายงานถึงความสัมพันธ์กับรอยโรคก่อนมะเร็งและมะเร็งหลายชนิด ดังนั้นการศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบระดับการแสดงออกของโมเลกุลยึดเกาะระหว่างเซลล์ในเนื้อเยื่อปกติกับเนื้อเยื่อที่เป็นโรคโดยวิธีทางอิมมูโนฮีสโตเคมีสตรี โดยใช้ชิ้นเนื้อที่ฝังในพาราฟิน ดังนี้ เนื้อเยื่อปกติ 10 ชิ้น ไลเคนพลานัส 20 ชิ้น ไลเคนพลานัสที่มีเนื้อเยื่อบุผิวเจริญผิดปกติ 4 ชิ้น ลิวโคเพลเคีย 10 ชิ้น ลิวโคเพลเคียที่มีเยื่อบุผิวเจริญผิดปกติ 7 ชิ้น และมะเร็งชนิดสแควมัส 10 ชิ้น โดยนับจำนวนของเซลล์ที่มีการแสดงออกของโมเลกุลยึดเกาะระหว่างเซลล์เปรียบเทียบกับจำนวนเซลล์ทั้งหมดในรูปของร้อยละ

ผลการศึกษาพบว่ามีการแสดงออกของโมเลกุลยึดเกาะระหว่างเซลล์ในระดับต่ำๆในเนื้อเยื่อปกติ โดยมีค่า เฉลี่ยร้อยละของเซลล์ที่แสดงออกของโมเลกุลยึดเกาะนี้เท่ากับ 8.92±1.04 และโมเลกุลนี้มีการแสดงออกมากขึ้นในกลุ่มของรอยโรค โดยพบว่าค่าเฉลี่ยร้อยละของเซลล์ที่มีการแสดงออกของโมเลกุลนี้มีการเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ได้แก่ ไลเคนพลานัส 15.05±5.01 ไลเคนพลานัสที่มีเนื้อเยื่อบุผิวเจริญผิดปกติ 17.01±1.06 ลิวโคเพลเคีย 17.88±2.16 ลิวโคเพลเคียที่มีเนื้อเยื่อบุผิวเจริญผิดปกติ 16.71±1.06 และมะเร็งชนิดสแควมัส 23.41±3.23 แต่ไม่พบความแตกต่างทางสถิติในกลุ่มของรอยโรค ซึ่งจากข้อมูลที่ได้สนับสนุนความสัมพันธ์ของโมเลกุลยึดเกาะระหว่างเซลล์ในพยาธิกำเนิดของรอยโรคไลเคนพลานัส และอาจเกี่ยวข้องกับขบวนการกลายเป็นมะเร็งของรอยโรคทั้งในรอยโรคไลเคนพลานัส และรอยโรคก่อนมะเร็ง

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LIST OF ABBREVIATIONS

CD	=	Cluster of differentiation
CEA	=	Carcinoembryonic antigen
HLA	=	Human leukocyte antigen
ICAM	=	Intercellular adhesion molecule
IFN-γ	=	Interferon gamma
Ig	=	Immunoglobulin
IL	=	Interleukin
LOH	=	Loss of heterozygosity
LFA	=	Leukocyte functional antigen
LP	=	Lichen planus
mAb	=	Monoclonal antibody
MHC	=	Major histocompatibility complex
MIF	=	Macrophage migratory inhibitory factor
MMP	=	Matrix metalloproteinase
N/C	=	Nuclear cytoplasmic
NCAM	=	Neural cellular adhesion molecule
OLP	=	Oral lichen planus
TGF	=	Tumor growth factor
TNF-α	=	Tumor necrosis factor alpha
TNF-β	=	Tumor necrosis factor beta
VCAM	=	Vascular adhesion molecule
VLA	=	Very late antigen

CHAPTER I
INTRODUCTION

Oral lichen planus (OLP) is the most common non-infectious chronic disease, which predominantly affects oral mucosa (1,2). Although this attracts many researchers performing their studies and abundant knowledge is revealed, the etiology and pathogenesis of the disease remain unclear. Nowadays, many studies focus on the mechanisms involved in OLP as well as several types of molecules which drive those mechanisms.

The histological features of OLP, two prominent characteristics are presentation of colloids bodies, which associate with the degeneration of basal keratinocytes, and the accumulation of leukocytes, predominantly T lymphocytes, along subepithelial layer. With this regard, molecules, especially adhesion molecules, which mediate interaction between keratinocytes and T lymphocytes are likely to be required for development of disease.

From the previous studies, the role of adhesion molecules in recruitment and adherence of lymphocytes in the active site of disease was proposed. There are various families of adhesion molecule possibly involved. Among those, intercellular adhesion molecule-1 (ICAM-1), which is the member of immunoglobulin (Ig) superfamily, was accepted as an important adhesion molecule in the inflammatory process, as well as the pathogenesis of OLP. ICAM-1, in addition, serves as a counter-receptor of leukocyte functional antigen-1 (LFA-1), which is members of integrin family of adhesion molecule expressed on all leukocytes. Hence, the upregulation of ICAM-1 on basal keratinocytes facilitates trafficking of lymphocytes to be involved in the pathogenesis of OLP.

Another aspect of OLP, which is the controversy issue, is whether OLP behave as premalignant lesion. Many studies attempted to identify malignant transformation of OLP. Because of the central roles in cell adhesion and cell signaling of the adhesion molecules, adhesion molecules, including ICAM-1, should have a role in

development of malignant transformation of OLP, and probably oral premalignant lesions. In this regard, abundant evidences exhibit the alteration in expression of those molecules in various premalignant and malignant lesions. Surprisingly, the role of adhesion molecules, particularly that of ICAM-1, in oral premalignant lesions have not been studied. Therefore, the data of expression of ICAM-1 in OLP, oral premalignant, and malignant lesions may provide more information in pathogenesis and malignant transformation of these diseases.

Objective of the study

The objective of the study was to evaluate the expression of intercellular adhesion molecule-1 (ICAM-1) on the keratinocytes of OLP lesion compared with that on normal mucosa. In addition, the expression of ICAM-1 in premalignant and malignant lesions, including OLP with dysplastic change, leukoplakia with and without dysplasia, and oral squamous cell carcinoma was determined.

Hypothesis

1. Expression of ICAM-1 on keratinocytes was higher in OLP tissue compared with those in normal mucosa.
2. Expression of ICAM-1 on keratinocytes in OLP tissues was lower than those in OLP with dysplasia, leukoplakia, and squamous cell carcinoma.

Benefits of this study

The result of ICAM-1 expression, may confirm the role of this molecule in the pathogenesis of OLP. Furthermore, it may affect on treatment modality of OLP. In order to downregulate the inflammatory process, inhibition of ICAM-1 function by anti-ICAM-1 application possibly gain benefits.

In addition, comparison of expression of ICAM-1 in OLP, OLP with dysplasia, as well as leukolplakia, and squamous cell carcinoma may reveal the association of this adhesion molecule in malignant transformation and progression.

CHAPTER II
LITERATURE REVIEW

ADHESIVE INTERACTION MEDIATED BY ADHESION MOLECULES: FOCUSED ON ICAM-1

Cell-cell interaction, which is direct interaction between cells, as well as between cells and the extracellular matrix, are critical to the development and function of multicellular organisms. Some cell-cell interactions are transient, such as the interactions between cells of the immune system and the interactions that direct white blood cells to sites of tissue inflammation. In other cases, stable cell-cell junctions play a key role in the organization of cells in tissues. For example, several different types of stable cell-cell junctions are critical to the maintenance and function of epithelial cell sheets.

Cell-cell adhesion is a selective process, such that cells adhere only to other cells of specific types. This selective cell-cell adhesion is mediated by transmembrane proteins called **cell adhesion molecules**, which can be divided into four major groups:

1. The selectins
2. The integrins
3. The immunoglobulin (Ig) superfamily
4. The cadherins

The cell-cell interactions mediated by the selectins, integrins, and members of the Ig superfamily are transient adhesion in which the cytoskeletons of adjacent cells are not linked to one another. Stable adhesion junctions involving the cytoskeletons of adjacent cells are instead mediated by the cadherins, in which cadherins are linked to cyto- skeletons of neighbor cells to form cell-cell junctions: adherens junctions and desmosomes.

Adhesion molecules in inflammatory process

In order to initiate and maintain inflammatory process of host defense system, it is clear that leukocyte-leukocyte and leukocyte-target cell adhesion are necessary events. There are three important steps of leukocyte attachment during inflammation, including

1. Leukocyte-endothelial cells adhesion must be occurred proir to migration of leukocytes from blood vessels to sites of inflammation.
2. Lymphocyte-antigen presenting cells adhesion must be taken place for subsequent antibody production or generation of T cell in the specific immunological processes.
3. Leukocytes-target cells adhesion must be occurred for leukocytes to perform their functions, as target cells lysis.

At the time of inflammation occurred in any location, initial attachment between leukocytes and endothelial cells are mediated by the selectin. There are two members of the selectins family involved: L-selectin, which is expressed on leukocytes, and E-selectin, which is expressed on endothelial cells. Because of unstability of these interactions, they must be followed by the formation of more stable adhesion. This process involves two types of adhesion molecule : the integrin on the surface of leukocyte and intercellular adhesion molecule (ICAM), which are members of the Ig superfamily expressed on the surface of endothelial cell. When leukocytes firmly attach to endothelial cells, they are able to penetrate the wall of blood vessel and migrate extravasation to the inflammatory site.

INTERCELLULAR ADHESION MOLECULES-1 (ICAM-1 or CD54)

ICAM-1 or CD54 is a member of the immunoglobulin (Ig) superfamily. It is a cell surface adhesion protein which serves as a counter receptor for the integrin leukocyte functional antigen-1 (LFA-1; CD11a/18), which is distributed on all leukocytes (14,15). Structurally, ICAM-1 is a single chain molecule expressing 5 tandem immunoglobulin-like domains followed by one transmembrane segment and short cytoplasmic tail (16). This molecule is constitutively expressed on unstimulated endothelial cells (17,18,29), but it is inducible *in vitro* on multiple cell types including

leukocytes (19), endothelial cells (21), epithelial cells (22-24), and fibroblasts (18) with cytokines such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), tumor necrosis factor-β (TNF-β), interferon-γ (IFN-γ) depending on cell types (18,20-22,26,29). The predominant function of ICAM-1 is in the recruitment and trafficking of leukocytes via interactions with leukocyte-expressed integrins

ICAM-1 in the inflammatory response

There are several evidence suggesting that ICAM-1 play a role in the inflammatory response, including :

1. ICAM-1 is expressed on multiple cell types at sites of inflammation (15,18,25-28).
2. ICAM-1 is markedly increased on venular endothelial cells at inflammation sites (18,25,26)
3. Anti-ICAM-1 monoclonal antibodies inhibit *in vitro* assays of inflammatory such as cytotoxic T cell activity (13), granulocyte and lymphocyte attachment to endothelium (26,30,36), and inhibition of leukocyte migration and aggregation (14,31-33,37).

In order to migrate to the inflammatory site, leukocytes must leave the vessel lumen and cross the endothelial barrier into tissue compartment. Before transendothelial migration of leukocytes, they must form the stable attachment to endothelial cell. In this regard, ICAM-1 on the endothelial surface plays a crucial role in the formation of a physical docking structure for adherent leukocytes (34,47). This interaction is composed of ICAM-1 on endothelial cell surface and LFA-1 which expressed on the surface of leukocytes. These informations suggest that ICAM-1 expression on endothelial cell and others cell types are critical for the normal function of host defense system.

In this recent years, in order to understand the role of ICAM-1 in the inflammatory process, there are many studies focused on the effects of anti-ICAM-1 monoclonal antibodies (mAb). The earliest experiments demonstrated that anti-ICAM-1 mAb inhibited neutrophil influx into rabbit lungs following systemic activation with phorbol ester (33), providing the evidence that anti-ICAM-1 mAb blocked function of neutrophil. Furthermore another study revealed that anti-ICAM-1

mAb also mitigated eosinophil influx and airway hyperresponsiveness in a non-human primate model of antigen-induced airway hyperresponsiveness (28). These suggested a potential clinical role for the use of anti-ICAM-1 mAb in both acute and chronic pulmonary diseases. More study on the anti-ICAM-1 mAb were tested in a model of immunological kidney allograft rejection. This experiment revealed that the time to rejection of allogeneic kidneys in non-human primate recipients was prolonged when anti-ICAM-1 mAb was given as the role form of immunosuppressive therapy (32). This finding provided the evidence of the ability of a mAb to ICAM-1 to inhibit lymphocyte function.

The findings revealed from many studies support a role for the use of anti-ICAM-1 in a number of diseases. The specific anti-ICAM-1 selected for clinical trials is an IgG2a, which binds to domain 2 of ICAM-1. This antibody, called BIRRI, blocks both lymphocyte and neutrophil adhesion, without disturbance of bactericidal or phagocytic functions of granulocytes (27,31). The first clinical areas selected for study with BIRRI have been solid organ transplantation and rheumatoid arthritis.

ICAM-1 expression in cutaneous epithelium

In normal condition, cutaneous epithelial cells are lacking of ICAM-1, but its expression can be induced in response to inflammatory cytokines such as IL-1β, TNF-α, IFN-γ and IL-6 (12,15,18,24,29,38). ICAM-1 is found to be induced on cutaneous keratinocytes from sensitized subject exposed to patch certain irritant (27,39). Along with the enhanced levels of ICAM-1, a monomuclear cell infiltration presented, followed by clinical inflammatory response. Moreover, ICAM-1 is also expressed on keratinocytes of cutaneous lesions such as lichen planus, pemphigoid, and pemphigus (12,27).

ICAM-1 expression in oral keratinocytes

In contrast to skin, oral mucosal keratinocytes constitutively express ICAM-1 at a high level with increased expression by IFN-γ and TNF-α (24). This finding is contrary to some investigators, whose result showed the absence of ICAM-1 expression on normally mucosal keratiocytes (12,40). However, in the inflammatory

condition of oral mucosa, such as in oral lichen planus (OLP) or contact lichenoid lesions, ICAM-1 showed upregulated expression on keratinocyts (12,24,40). Interestingly, one study demonstrated that there was no association between ICAM-1 expression and the present of OLP (25).

ICAM-1 and the role as a tumor suppressor

In order to damage tumor cells, the step of conjugation between tumor infiltrating lymphocytes and their target, induces tumor cell death and natural killer cell mediated cytolysis. Theoretically, upregulation of ICAM-1 enhances the host immune response against tumor, but tumor cell dissemination, in practical, is enhanced because of the attachment of tumor cell to circulating lymphocytes

ICAM-1 seems to render bladder tumor cells vulnerable to nonantigenic specific cytotoxicity mediated by activated lymphocytes. ICAM-1 is also involved in cell mediated lysis of transitional carcinoma cells after the binding of lymphokine activated killer cell. *In vitro* experiments showed that malignant cells in which abnormal ICAM-1 expression was not corrected by IFN-γ treatment had a more aggressive phenotype (96). Therefore, it seems that abnormal ICAM-1 expression facilitates the avoidance of immunological surveillance.

ICAM-1 and therapeutic implications

According to the role of cell adhesion in the pathogenesis of OLP, new treatment modalities are focused on the alteration of adhesion molecule expression.

1. The use of antibodies to proinflammatory cytokines such as TNF-α, IFN-γ may show benefits. The evidence demonstrated the effect of anti- IFN-γ on abrogation of experimentally-induced epidermal lichenoid reaction (42).
2. The use of antibodies to specific adhesion, such as anti-ICAM-1, which interfere leukocytes adhesion, may reduce tissue infiltration and damage as reported for chronic inflammation in asthma (28).
3. The use of cytokines which downregulate adhesion molecule expression, such as IL-8 (43), tumor growth factor- β (TGF-β) (44), and IL-1 inhibitor (45).

4. The use of immusuppressive agents which alter adhesion molecule expression and impaired lymphocyte function may be of benefit. For example, cyclosporine therapy is benefit on cutaneous and mucosal lichen planus (46).

ORAL LICHEN PLANUS: THE PATHOGENESISAND MALIGNANT TRANSFORMATION

Lichen planus (LP) is a chronic inflammatory disease with unclear etiology which either separately or in combination involving skin and mucous membrane. Particularly oral lesion is the most common non-infectious oral mucosal disease in patients referred to Oral Medicine clinic (1,2).

Epidemiology

Oral manifestation of LP is more common than cutaneous lesion. While cutaneous LP affects approximately 0.23%, oral lesion has incidence of 1.9% of general population (1-4). Most patients suffered from OLP are in midlife, predominantly in adults over 40, with sex predilection of female than male at a ratio of approximately 1.4:1 (1).

According to the survey of elderly dental patients in Thailand, OLP was presented in 2.8% of all subjects. Among that group, 4.3% of female subjects are affected, whereas only 0.5% in male groups were reported (5).

Clinical Features of OLP

OLP represents in different characters, including white reticular, papular, plaque-like, atrophic, erosive, or bullous. While reticular, atrophic, and erosive forms are the most commonly reported (50,53,56). Lesions often present as the mixture of clinical subtypes. As white linear or reticular pattern usually appears on the erythematous background (*Fig. 1*). In addition, most of OLP cases show reticular keratotic striation in some area of the oral mucosa. The presence of those characteristics are useful features in clinically distinguish OLP from other vesiculoerosive diseases, such as pemphigoid, pemphigus, and linear IgA disease.

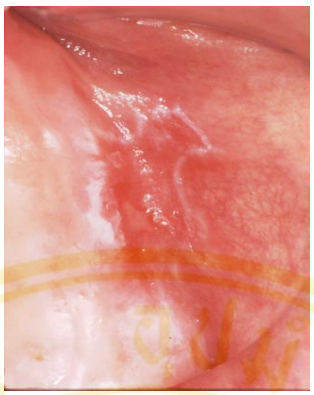

Figure 1 Clinical characteristic of oral lichen planus, reticular pattern on erythematous background.

Pathogenesis of OLP

Although the precise etiology of OLP remains unknown, the current evidence proves that immunological aspect is involved and plays a major role in pathogenesis of this disease. Several cell types and molecules, such as leukocytes, adhesion molecules, and cytokines, are altered their expressions at sites of OLP (6). In particular, T-cell lymphocyte is the predominant leukocyte taking part in the development of the disease (7). The two major subpopulations of T cells including CD4 and CD8 are presented in OLP, with greater numbers of CD8 as 1:2 in ratio of CD4 to CD8 (8). In addition to the role of T lymphocyte, many documents showed the alteration of expression of other elements. Among those, one type of cell probably plays a role in the disease is antigen presenting Langerhans cells, although there is no direct evidence proving the role of this cell in the antigen presentation process in OLP. From previous studies, the expression of Langerhans cell was vary, while some of those reported the increasing number (9), the others found no overall change (10) but with increasing only in areas of activated epithelium (11). In order to understand the pathophysiology of the disease, many studies have attempted to identify the complexities of cell surface adhesion receptors. Many types of adhesion molecule are identified as the adhesion receptors involved in the disease progression, such as intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), and some in the integrins family (12,13). Among those, significantly increased expression of ICAM-1 on endothelial cells and basal keratinocytes are reported in OLP (6,8,13). Expressions of those molecules are regulated by various types of

cytokines that are secreted by resident keratinocytes, Langerhans cells, and leukocytes. Those inflammatory cytokines include IFN-γ, TNF-α, IL-1α.

As mentioned above, the upregulations of adhesion molecule expressions are demonstrated in OLP (6,8,12,13). Those are the evidences indicating the important role of such molecules on the lymphocyte trafficking, which is the critical process of the disease. In particular, ICAM-1 is supposed to be an essential molecule for mediation of adhering interaction of lymphocytes and the other cells as well as the extracellular matrix proteins and keratinocytes.

Role of ICAM-1 in the pathogenesis of OLP

As mentioned previously, although the etiologic and pathogenesis knowledge of OLP is still unclear, immunopathogenetic aspect is believed to play a critical role. Most prominent in histopathology of OLP is the hydropic degeneration of the basal cell layer and lymphocytic trafficking to the submucosal-basal lamina-basal keratinocyte interface. In this regard, the pathogenesis of this process requires various types of adhesion molecule for migration and sticking of activated T cell.

Accordingly, ICAM-1 is often found to be enhanced expression on cutaneous and mucosal keratinocytes during inflammatory reaction of the diseases (12,13,24,27,40). In this respect, TNF-α, IFN-γ are functionally important mediators for upregulation of the expression of ICAM-1 by oral keratinocytes, whereas IL-1 has no effect on skin keratinocytes, but induced a small increase in oral keratinocytes (24). Activated T cells, which carry LFA-1, the ligand for ICAM-1, then may be able to adhere to basal cells of the epithelium. In addition, these same lymphocytes express ICAM-1 as well and thereby provide for lymphocyte-lymphocyte clustering. These processes allowed the localization of T cells. Then further pathogenesis of the disease proceeds, which are mediated by interaction between T cells and antigen presenting cells. However, some studies revealed lacking of ICAM-1 on basal keratinocytes in OLP, only expression on intraepithelial Langerhans cells and microvascular endothelial cells was demonstrated (25).

In addition to upregulation of expression of ICAM-1 on basal keratinocytes, OLP also exhibits increased levels of ICAM-1 on endothelial cells in the subepithelial vessels (12,13). These may facilitate transendothelial migration of leukocytes to the

active inflammatory sites in OLP, then resulting in the maintenance or persistence of LP lesion.

Moreover, it reveals not only the interactions between T lymphocytes and antigen presenting cells stimulate the proliferation of T cell. But also the cell signaling from T cell, which recognizes the antigen by the presentation of major histocompatibility complex (MHC), is able to induce the proliferation of T cell clone which could not recognize that antigen (41). This interaction requires T-T cell contact. In this regard, increased expression of T cell surface adhesion molecules LFA-1, ICAM-1, and LFA-3 during an inflammatory response found to be effected on rapid recruitment of T cells. These findings showed how the small amount of antigen/MHC-specific T cells can recruit large numbers of non-antigen-specific T cells in the generation of an inflammatory process.

Malignant Transformation in OLP

In 1978, the World Health Organization (WHO) proposed the definition of precancerous lesion as a tissue with altered morphology, at a higher risk of malignant transformation than the corresponding morphologically normal tissue, and the precancerous condition as a general process associated to a significantly greater risk of developing cancer (48,49). Accordingly to those, LP and other lesions, such as discoid lupus erythematosus, syphilis, and submucous fibrosis, are defined as precancerous conditions. Based on the previous studies, many documents demonstrated malignant potential of OLP. WHO has reported that 2-3% of LP cases undergo malignant transformation (49). While another studies established 0.14-3.33% of OLP cases with malignant transformation (50-59). Among those, squamous cell carcinoma showed the highest numbers, while the other type as verrucous carcinoma was reported in small numbers. Based on the clinical pictures, the erosive and atrophic forms of OLP are more frequently associated with malignant transformation, whereas the other forms are occasionally encountered, as plaque forms with a small numbers of reticular type (50,51, 53-55,56,58). The data of time from diagnosis of OLP to malignant recognition demonstrated the wide range of time, from less than 1 month to 17 years, with the most frequency of 6-8 years (50-59). Based on the location of OLP lesion, malignant transformation is more frequently found on the

tongue (50,51,54,55,56) and buccal mucosa (54,56,57,59) than the other sites of OLP.

The cause of increased risk of malignant transformation in OLP patient is still unclear. The possible etiological theories were proposed (59);

1. OLP itself transform into carcinoma, implicating the truly premalignant of OLP.
2. The altered surface epithelium in erosive or atrophic forms could increase susceptibility to carcinogen.
3. A carcinoma could appear coincidentally in the area affected by OLP.

According to those theories, it is still controversial issue whether OLP itself can transform to carcinoma. Although the affected area of OLP, either erosive or atrophic, may increased susceptibility to exogenous mutagens in tobacco, alcohol, and *Candida albicans*, recently report series of OLP cases undergoing malignant transformation had no known carcinogen (50,52-55,58). According to Krutchkoff and Eisenberg, they proposed the term *lichenoid dysplasia* for the lesion representing clinically as OLP but demonstrating epithelial dysplasia on histological finding (60), and they defined lichenoid dysplasia as a premalignant lesion. The concept of lichenoid dysplasia was supported by Lovas *et al.* (61). Therefore, the inflammatory response induced by an immune reaction against dysplastic epithelial in such premalignant lesion, leads to inflammatory process resemble to lichenoid lesion (60,61). According to Krutchkoff, topographic and cytologic features of dysplasia included two or more of the following characteristics (60):

1. Significantly increased nuclear size, which usually manifests by increased N/C ratios.
2. Cellular pleomorphism.
3. Altered or disturbed epithelial maturation.
4. Nuclear hyperchromasia.
5. More than sporadic foci of premature of abnormal keratinization.
6. Abnormal mitotic figures.
7. Lack of cellular cohesion which often demonstrates as notable intercellular fluid accumulation or edema that accompanies any of the six preceding parameters.

In contrast to Krutchkoff, several studies were focused on the determination of premalignant potential of OLP on the molecular basis. Alterations in genetic material seem to increase their interests. In particular, loss of heterozygosity (LOH) on chromosome 3, 9, and 17, which are found to be associated in progression to squamous cell carcinoma, were slightly found in OLP without dysplastic change (62-64). Whereas the greater numbers of LOH were demonstrated in the various degree of epithelial dysplasia (63). In contrast, the other study revealed the alteration of chromosome 9 on epithelial cells in OLP and lichenoid dysplasia, whereas no alteration on chromosome 17 was found among OLP, lichenoid dysplasia, and oral squamous cell carcinoma (65).

Because of the central role of basal cells in epithelial proliferation and differentiation, genetic instability of OLP is thought to be induced by the repetitive process of proliferation and destruction of basal cells which increased the risk of genetic error, though a carcinogen is exist or not (66).

Other studies focused on the expression of various types of cytokine in the role of carcinogenesis of OLP. Several cytokines released from subepithelial T cells, such as TGF-β1, TNF-α , IFN-γ , IL-12, are function as tumor suppressors (67-69), and probably inhibit carcinogenesis in OLP. In contrast, overexpression of keratinocyte-derived TGF-β1, normally weakly expressed by keratinocytes (67), was thought to enhance carcinogen-induced tumorigenesis (70). Furthermore, other cytokines such as macrophage migration inhibitory factor (MIF) stimulate tumor cell proliferation and suppress the transcription activity of p53 tumor suppressor proteins (71,72). This cytokine play a major role in delayed-type hypersensitivity reaction, although it has not been reported in OLP (73). In addition, matrix metalloproteinase-9 (MMP-9) derived from mast cells, neutrophils and macrophages also enhance cutaneous carcinogenesis (74).

EXPRESSION OF ICAM-1 IN PREMALIGNANT AND MALIGNANT LESIONS AND ITS ROLE IN MALIGNANT TRANSFORMATION

Role of Adhesion Molecules in Malignancies

Malignant transformation and the progression to metastasis are characterized by a severe aberration in cell-cell interactions. Connections to neighboring cells are disturbed and the tumor cells migrate away from their normal location, invade the surrounding connective tissue, enter the vascular and lymphatic systems, and then, exit to take up residence in foreign environments. Molecules controlling intercellular adhesion and directing cell interactions are likely to be important in many of these steps and might be expected to demonstrate dramatic changes in expression during tumor progression.

The changes of expression of adhesion molecules mediating homophilic adhesion have been observed. Some molecules such as E-cadherins, an important molecules in the organization of epithelial cell layers, were decreased their expression on a variety of carcinoma cell lines *in vitro* (75,76). This may contribute to the disruption of the normal epithelial-epithelial interactions, an important early step in freeing cells from contact-mediated positional and regulatory controls.

Tumor progression is characterized not only by the loss of normal cell interactions but also by the acquisition of new interactions. Intra-and extravasation, for example, depend on adhesion to endothelia, while the establishment of secondary growth depends on adhesive interactions with cells of a ‘foreign’ environment. Therefore, in addition to a downregulation of normally expressed adhesion molecules, as those of cadherins, one expected to find the expression of new cell adhesion molecules. Such newly expressed molecules might be expected to mediate heterotypic adhesion interacting with ligand present on non-tumor cells and serving to guide the tumor cells as they separate from the primary tumor and establish distant secondary growths. In support of this is the recent discovery that molecules mediating leukocyte adhesion to activated endothelium can appear as tumor-associated molecules in a variety of different solid tumors. The integrin very late antigen-4 (VLA-4) mediates adherence of lymphocytes to activated endothelium through its interaction with the inducible endothelial cell adhesion molecule VCAM-1. While expression of this β1

integrin in benign tissues is restricted to lymphoid cells, it has been found to characterize malignant melanocytes as well (77). Another study of metastasis of transfected rhabdomyosacroma cells by α2 integrin revealed increased metastatic properties of these tumor cells which is mediated by VLA-2 (78). This situation was the result of upregulation of adhesiveness to collagen and laminin in extracellular matrix. Hence, this molecule has been shown to mediate adhesion of the tumor cells to endothelium *in vitro* and are predicted to play a role in their extravasation *in vivo*. However, some types of integrins show the tumor suppression effect, as the overexpression of α5: β1 led to reduce tumerigenesis (79).

The identification of a progression–associated melanoma antigen as the intercellular adhesion molecule-1 (ICAM-1), provides another example of the expression by tumor cells of molecules mediating heterotypic cell adhesion. This molecule mediates leukocyte adherence and has been shown to enhance immune recognization and target effecter interactions of cells expressing it. The study measured the levels of ICAM antigen circulating in blood and correlated them with the behavior of melanoma. It had been revealed that expression of ICAM-1 by melanomas is associated with a poor prognosis and the development of metastatic disease (80).

The Expression of adhesion molecules and squamous cell carcinomas

In the defense mechanism against skin tumors, the recognition and presentation of tumor-associated antigens by antigen presenting cells to resident lymphocytes are requirement. These are followed by the induction of a specific immune response. Therefore, cell surface molecules are possibly important in suppression of tumor development.

The cadherins family

Cadherins are the most important of all adhesion molecules, and when they are expressed, the inactivation of other cell-cell adhesion molecules has little effect. E-cadherins and N-cadherins participate in the formation of adherens junctions, which interact with cytoskeletal elements of the cells via catenins α, β, γ. The study on expression of cadherins in oral cancer exhibited the downregulation of E-cadherins (81, 82). Also, downregulation of γ –catenin was reported, suggesting downregulation

of desmosomal proteins. Furthermore, some studies showed the tumor invasive suppressor property of this family of adhesion molecule (83).

The integrins family

Integrins, which mediate cell-cell and cell-matrix interaction, are expressed at very high density at the cell surface. The majority of them are substratum adhesion molecules, which function as receptors for proteins of the extracellular matrix. Other can function as cell adhesion molecules which are primarily found on leukocytes. They mediate the interaction to the members of immunoglobulin superfamily of adhesion molecules, such as ICAM-1, -2, and VCAM.

Integrins play the central role in cell adhesion and migration and their normal function is critical in the induction and differentiation of cells *in vitro*. The alteration of their expression are exhibited in transformed cell lines and in tumors. These molecules are implicated in tumor progression and metastasis**.**

In oral squamous cell carcinoma, downregulation of β1 integrins and α6β4 has been reported. The alteration of integrins implicate the role of extracellular in carcinogenesis. In a recent study, the expression of α2-6 integrins was strong in metastatic oral squamous cell carcinoma compared with non-metasatic cell lines (84). Additionally, the expression of αV is also altered, with αVβ6 being expressed in oral cancer (85). This result suggested the important role of such molecule in tumor spreading and migration.

Immunoglobulin Supergene family

The members of this family include intercellular adhesion molecules-1 (ICAM-1), vascular adhesion molecules-1 (VCAM-1), neural cellular adhesion molecule (NCAM) and carcinoembryonic antigen (CEA). VCAM, which is inducible by cytokines serves as a receptor for α4β1 integrin and VLA-4 on leukocytes and malignant cells, such as melanoma. Accordingly, VCAM-1 may serve as an adhesion receptor for malignant cells expressing VLA-4. ICAM-1 is also altered its expression in oral squamous cell carcinoma, but not VCAM-1. ICAM-1 was also being expressed by peritumoral endothelial cells and leukocytes (86). This result suggested that the upregulation of ICAM-1 possibly enhanced binding of leukocytes to tumor cells.

Expression of ICAM-1 in Malignant Diseases

ICAM-1, a member of the immunoglobulin superfamily, binds to the integrins LFA-1. The interaction of ICAM-1 with LFA-1 involves in inflammatory responses and may contribute to the onset and progression of numerous diseases including cancer. Expression of ICAM-1 has been examined in a number of neoplasms, including chronic lymphocytic leukemia (87), malignant melanoma (88), breast ductal carcinoma (89) and squamous cell carcinoma.

Expression of ICAM-1 relates to the progression of many malignant lesions. In malignant melanoma, ICAM-1 expression, encompassing with β3 integrins, is associated with enhanced metastatic capacity, possibly by permitting monocyte to reduce adhesion between the neoplastic cells. Interestingly, a study revealed that soluble ICAM-1 isolated from the sera of melanoma patients could inhibit effector cell lysis of melanoma cells, presumably by binding to LFA-1 on the effector cells (90). Furthermore, soluble ICAM-1 inhibits such melanoma cell-tumor-infiltrating lymphocyte cell interaction and blocks the induction of tumor-infiltrating lymphocyte cytokines.

Many studies in the expression of ICAM-1 in malignant cells demonstrated the upregulation of this molecule in the membrane of neoplasm cells. ICAM-1 is involved in leukocyte recognition and destruction of tumor cells. The presence of increased concentration of ICAM-1 render cells more liable to lysis by leukocyte-activated killer cells. In this regard, expressions of ICAM-1 on tumor cell surface facilitate the tumor cell lysis cytotoxic immune response, and possibly correlate with disease presentation and progression. Therefore, in case of downregulation of ICAM-1 expression on cell surface, tumor cells are able to escape from cytolytic effector leukocytes and undergo metastasis. In addition, the expression of ICAM-1 is in response to cytokine INF-γ and TNF-α produced by leukocytes (92).

Although many documents exhibited the upregulation of ICAM-1 of tumor cells, some studies demonstrated the downregulation of its expression on tumor cells. For instance, in oesophageal carcinoma, the decreased expression of MHC class I, class II, and ICAM-1 are reported (91). In this regard, it is possible that the neoplastic cells of

such cancer probably produce immunosuppressive factors, such as TGF-β1, which antagonizes or inhibits the production of inflammatory cytokines, such as IFN-γ.

For squamous cell carcinoma, a study on malignant transformation of keratinocytes demonstrated ICAM-1 positive keratinocytes in tissue of carcinoma, whereas occasional and absent expression of that in premalignant and normal tissue, respectively (93). According to the result, the modification in the immune response has an important role in the process of transformation from premalignancy into squamous cell carcinoma. Other studies focused on the adhesion molecules expression in oral cancer exhibiting consistent expression of ICAM-1 and other molecules, such as integrins and selectins. Such expression probably permits enhanced binding of leukocytes to tumor cells. The expression of ICAM-1, in contrast to human leukocyte antigen-DR (HLA-DR), is constitutively at low levels on squamous cell carcinoma cell line with upregulation of the antigen's expression occurring after IFN-γ treatment (92). In this regard, it could be proposed that low levels of ICAM-1 expressed on the surface of neoplastic cells serve to initiate lymphocyte movement into the tumor microenvironment, with subsequent local production of the cytokines IFN-γ and TNF-α resulting in induction of HLA-DR and ICAM-1. Moreover, the other cytokine involved in progression of oral cancer is IL-1, which is induced from monocytes by nicotine and arecoline. In the initial stages of malignant development, IL-1 probably serves as an attractant for leukocytes, and in established keratoses and 'leukoplakia' may be in part responsible for the increased number of lymphocytes present within the connective tissues. These actions are related to the ability of IL-1 to stimulate the expression of adhesion molecules, as well as their ability to stimulate the production of other cytokines that promote leukocyte infiltration (95).

CHAPTER III
MATERIALS AND METHODS

Tissue samples

In this study, six groups of sample tissues; normal mucosa, OLP with and without dysplasia, oral leukoplakia with and without dysplasia, and oral squamous cell carcinoma, were included. Normal mucosa specimens were obtained from 10 volunteers who gave informed consent to use the tissue specimens for research purposes. All specimens were taken from normal appearing buccal mucosa. They were fixed in 10% neutral buffered formalin and then embedded in paraffin for immunohistochemical analysis. Twenty specimens of OLP, four specimens of OLP with dysplastic change, ten specimens of oral leukoplakia without dysplastic change, seven specimens of oral leukoplakia with dysplastic change, and ten specimens of oral squamous cell carcinoma were retrieved as paraffin-embedded blocks from the Department of Oral Pathology, Faculty of Dentistry, Mahidol University and Khon Kaen University. The previous diagnosis was confirmed by reassessment of an oral pathologist .

Immunohistochemical study

Specimens were cut into 5 μm sections from paraffin-embedded blocks. Sections were deparaffinized in xylene and rehydrated through graded alcohol before quenching in 3% hydrogen peroxide for 15 minutes to block endogenous peroxidase activity. Then sections were treated with 0.4% pepsin in 0.01 M HCl at 37^0C for 40 minutes for antigen retrieval. The sections were cooled at room temperature for 10 minutes before equilibrating in phosphate-buffered saline (PBS) for 5 minutes, followed by application of 2% bovine serum albumin with 2% goat serum for 30 minutes to block nonspecific binding sites. The blocked sections were then covered with a mouse monoclonal anti-human CD54 (ICAM-1) antibody (20020951; CHEMICON International, Inc. 28835 Single Oak Drive, Temecula, CA 92590) in a

dilution of 1:500 in 0.05 M Tris buffer (pH 7.6) at room temperature for 2 hours. After washing in PBS, biotinylated IgG and streptavidin-biotin peroxidase complex (Strep ABComplex/ HRP Duet kit, Dako S/A Glostrup, Denmark) were applied to the sections for 30 minutes each and followed by three washes of PBS. Color was developed in freshly made diaminobenzidine (DAB chromogen tablets, Dako Corporation, Carpinteria, CA, USA). The sections were washed briefly in running tap water and lightly stained with Mayer's hematoxylin. Negative controls were obtained by omission of the primary antibody.

Evaluation

In each sample, 5 areas of epithelium were randomized as representation of the sample. By using the objective lens of 40 magnification and under a 100 mesh grid, the number of total cells and ICAM-1 positive cells in each area were collected. Although the representative areas were placed randomly, total cell count in each specimen had to be greater than 300 cells. Then, after complete collecting of data, the number of total cells and ICAM-1 positive cells from 5 areas were summed. The percentage of positive cells in total cells were presented. The degree of ICAM-1 expression was analyzed by comparison of percentage of positive cells from each group.

Statistical analysis

Mean percentage of positive cells in each specimen was tested normal distribution with Kolmogorov- Smirnov test. One way ANOVA was used to assess difference of positive cells in six groups. The difference of each couple in six groups was analyzed with Games-Howell. Statistical analysis was performed using a computer software package, SPSS version 13.0 (SPSS Inc., Chicago, IL). Statistical significance was considered at $p<0.05$.

CHAPTER IV
RESULTS

Characteristic and distribution of positive staining cells

ICAM-1 staining cells were found as dark brown staining line along cell membrane. On the normal epithelium, ICAM-1 was expressed in low level. Those positive staining cells were mostly basal keratinocytes, and occasionally spinous cells (*Fig.2*). The number of positive cells was increased in groups of OLP, OLP with dysplasia, leukoplakia with and without dysplasia. In OLP, positive cells were found in lower layer of prickle cells, and also in superficial layer (*Fig.3*). In group of leukoplakia, with and without dysplasia, positive cells were mostly found in basal layer, while some groups of spinous cells were also stained diffusely (*Fig.4,5*). ICAM-1 expression in squamous cell carcinoma was found both in normal appearing epithelium, which laid over cancer mass, and in cancerous area. (*Fig.6)*. In addition to epithelium, ICAM-1 was also expressed on membrane of lymphocytes, fibroblasts, and endothelial cells in subepithelial connective tissue (*Fig.7*).

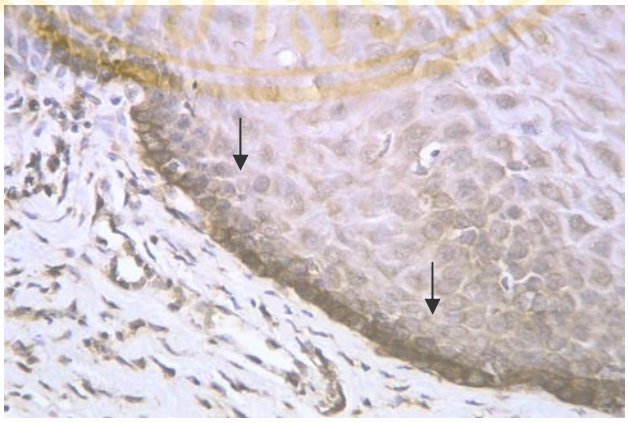

Figure 2. ICAM-1 positive staining cells in normal mucosa. ICAM-1 positive cells are noted along basal keratinocytes (arrows). Streptavidine peroxidase, x40

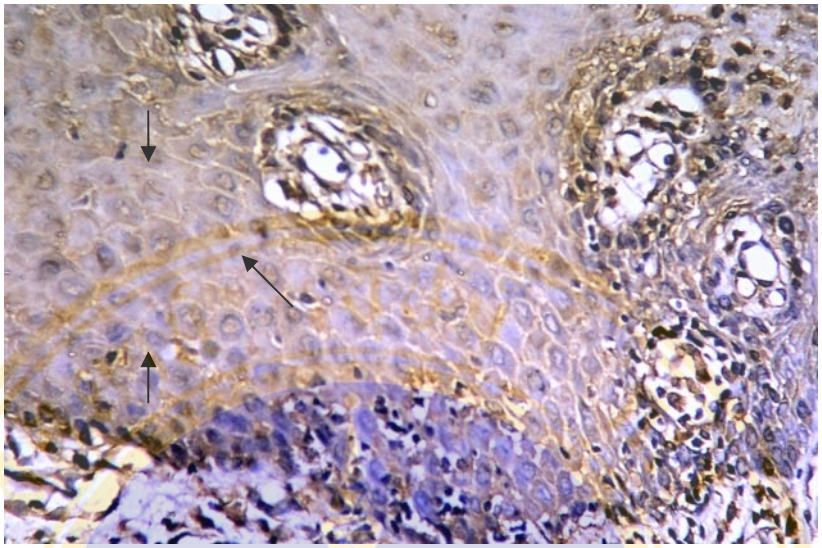

Figure 3. ICAM-1 positive staining cells in an OLP lesion (arrows). Streptavidine peroxidase, x40.

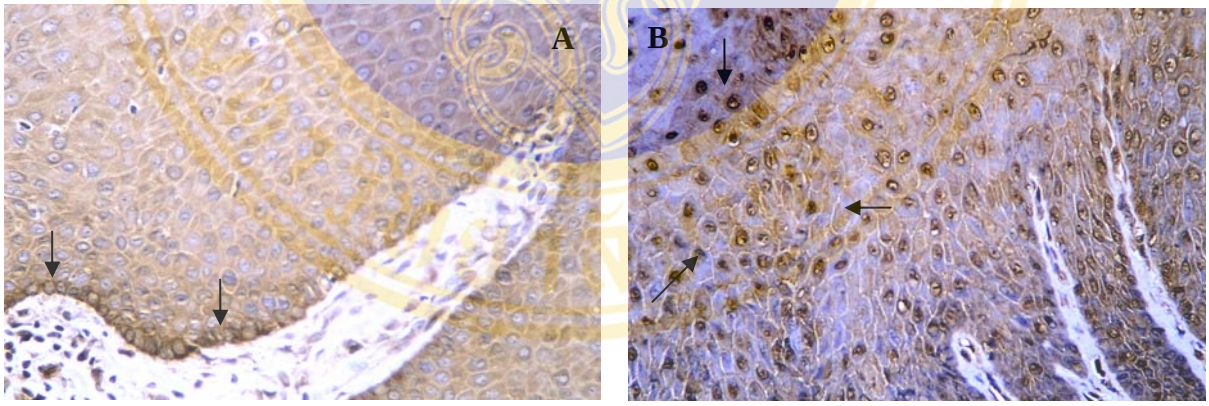

Figure 4. ICAM-1 expression in oral leukolplakia without dysplasia. Expression is found on both basal keratinocytes (A) and prickle cells (B). Streptavidine peroxidase, x40.

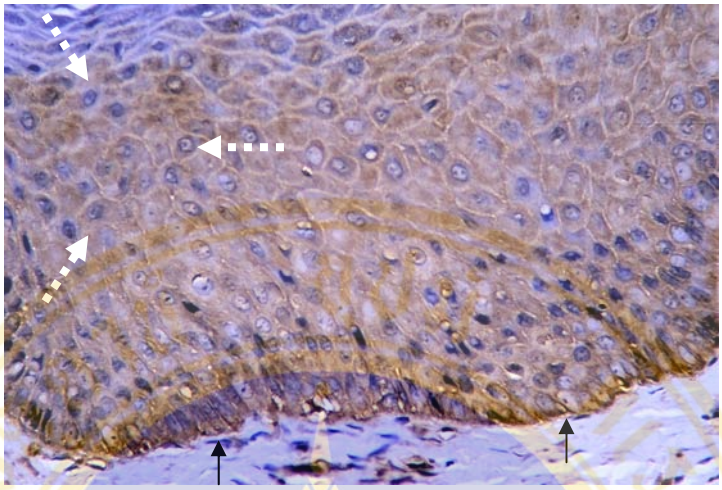

Figure 5. ICAM-1 expression in oral leukoplakia with dysplasia. Positive cells are basal keratinocytes (black arrows) and spinous cells (white arrows). Streptavidine peroxidase, x40.

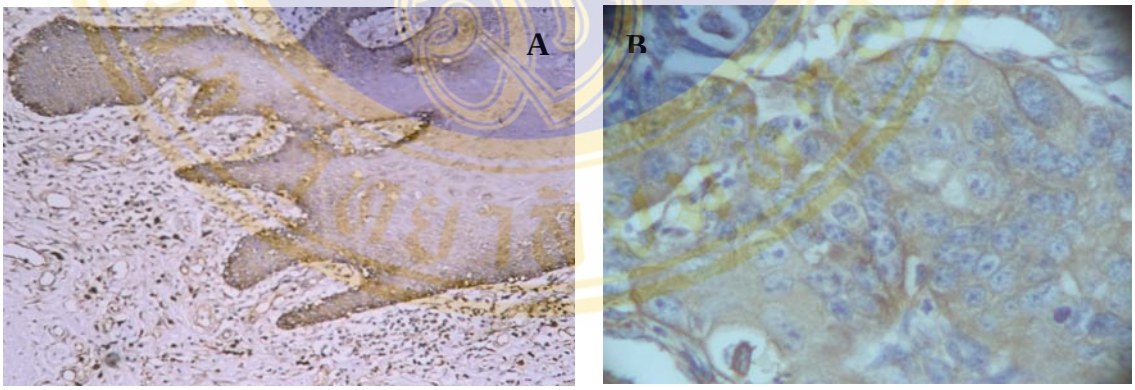

Figure 6. ICAM-1 expression in squamous cell carcinoma, keratinocytes in covering epithelium (A), and cancer cell (B). Streptavidine peroxidase, x40.

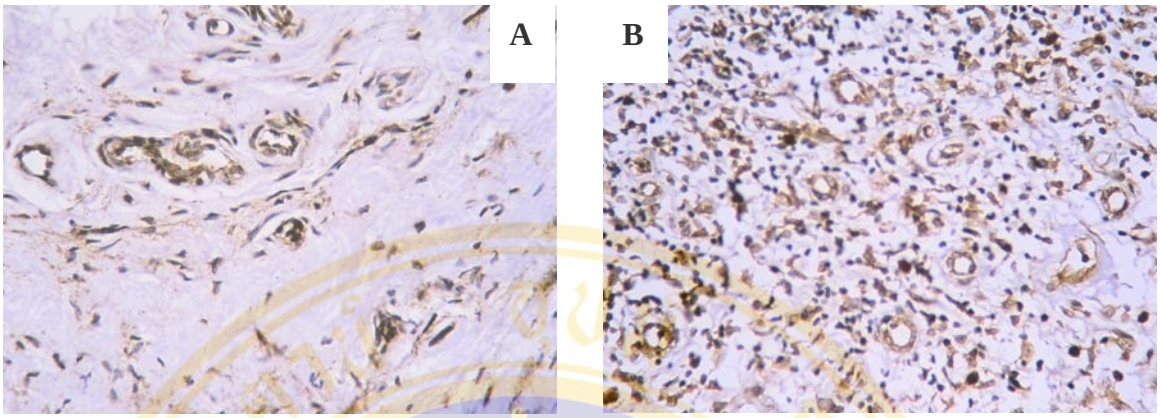

Figure 7. ICAM-1 expression in inflammatory cells, fibroblasts and endothelial cells of blood vessels in subepithelial connective tissue, in normal mucosa (A) and in OLP (B). Note markedly expression of ICAM-1 on endothelial cells both in normal mucosa and OLP. Streptavidine peroxidase, x40.

ICAM-1 expression in normal mucosa

Normal mucosa showed low level of expression of ICAM-1 on basal keratinocytes. Total cell count and number of positive cells were shown in Table 1.

In the specimens of normal mucosa, a mean total of 11,797 cells were counted. Among those, 1,052 cells were positive. The percentage of positive cells varied from 6.36-14.44 with a mean percentage of 8.92.

Table 1 Total cell count, number of positive cells, and percentage of positive cells of normal mucosa

Specimen no.	Total cell count	No. of Positive cells	Percentage of positive cells
1	1,039	93	8.95
2	1,035	134	12.95
3	1,396	114	8.17
4	1,230	140	11.38
5	1,786	93	5.21
6	1,098	79	7.19
7	1,084	83	7.66
8	912	58	6.36
9	1,011	146	14.44
10	1,233	112	9.08
Total	11,797	1,052	8.92
Mean (SD)	1,182.4 (80.03)	105.2 (9.15)	***8.92 (1.04)***

ICAM-1 expression in OLP and OLP with dysplasia

In OLP specimens, 20 specimens were included. Total cell count and positive cells were shown in Table 2. While group of OLP with dysplasia included 4 specimens, and the results were shown in Table 3.

A mean total cell count of OLP group was 14,724. Among those 2,040 cells were positively stained. A mean percentage of positive cells were 15.05, with a variety of percentage ranging from 7.35-36.15.

Likewise, in group of OLP with dysplasia, a mean total cell count was 2,604, and the mean number of positive cells was 443. A mean percentage of positive cells were 17.01.

Table 2 Total cell count, numbers of positive cells, and percentage of positive cells of OLP.

Specimen no.	Total cell count	No. of positive cells	Percentage of positive cells
1	843	62	7.35
2	1,088	82	7.54
3	738	94	12.74
4	685	99	14.45
5	1,049	175	16.68
6	552	96	17.39
7	525	104	19.81
8	645	44	6.82
9	814	106	13.02
10	533	94	17.64
11	811	136	16.77
12	632	102	16.14
13	763	104	13.63
14	1,179	103	8.74
15	865	99	11.45
16	928	79	8.51
17	426	154	36.15
18	706	137	19.41
19	499	67	13.43
20	443	103	23.25
Total	14,724	2,040	15.05
Mean (SD)	736.2 (48.01)	102 (6.83)	***15.05 (1.51)***

Table 3 Total cell count, number of positive cells, and percentage of positive cells of OLP with dysplasia.

Specimen no.	Total cell count	No. of positive cells	Percentage of positive cells
1	536	107	19.96
2	723	107	14.80
3	738	126	17.07
4	607	103	16.97
Total	2,604	443	17.01
Mean (SD)	651 (48.23)	110.75 (5.17)	***17.01 (1.06)***

Although the upregulation of ICAM-1 on keratinocytes was revealed in OLP groups, the correlation between degree of expression on keratinocytes and numbers of subjacent lymphocytes was not observed.

In addition to epithelium, the expression of ICAM-1 in various cells in subepithelial connective tissue was also observed. These cells included fibroblasts, and vascular endothelial cells. Furthermore, subepithelial infiltrating lymphocytes focally expressed ICAM-1 in some area.

ICAM-1 expression in leukoplakia and leukoplakia with dysplasia

In leukoplakia, positive cells were found on both basal and spinous keratinocytes. Positive-staining basal keratinocytes were observed in linear pattern along the basal layer, which similar to those in normal mucosa. However, 2 samples differently exhibited with focal expression in some area of basal keratinocytes. For 10 specimens of leukoplakia, the number of total cells and positive cells were shown in Table 4. Similar to leukoplakia, leukoplakia with dysplasia expressed ICAM-1 on basal and spinous keratinocytes. The results of 7 samples were shown in Table 5.

Table 4 Total cell count, the number of positive cells, and percentage of positive cells of leukoplakia.

Specimen no.	Total cell count	No. of positive cells	Percentage of positive cells
1	1,125	315	25.93
2	1,128	306	24.92
3	1,864	512	27.47
4	1,599	279	17.45
5	1,553	359	23.12
6	1,752	300	17.12
7	1,542	195	12.65
8	1,468	165	11.24
9	1,878	201	10.70
10	1,381	136	9.85
Total	15,480	2,678	17.88
Mean (SD)	1,529 (84.26)	276.8 (34.91)	***17.88 (2.16)***

Table 5 Total cell count, number of positive cells, and percentage of positive cells of leukoplakia with dysplasia.

Specimen no.	Total cell count	No. of Positive cells	Percentage of positive cells
1	1,308	246	18.81
2	1,149	149	12.97
3	877	126	14.37
4	1,291	244	18.90
5	1,365	268	19.63
6	994	134	13.48
7	861	144	16.72
Total	7,845	1,311	16.71
Mean (SD)	1,120.71 (79.8)	187.29 (23.46)	***16.71 (1.06)***

ICAM-1 in squamous cell carcinoma

For malignancy, 10 specimens of squamous cell carcinoma were included in the study. ICAM-1 positive cells were found on both covering epithelium and neoplastic cells. In epithelial layer, keratinocytes expressing ICAM-1 were mostly on basal layer, and in some specimens on prickle cells. The results were shown in table 6.

Table 6 Total cell count, numbers of positive cells, and percentage of positive cells of squamous cell carcinoma.

Specimen no.	Total cell count	No. of positive cells	Percentage of positive cells
1	1,206	165	13.68
2	1,017	236	23.21
3	809	201	24.85
4	1,072	170	15.46
5	644	251	38.98
6	1,070	259	24.21
7	880	312	35.45
8	1,213	172	14.18
9	979	195	19.92
10	857	321	37.46
Total	9,747	2,282	23.41
Mean (SD)	974.7 (56.79)	228.2 (18.12)	***23.41 (3.23)***

In additional, leukocytes infiltration also found in specimens of carcinoma, however, ICAM-1 positive-staining lymphocytes were not significant difference compared with normal mucosa.

Table 7 Total cells and number of positive cells of all 6 study groups.

Group	No. of specimens	Total cell count	No. of positive cells	Percentage of positive cells (SD)
Normal mucosa	10	11,797	1,052	8.92 (1.04)
OLP	20	14,724	2,040	15.05 (1.51)
OLP with dysplasia	4	2,604	443	17.01 (1.06)
Leukoplakia without dysplasia	10	15,480	2,678	17.88 (2.16)
Leukoplakia with dysplasia	7	7,845	1,311	16.71 (1.06)
Squamous cell carcinoma	10	9,747	2,282	23.41 (3.23)

Statistic analysis of expression of ICAM-1

ICAM-1 were differently expressed among the study groups (*Fig. 8*). The comparison of percentage of positive cells among 6 study groups was analyzed by ANOVA. The results showed significant difference in the percentage of positive cells among the 6 groups (*Table 8*).

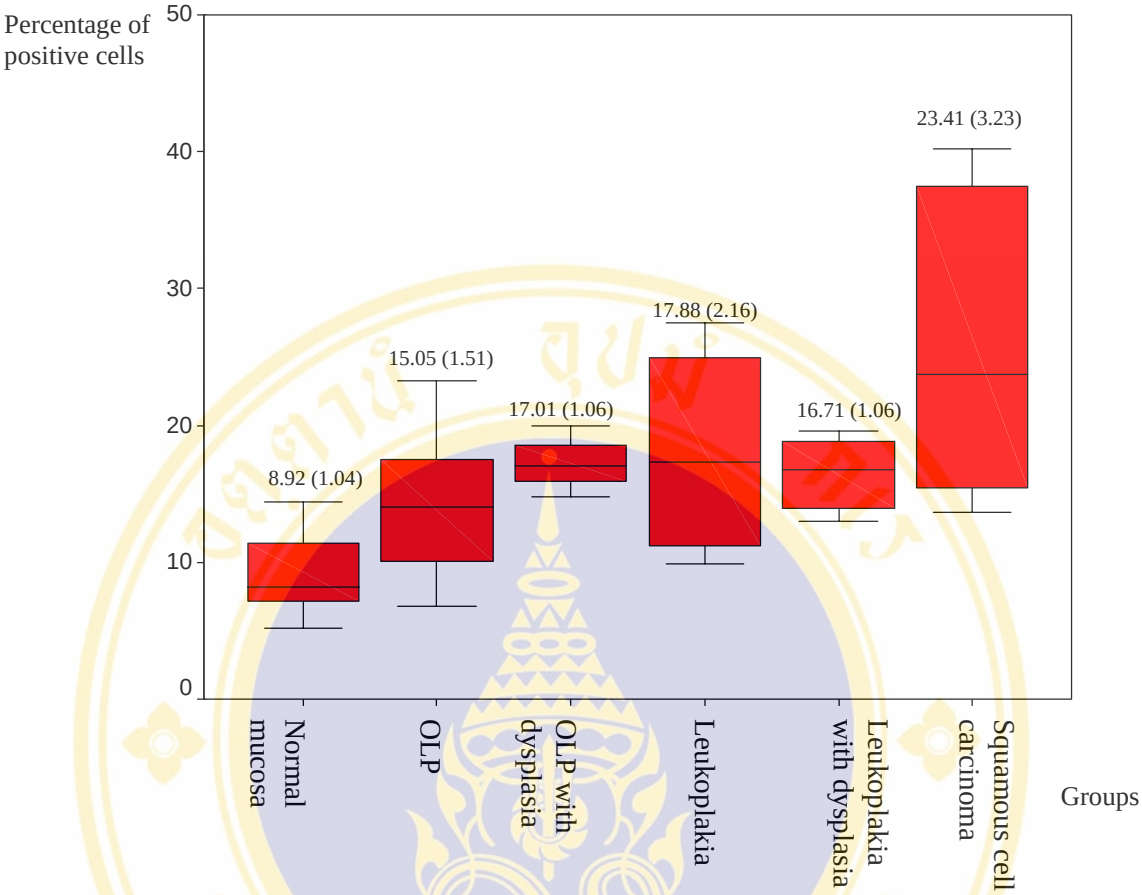

Figure 8 The expression of ICAM-1 in 6 sample groups. *Boxplots*, Mean (SD).

Table 8 One way ANOVA analysis of percentage of positive cells in 6 groups.

	Sum of squares	**df**	**Mean Square**	**F**	**Sig.**
Between groups	1308.297	5	261.659	5.971	.000
Within groups	2366.470	54	43.824		
Total	3674.767	59			

Then multiple comparison was performed by using Games-Howell analysis. The results revealed significant increased expression of ICAM-1 in OLP, OLP with dysplasia, leukoplakia, leukoplakia with dysplasia, and squamous cell carcinoma, compared with normal mucosa ($p<0.05$). However, there were no significant differences between groups of lesions (*Table 9*).

Table 9 Multiple comparison : Games-Howell analysis

(I) GROUP	(J) GROUP	Mean Difference (I-J)	p-value
Normal mucosa	**OLP**	-5.8959(*)	.036
	OLP with dysplasia	-8.0544(*)	.004
	Leukoplakia	-8.8994(*)	.026
	Leukoplakia with dysplasia	-7.2659(*)	.003
	Squamous cell carcinoma	-16.0404(*)	.006
OLP	**Normal mucosa**	5.8959(*)	.036
	OLP with dysplasia	-2.1585	.845
	Leukoplakia	-3.0035	.859
	Leukoplakia with dysplasia	-1.3699	.974
	Squamous cell carcinoma	-10.1445	.111
OLP with dysplasia	**Normal mucosa**	8.0544(*)	.004
	OLP	2.1585	.845
	Leukoplakia	-.8450	.999
	Leukoplakia with dysplasia	.7886	.993
	Squamous cell carcinoma	-7.9860	.255
Leukoplakia	**Normal mucosa**	8.8994(*)	.026
	OLP	3.0035	.859
	OLP with dysplasia	.8450	.999
	Leukoplakia with dysplasia	1.6336	.981
	Squamous cell carcinoma	-7.1410	.472
Leukoplakia with dysplasia	**Normal mucosa**	7.2659(*)	.003
	OLP	1.3699	.974
	OLP with dysplasia	-.7886	.993
	Leukoplakia	-1.6336	.981
	Squamous cell carcinoma	-8.7746	.182
Squamous cell carcinoma	**Normal mucosa**	16.0404(*)	.006
	OLP	10.1445	.111
	OLP with dysplasia	7.9860	.255
	Leukoplasia	7.1410	.472
	Leukoplakia with dysplasia	8.7746	.182

(*) The mean difference is significant at $p \leq 0.05$.

CHAPTER V
DISCUSSION

This study focused on the expression of intercellular adhesion molecule-1 or ICAM-1 in OLP, with and without dysplasia, oral leukoplakia, with and without dysplasia, and oral squamous cell carcinoma. Its expression was detected by immunohistochemical technique, and percentage of positive cells in each lesion was compared with that in normal mucosa.

Expression of ICAM-1 in normal mucosa

The results revealed various degree of expression in all sample groups, even normal mucosa. The present study exhibited low level of constitutive expression, although many studies reported no expression of ICAM-1 on keratinocytes in normal condition (12,40). This result was similar to others studies that reported the expression of ICAM-1 in normal condition (24,98,99). In this study, the expression of ICAM-1 in normal mucosa was mostly on basal layer of the epithelium, while the expression of keratinocytes in higher layer was rarely found.

To visualize the expression of ICAM-1 in keratinocytes, several techniques are performed. Some studies used the immunohistochemical technique in frozen biopsy sections (12,40), whereas some performed their studies on cell culture, which different results were noted (24,98,99). In normal mucosa, while the former technique revealed no expression of ICAM-1 in keratinocytes, the latter showed constitutive expression. However, Dorrego *et al.* which observed slight expression of ICAM-1 in normal tissues by using immunohistochemistry in frozen tissues (103). In this study, immunohistochemical technique was used in paraffin-embedded tissues, with the results that mentioned above. In this regard, this study revealed weak presence of lymphocytes

in submucosal layer of normal tissues which might be the result of ICAM-1 expression. Although the precise relationship between those was not revealed. The possible mechanisms probably either ICAM-1 played a role in trafficking lymphocytes, or its expression was secondarily due to lymphocytic infiltration.

In addition, ICAM-1 was also expressed by leukocytes, fibroblasts, and markedly found on vascular endothelial cells in submucosal area. This result was similar to the others studies which revealed the expression of ICAM-1 on vascular endothelial cells (13,40).

ICAM-1 in OLP

The OLP specimens which included in this study were clinically, histologically and immunofluorescent diagnosed as *oral lichen planus*. Furthermore, there was no evidence, clinical and historical, of lichenoid lesion, neither contact nor drug induced.

In contrast to normal mucosa, this study exhibited enhancement of expression of ICAM-1 in spinous layer of OLP specimens. This information was supported by the results of previous studies (12,40,103). According to those studies, ICAM-1 expression was required for activating and sticking of lymphocytes, that were the crucial mechanism for development of OLP. However, the level of expression of basal keratinocytes was lower than in normal, this may be related to indistinction of basal cells in the lesions.

In the opposite aspect, the expression of ICAM-1 by keratinocytes as the secondary to accumulation of lymphocytes and their cytokines were proposed (24,25). The proinflammatory cytokines IL-1, IFN-γ, and TNF-α have been shown to upregulate the expression of ICAM-1 by oral keratinocytes *in vitro* (24). Therefore the release of those cytokines by leukocytes during the development of OLP may account for the expression of ICAM-1 by keratinocytes. In this regard, ICAM-1 on keratinocytes were not required for lymphocytic migration.

The result from this present study could not identify whether ICAM-1 initiates the disease mechanism. ICAM-1, however, plays a role in assistance of trapping lymphocytes, reflecting to chronicity of disease, no matter expression of ICAM-1 initiates pathogenesis of OLP or not.

This study also revealed expression of ICAM-1 in some groups of lymphocytes among the abundant of lymphocytic infiltration in submucosal layer. This finding was similar to the others studies which also exhibited the expression of ICAM-1 on lymphocytes in OLP samples (13,40). According to study of Shiohara, proliferating process of T cells required the interactions between T lymphocytes and antigen presenting cells. In addition, the cell signaling from T cells recognizing the antigen by the presentation of MHC was able to induce the proliferation of T cell clones which did not recognize that antigen (42). This interaction requires T-T cell contact. In this regard, increased expression of T cell surface adhesion molecules LFA-1, ICAM-1, and LFA-3 during an inflammatory response found to be effected on rapid recruitment of T cells. These findings showed how the small amount of antigen/MHC-specific T cells can recruit large numbers of non-antigen-specific T cells in the generation of an inflammatory process. Therefore, the presence of ICAM-1 on lymphocytic infiltration in OLP probably contribute wide spread of inflammatory site of the disease.

Although the precise mechanism of ICAM-1 in development and progression of the disease is still unclear, the results from the previous and this study showed the strong relationship of this molecule and pathogenesis of OLP. Because of the central role of ICAM-1 in trafficking leukocytes into site of inflammation, level of expression of this molecule probably reflect degree of inflammation and chronicity of disease.

ICAM-1 in leukoplakia

In this study, leukoplakia exhibited significantly higher expression of ICAM-1 than in normal mucosa, although its expression was not statistically significant difference among the others groups, including OLP with and without dysplasia, leukoplakia with dysplasia, and squamous cell carcinoma.

As the previous study, nicotine, the major product of cigarette smoking, has capacity to raise level of prostaglandin E2 and IL-1 releasing from monocytes (101). Dustin *et al.* and Kvale *et al.* reported the effect of IL-1 in induction of ICAM-1 expression in various cell types (18,22). According to those, leukoplakia, which is closely associated with smoking habit, should reveal upregulation of ICAM-1 expression (104-106).

From the literature, the expression of ICAM-1 on keratinocytes in leukoplakia has not been reported. Therefore, the evidence of role of this molecule in the pathogenesis of disease was not available. However, based on the previous knowledge, this molecule is probably induced by IL-1, subsequently nicotine consumption. Furthermore, another mechanisms, as well as cytokines, may be involved in the mechanism of ICAM-1 expression in leukoplakia.

In addition to epithelial keratinocytes, leukoplakia also presented the larger numbers of chronic inflammatory cell infiltration in lamina propria. These cells rarely expressed ICAM-1. This finding probably associated with increased number of ICAM-1 expression on epithelial keratinocytes.

Another possible pathway contributing to expression of ICAM-1 focuses on the inflammatory process against the cellular transformation. According to the study of Wawryk *et al.*, ICAM-1 expression is enhanced by inflammatory cytokines, responsible to cellular transformation (100). Based on a previous study, oral leukoplakia without histological dysplasia was revealed molecular alteration, such as p53 protein and Ki-67 antigen (107). Hence, the upregulated expression of ICAM-1 in leukoplakia probably responsible to cellular transformation within lesion.

Although, the role of ICAM-1 in pathogenesis or development of leukoplakia could not be revealed in this study, ICAM-1, which induced its expression somehow, may trigger inflammation at site of lesion, as the evidence of increasing number of leukocytes in the specimens.

ICAM-1 in dysplastic tissue

This group included OLP with dysplasia and leukoplakia with dysplasia. The result showed that expression of ICAM-1 on epithelial keratinocytes in these lesions were significantly increased compared with normal epithelium. However, there was no significant difference among the others groups of lesion, including OLP, leukoplakia without dysplasia, and squamous cell carcinoma.

These results may be explained in two proposed different mechanisms. The first explanation, similar to leukoplakia without dysplasia, focused on the inflammatory response against cellular transformation that involved in dysplastic process (100). Many studies identified the alteration at cellular level of epithelial dysplasia,

including p53 protein and Ki-67 antigen, and Bcl-2 protein (108,109). Therefore, in this regard, OLP with the evidence of dysplastic change should have increased ICAM-1 expression, compared with OLP without dysplasia. In the same manner, leukoplakia with cellular atypia should show higher level of ICAM-1 expression in keratinocytes than in leukoplakia without dysplasia. From the results of this study, OLP with dysplasia slightly increased expression of ICAM-1 in keratinocytes, though it was not statistically significant. In contrast, groups of leukoplakia exhibited the different outcomes in this study, as leukoplakia with dysplasia showed slightly lower level of expression of ICAM-1 than in leukoplakia without dysplasia. This probably reflected from the size of sample, and severity grade of dysplasia. The group of leukoplakia with dysplasia included smaller sample size ($n=7$) than other study groups, and most of them were graded as mild dysplasia.

Another proposed mechanism is inflammation-induced cellular transformation. Many studies identified DNA damage and mutation resulted from chronic inflammation. These events increase risk of cellular dysplasia (109,110). In this regard, chronic inflammatory process which normally presents in both OLP and leukoplakia possibly contributes to dysplastic process. Therefore, in opposing to the mechanism mentioned above, ICAM-1 that involves in the chronicity of inflammation might be related to dysplastic changes.

From the present study, ICAM-1 in dysplastic tissues of OLP showed slight upregulation of expression, and slight decrement in leukoplakia with dysplasia, compared with OLP and leukoplakia without dysplasia, respectively. According to those, the information was not adequate for identification of whether its expression was either a subsequent effect of cellular transformation or a role in the pathogenesis of dysplasia.

ICAM-1 in squamous cell carcinoma

Many studies attempted to identify the malignant transformation and progression of cancer, by focusing on a group of adhesion molecules. Tucci *et al.* described the role of immune response modification in the process of transformation of premalignant lesion into cancer (93). They revealed upregulation of ICAM-1 expression in tissue of squamous cell carcinoma, when compared with premalignant

lesion and normal tissue. The result of present study was consistent to their findings. ICAM-1 expression in group of cancer was highest among all study groups, though there were no statistically significant differences between groups of lesions. The expression was found in covering keratinocytes, as well as neoplastic cells. Moreover, many studies that work on cell cultures also revelaed the similar results, which squamous cell carcinoma cell line was markedly found expression of ICAM-1 when compared to normal cells line and transformed cells (98,99).

The upregulation of ICAM-1 in malignant lesions is probably contributed by leukocyte-derived cytokines. According to the previous study, T lymphocyte could produce cytokines against tumor cells, including IFN-γ and TNF-α as well as nitric oxide that derived from macrophages (97,112). These mediators are responsible to enhance expression of ICAM-1 on cell membrane of squamous cell carcinoma (92,113).

Because ICAM-1 was proposed as an important molecule for conjugate formation between tumor infiltrating lymphocytes and their target, inducing tumor cell death and natural killer cell mediated cytolysis. Therefore, theoretically, upregulation of ICAM-1 could enhances the host immune response against tumor (96). Subsequently, cancer cells are rendered susceptible to be destroyed by leukocytes.

In malignant lesions, tumor cell spreading is one of the invasive property . As the study of Li *et al.*, implanted primary tumors showed strong expression of ICAM-1, whereas the tumor cells of metastatic lesions showed weak or negative expression of ICAM-1 (114). Therefore, upregulation of ICAM-1 expression on tumor cells may mediate the reduction of tumor cell invasiveness.

In addition, malignant tissues also exhibited the expression of ICAM-1 on membrane of infiltrating lymphocyte. The study of Kornfehl *et al*. reported strong expression of ICAM-1 in lymphocytes and macrophages (111). This expression of ICAM-1 enhance cytotoxic effect of lymphocytes against tumor cells (115).

Although the certain role of ICAM-1 in carcinogenesis is unclear, its part in development and progression of cancer is more obvious. The elevation of ICAM-1 expression in cancer cells provides the susceptible condition for lymphocytes migration and function on tumor cells.

CHAPTER VI
CONCLUSION

This study observed the expression of ICAM-1 in normal mucosa, OLP with and without dysplasia, leukoplakia with and without dysplasia, and squamous cell carcinoma by immunohistochemial technique in paraffin-embedded tissues. The results exhibited low level of constitutive expression of ICAM-1 in basal keratinocytes in normal condition. This molecule was significantly increased its expression in OLP, OLP with dysplasia, leukoplakia, leukoplakia with dysplasia, and squamous cell carcinoma, compared with normal mucosa. Although there were no significant differences between groups. However, squamous cell carcinoma showed the highest level of expression. While the expression of ICAM-1 was mostly found in basal keratinocytes in normal mucosa, it expressed in the basal and spinous layers in groups of lesion. Furthermore, in comparison of dysplastic and non-dysplastic lesions, OLP-OLP with dysplasia and leukoplakia-leukoplakia with dysplasia, there were not significant differences.

In OLP, the elevation of ICAM-1 expression, compared with normal mucosa, indicated its involvement in pathogenesis of this inflammatory disease, though the precise role was not revealed. ICAM-1 is also induced in premalignant lesions, as shown in OLP with dysplasia, leukoplakia with and without dysplasia, as well as squamous cell carcinoma. The data implied the role of ICAM-1 in immunoregulating response involved in development and progression of lesions.

Further studies are required for clarification of role of ICAM-1 in the process of cellular transformation and carcinogenesis in OLP and premalignant lesions, as well as progression and metastasis of malignant lesions. And these may be leading to development of treatment modalities of these lesions on the basis of ICAM-1.

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APPENDIX

GROUP	Kolmogorov-Smirnov(a)		
	Statistic	df	Sig.
Normal Mucosa	.192	9	.200(*)
OLP	.149	20	.200(*)
OLP with dysplaisa	.274	4	.
Leukoplakia	.185	10	.200(*)
Leukoplakia with dysplasia	.233	7	.200(*)
Squamous cell carcinoma	.230	10	.200(*)

* This is a lower bound of the true significance.

a Lilliefors Significance Correction

Appendix 1. Test of normality, by using Kolmogorov-Smirnov test. The result revealed normal distribution of data in all study groups.

F	df1	df2	Sig.
3.492	5	54	.008

Appendix 2. Levene's Test of Equality of Error Variances.

BIOGRAPHY

NAME	Mr. Nis Okuma
DATE OF BIRTH	15 September 1978
PLACE OF BIRTH	Chaingmai, Thailand
INSTITUTIONS ATTENDED	Mahidol University, 2002 Bachelor of Doctor of Dental Surgery Mahidol University, 2006 Master of Science (Oral Medicine)
POSITION & OFFICE	Department of Oral Medicine, Faculty of Dentistry, Mahidol University, Bangkok, Thailand Position : Lecturer Tel. (02) 6448644-6 ext. 3415-17 E-mail : harrypotter3938@yahoo.com
HOME ADDRESS	55/12 Soi Suparaj 1, Paholyothin Rd, Samsennai sub-district, Pyathai district, Bangkok, 10400 Tel. (02) 2726627