EXPRESSION OF KERATINOCYTE INTEGRINS IN ACUTE AND CHRONIC INFLAMMATION OF THE HUMAN PERIODONTIUM

by

KIRSI HAAPASALMI (LARJAVA)

Licentiate of Dental Science, The University of Turku, Finland 1993

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Department of Oral Biological and Medical Sciences)

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

September 1997

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Oral Biological & Medical Sciences

The University of British Columbia
Vancouver, Canada

Date Oct 6, 1997

DE-6 (2/88)

ABSTRACT

Epithelium plays a crucial role in protection, destruction and repair of human mucosal and epidermal tissues. Integrins compose a family of heterodimeric transmembrane glycoproteins that mediate cell adhesion and information transfer from extracellular milieu into the cytoplasm. In the present work, the expression of integrins in human mucosal and epidermal wound keratinocytes during acute (wound healing) and chronic inflammation (periodontitis) was investigated. Integrins and their ligands were localized in frozen sections using immunohistochemistry. During wound healing, expression of αvß6 integrin was induced relatively late during wound healing. Maximal expression was observed in 7-day-old wounds, in which epithelial sheets had joined and granulation tissue was present. Fibronectin and tenascin, both possible ligands for αvß6 integrin, were concentrated underneath the basal keratinocytes expressing this integrin. During chronic inflammation, focal loss of integrins and basement membrane components was common in periodontal pocket epithelium. In some local areas, however, upregulation of integrin expression was observed. The results suggest, that during acute inflammation, the reservoir of the keratinocyte integrins increases to correspond to new demands for keratinocyte migration and gene regulation. During chronic inflammation, however, the expression of integrins in keratinocytes is variable, suggesting focal areas of integrin up- and down-regulation that may possibly reflect the areas of healing and destruction in the periodontium.

TABLE OF CONTENTS

LIST OF FIGURES

ACKNOWLEDGEMENTS

I would like to thank Dr. Hannu Larjava and Dr. Veli-Jukka Uitto for their guidance and support during my studies. I would like to thank you for your patience that you showed during this long period of time that it took from me to finish this program.

I would like to thank Dr. Don Brunette for his suggestions and insight as a member of my research committee.

I would like to thank all the co-authors in the two publications that this work has produced. In particular, I would like to acknowledge Dr. Dean Sheppard and Dr. Kenneth Yamada for their generosity for providing some of the antibodies used in this study.

I am thankful for Mr. Cristian Sperantia for his technical support during this project.

I would like to thank all my friends at UBC who have supported me during these years.

I can not thank enough my loving husband, Hannu, for his endless love, patience and support he has given me during these years.

Last I would like to greatfully acknowledge the financial support that I have received during this program from following sources:
Finnish Cultural Foundation
Student Clinicians of American Dental Association, Henry M. Thornton Fellowship
Women Dentists of Finland
Faculty of Graduate Studies, UBC, Dr. George S. Beagrie Award

ABBREVIATION

LM-5	laminin-5
LM-1	laminin-1
RGD	arginine-glycine-aspartic acid
BPA	bullous pemphigoid antigen
FAK	focal adhesion kinase
TGFß	transforming growth factor ß
JE	junctional epithelium

INTRODUCTION

During healing and destruction of human epithelial tissues, cell adhesion receptors of the integrin family play an important role (Larjava et al., 1996). Integrins are responsible for interactions of epithelial cells with the extracellular matrix and to some extend for cell to cell adhesion. Integrins also convey signals from the extracellular mileu into the nucleus regulating gene expression. Alterations in the expression of integrins and cell adhesion proteins in keratinocytes during human and animal wound healing have been previously reported (Larjava et al., 1993; Gailit and Clark, 1994; Watt and Jones, 1993). Larjava and co-authors (1993) have shown that the expression of αv-integrins is induced in keratinocytes of mucosal wounds. Others have confirmed the induction of this integrin subunit in healing cutaneous wounds (Juhasz et al., 1993; Cavani et al., 1993). It has not been clear, however, which ß subunit is associated with the αv subunit in wound keratinocytes. The αv subunit can form heterodimers with multiple ß subunits, namely with ß1, ß3, ß5 and ß6. Vitronectin receptor αvß5 integrin has been detected in keratinocytes in an animal wound healing model (Gailit et al., 1994). In the present study, one of the goals was to find out which ß subunit forms a heterodimer with αv subunit in different stages of human mucosal wound healing and which extracellular matrix proteins could be found colocalized as its possible ligands.

Very little is known about epithelial cell behaviour during chronic inflammation. In

periodontal disease, epithelial cells proliferate into inflamed connective tissue forming long epithelial extensions. The basement membrane between the epithelium and connective tissue also shows alterations (Thilander, 1968; Freedman et al., 1968; Takarada et al., 1974 a, b; Peng et al., 1986). The mechanisms of epithelial adhesion and migration in chronic inflammation have not been adequately described. The distribution of cell adhesion receptors and proteins of developing and healthy periodontal soft and hard tissues have been examined in several animal studies (Thesleff et al., 1987; Caffesse et al., 1987; Steffensen et al., 1992), but there are no studies showing alterations of these molecules in conjunction with epithelial cells and chronically inflamed periodontal pockets. Therefore, the second goal of this study was to find out the alterations of epithelial integrins and cell adhesion proteins during the chronic inflammation of human periodontal tissues in order to elucidate the role of keratinocytes in periodontal disease.

CHAPTER ONE

Review of the literature

1.1. Integrins

Integrins are cell surface glycoproteins that function as cell-to-cell and cell-to-extracellular matrix adhesion receptors (Albelda and Buck, 1990; Ruoslahti, 1991; Hynes, 1992). Through binding to matrix proteins integrins mediate information transfer from the extracellular milieu to the cytoplasm and nucleus, leading to alterations in cell functions and ultimately in cell behaviour (Schwartz, 1992). Integrins are known to play important role in regulating a wide range of cellular interactions during growth, development, differentiation, and immune response (Hemler, 1990; Ruoslahti, 1991; Hynes, 1992). Integrins are composed of a single α and a single ß subunit that are non-covalently linked to each other. At least 15 different α and 8 ß subunits are currently known. These subunits can variously combine to form more than 20 different cell surface receptors that have distinct ligand binding specificities. It is known that some ß integrin subunits, namely ß1, ß2, ß3 and ß7, are able to associate with multiple α subunits. Some α subunits can become paired with more than one ß, namely α4, α6 and particularly αv, which appears to be able to combine with five different ß subunits. Both α and ß subunits are transmembrane glycoproteins that contain a single hydrophobic transmembrane segment. Usually integrins have relatively large extracellular domains and short cytoplasmic tails. Integrins seem to share a

distinctive shape containing mushroom-shaped head region that is thought to consist of both α and ß subunits, and mediate integrin binding to ligands. Integrins have long extracellular domains that hold the head approximately 20 nm above the cell surface and penetrate through the plasma membrane via hydrophobic transmembrane domains. Integrins have relatively short (15-60 amino acids in length) carboxy-terminal tails protruding into the cytoplasm. Integrin ß4 serves as an exception to this rule having an unusually long cytoplasmic domain, consisting of more than 1000 amino acids (Sastry and Horwitz, 1993). Extracellular domains of both α and ß subunits are large, over 75 kDa for ß subunits and over 100 kDa for α subunits. The ligand binding site of the integrins is formed of the NH_2-terminal extracellular sequences of both subunits. The transmembrane organization permits integrins to bind to extracellular ligands and to transmit signals into the cytoplasm. The intracellular tails interact with cytoplasmic molecules to regulate extracellular ligand-binding affinity. These functions have been termed "outside-in" and "inside-out" signalling, respectively (Ginsberg et al, 1992). The ligand binding process in which both subunits participate, is dependent on divalent cations (calcium, magnesium, manganese). Ligand binding causes clustering of the receptors that leads to cytoskeletal organization and signalling (Miyamoto et al., 1995). The cytoplasmic tail of ß subunit seems to be critical for the organization of actin microfilaments through its binding capacity to talin and α actinin. Hierarchy in signal transduction depends on ligand occupancy and receptor aggregation (Miyamoto et al., 1995). Integrin aggregation alone seems to

induce tensin and focal adhesion kinase (pp125FAK) accumulation. Aggregation and ligand occupancy leads to clustering of talin and α-actinin. A combination of these events results in accumulation of a large number of intracellular proteins. Specificity of integrin function can be attributed to the structures of both the extracellular and intracellular domains of integrins. Ligand specificity varies depending on the cell type and functional state, and a single cell can have several integrin receptors for the same ligand. Most integrins are expressed in many different cell types, but some integrin subunits are considered specific to a particular cell type, e.g. ß2 integrins to leukocytes and ß6 integrin to epithelial cells (Sheppard, 1996, for review).

1.2 Keratinocyte integrins and their ligands

The repertoire of integrins expressed by different cell types varies greatly. Human keratinocytes are known to express at least eight integrin heterodimers that mediate cell adhesion to various extracellular matrix proteins (Figure 1). Several integrins are able to bind multiple ligands in a cell line dependent fashion. The regulation of integrin function is complicated, since a single cell can express multiple integrins with apparently similar binding specificity. Keratinocytes are specialized cells that cover and protect the skin and mucosa (Eckert, 1989). Epidermal and mucosal keratinocytes undergo terminal differentiation when they move from basal layer to the surface.

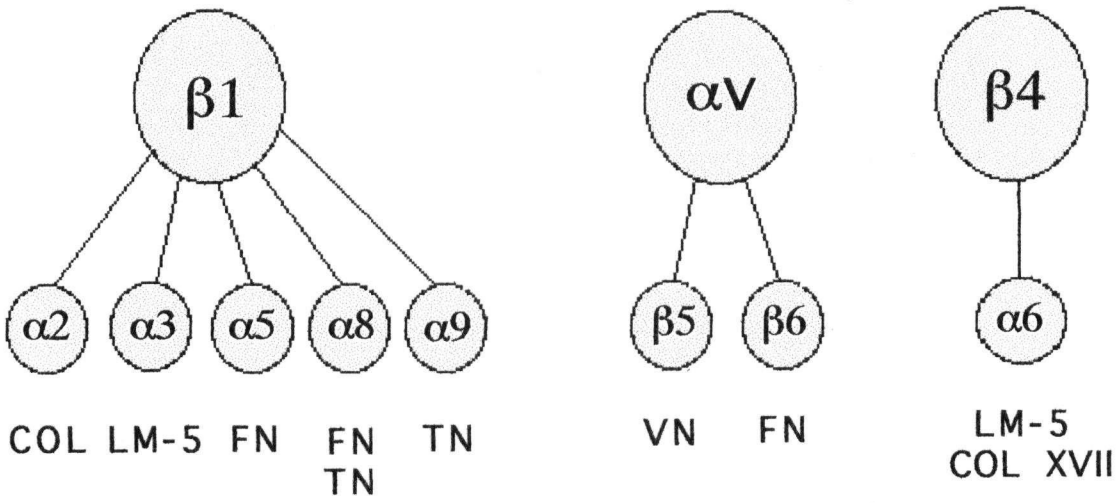

Figure 1. Keratinocyte integrin heterodimers and their known ligands (Larjava et al., 1996). COL = collagen; LM-5 = laminin-5; FN = fibronectin; TN = tenascin; VN = vitronectin

Polarized keratinocytes attach to the basement membrane through the basal cell membrane and to adjacent keratinocytes via lateral cell membranes. In normal cornified and stratified epithelia, integrins are expressed by basal keratinocytes but are absent in differentiating superfical layers (Hertle et al, 1991; Larjava et al., 1990; Konter et al., 1989; Peltonen et al., 1989). This suggests that integrins are important

in keratinocyte adhesion to the basement membrane, proliferation and differentiation. During wound healing, keratinocytes must be able to interact with various components of wound bed extracellular matrix. Based on immunolocalization studies, normal human keratinocytes residing in resting epithelium express only four different integrins; α2ß1, α3ß1, α9ß1 and α6ß4 (DeLuca et al., 1990; Hertle et al., 1991; Konter et al., 1989; Peltonen et al., 1989, Palmer et al., 1993).

Keratinocytes are shown to be activated when transferred from tissue to cell culture conditions (Grinnell, 1992). As a consequence of this process, they start to express integrins in addition to those found in vivo. Cultured keratinocytes express in addition to the integrins mentioned above, αvβ5, αvβ6 and α5β1 integrins that are also found to be present in keratinocytes during wound healing (Larjava et al, 1993; Cavani et al, 1993; Juhasz et al, 1993).

Many, but not all, integrins bind to extracellular matrix proteins containing the tripeptide arginine-glycine-aspartic acid (RGD) (Ruoslahti and Pierschbacher, 1987; Pierschbacher and Ruoslahti 1984; Pytela et al., 1985; Ruoslahti and Pierschbacher, 1986). First identified in fibronectin, it has since been shown to be a cellular recognition sequence in many extracellular matrix and platelet adhesion proteins. The conformation of the RGD site appears to determine which integrin an RGD protein or RGD peptide will bind (Ruoslahti and Pierschbacher, 1987). This tripeptide is the major binding site in fibronectin.

Fibronectin is a key component of extracellular matrix and plasma, thus

functioning in cell adhesion, cell and embryonic differentiation, cell migration, and thrombosis (see Ruoslahti, 1988 for review). Fibronectin is an important molecule in the provisional matrix during wound repair. Fibronectin has several functional domains that permit its interactions with a remarkably wide range of cell types, extracellular matrix molecules, and cytokines. The fibronectin molecule is composed of two polypeptides, with a total molecular mass of 560 kDa. Even though fibronectin is encoded by only a single gene, it exists in a number of variant forms. These forms differ in sequence at three general regions of alternative splicing of its precursor mRNA. If alternative splicing involves cell adhesion sequences, it provides posttranscriptional mechanism for potential regulation of fibronectin cell-type specificity. Depending on the particular combination of spliced sites, there can be 20 different variants of the human fibronectin subunit (Yamada and Clark, 1996, for review). Two separate forms of fibronectins, plasma fibronectins, present in blood as a soluble plasma glycoprotein, and cellular fibronectin, produced by a wide variety of cell types, have been described. Cellular fibronectins vary in composition and they typically contain considerably higher proportions of alternatively spliced sequences compared to plasma fibronectin. The alternative splicing of fibronectin can insert or delete cell-type-specific adhesion sites. Spliced sequences tend to be retained in cellular fibronectin, especially by certain embryonic and malignant cells (Vartio et al., 1987; Oyama et al., 1989) and healing wounds (ffrench-Constant et al., 1989; Brown et al., 1993). Fibronectin contains several peptide sites capable of mediating cell adhesion.

These are located in three general regions: the central cell-binding domain, the alternatively spliced IIICS region, and the heparin-binding region. Most cells can adhere to the centrally located "cell binding" domain (Akiyama et al., 1985, 1994). A crucial sequence in this domain is RGD (Pierschbacher and Ruoslahti, 1984, 1987; Yamada and Kennedy, 1984). Deletion or point mutation to this sequence leads to the loss of nearly all adhesive activity (Obara et al., 1988). It is known that human keratinocytes can express four receptors that are potentially able to bind to fibronectin, namely α5ß1, α3ß1, α6ß1 and αvß6 integrins. The classical fibronectin binding receptor is α5ß1 integrin that binds to the RGD sequence of the fibronectin molecule (Singer et al., 1988; Akiyama et al., 1989). α5ß1 is considered to be a specialized receptor for fibronectin and was originally characterized on fibroblasts (Singer et al., 1988). Integrin α5ß1 mediates fibronectin-matrix assembly, cell adhesion and migration on fibronectin (Akiyama et al., 1989).

Another fibronectin RGD site binding receptor in epithelial cells is the αvß6 integrin (Busk et al., 1992). ß6 integrin is expressed exclusively in epithelial cells, and only in a single integrin heterodimer, αvß6, a receptor for the extracellular matrix proteins fibronectin (Busk et al., 1992; Weinacker et al., 1994) and tenascin (Prieto et al., 1993). The αvß6 integrin is highly expressed in the lung, skin, and kidney during organogenesis (Breuss et al, 1995). In normal epidermis or oral mucosal epithelium this receptor is absent (Breuss et al., 1995) but becomes highly expressed in response to injury or inflammation, and in malignant epithelial neoplasms (Breuss et

al., 1995). Neither of the main fibronectin receptors, α5ß1 or αvß6 integrins, appears to be present in keratinocytes residing in the resting epithelium, but the expression of both of these integrins can be induced simply by placing epithelial cells in cell culture. Another integrin, α8ß1 can also bind to fibronectin in a RGD-dependent manner (Schnapp et al., 1995) and it has been localized in chicken epithelium but its expression in human keratinocytes is not clear. α3ß1 integrin can bind to fibronectin in some cell types, but it is believed to function as a receptor for laminin-5 in keratinocytes (Carter et al., 1991; Delwel et al., 1994).

In resting and wounded epithelia, keratinocytes are required to be able to interact with various collagen types. Different types of collagens are recognized mainly by one single receptor, the α2ß1 integrin. Unlike keratinocyte adhesion and motility on fibronectin and vitronectin, interactions with collagens are not mediated by RGD even though collagens contain these sequences. Collagens are extracellular matrix glycoproteins that provide structural support for the tissues and also regulate many cellular functions. Based on their genetic origin and primary structure, collagens can be divided into several groups. The basic structure of all collagens is triple-helical conformation that is formed of three α chains. Altogether 32 α chains have been identified, each having a common repetitive motif of Gly-X-Y, which allows α chains to wrap around each other and form the triple-helical structure. A total of 19 different collagen types have been identified and characterized in vertebrates (Eckes et al., 1996, for review). Collagens differ in their α chain structure as well as in their contents

of collagenous and noncollagenous regions in their chains. Collagens have been subdivided into two main subfamilies based on this ultrastructural organization; the fibril-forming and nonfibrillar collagens (van der Rest and Garrone, 1992).

Type IV collagen is the major collagenous component of the basement membrane. It forms a multilayered, highly insoluble structural network that interacts with other basement membrane molecules, such as laminins, nidogen, and heparan sulphate proteoglycan (Yurchenko and Schittny, 1990). Type IV collagen belongs to a subfamily of nonfibrillar collagens, that can also be called network-forming collagens. Type IV collagen is the component of lamina densa of the basement membranes (Figure 2). In vivo keratinocytes express $\alpha 2\beta 1$ which recognizes a variety of collagens. This is also the main receptor for type IV collagen in keratinocytes. Direct interactions of keratinocytes with type IV collagen are rare but may occur e.g. during healing of blister wounds in which case keratinocytes migrate on the components of lamina densa. Keratinocytes are more likely to interact with fibrillar collagens, such as types I, III and V collagens during healing of full thickness wounds. Also in this case, interaction of keratinocytes with collagens is mediated via $\alpha 2\beta 1$ integrin. One peculiar collagen type is localized as a transmembrane protein of keratinocyte cell surface. This type XVII collagen is better known as bullous pemphigoid antigen 2 (Uitto and Christiano, 1992). It appears to be interacting with $\alpha 6\beta 4$ integrin although the functional consequences of this interaction are not known.

Both $\alpha 2\beta 1$ and $\alpha 3\beta 1$ integrins can serve as receptors for laminin-1 (LM-1)

(Elices et al., 1991; Carter et al., 1990; Adams and Watt, 1991). These integrins may act in concert to mediate cell binding to LM-1. Antibodies against α2 and α3 individually inhibit attachment to LM-1, but together the antibodies inhibit more effectively (Carter et al., 1990; Adams and Watt, 1991). Although several studies point to the conclusion that normal epidermal and mucosal keratinocytes bind to LM-1, others seem to disagree (see above, Karecla et al., 1994; Zhang et al., 1996). Zhang and co-authors (1996) showed that normal keratinocytes adhere poorly to and become non-motile on LM-1 rich matrix and it could therefore act as an endogenous stop-signal for migrating keratinocytes. They also showed that integrin α6ß1 is an important LM-1 receptor that is not normally expressed in keratinocytes but becomes an important LM-1 receptor in malignant transformation of mucosal keratinocytes. This promotes their migration on LM-1, an important process in invasion and metastasis. It is questionable if keratinocytes in vivo are even required to adhere to LM-1. LM-1 can be found in the lamina densa of the basement membranes (Figure 2). Like all other members of the laminin-family, LM-1 is made of α, β and γ chains.

Laminin-5 (LM-5, also known as epiligrin, kalinin or nicein) is found in the basement membrane of skin and other epithelial tissues, and it serves as a component of anchoring filaments that span through the basement membrane (Figure 2) (Carter et al., 1991; Rousselle et al., 1991).

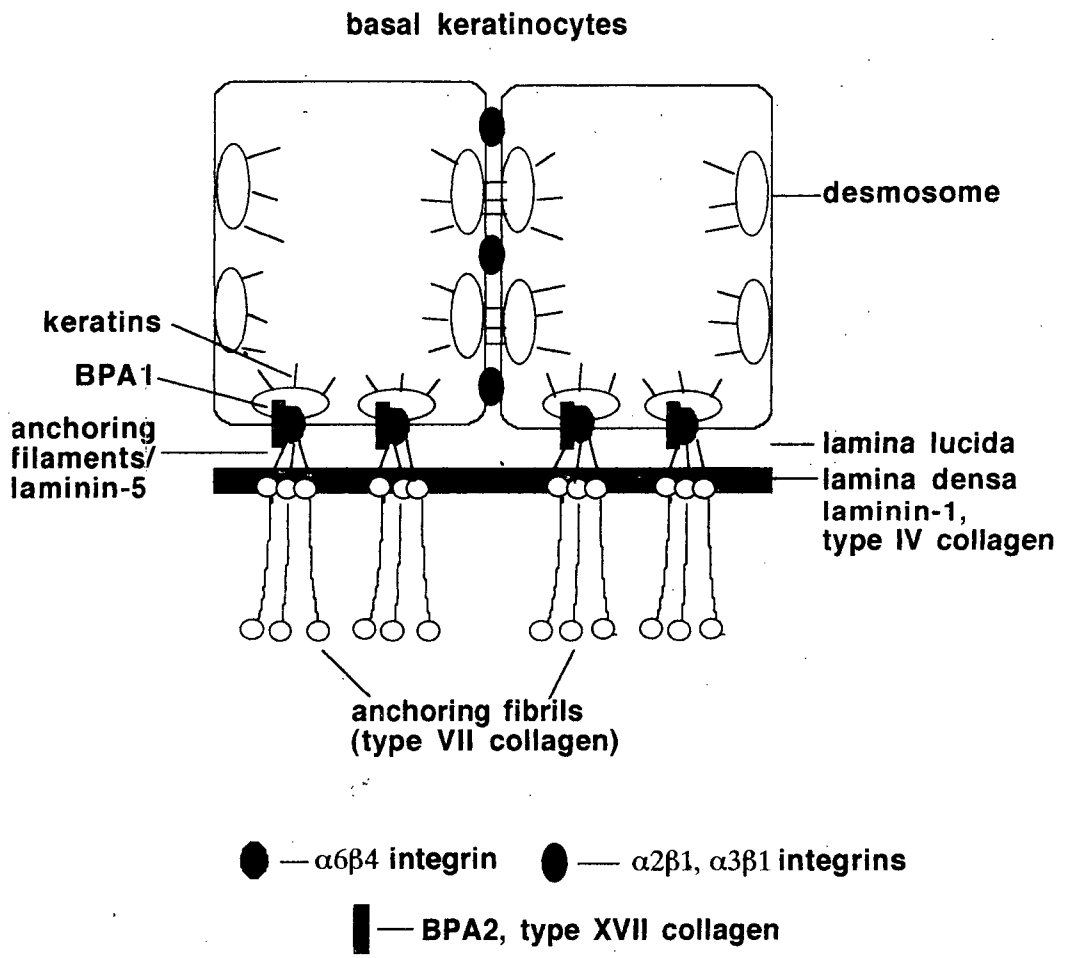

Figure 2. Ultrastructure of the basal keratinocytes and the underlying basement membrane (from Larjava et al., 1996).

LM-5 is a glycoprotein synthesized by keratinocytes (Carter et al., 1991). The extracellular domain of α6β4 integrin interacts with LM-5 (Rousselle et al., 1991, Niessen et al., 1994). This integrin is known to link the basal keratinocytes to the underlying basement membrane, and that this link mainly relies on the interaction with LM-5 in the anchoring filaments (Figure 2). The functional importance of α6β4 integrin is substantiated by in vitro experiments in which antibodies against it have been

shown to dissociate hemidesmosomes and lead to cell detachment (Kurpakus et al., 1991). Knockout mice lacking either α6 or ß4 integrin can not form hemidesmosomes and show blistering of skin (Fässler et al., 1996).

Besides playing an important function in keratinocyte cell-matrix adhesion, as discussed above, α2β1 and α3β1 integrins are believed to interact with each other (Symington et al., 1993). The α3ß1 and α2ß1 integrins localize commonly at the lateral surfaces of the stationary basal keratinocytes (Hertle et al., 1991; Konter et al., 1989; Larjava et al., 1990; 1992; Peltonen et al., 1989). It has been suggested that these receptors may play roles in homotypic cell-cell interaction in epithelia, supported by findings that α2ß1 and α3ß1 integrins can bind to each other (Carter et al., 1990; Symington et al., 1993) and that α3ß1 can bind to itself (Sriramarao et al., 1993). Some studies discount the functional importance of ß1 integrins in cell-cell binding of keratinocytes (Tenchini et al., 1993). LM-5 is also recognized by α3ß1 integrin (Carter et al., 1991). Transgenic mice lacking α3 integrin show localized blistering of the skin (DiPersio et al., 1997). In mice and man, α3ß1 integrin is occasionally found at the basal surface of the basal keratinocytes suggesting that α3ß1 integrin collaborates with α6ß4 integrin in LM-5 binding and they may be functionally interchangeable in some circumstances.

Vitronectin (also known as serum spreading factor, S-protein and epibolin) is an abundant protein present in blood plasma, extracellular matrices and fibrin clots. Vitronectin is known to be an active cell adhesion mediator as well as playing a role in

cell migration and invasion (Preissner, 1991, for review). The αvß1, αvß3, αvIIb3 and αvß5 integrins are all vitronectin receptors binding to the RGD cell attachment sequence of the protein (Cherny et al., 1993). Only αvß5 integrin is known to be expressed by the keratinocytes, thus being their only vitronectin recognizing receptor. It is important to note that all of these vitronectin receptors can also bind fibronectin.

Tenascins are a family of large extracellular matrix molecules that presently include tenascins -C, -R, and -X. Tenascin expression is tightly regulated, making this protein particularly interesting. The expression of tenascin is closely associated with morphogenetic events, including embryonic induction and migration, wound healing, and tumorigenesis (Chiquet-Ehrismann, 1990; Whitby and Ferguson, 1991; Koukoulis et al., 1991; Whitby et al., 1991). One study shows that even if tenascin gene is totally disrupted in developing mice, the animals seem to develop and grow normally (Saga et al., 1992). These mice lacking tenascin yet phenotypically normal, cast doubt on the theory that tenascin plays an essential role in normal development, never the less, as mentioned earlier, tenascin is generally considered an important protein during embryonic development. It is found in developing brain and in mesenchyme associated with epithelial-mesenchymal interactions (Chiquet-Ehrismann, 1990; Thesleff et al., 1991). In adult tissues tenascin is found in the connective tissue underneath epithelium (Lightner et al., 1989) but its expression is strongly upregulated during wound healing (Mackie et al., 1988; Chuong and Chen, 1991; Whitby et al., 1991). Several integrins have been found to bind tenascin, namely α2ß1, αvß6 and α9ß1 integrins (Prieto et al.,

1993; Yokosaki et al., 1994, 1996; Bourdon and Ruoslahti, 1989).

1.3 The function of integrins with special emphasis on keratinocytes

As mentioned earlier, cell behavior is dependent on the ligand-binding specificities of the integrins present on the cell surface. Characteristics of the integrins are their overlapping but nonidentical binding specificities. Several integrin heterodimers are able to bind multiple ligands depending of the cell type that is expressing them. Binding is based on protein-protein recognition, and in many cases a central element of the molecular recognition event involves only a short adhesive peptide sequence (Yamada, 1991), such as RGD (Arg-Gly-Asp) peptides in fibronectins. The types and concentrations of integrins on cell surfaces are important determinants of the specificity of binding to extracellular matrix proteins, although the activation state and spatial distribution (diffuse vs. aggregated) of the integrins also appear to be important (Yamada et al., 1996)

Integrins can become important signalling molecules after ligand binding or receptor clustering. For example, integrin-mediated adhesion of cells to a variety of extracellular matrix molecules including fibronectin, vitronectin, laminin, and collagen will lead to enhanced tyrosine phosphorylation of the focal adhesion kinase (FAK) (Guan et al., 1991; Kornberg et al. 1991, 1992; Guan and Shalloway, 1992). Since this protein localizes to focal contacts and may be involved in their organization, it has been very informative as a model for transmembrane signal transduction via integrins. Other

signaling molecules are likely to be identified in association with integrin cytoplasmic domains. Since the ligand binding affinity (Briesewitz et al., 1995) of an integrin can be modulated by activation of α and ß chains by various signalling molecules it has been suggested that both α and ß cytoplasmic domains can alter receptor assembly and function and hence, cell adhesion (O'Toole et al., 1994; Schwatz et al., 1995). The possible activation candidates, other than FAK, include calreticulin, α-actinin, and talin, because they have all been found to bind integrin cytoplasmic domains in vitro. Especially the cytoplasmic domain of integrin α subunit may function as affinity controller (O'Toole et al., 1994), but ß subunit seems to have a role in maintaining the receptor in a high-activity state (O'Toole et al., 1995). The activation of the cytoplasmic domains alters the relationship of the transmembrane α and ß domains and thus leads to conformational change and altered ligand binding affinity of the extracellular domains. The ligand binding affinity of integrins can also be modulated by divalent cations, such as calcium and manganese (Gailit and Ruoslahti, 1988). Calcium can inhibit and manganese can enhance the function of some ß1 integrins by affecting the appearance of a conformation favorable to ligand binding (Bazzoni et al., 1995). Integrin α5ß1 is suggested to contain distinct binding sites for magnesium, manganese and calcium, and their different usage modulates adhesion to fibronectin (Mould et al., 1995). The affinity of an integrin can also be modulated by co-operation with another type of cell adhesion receptor, e.g., cell surface proteoglycan (Iida et al., 1995). Integrin activation and functional regulation are very complex events and remain,

therefore, largely unknown.

Integrins are important not only in stable matrix anchorage and signalling but also in cell migration. The migratory state is found in embryonic cells as well as cells at the wound sites. The migratory phenotype is characterized by generally diffuse organization of integrins. Also migratory keratinocytes express integrins at different activity levels (Hertle et al., 1992, Larjava et al., 1993; Cavani et al., 1993; Juhasz et al., 1993). The stationary phenotype is recognized by integrin organization into focal adhesion sites with intracellular specialized cytoskeletal complexes composed of talin, α-actin and FAK (O'Toole et al., 1994). These two distinct molecular phenotypes most likely have an important functions in wound repair, which will be discussed later in detail.

In epithelium, integrins also function in cell differentation. Stem cells for keratinocytes appear to express the highest level of ß1 integrins (Jones and Watt, 1993). Keratinocytes with characteristics of stem cells can be isolated based on their high ß1 integrin expression (Jones and Watt, 1993). During the vertical migration of the epithelial cells from the basal cell layer to the suprabasal layers the integrin expression stops (Adams and Watt, 1991; Watt and Jones, 1993). Loss of functional integrins from the cell surface precedes epithelial differentiation (Hodivala and Watt, 1994). Integrin engagement to the ligand is believed to prevent differentiation (Watt and Jones, 1993). Fibronectin has been shown to inhibit keratinocyte differentiation by occupying integrins on keratinocyte cell surface (Watt and Jones, 1993). These

findings suggest that integrins have important role also in keratincyte proliferation and differentiation. New research data on keratinocyte integrins suggest that integrins are associated with the regulation of epithelial driven inflammation. Transgenic animals lacking ß6 integrin show normal early wound healing but develop inflammatory lesions in the lungs and skin (Huang et al., 1996). Suprabasal ß1 integrin expression is seen in many inflammatory skin diseases such as psoriasis (Watt and Hertle, 1994, for review). Carroll and his co-workers (1995) used forced expression of ß1 integrins in suprabasal layers using involucrin promoter. This resulted in skin inflammation resembling psoriasis. Both above mentioned studies indirectly suggest that integrins are associated with the regulation of inflammation that is controlled by epithelial cells.

1.4 Integrins and basement membrane

The basement membrane of the skin and mucosa consists of a large number of structural molecules that form an attachment zone at the epithelial-connective tissue interface. On the epithelial side of the basement membrane, basal keratinocytes are attached to underlying basement membrane structures via hemidesmosomes. Hemidesmosomes are known to contain three major protein components, namely bullous pemphigoid antigens 1 and 2 (BPA1 and BPA2) (Jones et al., 1994; Marinkovich, 1993; Uitto and Christiano, 1992) and integrin $\alpha 6\beta 4$. Integrin $\alpha 6\beta 4$ serves as a major receptor in the hemidesmosomal structure (Jones et al, 1991; Sonnenberg

et al, 1991; Stepp et al, 1990) that interacts with LM-5, a component of the anchoring filaments (Rousselle et al .,1991; Niessen et al., 1994). Other proteins, such as laminin-6 and uncein, may also be present in anchoring filaments (see figure 2.) (Zeng et al., 1994). Anchoring filaments interact with underlying lamina densa, that is composed of LM-1, nidogen and type-IV collagen. Type VII collagen is the major component of anchoring fibrils that are morphologically distinct attachment structures extending from the lower portion of the basement membrane, namely lamina densa, to the papillary dermis (Sakai et al, 1986).

In functional experiments in vitro antibodies against α6β4 integrin have been shown to cause dissociation of hemidesmosomes and led to cell detachment (Kurpakus et al, 1991). Transgenic animals lacking either α6 or ß4 integrins develop blisters in their skin (Fässler et al., 1996, for review). BPA2 has been sequenced and found to be a membrane anchored glycoprotein with unusual aminoterminal-intracellular domain and collagenous extracellular domain. Therefore the protein has been termed type XVII collagen (Giudice et al, 1991; Li et al, 1993). Recently, it has been demonstrated that the aminoterminal domain of BPA2 interacts with α6 subunit of the α6ß4 integrin (Hopkinson et al., 1995). It has been suggested that interaction of type XVII collagen with α6ß4 integrin may be crucial in mucocutaneous diseases that affect epithelial-connective tissue junction such as junctional epidermolysis bullosa (Uitto and Christiano, 1992). Extracellularly, α6ß4 integrin colocalizes and interacts with LM-5 (Niessen et al., 1994; Rousselle et al., 1991). Some patients with serious

skin conditions like lethal junctional epidermolysis bullosa have serum autoantibodies against LM-5 (Domloge-Hultsch et al., 1992). All mutations of this α6ß4 - LM-5 complex have also potential oral and dental implications beyond blistering since it is known that the adhesion of junctional epithelium to the tooth enamel is also mediated by α6ß4 integrin (Hormia et al., 1992). A split of junctional epithelium from the tooth due to mutations in this complex could weaken the host resistance in this area and possibly lead to early onset periodontal disease. Oral findings of these patients have not been carefully reported because attention has focused on their more serious skin condition. It should be noted that blisters may also raise from mutations in type VII collagen (dystrophic epidermolysis bullosa) (Uitto and Christiano, 1992).

1.5. Epithelial wound healing

Wound healing is a complex phenomenon that involves series of controlled events including: a) an inflammatory stage involving aggregation of platelets and later migration of macrophages, fibroblasts, and lymphocytes at the injury site; b) the formation of a provisional extracellular matrix, mainly composed of fibrinogen, fibrin, fibronectin, and vitronectin; c) the migration of epithelial cells from the edges of the wound, and; d) the local synthesis and secretion of a wide variety of regulating growth factors and cytokines (Pierce et al, 1989; Juhasz et al, 1993; Bennett and Schultz, 1993; Boyce, 1994). After the epithelium has been disrupted at the time of tissue injury, reepithelialization must occur as rapidly as possible in order to re-

establish tissue integrity. This process begins within hours after injury. Epithelial cells from residual epithelial structures loose their contact with basement membrane and move quickly across the wound defect. Clark and colleagues (1982) have shown that these keratinocytes migrate on provisional matrix in the newly created wound bed. Initially, the keratinocytes laterally migrate and cover the wound by a process of migration. Later, as the reepithelialization proceeds, the proliferation of keratinocytes that feed the advancing epithelial edge, becomes more important.

During wound healing, the function of keratinocytes is to rapidly cover the exposed connective tissue (Stenn and Malhorta, 1992). This process depends upon a variety of interactions between cells and the extracellular matrix. As the basal keratinocytes at the wound site are exposed to the new, provisional matrix, the phenotype of the cells is changed from stationary to migratory. In this process, keratinocytes detach themselves from basement membrane, migrate laterally into the wound bed and finally regenerate the basement membrane. In mucosa and in skin the migration seems to involve similar patterns although the source of migrating epithelial cells is different. In the mucosa the basal layers of the wound edge epithelium serves as a major source of the keratinocytes migrating into the wound area. In the skin, however, epithelial cells arise not only from the wound edge but also from hair follicles and sweat glands. Keratinocytes have been shown to synthesize most of the main components, such as type IV collagen, laminin and type VII collagen, that are present in the basement membrane zone (Stenn and Malhorta, 1992). During wound healing or

any other such conditions that can involve cell movement, such as cancer or some skin diseases, the interaction between epithelial cells and underlying tissues changes. Cells will be exposed to new extracellular matrix proteins and cytokines, which can lead to altered integrin expression of the basal cells. The ultimate goal of normal wound healing is to have epithelial cells to form new stable organized basement membrane structure that is essential for the integrity of the tissue.

During wound healing, most of the components of the basement membrane zone are missing underneath migrating keratinocytes (Larjava et al., 1993). Only LM-5 appears to be deposited against the wound bed matrix by keratinocytes during migration (Larjava et al., 1993). The role of LM-5 appears critical even for healing of the blister wounds where lamina densa remains intact (Kainulainen et al., submitted). Keratinocytes in chronic aphtous ulcers that have developed a healing response fail to deposit a proper LM-5 rich matrix (Richards et al., 1996).

1.6. Keratinocyte integrins and wound healing

As the epithelial cells undergo major environmental changes during the wound healing, they have to adjust their cell surface receptors for the new demands. During wound healing keratinocytes have to migrate on the provisional matrix different from their stationary matrix. This requires a change of distribution of some integrins and expression of some totally new integrins. Integrin $\alpha 6\beta 4$ is present in migratory epithelial cells although hemidesmosomes are absent. Expression of ß1 integrins is

strongly up-regulated during wound healing (Cavani et al, 1993; Gailit et al, 1994; Hertle et al, 1992; Juhasz et al 1993; Larjava et al, 1993). Both α2ß1 and α3ß1 integrins seem to be equally expressed. Integrin α3ß1 is known to be able to bind both fibronectin and LM-5, that are both present in provisional matrix (Carter et al, 1991; Larjava et al, 1993). Integrin α2ß1 is known to be able to bind many collagens, that form contacts with migrating keratinocytes, such as type I, III, V and VI collagens. During wound healing, keratinocyte ß1 integrins also localize in suprabasal cell layers of the epibolus. This may be due to transfer of nondifferentiated migrating cells into suprabasal cell layers. Migratory keratinocytes are induced to express α5ß1 integrin that is not present in stationary cells (Larjava et al., 1993). This induction seems to start relatively early after wounding and end shortly. Distribution of α6ß4 integrin change from hemidesmosomal to diffuse during cell migration (Kurpakus et al, 1991). Integrins of the αv-family are absent or expressed only weakly in normal epithelia. In contrast, αv-integrins have been shown to be present in wound keratinocytes (Cavani et al., 1993; Juhasz et al., 1993; Larjava et al., 1993). The partner ß-subunit associated with the αv-polypeptide has been unidentified in human wounds. Migrating keratinocytes in porcine epidermis have been reported to express the αvß5 integrin (Gailit et al., 1994). αvß5 integrin is present in cultured keratinocytes and has been shown to mediate keratinocyte migration on vitronectin (Kim et al., 1994). Vitronectin is present in the provisional wound bed matrix and it is, therefore, reasonable to assume that αvß5-vitronectin interaction can also exist during wound

healing.

1.7. TGF-ß in wound healing

In wounds proper regulatory signals are required for normal repair. Currently it is believed that transforming growth factor-betas (TGF-ßs) have a central role in the wound healing process. TGF-ßs are a family of polypeptides that have multiple regulatory actions in cell growth, differentiation, and developmental processes (Sporn and Roberts, 1992; Massaque et al., 1992). Three highly homologous TGF-ß genes have been identified in mammals, representing TGF-ß1, TGF-ß2 and TGF-ß3 polypeptides. Schmid et al (1993a) showed for the first time that human keratinocytes of intact skin express TGF-ß3. This growth factor seems to play an important role in epidermal maintenance (Scmid et al., 1993a). In animal studies only small amounts of TGF-ß2 and TGF-ß3 mRNAs had been found in keratinocytes of intact dermis (Scmid et al., 1993b). All TGF-ß isoforms are found in healing wounds of animals (Kane et al., 1991; Levine et al., 1993). However, Schmid et al. (1993a) did not find any detectable levels of TGF-ß2 mRNA in human wound keratinocytes. Wound fibroblasts and macrophages are known to express both TGF-ß1 and TGF-ß2 in cells adjacent to the wound (Obberghen-Schilling et al., 1988; Van Waes, 1995). TGF-ß1 is the major isoform in wound keratinocytes. Induction of TGF-ß1 in migrating keratinocytes is crucial for the successful re-epithelialization of skin wounds. In vitro studies have shown that TGF-ß3 inhibits growth of primary human keratinocytes

(Graycar et al. 1989) and TGF-ß1 seems to stimulate keratinocyte motility by switching the cells from differentiating to regenerative phenotype (Mansbridge and Hanawalt, 1988), and by inducing their production of fibronectin (Nickoloff et al., 1988). In cultured human keratinocytes, TGF-ß1 has also been shown to increase the levels of mRNA for some integrins, such as α5, αv and ß5, that may facilitate migration of wound keratinocytes (Gailit et al., 1994, Zambruno et al., 1995). TGF-ß1 may also stimulate the proliferation of wound keratinocytes at the wound margins indirectly by inducing the expression of other polypeptide growth factors, e.g. platelet-derived growth factor (PDGF) (Anatoniades et al., 1991; Chen et al., 1992).

1.8. Junctional and periodontal pocket epithelia

Human periodontium contains oral gingival, sulcular and junctional epithelia, the underlying connective tissue, periodontal ligament, cementum and alveolar bone. Oral gingival epithelium is continuous with the sulcular epithelium, which then apically changes to epithelium which is attached to the tooth, called junctional epithelium (JE). Based on the ultrastructure and cytochemical features the JE is a so-called simple, non-differentiating and non-keratinizing type (Nanci and Schroeder, 1996; Schroeder and Listgarten, 1997, for review). All JE cells are very much alike ultrastructurally, and characteristically all contain only few cytoplasmic filaments that are composed of keratins 5, 13, 14, and 19. Keratin 19 is a marker of simple epithelia, such as JE (Schroeder, 1981; Feghali-Assaly et al., 1994). In addition JE cells express epidermal

growth factor (EGF) and the intercellular adhesion molecule-1 (ICAM-1) and leukocyte function-associated antigen (LFA-3) on their surface (Crawford, 1992; Gao and Mckenzie, 1992). The turnover rate of junctional epithelium is exceptionally high. This is characterized by constantly coronally migrating daughter cells as well as high rate of exfoliating cells into sulcular fluid (Schroeder and Listgarten, 1997, for review). Proliferation appears to happen throughout the entire area of JE (Salonen, 1986). Another characteristic feature of JE is that the intercellular spaces are large and variable in size and there are less desmosomes than in other areas. During inflammation intercellular spaces further increase in size. In inflamed JE these enlarged spaces are increasingly occupied by transmigrating neutrophilic granulocytes and infiltrating mononuclear cells, such as macrophages and lymphocytes (Schroeder and Listgarten, 1997 for review).

The free surface of JE lines the bottom of the gingival sulcus. The structure separating the JE from the tooth enamel is called internal basal lamina whereas the external basal lamina separates the junctional epithelial cells from gingival connective tissue. Internal and external basal lamina (basement membrane) differ in composition. The external basal lamina contains typical constituents of the BM zone, while the internal basal lamina has been reported to contain type VIII collagen and LM-5 (Salonen et al 1991, Hormia et al, in press). LM-1 and type IV collagen appear to be absent in the internal basal lamina (Hormia et al., 1992). Hemidesmosomes connect basal cells to the internal basal lamina (also called DAT-cells) (Overman and Salonen,

1994). Integrin α6β4, a critical component of the hemidesmosome complex, is found in the internal basal lamina (Hormia et al, 1992). It is believed to mediate adhesion of DAT cells to the LM-5 substratum on the enamel surface (Hormia et al, in press). Cultured epithelial cell rests of Malassez are thought to resemble JE cells and are known to be able to attach on various extracellular matrix components (Uitto et al., 1992).

Periodontal pocket represents a pathologically deepened gingival sulcus in which the JE undergoes pathologic changes to form periodontal pocket epithelium. These changes include proliferation or disintegration of the internal basal lamina and are characteristic for periodontal disease. Based on keratin profile pocket epithelium resembles JE (MacKenzie et al., 1993).

Epithelial cell behavior is poorly characterized in chronic inflammation of the human periodontium. In inflamed tooth supporting tissues (periodontal disease), increased proliferation of surrounding epithelial cells into inflamed connective tissue stroma is commonly seen (Schroeder and Listgarten, 1997, for review). The healthy JE is transformed into periodontal pocket epithelium causing these epithelial cells to proliferate and form deep epithelial ridges into inflamed connective tissue. As a result, the relative area occupied by epithelial cell in the gingival tissue surrounding the tooth increases compared to healthy gingiva. The local immunologic activity in JE fails to prevent the bacterial intrusion and the resultant induced immunologic response that leads to release of extensive amounts of cytokines (see above) and a strong

inflammatory defence reaction. This is followed by an altered extracellular connective tissue matrix that the epithelial cells can adhere and migrate along. Basement membrane, or transformed external basal lamina, between the gingival epithelium and the connective tissue also shows some alterations, with discontinuities and other morphological changes seen during chronic inflammation (Thilander, 1968; Freedman et al., 1968; Takarada et al., 1974; Takarada et al., 1974; Peng et al., 1986). Loss of several heparan sulphate proteoglycans in chronically inflamed human periodontium has also been reported (Oksala et al., 1997). Perlecan, a structural component of the basement membrane zone disappears, as well as CD44 and syndecan-1, both of which are believed to function in cell-cell and cell-extracellular matrix interactions of epithelial cells (Oksala et al., 1997). The distribution of the adhesion receptors, integrins, of the chronically inflamed human epithelial cells have not been characterized before this study.

CHAPTER TWO

The aims of the study

The specific aims of this study were:

1. To find out which member of the αv integrin family is expressed during reepithelialization of healing periodontal wounds in humans.

The hypothesis was that the wound keratinocytes express αvß6 integrin as their fibronectin and tenascin receptor in healing wounds.

2. To characterize the alterations that occur in the expression of integrins and their ligands in epithelial tissue of chronically inflamed human periodontium.

The hypothesis was that the expression of integrins and their ligands change in periodontal pockets to make pocket epithelial cells capable of adhering and migrating on extracellular matrix modified by chronic inflammation.

CHAPTER THREE

KERATINOCYTES IN HUMAN WOUNDS EXPRESS αvß6 INTEGRIN

3.1. Introduction

Several reports have shown alterations in the expression of cell adhesion receptors, integrins, and their ligands during human and animal wound healing (Gailit and Clerk, 1994; Watt and Jones, 1993, for reviews,). In vivo, integrins are expressed by basal keratinocytes but are absent in differentiating upper cell layers (Larjava et al., 1990; Konter et al., 1989; Peltonen et al., 1989; Hertle et al., 1991; Larjava et al., 1992). This suggests that integrins have an important role in keratinocyte proliferation and differentiation.

Several recent reports have dealt with integrin distribution in human and animal wound healing (Hertle et al., 1992; Juhasz et al., 1993; Cavani et al., 1993; Gailit et al., 1994). Expression of ß1 integrins is generally strongly up-regulated in wounds. Besides α2ß1 and α3ß1, also α5ß1 is present in the migrating keratinocytes. Although hemidesmosomes are generally absent under the migrating cells both α6ß4 and LM-5 are present. Since also α3ß1 can bind LM-5 (Carter et al., 1991), it is conceivable that both α3ß1 and α6ß4 are involved in LM-5 binding in migrating keratinocytes.

Integrins of the αv-family are not stongly expressed in healthy human epidermis or mucosa (Larjava et al., 1993). We and others have recently shown that expression of αv-integrins is induced in wound keratinocytes (Larjava et al., 1993; Juhasz et al., 1993; Cavani et al., 1993). It is not clear which ß-subunit is associated with the αv-

subunit in wounds. In some models of wound healing, αvß5 appears to be the prominent mernber of the αv-integrin family in wound keratinocytes (Gailit et al., 1994) This integrin has been shown to mediate migration of cultured keratinocytes on vitronectin substrates (Kim et al., 1994). In this section I present evidence that αvß6 is abundant in wound keratinocytes during human mucosal and dermal wound healing. Furthermore, αvß6 expression occurs concomitantly with the expression of tenascin in wound granulation tissue. Since αvß6 may mediate cell adhesion to both fibronectin and tenascin (Prieto et al., 1993), I propose that this binding serve as a transient adhesion mechanism for keratinocytes prior to the assembly of the organized basement membrane.

3.2. Material and methods

Material

Experimental human palatal mucosal wounds (full thickness) were made in volunteers in Finland. Human experimental protocol was designed because most of the antibodies available against integrin polypeptides recognize human antigens. The experimental protocol was approved by the Ethical Committee of the University of Oulu, Finland. Incisional V-shaped wounds (about 1.5 cm long, 2 mm wide and deep) were created in human palate (distal from the first premolar tooth, about 4 mm from the gingival margin). Punch biopsies (4mm) were taken from wounds on day 3 and 7, rinsed in physiological saline, cut at an acute angle to the wound, embedded in

Tissuetek (Miles Inc, Elkhart, IN) and immediately frozen in liquid nitrogen. Biopsies from skin wounds were obtained from volunteers after wounding on day 1, 3, and 7. Biopsy blocks were stored at -70°C until used. Specimens were sectioned (6 μm) in a cryostat and fixed with -20°C acetone for 5 minutes and stored at -70°C until used. Morphological analysis was performed using toluidine blue staining.

Immunohistochemical studies

For immunolocalization studies, sections were incubated with primary antibodies diluted in phosphate buffered saline (PBS) containing 1mg/ml bovine serum albumin (BSA) for 60 minutes, after which sections were rinsed four times in 60 minutes with the PBS/BSA solution. After rinsing, sections were incubated with affinity-purified rhodamine-conjugated secondary antibodies (dilution 1:50, anti-mouse cat. #1214608, anti-rabbit cat. #1238841, both from Boehringer-Mannheim Biochemicals, Indianapolis, IN) for 60 minutes. Sections were rinsed and mounted using cyanoacrylate adhesive (Chemoco). Samples were examined using a Zeiss Axioskop 20 fluorescence microscope, and photographed using MC 80 Zeiss Microscope Camera. Antibodies against the following proteins were used in this study (Table 1); αv-integrin (Mab P3G8; Wayner et al., 1991), αvß5 integrin (Mab P1F6; Weinacker et al., 1994), αvß6 integrin (Mab E7P6; Breuss et al., 1993), ß6 integrin (Mab R6G9, Breuss et al., 1993) , ß1 integrin (antiserum 3847, Roberts et al., 1988), tenascin (Chemicon, Temecula, CA), and fibronectin (antiserum, Chen et al., 1985). We have previously shown that P3G8

and P1F6 react with human integrins in immunostaining (Nikkari et al., 1995).

Table 1. Antibodies used to recognize different integrins and their ligands in these studies.

Integrin/ligand	Antibody	Reference
α2	117	AMAC, Inc.
α3	J143	Kantor et al.
α5	mAb BIIG2	Werb et al.
α6	mAb GoH3	Sonnenberg et al.
αv	mAb P3G8	Wayner et al.
β1	Serum, 3847	Roberts et al.
β4	mAb 345-11A	Kennel et al
αvβ5	mAb P1F6	Weinacker et al.
β6	mAb R6G9	Breuss et al.
αvβ6	mAb E7P6	Breuss et al.
Type IV	mAb	ICN Biomedicals
Type VII	mAb	Chemicon
Laminin	Serum	Risteli et al
Kalinin	mAb GB3	Verrando et al

Control staining was performed without the primary antibodies and with nonimmune mouse serum. No specific reaction was obtained with either of the controls.

3.3. Results

Human mucosal wound keratinocytes express αvß6 integrin that is localized in areas of accumulation of fibronectin and tenascin.

Expression of αvß6 integrin was first investigated in experimental full thickness wounds of oral mucosa. Epithelial sheets showed marked migration into the wound bed on day 3, and all wounds were closed on day 7. Some of the wounds were already closed on day 3, which allowed us to study integrin distribution in fused epithelium during formation of early granulation tissue. Integrins of the ß1-family were present in normal keratinocytes of oral mucosa while αv, αvß5 or αvß6 integrins were absent (Fig. 3F). At the edge of the migrating epithelial sheet of 3-day-old wounds, antibodies against αv integrin and αvß6 integrin complex were reactive in some but not all specimens (Fig.3A, B, D, E). Antibody against the αvß5 integrin was nonreactive in all specimens (not shown). The leading epithelial front strongly expressed ß1 integrins (not shown). In those 3-day-old wounds in which epithelial sheets were fused, αvß6 integrin was present in the basal cell layer in the wound area (Fig. 3C), but absent from non-wounded site (Fig. 3F). Antibodies specific to αv integrin (P3G8) and αvß6 integrin complex (E7P6) reacted with basal keratinocytes in all 7-day-old wounds (Fig. 4A and B, respectively). Antibody (R6G9), recognizing ß6 polypeptide, gave staining results identical to those obtained with antibody reactive to αvß6 integrin complex (E7P6) (not shown).

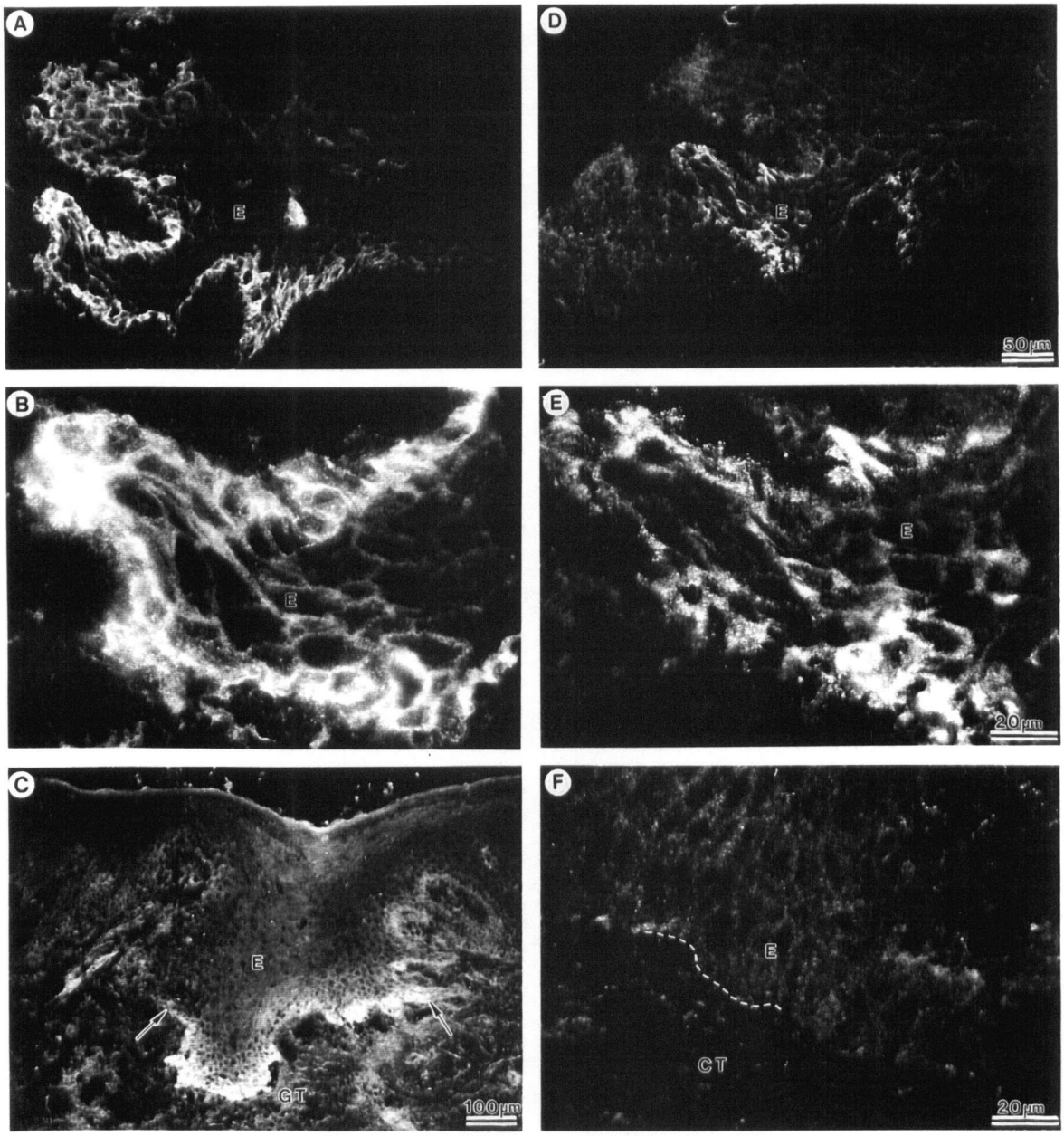

Figure 3. Integrin αvß6 is expressed in some but not all 3-day-old human mucosal wounds. Panels A, B, D, and E, separate sections of the same specimen of open 3-day-old mucosal wound. Panel C, closed 3-day-old wound. Panel F, nonwounded area of 3-day-old wound, same section as shown in panel C. Staining of αv integrin (panels A, B), or αvß6 (E7P6, panels C, D, E, F) integrin complex by immunofluorescence. Arrows mark the wound site and dotted line epithelial connective tissue interphase. E, epithelium; GT, granulation tissue; CT, connective tissue. Bar 50 μm: A, D; bar 20 μm: B, E, F; bar 100 μm: C.

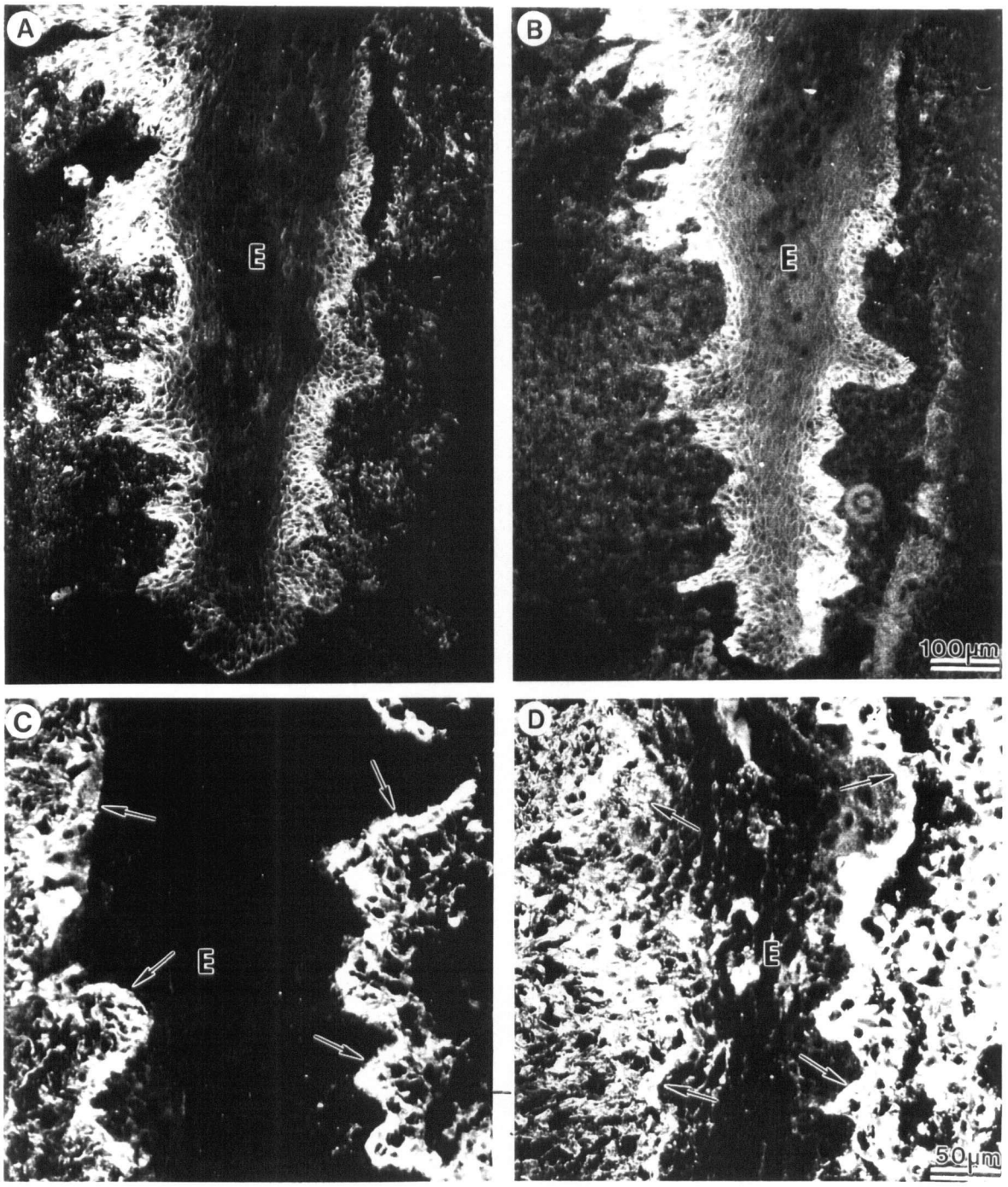

Figure 4. Integrin αvß6 and its putative ligands, fibronectin and tenascin, localize in similar areas in the 7-day-old wounds. Antibodies against αv integrin (panel A), αvß6 integrin complex (panel B), tenascin (panel C) and fibronectin (panel D) were used. Arrows indicate the epithelial connective tissue interphase. E, epithelium. Bar 100 µm: A, B; bar 50 µm: C, D.

We then localized the two putative ligands of αvß6 integrin, fibronectin and tenascin, in 7-day-old wounds that displayed high expression of this receptor. Fibronectin was present throughout the normal connective tissue and abundantly in the wound granulation tissue matrix, in which it showed a band-like distribution around the pseudo rete ridges of fused wound epithelium (Fig. 4D). Tenascin was present strongly in subepithelial connective tissue but weakly in deeper areas of the nonaffected tissue (not shown). Expression of tenascin was intense in the granulation tissue of 7-day-old mucosal wounds (Fig. 4C). Like fibronectin, the relative amount of tenascin appeared greatest underneath the cells expressing αvß6 integrin.

Wound keratinocytes in fused epidermal wounds express αvß6 integrin

Because cutaneous epithelium presumably has a different source of stem cells than mucosal epithelium do, we also investigated the expression of integrins and tenascin in full-thickness skin wounds. Stimulation of ß1 integrin expression was observed at the wound margin as soon as day 1 after wounding, and migrating epithelium remained strongly reactive up to day 7 (Fig. 5A-C). No expression of αv, ß5, or ß6 integrin was found in epithelial cells of nonwounded epidermis or in 1- or 3-day-old wounds (shown for αv in Fig. 5D,E). In 7-day-old wounds, basal epithelial cells covering the entire wound granulation tissue reacted strongly with antibodies against both αv integrin and αvß6 integrin complex (Fig. 5F and G, respectively) but not with antibodies to αvß5 integrin (not shown). In nonwounded areas, tenascin was present only at the

subepidermal connective tissue (Fig. 6A). This protein was absent from the coagulum of 1-day-old wounds and from the wound bed matrix of 3-day-old wounds (Fig. 6A-B). In contrast, the expression of tenascin was high in granulation tissue of 7-day-old wounds (Fig. 6C).

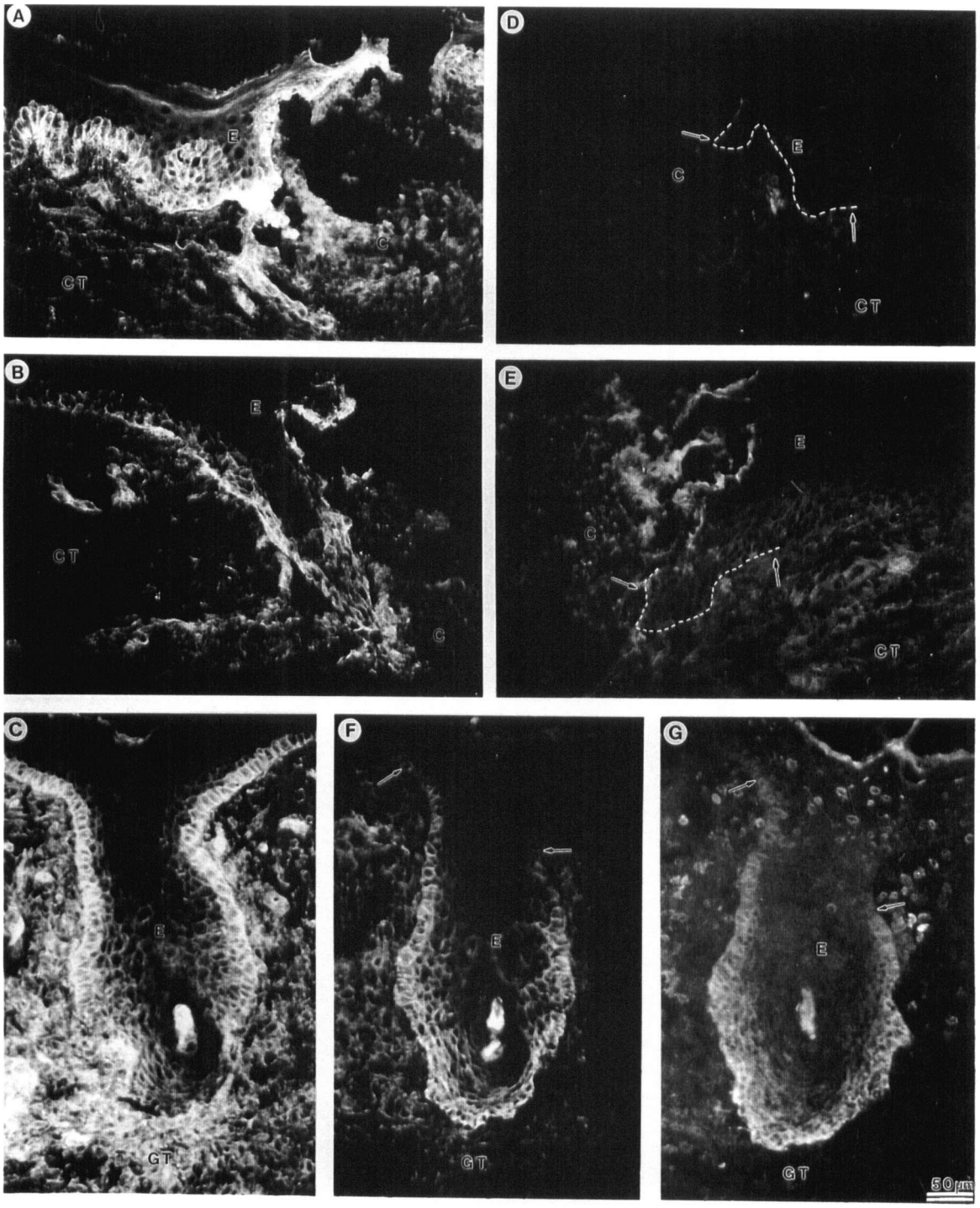

Fig. 5. In human dermal wounds ß1 integrin is expressed throughout the healing process but αv and αvß6 integrins are not expressed until day 7. 1-Dau old (panels A, D), 3-day-old (panels B, E) and 7-day-old (panels C, F, G) dermal wounds were stained with antibodies specific to ß1 integrin (panels A, B, C), αv integrin (panels D, E, F), and αvß6 integrin complex panel G). Epithelial-blood clot (E/C) interphase marked with dotted line in panels D and E. E, epithelium; CT, connective tissue; GT, granulation tissue. Bar 50 μm.

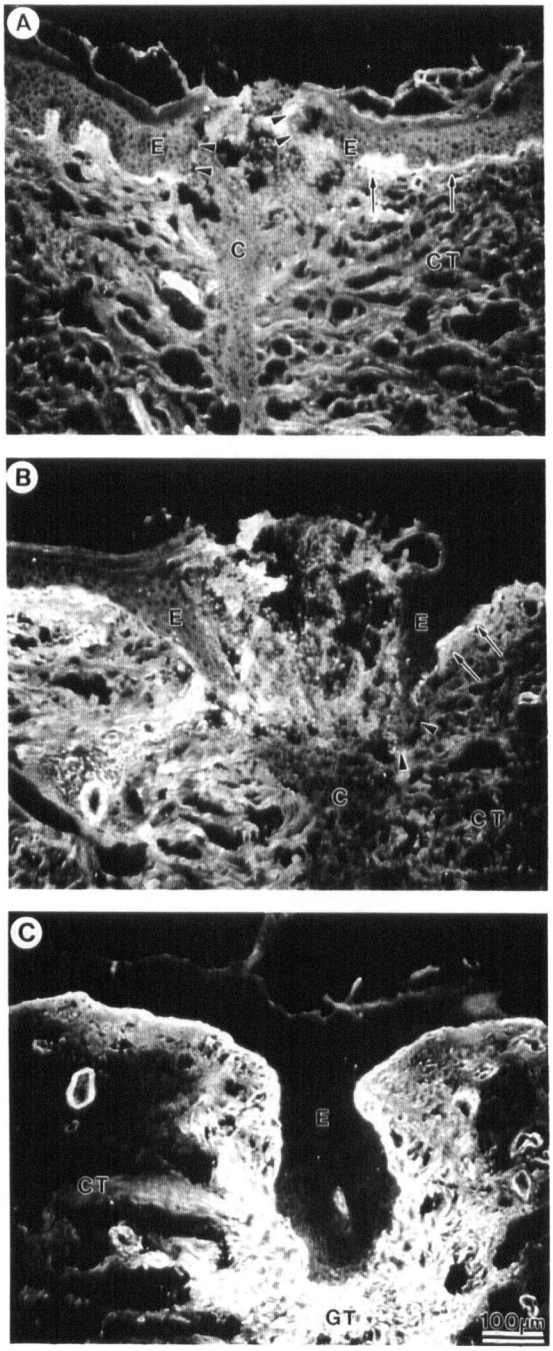

Fig. 6. Expression of tenascin is upregulated in dermal wound granulation tissue. Biopsy specimens were stained with antibody to tenascin by immunofluorescence as described in materials and methods. Distribution of tenascin in 1-day-old (A), 3-day-old (B), and 7-day-old (C) human dermal wounds. Arrowheads indicate wound margin and arrows tenascin containing subepithelial connective tissue in panels A and B. E, epithelium; C, clot; CT, connective tissue; GT, granulation tissue. Bar 100 μm

3.4. Discussion

We have reported previously that migrating wound keratinocytes start to express receptors of the αv integrin family (Larjava et al., 1993). Several recent studies have shown the presence of αv integrins in keratinocytes migrating in experimental wounds (Larjava et al., 1993; Juhasz et al., 1993; Cavani et al., 1993). The αv associated ß subunit has remained, however, unresolved. In the present study, I found that αvß6 is strongly expressed in wound keratinocytes.

In a porcine wound model, migrating keratinocytes appear to express αvß5 integrin (Gailit et al., 1994). In the human mucosal wound healing model, I have not found detectable levels of αvß5 integrin expression with immunofluorescence techniques. Further, I could not detect the αvß5 integrin in the healing epidermis of human skin. It is possible that porcine wound keratinocytes are different from human cells in their regulation of integrins. Alternatively, it is possible that the immunofluorescence method used was not sufficiently sensitive to detect minute levels of αvß5 in human skin. The antibody used here, was found to be reactive in immunofluorescence for αvß5 integrin in human synovium (Nikkari et al., 1995). The antibody recognizes the αvß5 complex. It is possible, therefore, that ß5 is present in wound keratinocytes but in another conformation not reactive with this monoclonal antibody. Cultured keratinocytes have been reported to express αvß5 integrin (Adams and Watt., 1991). Our results suggest that multiple αv-family integrins are expressed by cultured keratinocytes since the expression of ß6 was low compared to that of

total αv integrin expression. It is also possible that both ß5 and ß1 serve as partners for αv integrin subunit.

Expression of αvß6 was detected at the tip of migrating mucosal epithelium in some occasions and appeared to occur late during wound healing, since specimens were often nonreactive or weakly reactive in open 3-day-old wounds but became reactive in closed 7-day-old wounds. The age of the wound however, was less important than the stage of healing, since small 3-day-old wounds in which epithelial sheets had fused expressed αvß6 integrin. After seven days of healing, all wound specimens contained granulation tissue under the epithelial coverage and expressed strongly αvß6 integrin. These data agree with previous findings using αv specific antibody (Larjava et al., 1993), and also with findings using a graft healing model in which upregulation of αv occurs relatively late and persists for longer periods than induction of, for example, α5ß1 expression (Juhasz et al., 1993). This suggests that factors involved in regulating αvß6 expression are released after the initial movement of wound keratinocytes has already occured.

Initial assembly of hemidesmosomes and reconstruction of the basement membrane zone was observed in specimens that were strongly reactive with αvß6 antibody, suggesting that keratinocyte interaction with both basement membrane and granulation tissue matrix contributes to the upregulation of αvß6 expression. Deposition of basement membrane material and assembly of hemidesmosomes may not be necessary for the induction of αvß6 but may be required for optimal expression

of αvß6 in wounds. In normal tissues, expression of ß6 mRNA is found exclusively in epithelia (Breuss et al., 1993). Its expression is restricted however, to kidney tubule epithelium and endometrial epithelium, while in other epithelia, e.g., in normal skin, expression of ß6 mRNA is undetectable. Cultured cells use αvß6 as a fibronectin-binding integrin (Weinacker et al., 1994). It is possible, therefore, that wound keratinocytes use αvß6 for binding to fibronectin. It is puzzling that wound keratinocytes upregulate the expression of at least two fibronectin binding integrins. Both α5ß1 and αvß6 are expressed in different stages of wound healing. It is possible that α5ß1 is used mainly to bind cells to plasma-derived fibronectin during the early stage. αvß6 in contrast, could serve as a receptor for the differentially spliced embryonic type of fibronectin produced during formation of granulation tissue (ffrench-Constant et al., 1989). There is no experimental evidence, however, suggesting that differentially spliced fibronectins would function differently in this respect. Furthermore, both α5ß1 and αvß6 bind to the common RGD repeat present in all fibronectins (Busk et al., 1992). Alternatively, αvß6 and α5ß1 could mediate different cellular responses to fibronectin.

Tenascin was present underneath the epithelium both in normal mucosa and human dermis, as described earlier (Lightner et al., 1989). Its distribution extended deeper into the connective tissue stroma in mucosa compared to dermis. Expression of tenascin was strongly upregulated during granulation tissue formation both in mucosal and skin wounds, confirming previous reports from other wound healing

models (Mackie et al., 1988; Chuong and Chen, 1991; Whitby et al., 1991). Clearly, multiple integrins mediate cell adhesion to tenascin (Prieto et al., 1993; Bourdon and Ruoslahti, 1989). Evidently, αvß6 is also involved in recognition of the third fibronectin type repeat in tenascin, although direct data are still lacking (Prieto et al., 1993). Tenascin appears in wounds at about the same time as expression of αvß6 is optimal. In addition, tenascin is localized in proximity to basal keratinocytes associated with incompletely regenerated basement membrane. It is feasible therefore, that keratinocytes could utilize αvß6 for binding to tenascin. Basal keratinocytes express another integrin receptor, α9ß1, that could participate in tenascin binding (Palmer et al., 1993; Yokosaki et al., 1994) either alternatively or in conjunction with other integrins.

3.5. Summary

Cell adhesion receptors of the integrin family play a major role during re-epithelialization of human wounds. We have previously documented that the expression of αv family integrins is induced in keratinocytes of mucosal wounds (Larjava et al., 1993). In the present investigation, I extended these studies to determine whether αvß6 integrin is expressed during wound healing in humans. Mucosal and epidermal wound sections from 1- to 7-day-old wounds were used for immunolocalization of integrins and their putative ligands. Expression of αvß6 integrin appeared relatively late during mucosal and dermal wound healing. Maximal expression was seen in 7-day-old

wounds in which epithelial sheets had fused and granulation tissue was present. Fibronectin and tenascin, both possible ligands for αvß6 integrin, were found concentrated underneath the basal epithelial cells expressing this receptor, and the maximal expression of tenascin coincided with that of αvß6 integrin. These results suggest that the expression of αvß6 integrin, a putative binding integrin for fibronectin and tenascin, is induced in keratinocytes when epithelial sheets fuse during wound healing.

CHAPTER FOUR

EXPRESSION OF EPITHELIAL ADHESION PROTEINS AND INTEGRINS IN CHRONIC INFLAMMATION

4.1. Introduction

During the pathogenesis of periodontal disease, the JE between the tooth surface and gingival connective tissue moves apically and becomes transformed into periodontal pocket epithelium (Page and Schroeder, 1990). These epithelial cells proliferate and form epithelial ridges in inflamed connective tissue. As a result, the relative volume of gingival tissue occupied by epithelial cells increases compared to healthy tissue. This dynamic process of epithelial cell movement involves adhesion to and migration along an extracellular connective tissue matrix that is altered due to chronic inflammation. The basement membrane also shows alterations, with discontinuities and other morphological changes in chronic inflammation (Thilander, 1968; Freedman et al., 1968; Takarada et al., 1974; Peng et al., 1986). The adhesion and migration mechanisms of epithelial cells in chronically inflamed tissues are, so far, poorly characterized. It is likely, however, that the expression of specific cell surface adhesion receptors is altered as a result of changing functional demands and the effects of bioactive substances present in inflammation (Uitto and Larjava, 1991).

In this section I demonstrate alterations in the expression of ß1 and ß4 integrins and basement membrane zone proteins in chronic periodontal inflammation.

4.2. Material and methods

Material. Chronically inflamed periodontal tissue specimens were obtained from 22 subjects during extraction of periodontally involved teeth with a clinically hopeless prognosis. Specimens were rinsed in physiological saline, placed on a cork disk in a defined orientation, embedded in Tissue-Tek II O.C.T. compound (Miles Inc. Elkhart, IN), and immediately frozen in liquid nitrogen. Samples were stored at -70°C until used. Specimens were sectioned (6 μm) in a cryostat. Adjacent sections corresponding to those used for immunohistological studies were stained with hematoxylin and eosin for histopathological and morphological analyses. Gomori's periodic acid methenamine silver method was used to histochemically stain the basement membrane zone (Culling, 1963).

Immunohistochemical studies

Frozen sections (6 μm) were fixed and stored as described in the previous work. Immunolocalization of integrins and examination of stainings were performed as described previously. Antibodies against the following integrin subunits and their ligands were used (sources and references for antibodies are given in Table I): α2, α3, α5, α6, αv, ß1, ß4, ß5, ß6, fibronectin, type IV and VII collagens, tenascin, LM-1, and LM-5. Control stainings were performed omitting the primary antibody or with nonimmune mouse serum. No specific staining was obtained with either of these control staining procedures (not shown).

4.3. Results

Microscopic morphology of inflamed periodontal tissue

Chronically inflamed tissue sections were obtained from patients suffering from advanced adult periodontal disease. Specimens containing both relatively healthy and chronically inflamed tissue were investigated. Comparisons were always made between these two areas (Fig. 7 A and B) in the same section. Using hematoxylin-eosin staining, a heavy infiltrate of inflammatory cells was seen in close proximity to the epithelium of inflamed area. Cellular infiltrates were mainly composed of chronic inflammatory cells, i.e. lymphocytes and plasma cells (Fig. 7B). In chronically inflamed sites, nonkeratinized epithelium varied in thickness from several cell layers to long epithelial extensions into the inflamed connective tissue.

We then used Gomori's periodic acid methenamine silver method (Culling, 1963) to examine the basement membrane underlining the epithelium. This staining visualizes aldehydes generated by periodate treatment, and it can be used to evaluate the integrity of basement membrane in tissue sections. In relatively healthy areas of oral epithelium, basement membrane staining was continuous separating the oral epithelium from the underlying stroma (Fig. 7C). In contrast, in inflamed periodontal tissues only faint, discontinuous staining was observed (Fig. 7D).

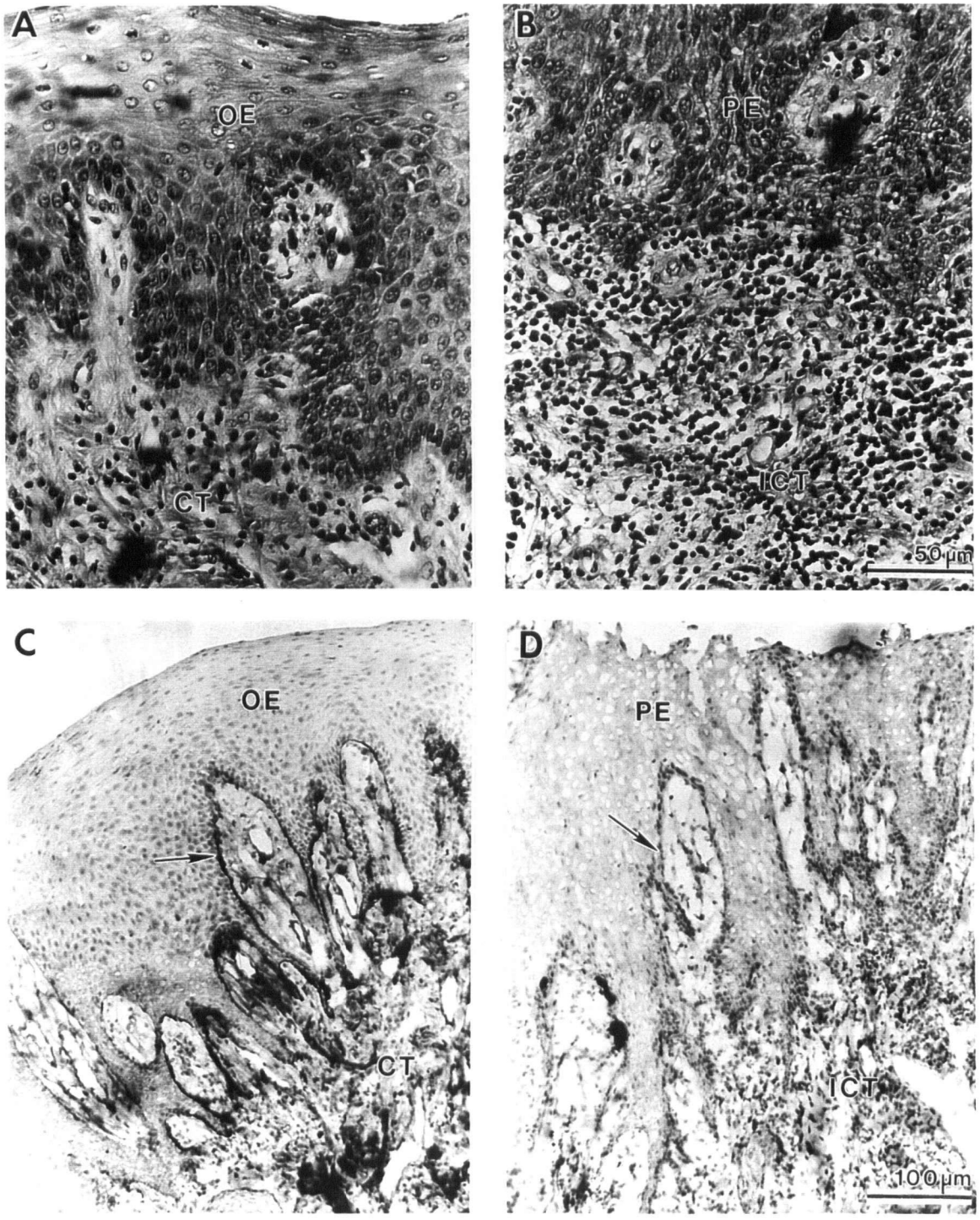

Figure 7. Hematoxylin and eosin staining of healthy (A), and chronically inflamed (B) periodontium. Gomori's periodic acid methenamine silver staining of basement membrane zone in normal (C) and inflamed periodontal tissue (D). Arrows indicate location of basement membrane (C and D). OE; oral epithelium; CT; connective tissue; PE; periodontal epithelium in chronic inflammation; ICT; inflamed connective tissue. Bar 50 μm: A, B; bar 100 μm: C, D.

Localization of LM-1 and type IV collagen

We then examined the putative adhesion molecules for epithelial cells, LM-1 (classical laminin) and type IV collagen using immunolocalization techniques. In healthy oral epithelium, both LM-1 and type IV collagen were found in epithelial and vascular basement membranes (Fig. 8A and B). In chronically inflamed tissue, both LM-1 and type IV collagen were still present at the epithelial basement membrane zone, although in somewhat reduced amounts (Fig. 8). Numerous blood vessels surrounded by LM-1 and type IV collagen were present in the inflamed connective tissue (Fig. 8C and D). In addition, anomalous localization of punctate extravascular laminin and to a lesser extent type IV collagen was found to be present in the inflamed connective tissue stroma. Visually, the total amount of basement membrane components therefore often appeared to be increased in the inflamed area due to the deposition of new vascular and extravascular basement membrane material. Using higher magnification, we were able to demonstrate several different patterns of extravascular LM-1 accumulation (Fig. 9 A-C). Clearly, LM-1 was often present surrounding blood vessels (Fig. 9A), but it was also found in punctate (Fig. 9B) and streak-like (Fig. 9C) distributions. Type IV collagen was also present in the stroma in a punctate pattern (Fig. 9D). Staining appeared to be specific, because only faint diffuse background staining was obtained with control antibodies (mouse nonimmune IgG or rabbit serum), or by omitting the first antibody (conjugate only) (not shown).

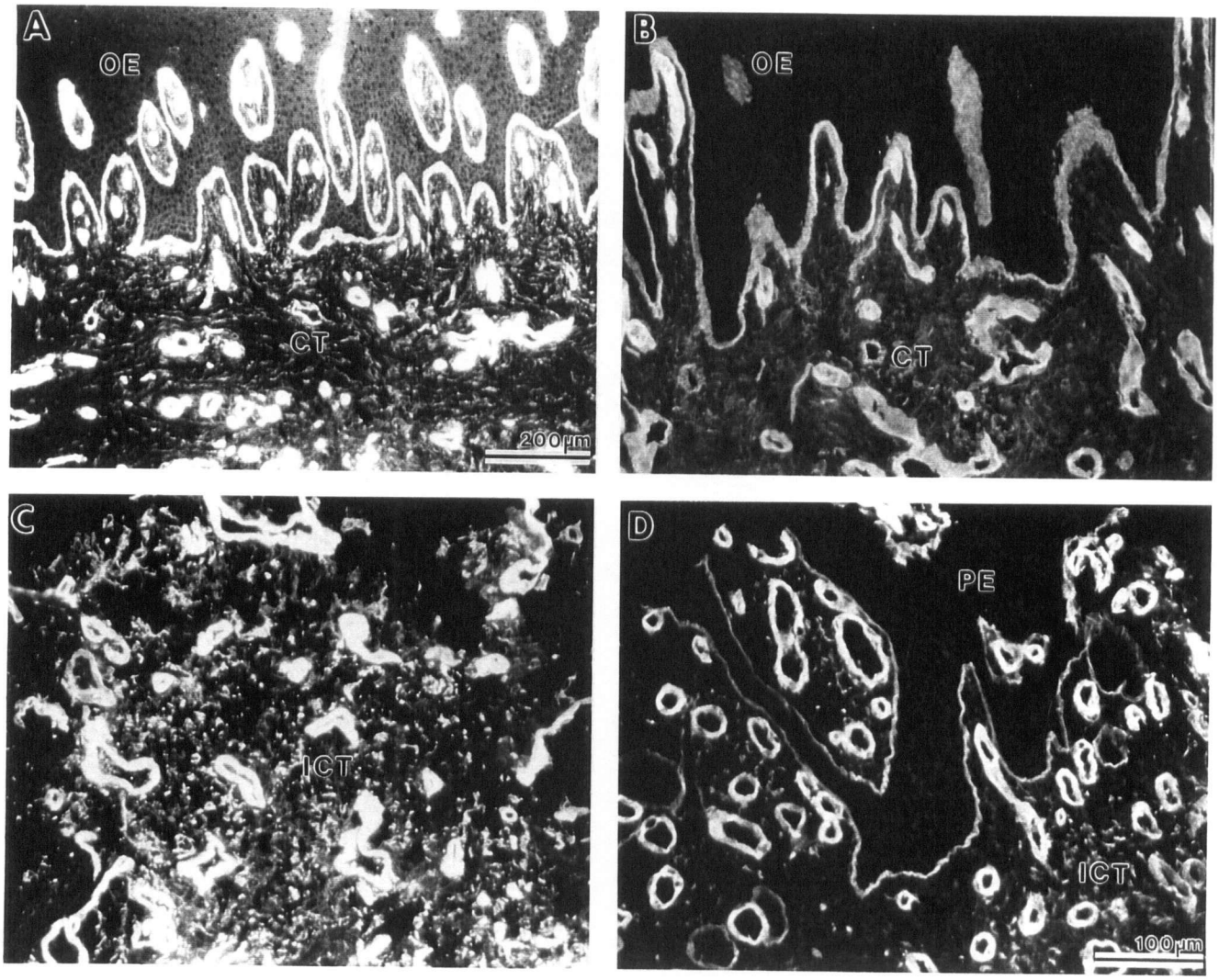

Figure 8. Immunolocalization of LM-1 (A and C) and type IV collagen (B and D) in healthy (A and B) and in chronically inflamed periodontal tissue (C and D), respectively. OE; oral epithelium; CT; connective tissue; PE; periodontal epithelium in chronic inflammation; ICT; inflamed connective tissue. Bar 200 μm: A; bar 100 μm: B, C, D.

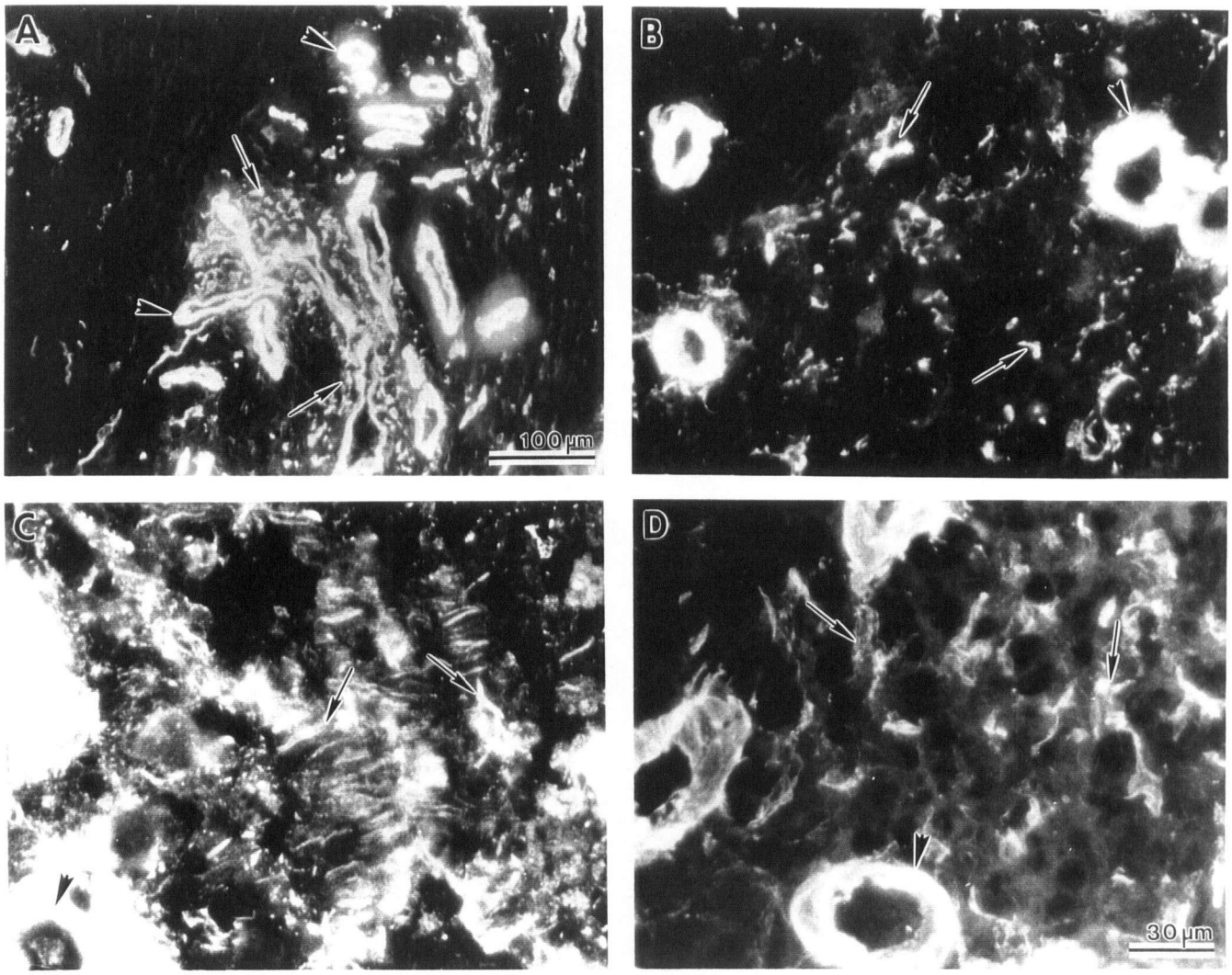

Figure 9. Distinct patterns of extravascular (arrows) LM-1 (A-C) and type IV collagen (D) in inflamed periodontal tissue. Arrowheads indicate LM-1 and type IV collagen associated with vascular basement membrane.

Localization of components of epithelial anchoring system

Epithelium is anchored to the underlying connective tissue via hemidesmosome structures in which anchoring filaments and anchoring fibers composed of LM-5 and type VII collagen, respectively, serve as linking elements (Rousselle et al., 1991; Keene et al., 1987). Since the α6ß4 integrin is believed to use some of these proteins as adhesive ligands, we compared their distributions in healthy and chronically inflamed tissues. Based on immunolocalization, type VII collagen was relatively well preserved at the basement membrane zone in chronic inflammation (Fig. 10). With higher magnification there was, however, a visible reduction of the intensity of staining in areas of chronic inflammation (compare Fig. 11C and D). In five out of eleven of the samples, type VII collagen was also found in unusual streak-like distributions in the subepithelial connective tissue stroma of inflamed tissue (compare Fig. 11C to 11D and 15F). In eight out of eleven samples the intensity of LM-5staining was generally reduced at the basement membrane zone in chronic inflammation, but in three samples it seemed to be relatively well-preserved (Fig. 11E and F). In four out of eleven samples LM-5 was found in similar streak-like patterns as type VII collagen in the connective tissue stroma of inflamed tissue (Fig. 15E and F).

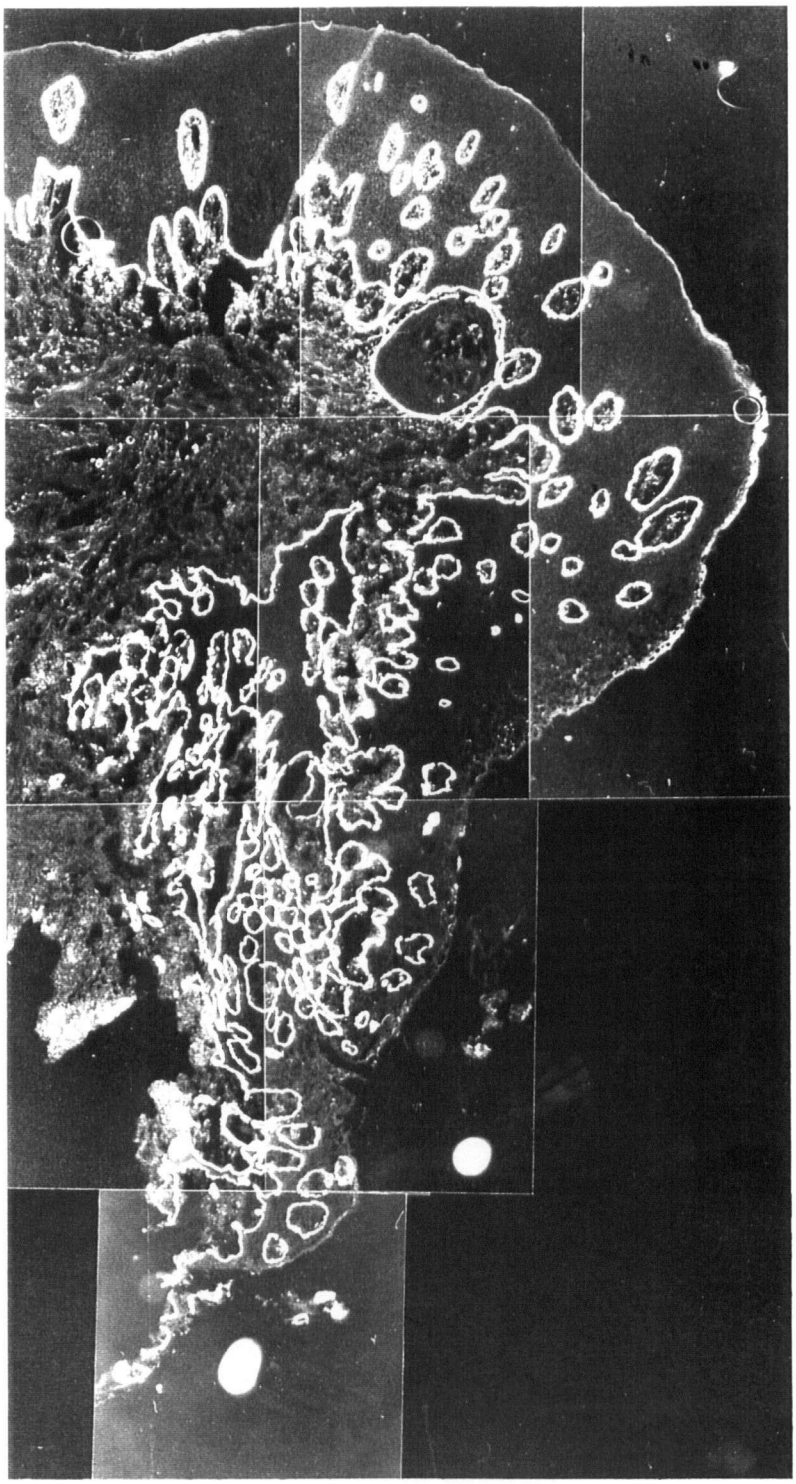

Figure 10. Immunostaining of type VII collagen associated with oral epithelium (OE) and with periodontal epithelium in chronic inflammation (PE).

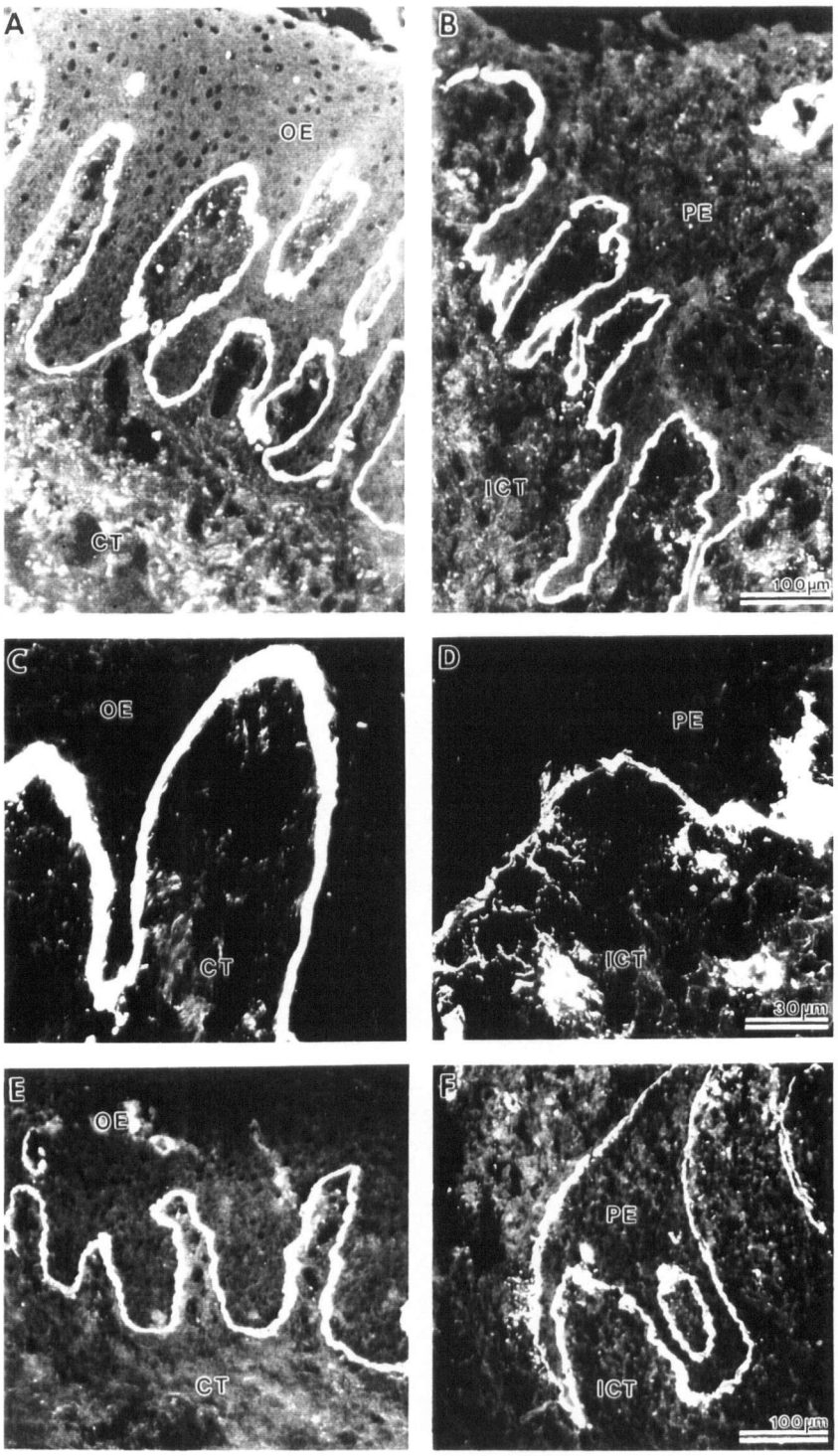

Figure 11. Immunolocalization of type VII collagen (A-D) and LM-5 (E, F) in healthy oral mucosa (A, C, E) and in chronically inflamed periodontal tissue (B, D, F). OE; oral epithelium; CT; connective tissue; PE; periodontal epithelium in chronic inflammation; ICT; inflamed connective tissue. Bar 100 μm: A, B, E, F; bar 30 μm: C, D.

Localization of fibronectin and tenascin

Fibronectin and tenascin are also putative ligands for epithelial cell adhesion receptors. Fibronectin was present throughout the connective tissue adjacent to healthy oral epithelium, but it was clearly diminished in inflamed stroma (Fig. 12A and B). A fragmented and punctate staining pattern of fibronectin surrounded the inflammatory infiltrate. Tenascin was enriched in the subepithelial zone of healthy oral epithelium (Fig. 12C). In chronically inflamed tissue, tenascin was almost completely absent (Fig. 12D) and positive staining for tenascin was only seen at the basement membrane zone.

Distribution of ß1 and α6ß4 integrins in epithelial cells

Staining for ß1 integrins was found around the periphery of basal keratinocytes of healthy oral epithelium (Fig. 13A). In chronic inflammation, two distinct patterns were seen. Most often, a focal loss of epithelial ß1 integrins was seen in inflamed areas (Fig. 13D). Most of the samples, however, also included areas where the full thickness of epithelium (Fig. 13G) or the extending tip of it (Fig. 13H) was found to be positive for ß1 integrins. In both cases, cells in the inflamed stroma showed bright staining for ß1 integrins (see Fig. 13H). Immunolocalization of α2ß1 and α3ß1 integrins by specific antibodies confirmed the variable staining pattern of ß1 integrins observed with ß1 specific antibodies (Fig. 14). Epithelial cells showed a loss of integrin expression in some areas of the section, while up-regulation was observed in others. During inflammatory stimulation, the staining pattern also changed from localization around

basal cells to a pattern encompassing cells in all cell layers (Fig. 14G, H, I). The transition from a nearly normal staining pattern to that of strongly induced expression was often remarkably abrupt sharp (e.g. see Fig. 14G). A continuous staining pattern was observed for α6 and ß4 integrins at the basal aspect of basal keratinocytes in noninflamed areas (Fig. 13B, C). Focal losses of both α6 and ß4 integrin subunits were common in epithelial cells at inflamed sites (Fig. 13E, F). Double staining for ß1 integrins and type VII collagen permitted better visualization of the alterations, since the loss of integrin staining sometimes made it difficult to distinguish epithelial cells from other cells in the stroma (Fig. 13H, I). It was noted that in areas of relatively high ß1 integrin expression in epithelium, also type VII collagen often became localized in streaks in the subepithelial connective tissue (Fig. 13H and I). This view was supported by findings from serial parallel sections that were stained for ß1, α6, ß4, type IV collagen, LM-5, and type VII collagen (Fig. 15A-F). In areas which showed streak-like distributions of type IV and VII collagen and LM-5, all cell layers of epithelium were strongly positive for ß1 integrins. In addition, staining for both α6 and ß4 integrins was relatively intense.

Members of the αv-integrin family were not detected in epithelia of healthy or inflamed tissue. Attempted immunolocalization with specific antibodies recognizing αvß5 and αvß6 integrins gave negative results in all samples (data not shown).

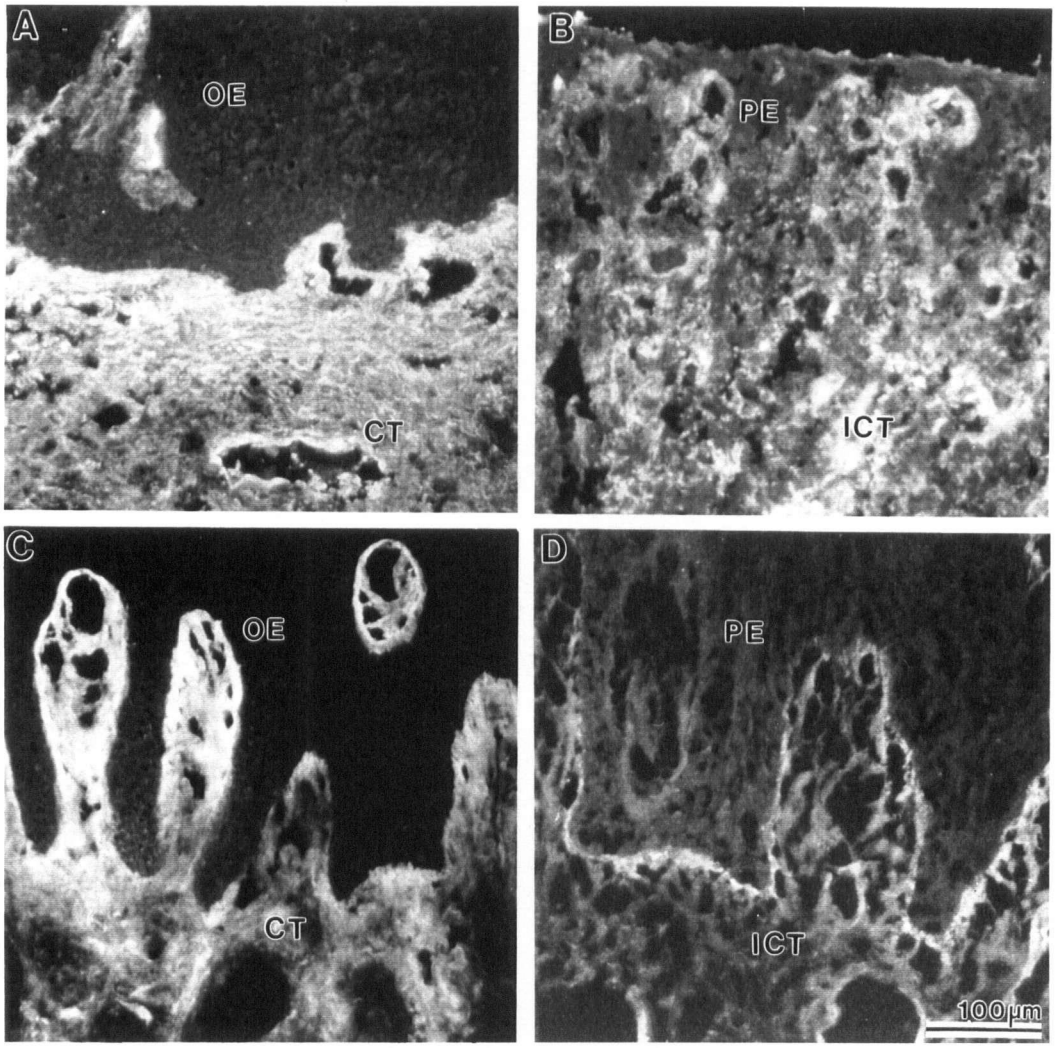

Figure 12. Immunolocalization of fibronectin (A, B) and tenascin (C, D) in healthy oral mucosa (A, C) and in chronically inflamed periodontium (B, D). Note the reduction in intensity of staining for each molecule in inflamed subepithelial connective tissue. OE; oral epithelium; CT; connective tissue; PE; periodontal epithelium in chronic inflammation; ICT; inflamed connective tissue. Bar 100 μm: all figures.

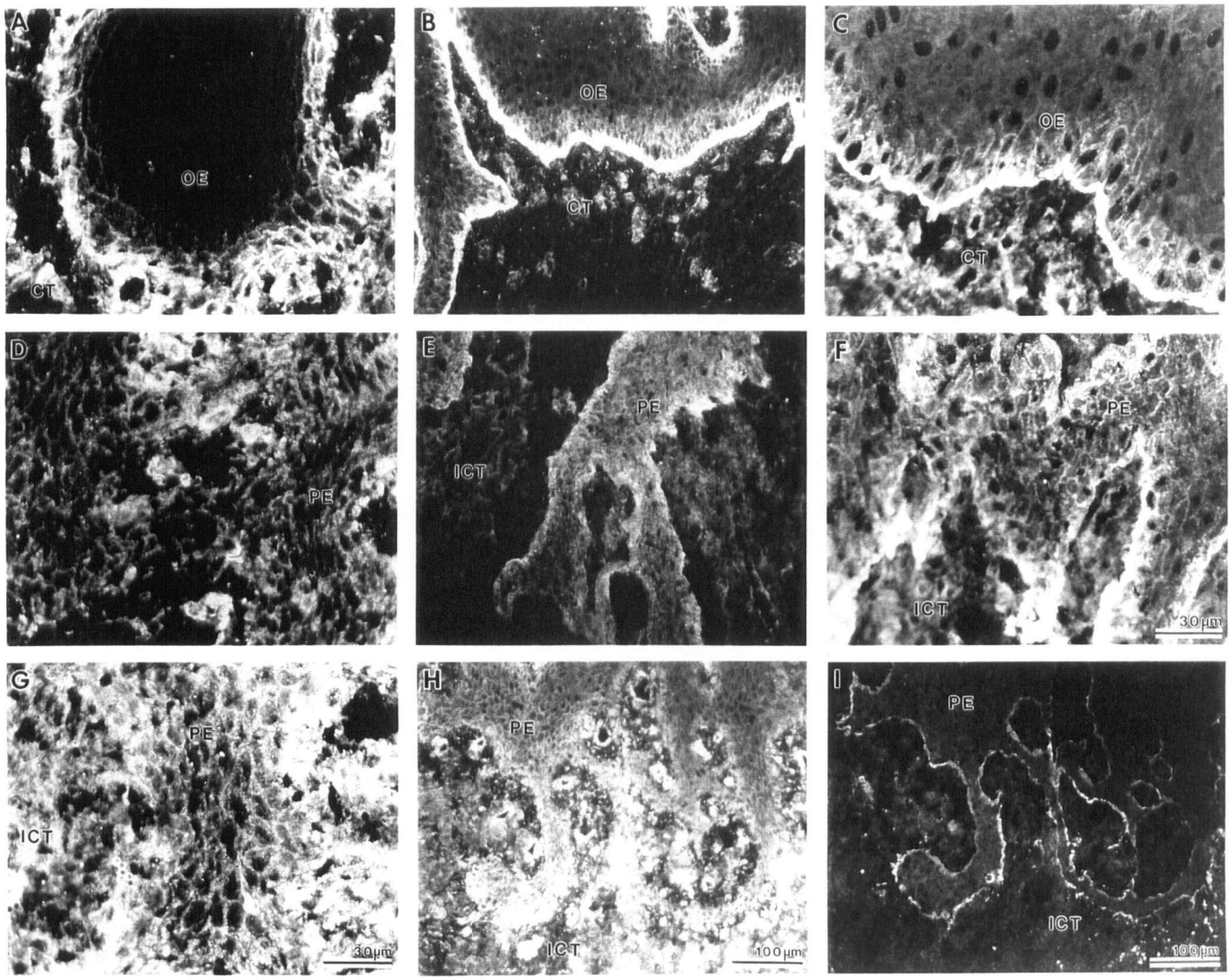

Figure 13. Immunolocalization of ß1-integrin (A, D, G), α6-integrin (B, E), ß4-integrin (C, F) in healthy oral epithelium (A-C) and in chronically inflamed periodontal tissue (D-G). Double-immunostaining of ß1-integrin (H) and type VII-collagen (I) in inflamed periodontal tissue. OE; oral epithelium; CT; connective tissue; PE; periodontal epithelium in chronic inflammation; ICT; inflamed connective tissue. Bar 30 µm: A, C, D, F, G; bar 100 µm: B, E, H, I.

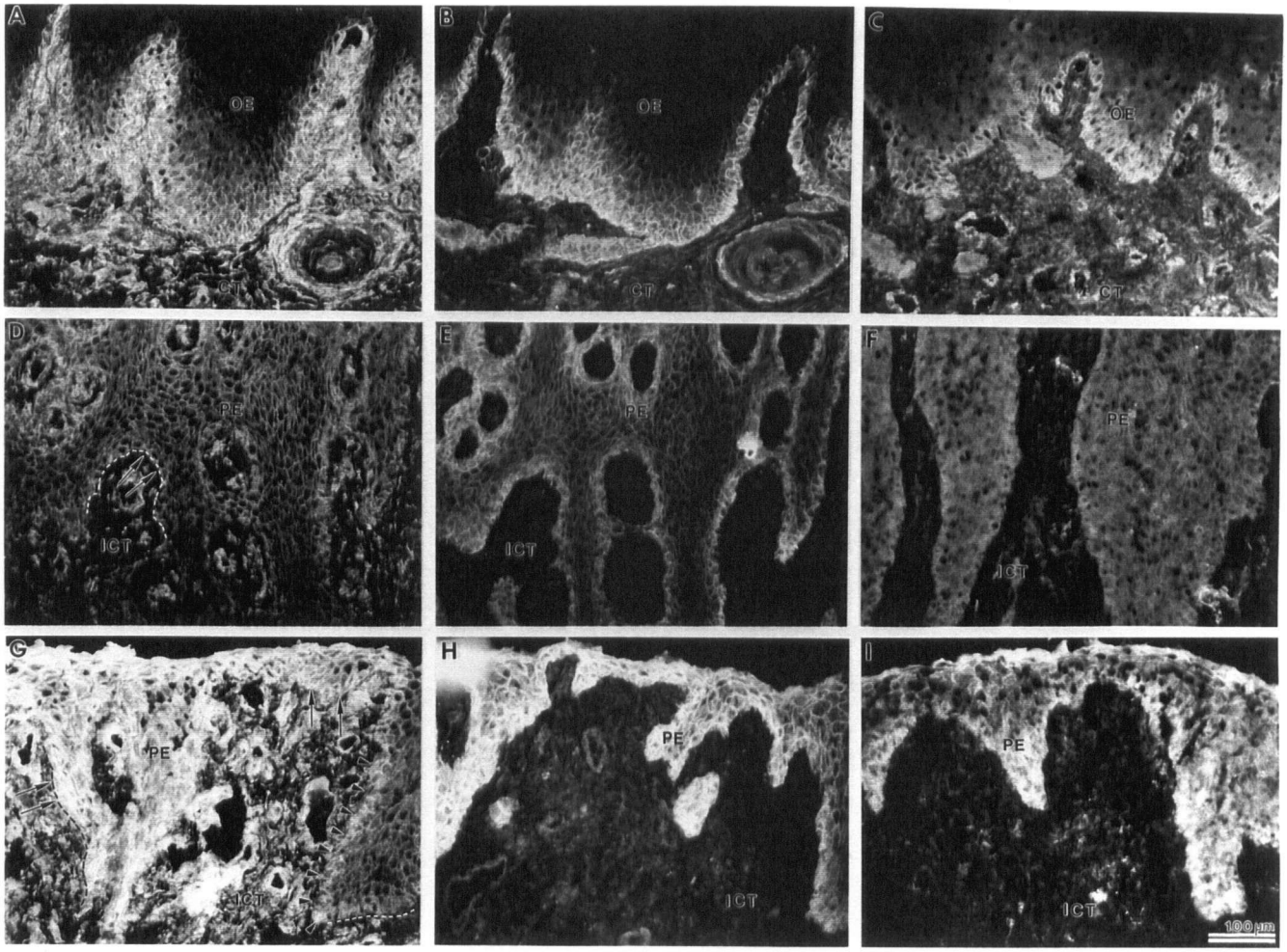

Figure 14. Immunolocalization of ß1 (A, D, G), α3 (B, E, H) and α2 (C, F, I) integrins in healthy oral epithelium (A-C), and in periodontal epithelium in chronic inflammation (D-I). In inflammation, some areas of periodontal epithelium show focal loss of these integrins (D-F) whereas in others the full thickness of inflamed periodontal epithelium is stongly positive for ß1, α3 and α2 integrins (G-I). Arrows mark epithelial-connective tissue boundary (dashed black line, panel G). Arrowheads (panel G) mark ß1 staining which is comparable to normal oral epithelium, and dashed white line indicates area of epithelium that demonstrates a loss of ß1 staining. OE; oral epithelium; CT; connective tissue; PE; periodontal epithelium in chronic inflammation; ICT; inflamed connective tissue. Bar 100 μm.

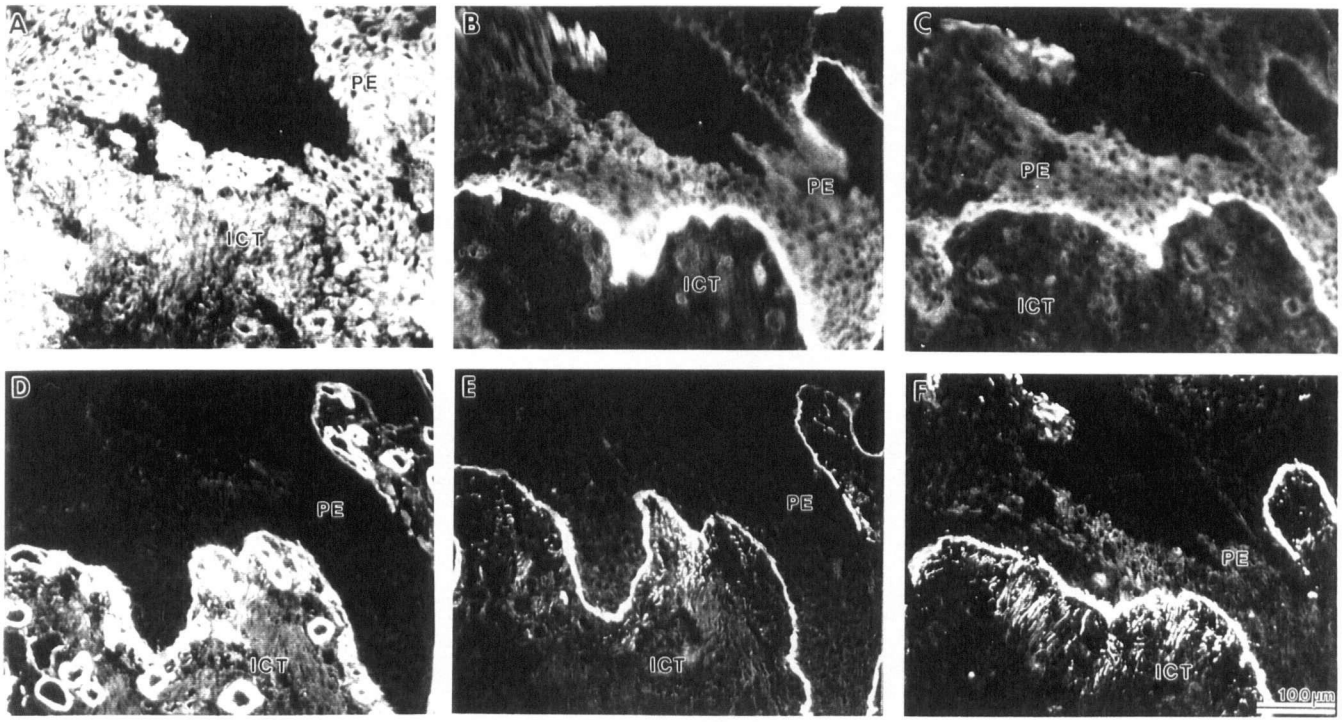

Figure 15. Immunolocalization of ß1 (A), α6 (B), ß4 (C), type IV (D), LM-5 (E), and type VII (F) in parallel serial sections of chronically inflamed periodontal tissue. Note the strong expression of ß1 integrin (A) in epithelium and also the streak-like appearance of LM-5 and type VII collagen (E,F) in connective tissue. PE; periodontal epithelium in chronic inflammation; ICT; inflamed connective tissue. Bar 100 μm.

4.4. Discussion

Epithelial cell adhesion, migration and proliferation have been studied in acute inflammatory conditions such as wound healing (Stenn and Malhorta, 1992, for review). However, epithelial cell behavior in chronic inflammatory conditions is much less characterized. Periodontal disease is characterized by chronic inflammation and slowly progressive tissue loss and provides, therefore, an interesting model to study epithelial cell behavior during chronic inflammation. The epithelium undergoes alterations that lead to ulceration, degeneration, and also increased proliferation and formation of epithelial rete ridges into inflamed connective tissue stroma. Our study demonstrates that major alterations take place in the expression of epithelial adhesion molecules and their receptors in the chronic inflammation of periodontal disease.

General structural alterations of the basement membrane zone in inflammatory periodontal lesions have been previously well-characterized. Several studies describe a diffuse appearance (Thilander, 1968), localized discontinuities (Freedman et al., 1968) or degradation (Takarada et al., 1974a, b) of the basement membrane facing inflamed connective tissue. Our study showed major alterations of individual adhesion molecules at the basement membrane zone. Despite extensive loss of subepithelial collagen, most of the matrix components associated with the basement membrane zone were partially retained in chronic inflammation. Generally, all components of the basement membrane zone including type IV collagen and laminins demonstrated

reduced staining intensity compared to normal noninflamed tissue. These proteins were, however, better preserved than stromal proteins fibronectin and tenascin, quantities of which were found to be markedly reduced in inflamed areas. In vitro experiments have shown that all of the above mentioned proteins are readily cleaved by matrix metalloproteinases (Birkedal-Hansen, 1993) which are present in high amounts in inflamed tissues (Mäkelä et al., 1994). Fragments of fibronectin have been demonstrated directly in fluids collected from sites of chronic inflammation (Carsons et al., 1985; Talonpoika et al., 1989; Wysocki and Grinnell, 1990). Our results suggest, however, that proteins of the basement membrane may be woven into a meshwork in which protein epitopes persist although proteins are partially degraded. Focal loss of type IV collagen, LM-1 and LM-5 was observed in some samples, similar to data obtained in a previous study of type IV collagen immunolocalization in inflamed periodontal tissue (Peng et al., 1986). In many specimens, however, type VII collagen and to a lesser extent LM- 5 were found in unusual locations in inflamed stroma. It is believed that type VII collagen is synthesized by keratinocytes, although stimulus from fibroblasts seems to be needed for optimal synthesis (Regauer et al., 1990; König et al., 1991). It is also possible, however, that fibroblasts in chronic inflammation could contribute to collagen VII accumulation, since TGF-ß is able to induce type VII collagen expression in fibroblasts (König et al., 1992).

Chronic lesions of periodontal disease contain large numbers of newly formed capillaries surrounded by basement membrane, as demonstrated in our samples

stained with antibodies against LM-1 and type IV collagen. These basement membrane components associated with new vessels were not found to be degraded at sites of inflammation. In fact, significant amounts of extravascular LM-1 and to lesser extent type IV collagen were found in the inflammatory sites. This atypical localization seems to be rather specific to chronic inflammation of periodontal disease and is not found during acute inflammation of wound healing (Larjava et al., 1993) or during chronic inflammation of rheumatoid arthritis (Nikkari et al., 1993), where type IV collagen and laminin are found exclusively in the basement membrane area. Electron microscopic studies have earlier demonstrated the presence of extra layers of basement membrane-like material surrounding the capillaries of inflamed gingival lesions (Gavin, 1970). It is not known which cells deposit this material, but sprouting endothelial cells are candidates for this activity. In addition granulation tissue fibroblasts may be responsible, because they have been found to synthesize basement membrane components in vivo (Betz et al., 1992). Our findings point to the conclusion that during chronic periodontal inflammation, the basement membrane zone proteins type VII collagen and laminin partly replace the connective tissue stroma. These proteins may then serve as adhesive ligands for epithelial cells, granulation tissue fibroblasts and inflammatory cells.

We have shown earlier that ß1 integrins localize mainly at lateral borders of basal cells in normal mucosa and skin (Peltonen et al., 1989; Larjava et al., 1992; Larjava et al., 1993) and that these integrins could mediate cell-cell adhesion of

keratinocytes (Larjava et al., 1990; Carter et al., 1990). The hemidesmosomal integrin α6ß4 is exclusively localized at the basal aspect of basal cells in normal epithelia. Both of these integrin types are found in an altered distribution surrounding suprabasal cells during wound healing (Larjava et al., 1993; Hertle et al., 1992) and in psoriatic epidermis (Hertle et al., 1992; Pellgrini et al., 1992). In the present study, focal loss of integrins was a common finding in epithelium of chronically inflamed periodontal lesions. This loss may result from specific downregulation of integrin expression, since the cells in the same area seemed to deposit normal amounts of basement membrane components. There were, however, also areas in which the expression of both α2ß1 and α3ß1 integrins appeared to be paradoxically markedly upregulated. Integrin expression is clearly up-regulated during wound healing (Larjava et al., 1993; Cavaniet al., 1993; Juhasz et al., 1993; Clark, 1990). Stimulated expression of ß1 integrins by keratinocytes has been found in many inflammatory dermal lesions such as psoriasis (Pellegrini et al., 1992), lichen (Konter et al., 1990; Ralfkiaer et al., 1991) and cutaneous lymphoma (Ralfkiaer, 1992). In contrast, integrin expression has been reported to be relatively unchanged in dystrophic or systemic diseases such as epidermolysis bullosa (Nazzaro et al., 1990) and systemic sclerosis (Sollberg et al., 1992). Periodontal lesions are known to contain areas with variable disease involvement as judged by varying gingival surface topography and tissue histology (Saglie et al., 1982). Cytokines are known to exert potent cell-specific effects on integrin expression (Heino, 1993; Heino et al., 1989; Milam et al., 1991). It is therefore

likely that integrin expression is regulated locally in pocket epithelial cells by combinations of inflammatory cytokines.

4.5. Summary

Epithelial cell behavior in chronic inflammation is poorly characterized. During inflammation of periodontal tissues, increased proliferation of epithelial cells into the inflamed connective tissue stroma is commonly seen. In some areas ulceration and degeneration take place. We studied alterations in the expression of adhesion molecules and integrins during chronic periodontal inflammation. In inflamed tissue, LM-1 and type IV collagen were still present in the basement membrane and surrounding blood vessels, but they were also found extravascularly in inflamed connective tissue stroma. Type VII collagen and LM-5 were poorly preserved in the basement membrane zone, but both were found in unusual streak-like distributions in the subepithelial connective tissue stroma in inflamed tissue. Both fibronectin and tenascin were substantially decreased in chronically inflamed connective tissue, showing only punctate staining at the basement membrane zone. Integrins of the ß1 family showed two distinct staining patterns in epithelial cells during chronic inflammation; focal losses of ß1 integrins ($\alpha 2\beta 1$ and $\alpha 3\beta 1$) were found in most areas, while in other areas the entire pocket epithelium was found to be strongly positive for ß1 integrins. No members of the αv integrin family were found in any epithelia studied. Expression of the $\alpha 6$ß4 integrin was high in basal cells of healthy tissue, but weak in epithelium

associated with chronic inflammation.

Chronic inflammation therefore involves alterations in both adhesion proteins and integrins expressed by epithelial cells. Basement membrane components found at abnormal sites in stroma in chronic inflammation might serve as new adhesive ligands for various cell types in inflamed stroma.

CHAPTER FIVE

GENERAL DISCUSSION AND CONCLUSIONS

Periodontal epithelia play a critical role in protection, destruction and repair of human periodontium. During optimal repair, epithelium migrates and covers the wound surface to prevent the infection and damage of vulnerable underlying connective tissue. During destruction, JE undergoes transformation to the pocket epithelium that increases in volume and changes architecture. Both scenarios, regeneration and destruction, involve active epithelial migration either in the wound provisional matrix in the case of regeneration, or in the inflamed connective tissue matrix during periodontal disease, respectively.

During the last decade information on the cell adhesion receptors and their ligands in the extracellular matrix has explosively increased. Even though there are still many unanswered questions some clear patterns have emerged from these studies. Integrins that form a large family of cell surface receptors are mainly responsible for the cell-matrix interactions. Cells react rapidly to the changes in their environment by relocation of exisiting integrins and expressing new integrins specifically targeting the pericellular matrix proteins. Matrix binding is a prerequisite for cell migration and proliferation of epithelial cells. During wound healing, migrating epithelial cells of the wound margin intensely express integrins binding to fibronectin, vitronectin, tenascin and collagens. Only after epithelial confrontation and formation of new basement membrane do the cells revert to their normal integrin expression pattern.

Epithelial cell adhesion undergoes marked modulation during keratinocyte migration and basement membrane reconstruction during wound healing (Larjava et al., 1996, for review). Receptors that already exist in stationary cells change their cell surface distribution and probably also function. Alpha5ß1 fibronectin receptor is induced and it is thought to mediate binding to serum fibronectin during the early stage of healing. At later stage of wound healing, αvß6 integrin could replace α5ß1 as the fibronectin binding receptor to transfer epithelial cells to lower affinity binding that would then allow basement membrane reconstruction. Alternatively, αvß6 integrin could participate in regulation of connective tissue deposition by wound fibroblasts. Temporal and spatial expression pattern of αvß6 integrin matches with collagen deposition by wound fibroblasts that are known to crosstalk with the neighboring epithelial cells. Although this thesis deals with αvß6 integrin, it should be remembered that other integrins are also important during wound healing. Integrins α6ß4 and α3ß1 mediate keratinocyte adhesion to LM-5 that migrating cells actively deposit during wound healing. LM-5 appears to be always present under the migrating epithelial cells. LM-5 can not be providing its normal function as a constituent of the hemidesmosomes since they are missing. It is possible that proteolytic cleavage of LM-5 by gelatinase A makes it a migratory substrate for keratinocytes while intact LM-5 would then support the formation of stable anchoring contacts, the hemidesmosomes (Giannelli et al., 1997). These new data emphasize how complex the wound healing process is. Not only alterations of integrins and their functions but also

processing of the wound bed matrix play important roles in recruitment of epithelial cells into the wound. During wound healing, α2ß1 and α9ß1 integrins could mediate epithelial adhesion to collagen and tenascin, respectively. Mucosal keratinocyte migration seems to occur similarly to that of the epidermal keratinocytes although the source of epithelial cells is different. In the skin, epithelial cells arise not only from the edge of wound but also from hair follicles and sweat glands. In mucosa, the wound edge epithelium serves as a major source of keratinocytes that migrate into the wound bed. How the expression of integrins is modulated during healing of epithelial-hard tissue interphase during formation of long JE is not known. Without doubt cell adhesion mechanisms are instrumental for healing of mucosa and skin. Understanding of these mechanisms would contribute to our understanding of clinical treatment modalities such as guided tissue regeneration.

During chronic inflammation of periodontal disease, several changes occur in the extracellular matrix molecules and integrin expression of the structural cells. While the amount of fibronectin and tenascin is largely decreased, several other basement membrane proteins are abnormally expressed in connective tissue stroma. Wide variation in integrin expression follows. In some areas a strong increase takes place in the pocket epithelium while in other sites the expression is depressed. We have only started to understand the regulatory factors of integrin expression. It is clear, however, that both composition of the pericellular matrix and a variety of cytokines are responsible for this regulation. Chronic inflammation of periodontal disease

produces a variety of cytokines that potentially regulate integrin expression in epithelial cells. High concentration of interleukin-1 (IL-1), interleukin-4 (IL-4), interleukin-6 (IL-6), granulocyte colony stimulating factor (G-CSF), and tumor necrosis factor-α (TNFα) have been found in chronic inflammation of oral mucosa (Yamamoto and Osaki, 1995). Although the role of some of these cytokines in the regulation of integrins is known in some cell types, the complex synergistic and antagonistic action of these compounds in integrin expression remains unsolved. Additional complexicity is created by altered extracellular matrix of chronic inflammation which may contribute to the regulation of expression of integrins. It is also possible, that bacterial components of the periodontal pocket directly act on epithelial cells inducing changes in their integrin expression. This new phenotype of epithelial cells could be proinflammatory and be potentially responsible for the maintenance of chronic inflammation by secretion of multitude of cytokines. The role of integrins in regulating epithelial cell phenotype and inflammation has been proposed (Carroll et al., 1995). It is possible that periodontal pocket epithelium plays a much more active role in periodontal destruction than previously thought. Further studies on genetic defects in the integrin and matrix molecule expression predisposing to pathologic conditions such as periodontal diseases has only recently been initiated. Only when details of cell-matrix interactions and their control processes are understood we can effectively tackle problems related to therapeutic tissue manipulation aimed at periodontal disease prevention and optimal tissue regeneration.

BIBLIOGRAPHY

Adams JC, Watt FM: Changes in keratinocytes adhesion during terminal differentiation: reduction in fibronectin binding precedes $\alpha 5\beta 1$ integrin loss from the cell surface. Cell 1990, 63:425-435

Adams JC, Watt FM: Expression of ß1, ß3, ß4, and ß5 integrins by human epidermal keratinocytes and non-differentiating keratinocytes. J Cell Biol 1991, 115:829-841

Akiyama SK, Hasegawa E, Hasegawa T, Yamada KM: The interaction of fibronectin fragments with fibroblastic cells. J Biol Chem 1985, 260:13256-60

Akiyama SK, Yamada SS, Chen W-T, Yamada KM: Analysis of fibronectin receptor function with monoclonal antibodies: Roles in cell adhesion, migration, matrix assembly, and cytoskeletal organization. J Cell Biol 1989, 109:863-875

Akiyama SK, Aota S, Yamada KM: Function and receptor specificity of a minimal 20 kilodalton cell adhesive fragment of fibronectin. Cell Adhesion Commun 1994, 3:13-25

Albelda SM, Buck CA: Integrins and other cell adhesion molecules. FASEB J 1990, 4:2868-2880.

Anatoniades HN, Galanopoulos T, Neville-Golden J, Kiritsy CP, Lynch E: Injury induces in vivo expression of platelet-derived growth factor (PDGF) and PDGF receptor mRNAs in skin epithelial cells and PDGF mRNA in connective tissue fibroblasts. Proc Natl Acad Sci 1991, 88:565-569

Bazzoni G, Shih DT, Buck CA, Hemler ME: Monoclonal antibody 9EG7 defines a novel beta 1 integrin epitope induced by soluble ligand and manganese, but inhibited by calcium. J Biol Chem 1995, 270(43):25570-25577

Bennet NT, Schultz GS: Growth factors and wound healing, II. Role in normal and chronic wound healing. Am J Path 1993, 166:74-81

Betz P, Nerlich A, Wilske J, Tübel J, Wiest I, Penning R, Eisenmenger W: Time-dependent pericellular expression of collagen type IV, laminin, and heparan sulfate proteoglycan in myofibroblasts. Int J Legal Med 1992, 105:169-172

Birkedal-Hansen H: Role of matrix metalloproteinases in human periodontal diseases. J Periodontol 1993, 64:474-484

Bourdon MA, Ruoslahti E: Tenascin mediates cell attachment through an RGD-dependent receptor. J Cell Biol 1989, 108:1149-1155

Boyce ST: Epidermis as a secretory tissue. J Invest dermatol 1994, 102:8-10

Breuss JM, Gillett N, Lu L, Sheppard D, Pytela R: Restricted distribution of integrin β6 mRNA if primate epithelial tissues. J Histochem Cytochem 1993, 41:1521-1527

Breuss JM, Gallo J, DeLisser HM, Klimanskaya IV, Folkesson HG, Pittet JF, Nishimura SL, Aldape K, Landers DV, Carpenter W, Gillett N, Sheppard D, Matthay MA, Albelda SM, Kramer RH, Pytela R. Expression of the ß6 integrin subunit in development, neoplasia and tissue repair suggests a role in epithelial remodeling. J Cell Sci 1995; 108:2241-2251.

Briesewitz R, Kern A, Marcantonio EE: Assembly and function of integrin receptors is dependent on opposing α andß cytoplasmic domains. Mol Biol Cell 1995, 6:997-1010

Brown LF, Lanir N, McDonagh J, Tognazzi K, Dvorak AM, Dvorak HF: GFibroblast migration in fibrin gel matrices. Am J Path 1993, 142:273-283

Busk M, Pytela R, Sheppard D: Characterization of the integrin $\alpha_v\beta_6$ as a fibronectin-binding protein. J Biol Chem 1992, 267:5790-5796

Caffesse RG, Smith BA, Nasjleti CE, Lopatin DE: Cell proliferation after flap surgery, root conditioning and fibronectin application. J Periodontol 1987, 58:661-666.

Carroll MC, Romero MR, Watt FM: Suprabasal integrin expression in the epidermis of transgenic mice results in developmental defects and a phenotype resembling psoriasis. Cell 1995, 83:957-968

Carsons S, Lavietes BB, Diamond HS, Kinney SG: The immunoreactivity, ligand, and cell binding characteristics of rheumatoid synovial fluid fibronectin. Arthritis Rheum 1985, 28:601-612

Carter WG, Wayner EA, Bouchard TS, Kaur P: The role of integrins $\alpha 2\beta 1$ and $\alpha 3\ \beta 1$ in cell-cell and cell-substrate adhesion of human epidermal cells. J Cell Biol 1990a, 110:1387-1404

Carter WG, Kaur P, Gil SG, Gahr PJ, Wayner EA: Distinct functions for integrins $\alpha 3\beta 1$ in focal adhesions and $\alpha 6\beta 4$/bullous pemphigoid antigen in a new stable anchoring contact (SAC) of keratinocytes: relation to hemidesmosomes. J Cell Biol 1990b,

111:3141-3154

Carter WG, Ryan MC, Gahr PJ: Epiligrin, a new cell adhesion ligand for integrin $\alpha 3\beta 1$ in epithelial basement membranes. Cell 1991, 65:599-610

Cavani A, Zambruno G, Marconi A, Manca V, Marchetti M, Giannetti A: Distinctive integrin expression in the newly forming epidermis during wound healing in humans. J Invest Dermatol 1993, 101:600-604

Chen W-T, Hasegawa E, Hasegawa T, Weinstock C, Yamada KM: Development of cell surface linkage complexes in cultured fibroblasts. J Cell Biol 1985, 100:1103-1114

Chen JWC, Rogers AA, Lydon MJ: Characterization of biologic properties of wound fluid collection during early stages of wound healing. J Invest Dermatol 1992, 99:559-564

Cherny RC, Honan MA, Thiagaran P: Site-directed mutagenensis of the arginine-glycine-aspartic acid in vitronectin abolishes cell adhesion. J Biol Chem 1993, 268:9725-9729

Chiquet-Ehrismann: What distinguishes tenascin from fibronectin? FASEB J 1990, 4:2598-2604

Chuong CM, Chen HM: Enhanced expression of neural cell adhesion molecules and tenascin (Cytotactin) during wound healing. Am J Path 1991, 38:427-440

Clark RAF, Lanigan JM, DellaPelle P, Manseau E, Dvorak HF, Colvin RB: Fibronectin and fibrin provide a provisional matrix for epidermal cell migration during wound reepithelialization. J Invest Dermatol 1982, 70:264-269

Clark RAF: Fibronectin matrix deposition and fibronectin receptor expression in healing and normal skin. J Invest Dermatol 1990, 96:128S-134S

Crawford JM: Distribution of ICAM-1, LFA-3 and HLA-DR in healthy and diseased gingival tissues. J Periodont Res 1992, 27:291-298

Culling CFA: Handbook of Histopathological Techniques. 2nd Edition, Butterworths, London. 1963:230-231

De Luca M, Tamura RN, Kajiji S, Bondanza S, Rossino P, Candecca R, Marchisio PC, Quaranta V: Polarized integrin mediates human keratinocyte adhesion to basal lamina. Natl Acad Sci USA 1990, 87:6888-6892

Delwel GO, de Melker AA, Hogervorst F, Jaspars LH, Fles DL, Kuikman I, Lindblom A, Paulsson M, Timpl R, Sonnenberg A: Distinct and overlapping ligand specificities of the α3Aß1 and α6Aß1 integrins: Recognition of the laminin isoforms. Mol Biol Cell 1994, 5:203-215

DiPersio CM, Hodival-Dilke KM, Jaenisch R, Kreidberg JA, Hynes RO: α3ß1 integrin is required for normal development of the epidermal basement membrane. J Cell Biol 1997, 137:729-742

Domloge-Hultsch N, Gammon WR, Briggaman RA, Briggaman RA, Gil SG, Carter WG, Yancey KB: Epiligrin, the major human keratinocyte integrin ligand, is a target in both an acquired autoimmune and an inherited subepidermal blistering skin disease. J Clin Invest 1992, 90:1628-1633.

Eckert RL: Structure, function, and differentiation of the keratinocyte. Physiol Rev 1989, 69:1316-1346

Eckes B, Aumailley M, Krieg T: Collagens and the reestablishment of dermal integrity. In The molecular and cellular biology of wound repair. Clark RAF, editor. Plenum Press, New York. 1996:493-512

Elices MJ, Urry LA, Hemler ME: Receptor functions for the integrin VLA-3: Fibronectin, collagen, and laminin binding are differentially influenced by Arg-Gly-Asp peptide and by divalent cations. J Cell Biol 1991, 112:169-181.

Feghali-Assaly M, Sawaf MH, Serres G, Forest N, Ouhayoun JP: Cytokeratin profile of the junctional epithelium in partially erupted teeth. J Periodont Res 1994, 29:185-195

ffrench-Constant C, Van De Water L, Dvorak HF, Hynes RO: Reappearance of an embryonic pattern of fibronectin splicing during wound healing in the adult rat. J Cell Biol 1989, 109:903-914

Freedman HL, Listgarten MA, Taichman NS: Electron microscopic features of chronically inflamed human gingiva. J Periodont Res 1968, 3:313-319

Fässler R, Georger-Labouesse E, Hirsch E: Genetic analysis of integrin function in mice. Curr Opin Cell Biol 1996, 7:300-318

Gao Z, Mckenzie IC: Patterns of phenotypic expression of human junctional, gingival and reduced enamel epithelia in vivo and in vitro. Epith Cell Biol 1992, 1:156-167

Gailit J. Ruoslahti E. Regulation of the fibronectin receptor affinity by divalent cations. J Biol Chem 1988; 263:12927-12932.

Gailit J, Clark RAF: Wound repair in the context of extracellular matrix. Curr Opin Cell Biol 1994, 6:717-725

Gailit J, Welch MP, Clark RAF: TGF-β1 stimulates expression of keratinocyte integrins during re-epithelialization of cutaneous wounds. J Invest Dermatol 1994, 103:221-227

Gavin JB: Ultrastructural features of chronic marginal gingivitis. J Periodont Res 1970, 5:19-29

Giancotti FG, Mainiero F: Integrin-mediated adhesion and signalling in tumorigenesis. Biochim Biophys Acta 1994, 1198:47-64

Giannelli G, Falk-Marzillier, Schiraldi O, Stetler-Stevenson WG, Quaranta V: Induction of cell migration by metrix metalloprotease-2 cleavage of laminin-5. Science 1997, 277:225-228

Ginsberg MH, Du X, Plow EF: Inside-out integrin signalling. Curr Opin Cell Biol 1992, 4:766-771.

Giudice GJ, Squiquera HL, Elias PM, Diaz LA: Identification of two collagen domains within the bullous pemphigoid autoantigen, BP-180. J Clin Invest 1991, 87:734-738.

Graycar JL, Miller DA, Arrick BA, Lyons RM, Moses HL, Derynck R: Human transforming growth factor-ß3: recombinant expression, purification, and biological activities in comparison with transforming growth factors-ß1 and ß2. Mol Endocrinol 1989, 3:1977-1986

Grinnell F: Cell adhesion. In Wound Healing. Biochemical and Clinical Aspects. IK Cohe, RF Diegelmann and WJ Lindblad, editors. WB Saunders Co., Philadelphia. 1992:209-222

Guan JL, Trevithick JE, Hynes RO: Fibronectin/integrin interactions induces tyrosine phosphorylation of a 120-kDa protein. Cell Regul 1991, 2:951-964

Guan JL, Shalloway D: Regulation of focal adhesion-associated protein tyrosine kinase by both cellular adhesion and oncogenic transformation. 1992, 358:690-692

Heino J, Ignotz RA, Hemler ME, Crouse C, Massagué J: Regulation of cell adhesion receptors by transforming growth factor-ß. Concomitant regulation of integrins that

share a common ß1 subunit. J Biol Chem 1989, 262:380-388

Heino J: Integrin-type extracellular matrix receptors in cancer and inflammation. Ann Med 1993, 25:335-342

Hemler ME: VLA proteins in the integrin family: structures, functions, and their role on leukocytes. Annu Rev. Immunol. 1990, 8:365-400

Hertle MD, Adams JC, Watt FM: Integrin expression during human epidermal development in vivo and in vitro. Development 1991, 112:193-206

Hertle MD, Kubler M-D, Leigh IM, Watt FM: Aberrant integrin expression during epidermal wound healing and in psoriatic epidermis. J Clin Invest 1992, 89:1892-1901

Hodivala KJ, Watt FM: Evidence that cadherins play a role in the downregulation of integrin expression that occurs during keratinocyte terminal differentation. J Cell Biol 1994, 124:589-600

Hopkinson SB, Baker SE, Jones JCR: Molecular genetic studies of a human epidermal autoantigen (the 180 kd cullous pemphigoid antigen/BP180): Identification of functionally important sequences within the BP180 molecule and evidence for an interaction between BP180 and α6 integrin. J Cell Biol 1995, 130:117-125

Hormia M, Virtanen I, Quaranta V: Immunolocalization of integrin alpha 6 beta 4 in mouse junctional epithelium suggests an anchoring function to both the internal and the external basal lamina. J Dent Res 1992, 71:1503-1508

Hormia M, Sahlberg C, Thesleff I, Airenne T: The epithelium-tooth interface -a basal lamina rich in laminin-5 and lacking other known laminin isoforms. J Dent Res, in press

Huang XZ, Wu JF, Cass D, Erle DJ, Corry D, Young SG, Farese RV Jr., Sheppard D: Inactivation of the integrin beta 6 subunit gene reveals a role of epithelial integrins in regulating inflammation in the lung and skin. J Cell Biol 1996, 133:921-8

Hynes RO: Integrins: versatility, modulation, and signaling in cell adhesion. Cell 1992, 69: 11-25

Iida J, Meijne AML, Spiro RC, Roos E, Furcht LT, McCarthy JB: Spreading and focal contact formation of human melanoma cells in response to the stimulation of both melanoma-associated proteoglycan (NG2) and α4β1 integrin. Cancer Res 1995, 55:2177-2185

Jones JCR, Kurpakus MA, Cooper HM, Quaranta V: A function for the integrin α6β4 in the hemidesmosome. Cell Regul 1991, 2:427-438

Jones J, Sugiyama M, Watt FM, Speight PM: Integrin expression in normal, hyperplastic, and malignant oral epithelium. J Pathol 1993, 169:235-243

Jones JCR, Asmuth J, Baker SE, Langhofer M, Roth SI, Hopkinson SB: Hemidesmosomes: extracellular matrix/intermediate filament connectors. Exp Cell Res 1994, 213: 1-11

Juhasz I, Murphy GF, Yan HC, Herlyn M, Albelda SM: Regulation of extracellular matrix proteins and integrin cell substratum adhesion receptors on epithelium during cutaneous human wound healing in vivo. Am J Path 1993, 43:1458-1469

Kane CJM, Hebda PA, Mansbridge JN, Hamawalt PC: Direct evidence for spatial and temporal regulation of transforming growth factor-ß1 expression during cutaneous wound healing. J Cell Physiol 1991, 148:157-173

Kantor RRS, Mattes MJ, Lloyd KO, Old LJ, Albino AP: Biochemical analysis of two cell surface glycoprotein complexes, very common antigen 1 and very common antigen 2. J Biol Chem 1987, 262:15158-15165

Karecla PI, Timpl R, Watt FM: Adhesion of human keratinocytes to laminin. Cell Adh Commun 1994, 2:309-318

Keene DR, Sakai LY, Lundstrum GP, Morris NP, Burgeson RE: Type VII collagen forms an extended network of anchoring fibrils. J Cell Biol 1987, 104:611- 616

Kennel SJ, Foote LJ, Falcioni R, Sonnenberg A, Stringer CD, Crouse C, Hemler ME: Analysis of the tumor-associated antigen TSP-180. J Biol Chem 1989, 264:15515-15521

Kim JP, Zhang K, Chen JD, Kramer RH, Woodley DT: Vitronectin-driven human keratinocyte locomotion is mediated by the αvβ5 integrin receptor. J Biol Chem 1994, 269:26926-26932

König A, Bruckner-Tuderman L: Epithelial-mesenchymal interactions enhance expression of collagen VII in vitro. J Invest Dermatol 1991, 96:803-808

König A, Bruckner-Tuderman L: Transforming growth factor-β stimulates collagen VII expression by cutaneous cells in vitro. J Cell Biol 1992, 117:679-685

Konter U, Kellner I, Klein E, Kaufmann R, Mielke V, Sterry W: Adhesion molecule mapping in normal human skin. Arch Dermatol Res 1989, 281:454-462

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

Kornberg LJ, Earp HS, Turner CE, Prockop C, Juliano RL: Signal transduction by integrins: Increased protein tyrosine phosphorylation caused by clustering of ß1 integrins. Proc Natl Acad Sci USA 1991, 88:8392-8396

Kornberg LJ, Earp HS, Parsons JT, Schaller M Juliano RL: Cell adhesion or integrin clustering increases phosphorylation of a focal adhesion-associated tyrosine kinase. J Biol Chem 1992, 267:23439-23442

Koukoulis GK, Gould VE, Bhattacharyya A, Gould JE, Howeedy AA, Virtanen I: Tenascin in normal, reactive, hyperplastic, and neoplastic tissues: biologic and pathologic implications. Human Path 1991, 22:636-643

Kurpakus MA, Quaranta V, Jones JCR: Surface relocation of alpha6beta4 integrins and assembly of hemidesmosomes in an in vitro model of wound healing. J Cell Biol 1991, 115:1737-175030.

Larjava H, Peltonen J, Akiyama SK, Yamada SS, Gralnick HR, Uitto J, Yamada KM: Novel function for ß1 integrins in keratinocyte cell-cell interactions. J Cell Biol 1990, 110:803-815

Larjava H: Expression of ß1 integrins in normal human keratinocytes. Am J Med Sci 1991, 301:63-68

Larjava H, Zhou C, Larjava I, Rahemtulla F: Immunolocalization of ß1 integrins in human gingival epithelium and cultured keratinocytes. Scand J Dent Res 1992,100:266-273

Larjava H, Lyons JG, Salo T, Mäkelä M, Koivisto L, Birkedal-Hansen H, Akiyama SK, Yamada KM, Heino J: Anti-integrin antibodies induce type IV collagenase expression in keratinocytes. J Cell Physiol 1993, 157:190-200

Larjava H, Salo T, Haapasalmi K, Kramer RH, Heino J: Expression of integrins and basement membrane components by wound keratinocytes. J Clin Invest 1993, 92:1425-1435

Larjava H, Haapasalmi K, Salo T, Wiebe C, Uitto V-J: Keratinocyte integrins in wound

healing and chronic inflammation of the human periodontium. Oral Dis 1996 2:77-86

Levine JH, Moses HL, gold LI, Nanney LMB: Spatial and temporal patters of immunoreactive transforming growth factor beta 1, beta 2 and beta 3 during excitional wound repair. Am J Path 1993, 143:368-380

Li K, Tamai K, Tan EML, Uitto J: Cloning of type XVII collagen. Complementary and genomic DNA sequences of mouse 180-kilodalton bullous pemphigoid antigen (BPAG2) predict an interrupted collageneous domain, a transmembrane segment, and unusual features in the 5'-end of the gene and the 3'-untranslated region of the mRNA. J Biol Chem 1993, 268:8825-8834

Lightner VA, Gumkowski F, Bigner DD, Erickson HP: Tenascin/hexabrachion in human skin: biochemical identification and localization by light and electron microscopy. J Cell Biol 1989, 108:2483-2493

MacKenzie IC, Gao Z: Patterns of cytokeratin expression in the epithelia of inflamed human gingiva and periodontal pockets. J Perio Res 1993, 28:49-59

Mackie EJ, Halfter W, Liverani D: Induction of tenascin in healing wounds. J Cell Biol 1988, 107:2757-2767

Mäkelä M, Salo T, Uitto V-J, Larjava H: Matrix metalloproteinases (MMP-2 and MMP-9) of the oral cavity: cellular origin and relatioship to periodontal status. J Dent Res 1994, 73:1397-1406

Mansbridge JN, Hanawalt PC: Role of transforming growth factor-beta in the maturation of human epidermal keratinocytes. J Invest Dermatol 1988, 90:336-341

Marchisio PC, Bondanza S, Cremona O, Canceda R, De Luca M: Polarized expression of integrin receptors ($\alpha 6\beta 4$, $\alpha 2\beta 1$, $\alpha 3\beta 1$, and $\alpha v\beta 5$) and their relationship with the cytoskeleton and basement membrane matrix in cultured human keratinocytes. J Cell Biol 1991, 112:761-773

Massaque J, Cheifetz S, Laiho M, Ralph DA, Weis FM, Zentella A: Transforming growth factor-ß. Cancer Sur 1992, 12:81-103

Marinkovich MP . The molecular genetics of basement membrane diseases. Arch dermatol 1993, 129:1557-1565

Milam SB, Magnuson VL, Steffensen B, Chen D, Klebe RJ: IL-1 beta and

prostaglandins regulate integrin mRNA expression. J Cell Physiol 1991, 149:173-183

Miyamoto S, Akiyama SK, Yamada KM: Synergistic roles for receptor occupancy and aggregation in integrin transmembrane function. Science 1995, 267:883-885

Mould AP, Akiyama SK, Humphries MJ: Regulation of integrin α5β1-fibronectin interactions by divalent cations. J Biol Chem 1995, 270:26270-26277

Nanci A, Schroeder HE: The junctional epithelium: origin, structure and significance. Acta Med Dent Helv 1996, 1:155-167

Nazzaro V, Berti E, Cerri A, Brusasco A, Cavalli R, Caputo R: Expression of integrins in junctional and dystrophic epidermolysis bullosa. J Invest Dermatol 1990, 95:60-64

Nickoloff BJ, Mitra RS, Riser BL, Dixit VM, Varani J: Modulation of keratinocyte motility. Correlation with production of extracellular matrix molecules in response to growth promoting and antiproliferative factors. Am J Pathol 1988, 132:543-551

Niessen CM, Hogervorst F, Jaspars LH, de Melker AA, Delwel GO, Hulsman EH, Kuikman I, Sonnenberg A: The α6β4 integrin is a receptor for both laminin and kalinin. Exp Cell Res 1994, 211:360-367.

Nikkari L, Aho H, Yli-Jama T, Larjava H, Jalkanen M, Heino J: Expression of integrin family of cell adhesion receptors in rheumatoid synovium. α6 integrin subunit in normal and hyperplastic synovial lining cell layer. Am J Pathol 1993, 142:1019-1027

Nikkari L, Haapasalmi K, Aho H, Torvinen A, Sheppard D, Larjava H, Heino J: Localization of the αv subfamily of integrins and their putative ligands in synovial lining cell layer. J Rheumatol 1995, 22:16-23

Obara M, Kang MS, Yamada KM: Site-directed mutagenesis of the cell-binding domain of human fibronectin: Separable, synergistic sites mediate adhesive function. Cell 1988, 53:649-657

Obberghen-Schilling EV, Roche NS, Flanders KC, Sporn MB, Roberts AB: Transforming growth factor ß1 positively regulates its own expression in normal and transformed cells. J Cell Chem 1988, 263:7741-7746

Oksala O, Haapasalmi K, Hakkinen L, Uitto V-J, Larjava H: Expression of heparan sulphate and small dermatan/chondroitin sulphate proteoglycans in chronically inflamed human periodontium. J Dent Res 1997, 76:1250-1259

O'Toole TE, Katagiri Y, Faull RJ, Peter K, Tamura R, Quaranta V, Loftus JC, Shattil SJ, Ginsberg MH: Integrin cytoplasmic domains mediate inside-out signal transduction. J Cell Biol 1994, 124:1047-1059

O'Toole TE, Ylänne J, Culley BM: Regulation of integrin affinity states through an NPXY motif in the ß subunit cytoplasmic domain. J Biol Chem 1995, 270:8553-8558

Overman DO, Salonen JI: Characterization of the human junctional epithelial cells directly attached to the tooth (DAT cells) in periodontal disease. J Dent Res 1994, 73:1818-1823

Oyama F, Murata Y, Suganuma N, Kimura T, Titani T, Sekiguchi K: Patterns of alternative splicing of fibronectin pre-mRNA in human adult and fetal tissues. Biochemistry 1989, 28:1428-1434

Page RC, Schroeder HE: Structure and pathogenesis. In Periodontal Diseases. S Schluger, R Yuodelis, RC Page, RH Johnson, editors. Lea & Febiger, Philadelphia, London. 1990:183-220

Palmer EL, Rüegg C, Ferrando R, Pytela R, Sheppard D: Sequence and tissue distribution of the integrin α9 subunit, a novel partner of ß1 that is widely distributed in epithelia and muscle. J Cell Biol 1993, 123:1289-1297

Pellegrini G, De Luca M, Orecchia G, Balzac F, Cremona O, Savoia P, Cancedda R, Marchisio PC: Expression, topography, and function of integrin receptors are severely altered in keratinocytes from involved and uninvolved psoriatic skin. J Clin Invest 1992, 89:1783-1795

Peltonen J, Larjava H, Jaakkola S, Grainick H, Akiyama SK, Yamada SS, Yamada KM, Uitto J: Localization of integrin receptors for fibronectin, collagen, and laminin in human skin: variable expression in basal and squamous cell carcinomas. J Clin Invest 1989, 84:1916-1923

Peng TK, Nisengard RJ, and Levine MJ: The alteration in gingival basement membrane antigens in chronic periodontitis. J Periodontol 1986, 57:20-24

Pierce GF, Mustoe TA, Lingelbach J, Masakowski VR, Griffin GL, Senior RM, Deuel TF: Platelet-derived growth factor and transforming growth factor-ß enhance tissue repair activities by unique mechnism. J Cell Biol 1989, 109:429-440

Pierschbacher MD, Ruoslahti E:Call attachment activity of fibronectin can be duplicated

by small synthetic fragments of the molecule. Nature 1984, 309:30-33

Pierschbacher MD, Ruoslahti E: Influence of stereochemistry of the sequence arg-gly-asp-xaa on binding specificity in cell adhesion. J Biol Chem 1987, 262:17294-17298

Preissner KT: Structure and biological role of vitronectin. Annu Rev Cell Biol 1991, 7:275-310

Prieto AL, Edelman GM, Crossin KL: Multiple integrins mediate cell attachment to cytotactin/tenascin. Proc Natl Acad Sci 1993, 90:10154-10158

Pytela R, Pierschbacher MD, Ruoslahti E: Identification and isolation of a 140 kd cell surface glycoprotein with properties expected of a fibronectin receptor. Cell 1985, 40:191-198.

Ralfkiaer E, Thomsen K, Vejlsgaard GL: Expression of a cell adhesion protein (VLA ß) in normal and diseased skin. Br J Dermatol 1991, 124:527-532

Regauer S, Seiler GR, Barrandon Y, Easley KW, Compton CC: Epithelial origin of cutanous anchoring fibrils. J Cell Biol 1990, 111:2109-2115

Richards DW, MacPhail LA, Dekker N, Greenspan D, Greenspan JS, Lozada-Nur F, Regezi JA: Expression of laminin 5, fibronectin, and epithelium-associated integrins in recurrent aphthous ulcers. J Dent Res 1996, 75:1512-1517

Risteli L, Timpl R: Isolation and characterization of pepsin fragments of laminin from human placental and renal basement membranes. Biochem J 1981, 193:749-755

Roberts CJ, Birkenmeier TM, McQuillan JJ, Akiyama SK, Yamada SS, Chen W-T, Yamada KM, McDonald JA: Transforming growth factor ß stimulates the expression of fibronectin and of both subunits of the human fibronectin receptor by cultured human lung fibroblasts. J Biol Chem 1988, 263:4586-4592

Rousselle P, Lunstrum GP, Keene DR, Burgeson RE: Kalinin: an epithelium-specific basement membrane adhesion molecule that is a component of anchoring filaments. J Cell Biol 1991, 114:567-576

Ruoslahti E, Pierschbacher MD: Arg-Gly-Asp: A versatile cell recognition signal. Cell 1986, 44:517-518

Ruoslahti E, Pierschbacher MD: New Perspectives in cell adhesion" RGD and integrins.

Science 1987, 238:491-497

Ruoslahti E. Fibronectin and its receptors. Ann Rev Biochem 1988; 57:375-413

Ruoslahti E: Integrins. J Clin Invest 1991, 87:1-5

Saga Y, Yagi T, Ikawa Y, Sakakura T, Aizawa S: Mice develop normally without tenascin.Genes Develop1992, 6:1821-31

Saglie R, Carranza FA Jr, Newman MG, Pattison GA: Scanning electron microscopy of the gingival wall of deep periodontal pockets in humans. J Periodont Res 1982, 17:284-293

Sakai LY, Keene DR, Morris NP, Burgeson RE: Type VII collagen is major structural component of anchoring fibrils. J Cell Biol 1986, 103:1577-1586

Salonen J: Basal cell aharacteristics in human oral epithelium adhered to the tooth in vivo and to an inert substrate in vitro. J Periodont Res 1986, 21:22-29

Salonen J, Oda D, Funk SE, Sage H: Synthesis of type VIII collagen by epithelial cells of human gingiva. J Periodont Res 1991, 26:355-60

Sastry SK, Horwitz AF: Integrin cytoplasmic domains: Mediators of cytoskeletal linkages and extra- and intracellular initiated transmembrane signaling. Curr. Opin. Cell Biol. 1993, 5:819-831

Schmid P, Kunz S, Cerletti N, McMaster G, Cox D: Injury induced expression of IGF-ß1 is enhanced by exogenously applied TGF-ßs. Biochem Biophys Res Commun 1993a, 194:399-406

Schmid P, Cox D, Bilbe G, McMaster G, morrison C, Stähelin H, Lüscher N, Seiler W: TGF-ßs and TGF-ß type II receptor in human epidermis: differential expression in acute and chronic skin wounds. J Path 1993b, 171:191-197.

Schroeder HE, Listgarten MA: Fine structure of the developing epithelial attachment of human teeth. Monogr Dev Biol 1977, 2

Schroeder HE: Differentiation of human oral stratified epithelia. Kager, Basel 1981

Schroeder HE, Listgarten MA: The gingival tissues: the architecture of periodontal protection. Periodont 2000 1997, 13:91-120

Schwartz MA: Transmembrane signalling by integrins. Trends Cell Biol 1992, 2:304-308

Schwartz MA, Schaller MD, Ginsberg MH: Integrins: Emerging paradigms of signal transduction. Annu Rev Cell Dev Biol 1995, 11:549-599

Schnapp LM, Hatch N, Ramos DM, Klimanskaya IV, Sheppard D, Pytela R: The human integrin α8ß1 functions as a receptor for tenascin, fibronectin, and vitronectin. J Biol Chem 1995, 270:23196-23202

Sheppard D: Epithelial integrins. BioEssays 1996, 18:655-660

Singer II, Scott S, Kawka DW, Kazazis DM, Gailit J, Ruoslahti E: Cell surface distribution of fibronectin and vitronectin receptors depends on substrate composition ad extracellular matrix accumulation. J Cell Biol 1988, 106:2171-2182

Sollberg S, Peltonen J, Uitto J, Jimenez SA: Elevated expression of ß1 and ß2 integrins, intercellular adhesion molecule 1, and endothelial leukocyte adhesion molecule 1 in the skin of patients with systemic sclerosis of recent onset. Arthritis Rheum 1992, 35:290-298

Sonnenberg A, Hogervorst F, Osterop A, Veltman FEM: Identification and characterization of a novel antigen complex on mouse mammary tumor cells using a monoclonal antibody against platelet glycoprotein Ic. J Biol Chem 1988, 263:14030-14038

Sonnenberg A, Calafat J, Janssen H, Daams H, van der Raaij-Hemler LMH, Falcioni R, Kennel SJ, Aplin JD, Baker J, Loizidou M, Garrod, D: Integrin α6β4 complex is located in hemidesmosomes, suggesting a major role in epidermal cell-basement membrane adhesion. J Cell Biol 1991, 113:907-917

Sporn MB, Roberts AB: Transforming growth factor-ß: recent progress and new challenges. J Cell Biol 1993, 119:1017-1021

Sriramarao P, Steffner P, Gehlsen KR: Biochemical evidence for a homophilic interaction of the α3ß1 integrin. J Biol Chem 1993, 268:22036-22041

Steffensen B, Duong AH, Milam SB, Potempa CK, Winborn WB, Magnuson VL, Chen D, Zardeneta G, Klebe RJ: Immunohistological localization of cell adhesion proteins and integrins in the periodontium. J Periodontol 1992, 63:584-592

Stenn KS, Malhotra R: Epithelialization. In Wound Healing. Biochemical & Clinical Aspects. IK Cohe, RF Diegelmann, WJ Lindblad, editors. WB Saunders Co, Philadelphia. 1992:115-127

Stepp MA, Spurr-Michaud S, Tisdale A, Elwell J, Gipson IK: α6β4 integrin heterodimer is a component of hemidesmosomes. Proc Natl Acad Sci USA 1990, 87:8970-8974

Symington BE, Takada Y, Carter WG: Interaction of integrins α3ß1 and α2ß1: Potential role in keratinocyte intercellular adhesion. J Cell Biol 1993, 120:523-535

Takarada H, Cattoni M, Sugimoto A, Rose GG:. Ultrastructural studies of human gingiva. II. The lower part of the pocket epithelium in chronic periodontitis. J Periodont Res 1974a, 45:155-169

Takarada H, Cattoni M, Sugimoto A, Rose GG:. Ultrastructural studies of human gingiva. III. Changes of the basal lamina in chronic periodontitis. J Periodont Res 1974b, 45:288-302

Talonpoika J, Heino J, Larjava H, Häkkinen L, Paunio K: Gingival crevicular fluid fibronectin degradation in periodontal health and disease. Scand J Dent Res 1989, 97:415-421

Tenchini ML, Adams JC, Gilberty C, Steel J, Hudson DL, Malcovati M, Watt FM: Evidence against a major roel for integrins in calcium-dependent intercellular adhesion of epidermal keratinocytes. Cell Adhesion Commun 1993, 1:55-66

Thesleff I, Mackie E, Vainio S, Chiquet-Ehrismann R: Changes in the distribution of tenascin during tooth development. Devel 1987, 101:289-296.

Thesleff I, Partanen AM, Vainio S: Epithelial-mesenchymal interactions in tooth morphogenesis: the roles of extracellular matrix, growth factors, and cell surface receptors.Journal of Craniofac Genet Devel Biol 1991, 11:229-37

Thilander H: Epithelial changes in gingivitis. J Periodont Res 1968, 3:303-312

Uitto V-J, Larjava H: Extracellular matrix molecules and their receptors: an overview with special emphasis on periodontal tissues. Critical Rev Oral Biol Med 1991, 2:323-354

Uitto V-J, Larjava H, Peltonen J, Brunette DM: Expression of fibronectin and integrins in cultured periodontal ligament epithelial cells. J Dent Res 1992, 71:1203-1211

Uitto J, Christiano AM: Molecular genetics of the cutaneous basement membrane zone. Perspectives on epidermolysis bullosa and other blistering skin diseases. J Clin Invest 1992, 90:687-692

van der Rest M, Garrone R: The collagen family of proteins. FASEB J 1992, 5:2814-2823

Van Waes C: Cell adhesion and regulatory molecules involved in tumor formation, hemostasis, and wound healing. Head & Neck 1995, 17:140-147

Verrando P, Hsi BL, Yeh C-J, Pisani A, Serieys N, Ortonne J-P: Monoclonal antibody GB3, a new probe for the study of human basement membranes and hemidesmosomes. Exp Cell Res 1987, 170:116-128

Vartio T, Laitinen L, Nrvnen O, Cutolo M, Thornell LE, Zardi L, Virtanen I: Differential expression of the ED sequence -containing form of cellular fibronectin in embryonic and adult human tissues. J Cell Sci 1987, 88:419-430

Watt FM, Hertle MD: Keratinocyte integrins. In the keratinocyte handbook. IM Leigh, EB Lane, FM Watt, editors. Cambridge University Press, Cambridge. 1994:153-164

Watt FM, Jones PH: Expression and function of the keratinocyte integrins. Development, Suppl 1993,185-192

Wayner EA, Orlando RA, Cheresh DA: Integrins $\alpha v\beta 3$ and $\alpha v\beta 5$ contribute to cell attachment to vitronectin but differentially distribute on the cell surface. J Cell Biol 1991, 113:919-929

Weinacker A, Chen A, Agrez M, Cone RI, Nishimura S, Wayner E, Pytela R, Sheppard D: Role of integrin $\alpha v\beta 6$ in cell attachment to fibronectin: heterologous expression of intact and secreted forms of the receptor. J Biol Chem 1994, 269: 6940-6948

Werb Z, Tremble PM, Behrendtsen O, Crowley E, Damsky CH: Signal transduction through the fibronectin receptor induces collagenase and stromelysin gene expression. J Cell Biol 1989, 109:877-889

Whitby DJ, Longaker MT, Harrison MR, Adzick NS, Ferguson MWJ: Rapid epithelialization of fetal wounds is associated with the early deposition of tenascin. J Cell Sci 1991, 99:583-586

Whitby DJ, Ferguson MW: The extracellular matrix of lip wounds in fetal, neonatal and

adult mice. Development 1991, 112:651-68

Wysocki AB, Grinnell F: Fibronectin profiles in normal and chronic wound fluid. Lab Invest 1990, 63:825-831

Yamada KM, Kennedy DW: Dualistic nature of adhesive protein function: fibronectin and its biologically active peptide fragments can autoinhibit fibronectin function. J Cell Biol 1984, 99:29-36

Yamada KM: Adhesive recognition sequences. J Biol Chem 1991, 266:12809-12812

Yamada KM, Gailit J, Clark RAF: Integrins in wound repair. In The molecular and cellular biology of wound repair. RAF Clark, editor. Plenum Press, New York.1996:311-338

Yamada KM, Clark RAF: Provisional matrix. In The molecular and cellular biology of wound repair. RAF Clark, editor. Plenum Press, New York. 1996:51-93.

Yamamoto T, Osaki T: Dcharacteristic cytokines generated by keratinocytes and mononuclear infiltrates in oral lichen planus. J Invest Dermatol 1995, 5:784-788

Yokosaki Y, Palmer EL, Prieto AL, Crossin KL, Bourdon MA, Pytela R, Sheppard D: The integrin α9ß1 mediates cell attachment to a non-RGD site in the third fibronectin type III repeat of tenascin. J Biol Chem 1994, 269: 26691-26696

Yokosaki Y, Monis H, Chen J, Sheppard D: Differential effects of the integrins alpha9beta1, alphavbeta3, alphavbeta6 on cell proliferative responses to tenascin. Roles of the beta subunit extracellular and cytoplasmic domains. J Biol Chem 1996, 271:24144-24150

Yurchenco PD, Schittny JC: Molecular architecture of basement membrane. FASEB J 1990, 4:1577-1590

Zambruno G, Marchisio PC, Marconi A, Vaschieri C, Melchiori A, Giannetti A, De Luca M:Transforming growth factor-ß1 modulates ß1 and ß5 integrin receptor and induces the de novo expression of the αvß6 heterodimer in normal keratinocytes: Implications for wound healing. J Cell Biol 1995, 129:853-865

Zeng L, Daniels A, Riddelle K, Pittelkow, Fine J-D . Uncein, an anchoring filament component, differs from kalinin biochemically and in its in vitro expression by normal and junctional epidermolysis bullosa (JEB) human keratinocyte monolayers. Mol Biol Cell 1994, 5: 305a

Zhang K, Kim JP, Woodley DT, Waleh NS, Chen YQ, Kramer RH: Restricted expression and function of laminin-1 binding integrins in normal and malignant oral mucosal keratinocytes. Cell Adh Commun 1996, 4:159-174