

EARLY ONSET PERIODONTITIS ASSOCIATED WITH KINDLER SYNDROME

by

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ABSTRACT

Kindler syndrome is an extremely rare genetic disorder with features of epidermolysis bullosa and poikiloderma congenitale. Approximately 70 cases have been documented in the past 50 years but oral findings have only been superficially mentioned. The aims of this study were to 1) accurately document oral findings with an emphasis on the periodontal condition, 2) examine the components of the basement membrane zone in search of the pathobiological defect(s) responsible for the clinical findings, and 3) determine the effectiveness of periodontal treatment and other dental treatment for a patient with Kindler syndrome. A female 16-year-old patient with a history of prepubertal periodontal disease and loss of permanent molars and incisors in the mandible presented with localized, early onset periodontal disease, severe gingival bleeding, and gingival fragility. The basement membrane zone of normal gingiva and that with the characteristic vesiculobullous lesions were examined using immunofluorescence microscopy to bullous pemphigoid antigens 1 and 2, collagen types IV and VII, laminins -1 and -5, integrins $\alpha 3\beta 1$, $\alpha \nu\beta 6$, and $\alpha 6\beta 4$. Biopsies studied revealed blistering with trauma at the level of lamina lucida on distribution of type IV collagen and laminin-1 at the blister floor. In the non-inflamed tissue, discontinuous areas of the basement membrane zone were found. Distribution of basement membrane zone components and integrins studied appeared normal except for type VII collagen which was found in abnormal locations deep in the connective tissue stroma and integrin $\alpha \nu\beta 6$ which was unexpectedly present in localized areas of the epithelium. Our results suggest that Kindler syndrome is associated with abnormalities in the construction of the basement membrane, especially in the expression of type VII collagen. These alterations are likely to play a role as etiological factors leading to a weakened resistance at the tooth-periodontal tissue interface. Oral polymorphonuclear leukocyte counts and elastase levels were high suggesting normal leukocyte function. The patient responded well to nonsurgical periodontal therapy with reduced probing depths,

bleeding on probing, mobility and dental pain. Successful dental treatment involving orthodontics and fixed and removable prosthodontics was carried out demonstrating that complex oral therapy can be performed in Kindler syndrome patients regardless of the systemic condition.

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GLOSSARY OF TERMS

Acanthosis	Increased thickness of the prickle cell layer.
Acral	Pertaining to the extremities.
Acrogeria	A condition wherein the skin of the hands and feet show signs of premature aging.
Acrokeratoses	Hereditary, wart-like growths on the extremities.
Alopecia	Absence or loss of hair; especially of the head.
Anhidrosis	Diminished or complete absence of secretion of sweat.
Ankyloglossia	Abnormal shortness of the lingual frenum that restricts normal tongue movements.
Anodontia	Absence of teeth.
Consanguinity	Relationship by blood, i.e., being descended from a common ancestor.
Ectropion	Eversion of an edge or margin, as the edge of an eyelid.
Hyperhidrosis	Sweating greater than would be expected in the temperature of the environment.
Hyperkeratosis	Overgrowth of the keratinized layer of the epithelium.
Integrins	Heterodimeric cell surface glycoproteins that are responsible for most cell-cell and cell-extracellular matrix interactions and link the extracellular matrix to the cytoskeleton.
Keratoderma	Hypertrophy of the stratum corneum of the epidermis.
Microcephaly	Congenital, abnormal smallness of the head.
Microdontia	Abnormal smallness of the teeth or of a tooth.
Milia	Pinhead-sized keratin filled cysts that occur on newborns and usually disappear in several weeks.

Nikolsky's sign	A condition of the external layer of the skin in which it can be rubbed off by slight friction or injury. Typically seen, for example, in pemphigus.
Photodistribution	Skin reaction in areas exposed to sunlight.
Photophobia	Unusual intolerance of light by the eyes.
Photosensitivity	Condition in which the skin reacts abnormally to sunlight. This can be congenital or due to drugs, hormones or heavy metals in the system.
Poikiloderma	A skin disorder characterized by pigmentation, telangiectasia, purpura, pruritus and atrophy.
Prognathism	Abnormal projection of the jaw(s) forward.
Pruritus	Severe itching.
Purpura	Hemorrhage into the skin, mucous membranes, internal organs or other tissues. Hemorrhage into the skin shows red, darkening into purple, then brownish-yellow and finally disappearing in two to three weeks.
Syndactyly	A fusion of two or more fingers or toes.

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Chapter 1 - Introduction

Vesiculobullous diseases are commonly divided into three etiologies: viral diseases, conditions associated with immunologic defects and hereditary diseases. Pemphigus, pemphigoid and linear IgA bullous dermatosis (subepithelial bullae and microabscesses, IgA and fibrin in the basement membrane, may present as desquamative gingivitis) are thought to be associated with immunologic defects. Epidermolysis bullosa is a group of diseases characterized by the development of blisters or erosions following minor trauma to the skin. Most of these mechanobullous diseases are hereditary but some are of uncertain etiology.

Acquired bullous diseases of children are rare and can be difficult to distinguish clinically as there is considerable overlap which can be misleading when determining a diagnosis. The oral cavity is often overlooked as a source of diagnostic signs dermatologic disease.

Periodontal disease is a family of related but distinct diseases that differ in etiology, natural history, progression and response to therapy. Of particular interest are the groups of periodontal diseases classified under the categories of Early-Onset Periodontitis, many of which show a familial tendency although the genetic basis is unclear, and Periodontitis Associated With Systemic Disease which has been associated with such disorders such as scleroderma, cyclic and chronic neutropenias, leukemias, type I diabetes, histiocytosis X and in disorders of the immune system and mucocutaneous structures (Proceedings of the World Workshop in Clinical Periodontology, 1989).

Genetic and hereditary diseases have effects on the oral soft tissues. Severe periodontal destruction is a common feature of Down's Syndrome. The etiology of the increased periodontal destruction remains unknown but contributing factors appear to be both local

(occlusal problems, oral habits and changes in salivary composition) and systemic (defects in polymorphonuclear leukocytes, monocytes, B and T lymphocytes). (Reuland-Bosma, 1988a, b and c). Hypophosphatasia is a rare familial metabolic disorder with a deficiency in alkaline phosphatase resulting in hypomineralization of bone and dental hard tissues. Dental presentation includes premature exfoliation of primary teeth in the absence of severe gingival inflammation and loss of alveolar bone (Jedrychowski, 1979). Papillon-Lefevre Syndrome is an autosomal recessively inherited disorder involving palmar-plantar keratosis and alveolar bone loss resulting in premature loss of the primary and permanent dentition. Skin lesions involve diffuse, erythematous, keratotic areas on the palms and soles, hyperhydrosis of the palms and also dystrophic changes of the nails (Preus, 1988). Oral and periodontal symptoms are found in numerous other genetic and hereditary disorders including Ehlers-Danlos syndrome, hereditary gingival fibromatosis, mucopolysaccharidoses, Crohn's disease, and Chediak-Higashi syndrome (Seymour and Heasman, 1992).

Kindler syndrome is an extremely rare and poorly understood disorder. Oral complications such as bright red mucous membranes of unusual fragility (Kindler, 1954) and dystrophic teeth (Alper, 1978) have been mentioned in case reports but never thoroughly described and documented. Oral findings will be examined in related disorders as well as in Kindler syndrome and presented here along with pathobiological examination of the oral soft tissue. In addition, treatment of the oral symptoms found in Kindler syndrome is described.

Chapter 2 - Literature Review

2.1 Kindler Syndrome

In 1954, Kindler described a 14-year-old girl who had cutaneous manifestations of both epidermolysis bullosa and poikiloderma congenitale associated with extreme photosensitivity and questioned whether this was the simultaneous occurrence of two rare congenital conditions or a single, previously undescribed disorder (Kindler, 1954). Blisters appeared from birth and formed either following trauma or spontaneously. As the child aged her face became mottled with abnormal pigmentation. Her condition was exaggerated in the summer months. Blister formation and photosensitivity almost ceased by age nine years and reticular changes that started in sun-exposed areas spread to non-exposed areas. Pitted, atrophic spots started to form on the abdomen and spread to the chest and thighs. The patient had swollen and bleeding gums since infancy but the teeth appeared normal. No consanguinity was noted in the two previous generations. The patient was physically underdeveloped for her chronological age but of average mental development. Perspiration was normal except for hyperhidrosis of the palms and soles. Blistering was not induced by rubbing and the Nikolsky sign (i.e. skin can be rubbed off by slight friction) was negative (Kindler, 1954).

The poikiloderma consisted of raised pigmented nevi on the face and forearms and telangectasias around the nose, eyes, ears and on the buccal mucosa. The cheeks, chin and neck had a reticular pattern with hypo- and hyperpigmented areas. Over the cheekbones a more diffuse erythematous appearance existed that was also present on the ventral aspects of the arms and thighs. Dark brown reticular pigmentation was present in the axillary folds and groin area. The gingiva was bright red, fragile and the patient experienced repeated oral infections (Kindler, 1954).

As the blistering decreased with age the atrophy of the skin increased. An atrophic appearance with fine scaling and wrinkling was present on the abdomen, arms and thighs. The dorsal surfaces of the hands, wrists, elbows, feet, ankles and knees were thin, wrinkled and exhibited a purplish-red color. Symmetrical webbing between the second and third toes was present. (Kindler, 1954).

Histopathology of the original Kindler patient's skin showed moderate hyperkeratosis, alternating areas of thickened and atrophied basal cell layer with corresponding increased or lacking pigmentation. Blistering occurred between the dermis and epidermis. Acanthosis was present at the edges of the lesions. There was edema, and proliferation and dilatation of capillaries in the dermis as well as homogenization of the collagen fibers (rather than distinct fibers) but no inflammatory infiltrate. The hair follicles, sebaceous and sweat glands appeared normal. (Kindler, 1954).

Kindler suggested that syndrome she observed appeared to be a combination of dystrophic epidermolysis bullosa and poikiloderma congenitale as described by Thomson (1923). The epidermolysis bullosa explained the blistering and its subsequent decrease with age. The poikiloderma explained the syndactyly of fingers and toes, keratoses, photosensitivity, dystrophy of nails and atrophic changes. Kindler considered the possibility of two congenital conditions arising in the same individual but suggested this would be unexpected due the rarity of both of the disorders. She also suggested that there could be a relationship between the two disorders.

In 1971, Weary *et al.* reported a dominantly inherited disorder affecting 10 members of a Caucasian family group with features similar to those reported in Kindler's patient and named it Hereditary Acrokeratotic Poikiloderma (HAP). Expression was highly variable

and fell into four categories 1) blister formation which remains confined to the hands and feet, beginning at 1-3 months of age and resolving in late childhood, 2) widespread eczematoid dermatitis starting at 3-6 months of age and resolving by five years of age, 3) gradual appearance of diffuse poikiloderma with striate and reticulate atrophy which spares only the face, scalp and ears and persists into adulthood, and 4) development of keratotic papules on the hands, feet, elbows and knees which develop prior to age five years and persist throughout life. A pigmentary anomaly was present in the majority of cases. There were no oral findings reported except for poor oral hygiene and numerous carious teeth. Lifespan appears to be normal. Routine laboratory tests were normal but an elevated IgG level intrigued Weary and caused him to postulate that a underlying immunologic abnormality may be responsible (Weary, 1971).

Biopsies of various cases produced the following picture. Areas of epidermal thinning and alternating areas of high and low density of dermal melanin deposits existed. Degeneration of the basal layer occurred in focal areas. Many pigment-laden macrophages were observed in the upper dermis and some mild perivascular inflammatory infiltrate existed. Sweat glands and hair follicles appeared normal (Weary, 1971).

All but two of Weary's kindred developed blister formation as the initial symptom. Age of onset was between five weeks and six months after birth and were present and their highest levels three-to-nine months following onset. The blisters gradually resolved and stopped in late childhood. Parents said that trauma, heat and sunlight did not contribute to the acral blistering and this is supported by the fact that age of onset was prior to the time of crawling and significant exposure to sunlight (Weary, 1971).

Widespread eczematous dermatitis was noted in three of the older children prior to onset of poikiloderma. This eczema rarely involved the face but was worst in the flexural areas.

Poikiloderma arose both in sites that had previous dermatitis and in sites that did not. Poikiloderma started as mottled discoloration and reticulated hyperpigmentation. Reticular fine atrophy was also present. Acrokeratoses, a series of keratotic papules on the extremities, arose in the hands and feet as well as over the elbows and knees (Weary, 1971).

Weary (1971) reviewed differential diagnoses for HAP and ruled out poikiloderma congenitale (Rothmund-Thomson syndrome) on the basis of HAP being dominantly inherited as opposed to the recessive pattern of poikiloderma congenitale (PC). Clinical presentations also differed in that PC has more poikilodermatous involvement of the face and sun-exposed areas and little involvement of the flexural areas, partial alopecia, cataract formation and photosensitivity. Weary (1971) also ruled out epidermolysis bullosa. Certain forms of EB display poikiloderma but trauma does not appear to precipitate the blistering seen in HAP.

A number of other cases of HAP and Kindler syndrome have been reported and are well summarized by Forman *et al.* (1989). Forman also reports a case treated by her group of an 11-year-old boy with Kindler syndrome who presented with 1) predominantly acral blistering that occurred in infancy and early childhood and that diminished with age, 2) diffuse cutaneous atrophy with mottled pigmentation and distinctive hyperpigmented macules on the neck and trunk, 3) nail dystrophy, 4) mild photosensitivity, 5) eczema in the axillae, 6) syndactyly of the fingers and toes, 7) urethral stenosis, and 8) severe dental caries.

No thorough documentation of dental findings in Weary-Kindler patients appears to have been published. Kindler noted oral mucosal lesions in her examination of the original patient. Weary *et al.* did not report any oral findings except for poor oral hygiene in two

of the kindred. Forman *et al.* discussed two cases of Kindler syndrome and reported severe dental caries in an 11-year-old Mexican-American boy. Hacham-Zadeh and Garfunkel (1985) examined four cases of Kindler syndrome from two related Kurdish families. Dental findings included normal tooth morphology, an overjet malocclusion, a high palatal vault, limited jaw opening, ankyloglossia, atrophy of the buccal mucosa with widespread white macules and easily bleeding gums. Draznin *et al.* (1978) found the teeth and oral mucous membranes to be normal in the case of a 14-year-old with features consistent with HAP. Bordas *et al.* (1982) and Alper *et al.* (1978) each reported single cases of Kindler syndrome and found all abnormal findings were limited to the skin and Kapasi *et al.* (1993) made no mention of dental findings. It is difficult to draw conclusions on the oral conditions of the patients in the various reports as standardized documentation was not carried out or published. Some of the dental findings may simply have been due to factors such as poor oral hygiene or diet. Large amounts of bleeding upon brushing or pain in the hands or oral soft tissues upon brushing could also discourage these young patients from practicing proper oral hygiene.

2.2 Individual Diseases Found in Kindler Syndrome

Epidermolysis Bullosa

Epidermolysis bullosa is a general term encompassing a group of dermatologic diseases in which bullae or erosions occur on the skin or mucous membranes spontaneously or after minor trauma. Several different forms of the disease are recognized including one acquired and several genetic varieties. Pearson (1988) has provided an overview of the classification and diverse findings of epidermolysis bullosa syndromes. The most common classification used in mechanobullous diseases contains subgroups that specify the level of tissue separation that occurs during blister formation. This level of separation can be

determined by diagnostic electron microscopy and histopathology with immunostaining. In its simplest form of classification, inherited epidermolysis bullosa can be divided into three major subgroups:

- 1) Simplex (epidermolytic) - Tissue cleavage occurs within the epidermal cell layer or at the level of the basal keratinocytes.
- 2) Junctional (junctionolytic) - Tissue cleavage occurs at the level of the basal lamina separating the dermis from the epidermis and is mostly manifested as atrophy of the skin. A more precise designation that is also used is lamina lucidolytic which specifies the split is at the level of the lamina lucida.
- 3) Dystrophic (dermolytic) - Tissue cleavage occurs below the level of the basal lamina and is usually followed by atrophy and scarring.

These major subgroups are further classified depending on ultrastructural and phenotypic features, mode of inheritance, expression of certain basement membrane proteins and molecular genetic studies. The acquired form of epidermolysis bullosa known as epidermolysis bullosa acquisita is an autoimmune disease in which the patients' sera contains circulating IgG antibodies that recognize type VII collagen epitopes and whose clinical features are similar to those found in dystrophic epidermolysis bullosa.

Pearson (1988) provides a comprehensive list of hereditary diseases meeting the criteria for epidermolysis bullosa (Table 1).

Table 1 - Mechanobullous Group of Diseases

1) Epidermolytic Subgroup (Simplex)

Type	Inheritance
Epidermolysis bullosa simplex (EBS)	AD
- generalized Koebner (EBS-K)	
EBS, localized, Weber-Cockayne (EBS-WC)	AD
EBS, herpetiformis, Dowling-Meara (EBS-DM)	AD
EBS, mottled pigmentation (EBS-M)	AD
EBS, herpetiformis, mottled pigmentation, punctate keratoderma (EBS-MPPK)	AD
EBS, localized, anodontia, Kallin syndrome (EBS-LK)	AR
EBS, Ogna (EBS-O)	AD
EBS, Bart (EBS-Bart)	AD
EBS, "letalis" (EBS-L)	AR
EBS, Mendes da Costa (EBS-MC)	XR
Epidermolytic hyperkeratosis, Brocq (EH-B)	AD
Epidermolytic hyperkeratosis, Siemens (EH-S)	AD
Pachyonychia congenita, subtypes 1, 2 and 3 (PC-1, 2 & 3)	AD
Pachyonychia congenita Haber-Rose (PC-HR)	AR
Benign familial pemphigus, Hailey-Hailey (BFP)	AD
Acantholytic EB (AEB)	AD
Peeling skin syndrome (PSS)	AD

2) Junctionolytic subgroup (Junctional)

Type	Inheritance
JEB Herlitz (Gravis) (HJEB)	AR
JEB-mitis (JEB-M) generalized atrophic EB, mitis	AR
JEB-localized, minimus (JEB-L, M)	AR
JEB-cicatricial (JEB-C)	AR
JEB-inversa (JEB-I)	AR
JEB-progressiva (JEB-P) EB neurotropica	AR

3) Dermolytic subgroup (Dystrophic)

Type	Inheritance
DEB Cockayne-Touraine (DEB-CT)	AD
DEB-minimus (DEB-Min)	AD
DEB-pretibial (DEB-Pt)	AD
DEB-albopapuloides (DEB-AP)	AD
DEB-Hallopeau-Siemens (DEB-HS)	AR
DEB-inversa (DEB-I)	AR

AD indicates autosomal dominant; AR, autosomal recessive; and XR, X-linked recessive.

The basic classification has been extensively subdivided, mainly on the basis of the extent and location of lesions on the skin, and age of onset. The large range of clinical presentations, from occasional localized blistering to widespread blistering of the skin and mucosa that can be life-threatening, results in a very complex descriptive nomenclature for epidermolysis bullosa and the lines between types is sometimes debatable and the exact etiology for each of the subtypes remains obscure. In this section the clinical presentations are discussed under the above classification but later in the chapter (2.5) a future method of classification based on molecular biology and underlying mutations will be discussed.

Inheritance patterns vary for all three subgroups of epidermolysis bullosa (EB) but generally speaking, most epidermolysis bullosa simplex (EBS) types are autosomal dominant, junctional epidermolysis bullosa (JEB) types are autosomal dominant, mild forms of dystrophic epidermolysis bullosa (DEB) are autosomal dominant and the severe forms tend to be autosomal recessive (Fine *et al.*, 1991).

Epidermolysis bullosa acquisita stands out in a category on its own as it is the only known nonheritable form of the disease. This acquired form is an autoimmune disease whose clinical presentation is similar to dystrophic forms of epidermolysis bullosa and splitting occurs below the lamina densa. Patient's sera contain circulating IgG autoantibodies that bind to type VII collagen of the anchoring fibrils. This is a similar etiology as is seen in other acquired blistering diseases such as bullous pemphigoid (against BPA 1 and/or BPA 2), pemphigus vulgaris (against E-cadherin) (Uitto and Christiano, 1992a).

Epidermolysis Bullosa Simplex

Patients with epidermolysis bullosa simplex (EBS) generally follows a benign clinical course and mucosal involvement is mild if present at all. Blisters heal without scarring. The Koebner (EBS-K) type generally has blistering developing within the first few weeks

of life and may even be present at birth. The degree of trauma to cause blistering varies greatly between individuals. Generalized blistering occurs at young ages but tends to become localized to the hands, feet and acral areas later in life. There is often noted improvement in symptoms after puberty, especially in females. Blistering is aggravated by warm weather (possibly due to sweating) and can be triggered by physical stress such as friction under clothing. The teeth are normal and nails may show mild involvement. Oral and nasopharyngeal mucosal involvement is usually mild to moderate and improves with time. Eye abnormalities have been reported in some cases. Cytolysis of basal cells results in blisters at the basal cell level and dyskeratosis is minimal to mild (Pearson, 1988).

The onset of blistering in EBS Weber-Cockayne (EBS-WC) arises later than in EBS-K and is usually limited to trauma-induced blistering on the hands and feet. This is aggravated when children learn to walk. In many patients substantial trauma, such as active sports, is required to cause blistering and Leigh and Lane (1993) mention that placing shoes in the refrigerator prior to sporting events can minimize this blistering on the feet. As with EBS-K, excessive sweating of the feet is common in this subtype. The lesions heal without scarring and noncutaneous findings have not been reported. Basal cell cytolysis with mild dyskeratosis has been observed pathologically (Pearson, 1988).

Blisters develop between birth and early childhood in patients with EBS-Mottled Hyperpigmentation (EBS-M). The feet, ears, hands and buttocks are most commonly involved and the lesions heal without scarring. Mottled hyperpigmentation and hypopigmentation are present at birth but decreases with age. The nails may be thickened and curved and severe dental caries have been reported. Again, blister formation is due to cytolysis at the basal cell layer (Pearson, 1988).

EBS-Dowling Meara (EBS-DM) is an unusual variant also known as EBS-Herpetiformis. The original four patients displayed hereditary blistering, palmoplantar hyperkeratosis, nail dystrophy or shedding of nails and oral ulcerations. Blisters and erosions are usually present at birth or develop within a few days thereafter. Death may occur in the neonatal period and this form of EBS has often been misdiagnosed as junctional or dystrophic epidermolysis bullosa. Diffuse blistering may occur in addition to the herpetiform pattern and the most common sites are on the hands and feet. True scarring rarely occurs but keratin filled cysts (milia) may persist for some time before resolving. Incidence and severity of blistering tends to decrease starting in late childhood (10-12 years old) and reversal of nail dystrophy may also occur. Noncutaneous findings include oral and esophageal blistering and suggestions of dental abnormalities (natal teeth, anodontia). Dowling and Meara (1954) suggested that the blisters appear subepidermally from their light microscopy investigations but electron microscopy, which is now accepted method of pathological diagnosis reveals that tissue cleavage is actually low within the basal cells. Tonofilament clumping in the basal cells is also a common finding (Pearson, 1988 and Leigh and Lane, 1993).

Many other forms of EBS have been identified that have symptoms that are combinations of those seen in the better studied subtypes described above. EBS-Mottled Pigmentation, Punctate Keratoderma (EBS-MPPK) has widespread blistering that presents with postblistering hyper- or hypopigmentation. As the child ages the blistering decreases but the altered pigmentation continues to worsen. Nails are repeatedly lost and there is hyperkeratosis of the soles and palms. Oral blistering occurs only during the natal period and a high dental caries rate has been reported. EBS-Localized, Anodontia, Kallin Syndrome (EBS-LK) affects children starting at less than one year old with hair (brittle, alopecia) and nail involvement. There are dental findings of abnormal tooth mineralization or anodontia. Vision and hearing disorders may also occur. EBS-Ogna (EBS-O) has

generalized blistering that is worse on the hands and feet. No noncutaneous findings have been reported. EBS-Bart (EBS-B) presents with blisters at birth that heal with fine scarring but the widespread blistering that develops postparturition heal without scarring. The oral mucosa is commonly involved. EBS-B may be related to EBS-DM rather than being a distinct disease. EBS-Letalis (EBS-L) has blistering that starts on the hands and feet but later becomes more generalized. Oral ulcerations can occur but teeth and nails appear normal. Numerous patients in the past have died prematurely and esophageal obstruction, septicemia and gastrointestinal disease have all been suggested as possible causes. EBS-Mendes da Costa (EBS-MC) involves blistering of the extremities, alopecia, atrophy of the face and extremities, hyperpigmentation and microcephaly (Pearson, 1988).

Junctional Epidermolysis Bullosa

The subtypes of junctional epidermolysis bullosa (JEB) are based on blister distribution, extracutaneous involvement and severity of the disease. The most severe form of the JEB subtypes is the Herlitz (aka Gravis) subtype and death usually occurs within a few weeks following birth although the occasional patient will survive a number of years.

Generalized blistering occurs from birth and lesions are slow to heal. Atrophy and scarring is common. Vegetating lesions are common in the perioral area. All mucosa is susceptible to involvement as are numerous organ systems. The nails are repeatedly lost, the teeth are dystrophic and growth is retarded (Pearson, 1988).

JEB-Mitis (JEB-M) is similar to JEB-H except it is less severe and growth and lifespan is close to the normal range for healthy individuals. JEB-Localized, Minimus (JEB-LM) has blistering limited to the hands and feet. Growth and development is normal although nails are repeatedly lost and teeth are dystrophic. JEB-Cicatricial (JEC-C) is present from birth with generalized blistering that is most severe on the hands and feet. Scarring occurs upon healing and sometimes results in syndactyly (mitten type) and contractures. Oral and

esophageal blistering, stenosis of the nares, dysplastic teeth and anemia have been reported. JEB-Inversa (JEB-I) has generalized neonatal erosions and blisters that later become restricted to the axillary and groin regions. Nail dystrophy is seen and mild to moderate mucosal involvement. In JEB-Progressiva (JEB-P) nail dystrophy is the first sign of this subtype and is seen between ages five to eight years. Blistering follows mainly on the feet and hands and mild finger contractures may develop. Oral blistering and loss of lingual papillae is also known to occur (Pearson, 1988).

Dystrophic Epidermolysis Bullosa

Dystrophic epidermolysis bullosa (DEB) is characterized by localized or widespread blisters and erosions on the skin. DEB-Cockayne-Touraine (DEB-CT) has generalized blistering and erosions at birth or soon after. The nails are often affected and patients seem more susceptible to developing squamous cell carcinomas. Oral mucosal involvement is mild but the teeth may be malformed. The thin and uneven pattern of enamel is theorized to result from vascular changes in the stellate reticulum that result in early degeneration of the outer enamel epithelium during tooth development. Also, it has been suggested that some ameloblasts have reduced activity at the time of matrix formation and calcification (Koshiha *et al.*, 1977). Keratitis (inflammation of the cornea) may also occur. DEB-Minimus (DEB-M) has mild blistering limited to the childhood years and in adulthood the only remaining sign may be nail dystrophy. This may be a mild variant of DEB-CT. DEB-Pretibial (DEBP) has blisters localized to the pretibial area and dorsum of the feet. There is minimal extracutaneous involvement. DEB-Albopapuloidea (DEB-A) has generalized blistering at birth although these lesions may concentrate in the acral areas at a later age. Mucosal involvement is minimal and the teeth and nails have mild, if any, involvement. DEB-Hallopeau-Siemens (DEB-HS) is a recessively inherited, mutilating form that has blisters and scarring frequently present at birth and gradual disabling fusion of the fingers (mitten) and toes occurs. These patients are more susceptible to bacterial infections and

have an increased incidence of squamous cell carcinomas. The mucosa is frequently involved and the teeth are dystrophic. Keratitis and anemia are also common findings. Growth and development is retarded. Lifespan does not usually exceed 20 to 30 years. DEB-Inversa (DEB-I) often has more severe oral and esophageal involvement than the cutaneous findings of blistering in the axillary, groin and neck regions. Teeth are mildly dystrophic and the tongue may be bound to the floor of the mouth (Pearson, 1988).

There is presently no cure and little treatment for any form of epidermolysis bullosa. Cutaneous hygiene is emphasized to minimize the risk of scarring and infections. Blisters are to be drained with a sterile needle and open erosions cleaned and covered without placing tape directly onto the skin. Indurated or hyperkeratotic growths should be biopsied as these patients are at risk for developing squamous cell carcinoma (Lin and Carter, 1993).

Many forms of EB are associated with extracutaneous involvement, including diverse oral manifestations. Oral findings can involve soft and/or hard tissues and varies greatly between different subtypes of EB. Patients with EB Simplex generally have only mild oral involvement whereas more severe soft and hard tissue abnormalities are seen in junctional and dystrophic EB.

Table 2 - Oral Findings Associated With Epidermolysis Bullosa

<u>Oral Finding</u>	<u>Reference</u>
Intraoral milia	Pearson, 1988
Blistering of buccal mucosa, tongue and lips (gingiva and hard palate less frequently)	Pratilas <i>et al.</i> , 1975, Wright, 1990
Perioral carcinomas	Sedano and Gorlin, 1989
Ankyloglossia	Nowak, 1988
Lingual papilla atrophy	Nowak, 1988
Dental caries	Crawford <i>et al.</i> , 1976, Wright <i>et al.</i> , 1994
Enamel hypoplasia	Wright <i>et al.</i> , 1994, Nowak, 1988
Rapid Attrition	Holbrook, 1988
Obliteration of the oral vestibule	Nowak, 1988

Traditionally, patients with mild oral symptoms from EB have been treated under local anesthetic for routine dental treatment but patients with more severe oral involvement and/or greater restorative needs have received treatment under general anesthetic.

Intubating EB patients has been a concern for fear of traumatizing the airway and inducing blister formation that may impede air exchange. Wright (1990) retrospectively reviewed the general anesthetic management of nine recessive DEB, one dominant DEB, and four JEB patients. Oral intubation was used in all but one case and any device that contacted the patient was first well-lubricated. The operating room table was padded with sheepskin bedding and no adhesives were placed directly onto the patient's skin. The pharynx did not blister in any of the cases following intubation or throat-packing although extraoral blistering and blistering at the commissure of the lip was common following tissue manipulation. High volume suction tips used during restorative work were found to cause extensive tissue damage and were replaced with smaller, surgical suction tips. Broad malleable retractors helped disperse pressure on the tissue and in the posterior sextants of the mouth where access was limited, stainless steel crowns were attached to a curved bone file with wax as this allowed delivery without excessive stretching of the tissues. Although the frequency of intraoral and/or extraoral blistering was 100%, there were no serious complications following the general anesthetic or dental treatment. Also of interest is that

the author reports examining two patients with severe recessive DEB who tolerate complete upper dentures even though this would be expected to create unbearable trauma to the mucosa (Wright, 1990).

Wright *et al.* (1991a) conducted a prospective study of 216 patients representing all three major subclasses of EB to determine the incidence and extent of soft tissue involvement. Virtually all patients with dystrophic and junctional EB demonstrated oral blistering as well as 35% of those with localized EBS and 59% of those with generalized EBS. No pathognomonic soft tissue manifestations were found for the subtypes of EB but obliteration of the oral vestibule, ankyloglossia and microstomia, which are known to result from continuous oral blistering, were most commonly associated with generalized recessive DEB. Oral milia (small keratin-filled cysts) were found in all three EB subtypes but were most common in DEB. No studies appear to have been conducted as to the degree of periodontal disease in epidermolysis bullosa.

The prevalence of dental caries in EB versus control patients has been examined in 252 patients with all three major subtypes and nearly all known varieties of the disease (Wright *et al.*, 1994). Enamel hypoplasia was reported in recessive dystrophic DEB (18.1%), dominant dystrophic DEB (15.4%), JEB (100%) and EBS (21.9%) versus 17.5% of the controls. This shows that development enamel defects are highly prevalent in JEB but are similar to healthy patients in the other EB subtypes. Only recessive DEB (37.6%) and JEB (58.6%) had statistically higher Decayed, Missing or Filled Surfaces (DMFS) than the controls (23.2%). There was no significant relationship between enamel hypoplasia and dental caries or dental caries and oral blistering in this study. Even with a DMFS score of >25 in the recessive DEB group there was no relationship with ankyloglossia or vestibular obliteration which are associated with continual blistering and scarring of the oral soft tissue. The standard deviations of the DMFS scores were very high indicating large

variations between individuals with the same type of EB. Even in groups where the DMFS score was high, there were still individuals who did not develop any caries. This suggests that certain types of EB (i.e. Recessive DEB and JEB) predispose a person to dental caries but that other factors are involved (ex. diet, fluoride, microbial flora and oral hygiene). Wright *et al.* (1991b) did not find any changes in salivary flow rates or composition that could explain an increased susceptibility to dental caries.

Poikiloderma Congenitale (Rothmund-Thomson Syndrome)

Poikiloderma refers to a skin disorder characterized by pigmentation, telangiectasia (a vascular lesion formed by dilatation of a group of small blood vessels), purpura (hemorrhage into the skin, mucous membranes or other internal organs), pruritus (severe itching), and atrophy. There are numerous poikilodermatous disorders that are of congenital origin including Rothmund-Thomson syndrome, acrogeria (Gottron syndrome), pangeria (Werner's syndrome), Kindler syndrome, acrokeratotic poikiloderma of Weary, Mendes da Costa's syndrome, sclerosing poikiloderma, cachectic dwarfism (Cockayne's syndrome), xeroderma pigmentosum and dyskeratosis congenita. Rothmund (1868) described ten related patients from an isolated village with poikiloderma and cataracts from a young age. Thomson (1923), unaware of Rothmund's publication 50 years earlier, described another two cases that were very similar to the original ten cases but lacked cataracts and he coined the term poikiloderma congenitale. It has been suggested by numerous investigators that the cutaneous changes represented the same disease and it has since been referred to as poikiloderma congenitale, Rothmund-Thomson syndrome and poikiloderma atrophicans (Vennos *et al.*, 1992).

Poikiloderma congenitale (PC) involves poikiloderma of the face and extremities which coincides with sun-exposed areas (photodistributed) but other areas, such as the buttocks, are also commonly affected. Associated findings include cataracts, alopecia (loss of hair),

dwarfism, hypogonadism, poor dentition, skeletal anomalies, cutaneous malignancy and hyperthyroidism. There are approximately 200 published cases of PC and Vennos *et al.* (1992) have reviewed the world literature. They report that 27% of the probands had consanguinity between parents or other ancestors who were not affected with PC. 59% of the probands had relatives other than parents who also had findings of PC. This is suggestive of a recessive mode of inheritance although cases have also been described that fit an autosomal dominant pattern of inheritance (an affected parent and child with no other affected relatives or history of consanguinity). PC is not sex-linked as a 51%:49% female:male ratio was found upon review. Age of onset is in the first year of life in the majority of cases and does not appear to correlate with disease severity (Vennos *et al.*, 1992).

Cutaneous manifestations generally begin as erythematous patches on the cheeks and spreads to the forehead, ears, neck and then appears on the dorsal surface of the hands, extensor surfaces of the arms and legs and on the buttocks. This active phase can involve blistering and lasts for months to years and then develops into the characteristic poikilodermatous appearance of atrophy, abnormal pigmentation and telangiectases. Warty or verrucous hyperkeratotic lesions have been reported in 30% of cases. (Vennos *et al.*, 1992)

Histopathological examination reveals epidermal flattening with edema at the dermoepidermal junction during the erythematous active phase. Later, epidermal atrophy and hyperkeratosis result in the poikilodermatous appearance. Pigment incontinence and fragmentation of elastic fibers is seen in the dermis. Glycogen deposits in the spinous and granular keratinocyte layers have been reported. (Vennos *et al.*, 1995)

Most patients develop some degree of alopecia and this can continue to progress until the third decade. A third of the patients have dystrophic nails and a few cases of anhidrosis (diminished or complete absence of secretion of sweat) have been reported. (Vennos, 1992). Oral defects described in PC include microdontia, hypoplastic teeth, impacted teeth, increased dental caries and prognathism (Bottomley, 1976 and Vennos *et al.*, 1992).

Skeletal abnormalities were found in 68% of cases with the most common being frontal bossing, saddle nose, prognathism, proportionately small hands and feet and missing or malformed radii. Syndactyly of the fingers and toes as well as delayed bone development (Vennos *et al.*, 1992).

Cataracts have been found in one-third of PC patients with most arising before six years of age. All were bilateral and of rapid onset (two-to-three months). If one PC sibling develops cataracts then it can be expected with very high certainty that other affected siblings will also develop cataracts. Addition ocular abnormalities such as exophthalmos and photophobia have been reported (Vennos *et al.*, 1992).

Most infants with PC are of low birth weight but are born after a normal period of gestation. Delayed physical development is common and most have normal intelligence. Infertility is common. Immunologic function appears normal although some reports of cases with frequent infections exist. Growth hormone and thyroid studies indicate normal levels of these hormones although abnormalities in gonadotrophins have been reported (Vennos *et al.*, 1992).

Skin malignancies have been reported in Caucasian patients with PC. The incidence of squamous cell carcinomas are considerably higher than in the overall population and age

of onset is earlier. Bone malignancies are also more prevalent in PC patients (Vennos *et al.*, 1992).

It has been proposed that mutations in developmental genes and DNA repair genes may cause PC and this would also explain the increased prevalence of neoplasms. To date, post-irradiation and phototesting studies have, for the most part, been normal. Tests also do not provide a picture that could consistently trace clinical findings to defects in an endocrine gland (Vennos *et al.*, 1995).

2.3 Differential Diagnosis of Kindler Syndrome

Hereditary sclerosing poikiloderma - an autosomally inherited progressive poikilodermatous disease. It lacks bullae formation, acrokeratoses, eczematous dermatitis and photosensitivity thereby differentiating itself from Kindler syndrome and HAP (Weary *et al.*, 1969).

Poikiloderma congenitale (Rothmund-Thomson syndrome) - autosomal recessive disease with poikiloderma and bullae that are limited to sun-exposed areas and not necessarily in acral locations thereby differentiating itself from HAP. The poikiloderma starts prior to age two years and is not of the diffuse type seen in Kindler syndrome (Vennos *et al.*, 1992).

Xeroderma pigmentosum - recessively inherited photodistributed poikilodermatous disease with photosensitivity. Acral bullae are rare. Diagnosis is confirmed by a fibroblast defect in DNA repair following exposure to ultraviolet light (Forman *et al.*, 1989).

DaCosta's syndrome - X-linked recessive inheritance with bullae and poikiloderma producing a clinical picture similar to Kindler syndrome but the distribution and inheritance patterns are different. DaCosta's syndrome is also associated with

mental retardation and dwarfism unlike HAP and Kindler syndrome (Forman *et al.*, 1988).

2.4 Basement Membrane Zone

The epithelium is organized into basal, spinous, granular and keratinized cell layers. Basal cells migrate to the surface of the epithelium as they mature. Neighboring keratinocytes attach to one another via desmosomes. Keratins are the major structural protein of these cells and form in the cytoskeleton. Basal cells of the epithelium attach to the basement membrane via a hemidesmosomal complex. The basement membrane separates the epithelium from the underlying dermis in a polarized fashion (Fig. 1) and compartmentalizes various types of cells and tissue structures including muscle, fat and nerve axons. Basement membranes are specialized types of extracellular matrices laid down by epithelial and endothelial cells and consist mainly of collagenous and noncollagenous glycoproteins (Timpl and Aumailley, 1989). Basement membranes also contain the heparan sulphate proteoglycan (Paulsson *et al.*, 1986) which has been shown to bind to laminin-1, laminin-5 collagen type IV and fibronectin (Battaglia *et al.*, 1992).

Basement membranes are highly organized molecular aggregates whose primary functions are to provide a physical support for the epithelium and separate it from the underlying mesenchyme. Basement membranes form early during development and are important in embryogenesis, cell differentiation and as a substrate for cell anchorage and migration (Engvall, 1995). Similarly, remaining basement membrane after wounding serves as a substrate guiding the migration of cells from the wound margin to regain epithelial continuity. Basement membranes also represent barriers that selectively filter macromolecules as well as migrating and neoplastic cells (Paulsson, 1992).

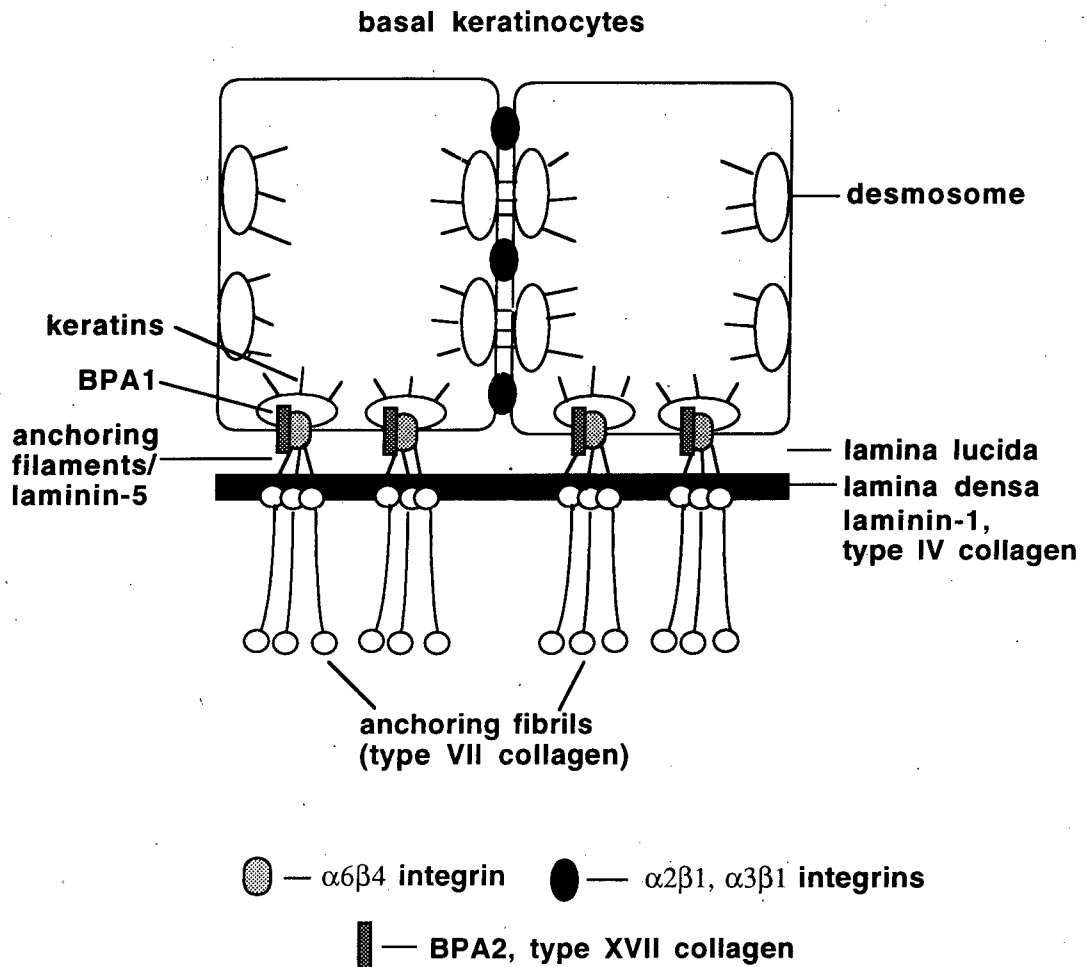


Figure 1 - Ultrastructure of the Basement Membrane Zone (from Larjava *et al.*, 1996).

Intermediate filaments of the basal keratinocytes contain keratin filaments made up of keratins K5 (basic) and K14 (acidic) in the cytoplasm that connect to desmosomes and hemidesmosomes. All keratins contain a central α -helical coil and the primary sequences of the keratin peptides causes their self-assembly. Heterodimers are formed by the combining of an acidic and basic keratin peptide. Two heterodimers combine in a head-to-toe fashion to form a heterotetramer. These heterotetramers combine end-to-end and laterally to create protofilaments (3 nm diameter), protofibrils (4.5 nm) and keratin filaments (10 nm) (Marinkovich, 1993). As basal cells mature and migrate to more

superficial layers the keratin expression changes (e.g. K1 and K10 are synthesized in the spinous layer) and the amount keratin fiber bundles increase (Fuchs, 1990). The keratin filament attachment to the desmosomes and hemidesmosomes contributes to the integrity of the epithelium.

Basal epithelial cells synthesize and secrete the components of the basement membrane that facilitate attachment of the epidermis to the dermis. This includes the hemidesmosomes, basal membrane, anchoring filaments and anchoring fibrils.

While desmosomes link keratinocytes to themselves the hemidesmosomes attach basal epithelial cells to the basement membrane (Stepp *et al.*, 1990). Three major protein components that are associated with hemidesmosomes include bullous pemphigoid antigen 1 (BPA 1 or gp 230) and 2 (BPA 2 or gp 180) and $\alpha 6\beta 4$ integrin (Uitto and Christiano, 1992). BPA 1 is a 230-kD noncollagenous glycoprotein that derives its name from being the major autoantigen identified in the sera of patients with bullous pemphigoid, a vesiculo-bullous disease that results in tissue separation at the level of the lamina lucida. BPA 1 has been cloned and it consists of a central helical component that suggests it may have an alternate dimeric form and globular ends that are similar to the desmosomal protein desmoplakin I. Immunoelectron microscopy has shown that BPA 1 is located intracellularly in the basal keratinocytes (Shimizu *et al.*, 1989). The BPA 1 gene is located on chromosome 6 at the locus 6p11-12 (Sawamura *et al.*, 1990).

BPA 2 is a 180-kD collagenous protein (also called type XVII collagen) that is also recognized by autoantigens in the sera of some patients with bullous pemphigoid (Uitto *et al.*, 1992c). It is a transmembrane protein containing repeated Gly-X-Y triplets that are occasionally separated by noncollagenous peptide sequences and the amino-terminal end is

a globular protein (Li *et al.*, 1992). The BPA 2 gene has been mapped to chromosome 10 (locus 10q24.3) (Li *et al.*, 1991).

Integrin $\alpha 6\beta 4$ plays a crucial role in the assembly of hemidesmosomes (Stepp *et al.*, 1990, Jones *et al.*, 1991, Sonnenberg *et al.*, 1991). Integrins are heterodimeric cell surface glycoproteins that are, in general, responsible for most cell-cell and cell-extracellular matrix interactions and link the extracellular matrix to the cytoskeleton. Integrins are composed of an α and β subunit and there are at least 15 different α and 8 β subunits currently known. These subunits combine to form more than 20 different cell surface receptors. The subunits are membrane-anchored glycoproteins that generally have large extracellular domains and short cytoplasmic domains. The $\beta 4$ subunit is an exception having a long cytoplasmic domain. The extracellular domain of $\alpha 6\beta 4$ integrin interacts with laminin-5, a component of the anchoring filaments in the lamina lucida. It is possible that $\alpha 6\beta 4$ integrin may also bind to other components of the lamina lucida. Kurpakus *et al.* (1991) have shown that antibodies against $\alpha 6\beta 4$ integrin will result in dissociation of hemidesmosomes and cell detachment. The cytoplasmic tail of the $\beta 4$ integrin subunit along with BPA1 are believed to be important components of the intracellular hemidesmosomal plaque. It has also been determined that the amino-terminal end of BPA2 interacts with $\alpha 6\beta 4$ integrin; probably through the $\alpha 6$ integrin subunit (Hopkinson *et al.*, 1995). Adhesion of the junctional epithelium to tooth enamel is also mediated by $\alpha 6\beta 4$ integrin (Hormia *et al.*, 1992).

The lamina lucida is located between the basal plasma membrane and the lamina densa. Anchoring filaments span through the lamina lucida. It is not known how or even whether the anchoring filaments insert into the anchoring fibrils of the lamina densa but electron microscopy gives this type of appearance (Gipson *et al.*, 1988). The monoclonal antibody GB3 was found to localize in the lamina lucida and bind to a large glycoprotein which has

gone through a series of names (BM 600, nicein, kalinin and epiligrin) and is now called laminin-5. This protein has a similar structure to classical laminin which is now designated laminin-1 (Bürgeson *et al.*, 1994).

Laminins are a family of cross-shaped heterotrimeric glycoproteins found in the basement membrane that regulate/modulate cell adhesion, migration, proliferation and differentiation. Eight laminin peptide chains have been identified thus far that combine to form different heterotrimeric isoforms. Each chain is from a different class ($\alpha\beta\gamma$) in the trimeric form. Laminin-1 has the composition $\alpha1\beta1\gamma$ whereas laminin-5 is $\alpha3\beta3\gamma2$. Laminin-6 consists of $\alpha3\beta1\gamma1$ (Sugiyama *et al.*, 1995). Sugiyama *et al.* (1995) have mapped the $\gamma2$ chain of laminin-5 and found it to be a homologue of, and its gene in close proximity on chromosome 1, to the $\gamma1$ chain of laminin-1. The interaction of cells with laminins is mediated by integrins $\alpha1\beta1$, $\alpha2\beta1$, $\alpha3\beta1$ and $\alpha6\beta1$ (Engvall, 1993). Antibody blocking experiments have shown that neural cell adhesion and process outgrowth on laminin-1 is mainly mediated by integrin $\alpha1\beta1$ (Smith *et al.*, 1996), whereas $\alpha3\beta1$ mediates binding to laminin-5 in keratinocytes (Carter *et al.*, 1991).

The lamina densa contains laminin-1, heparan sulfate proteoglycan and type IV collagen. Laminin-1 is a glycoprotein of about 850,000 molecular weight that is composed of three polypeptide chains that are arranged in a cruciform tertiary structure. Laminin-1 has globular regions that react with collagens, proteoglycans and other molecules and is therefore an important regulatory substance in attachment of epithelial cells. Heparan sulfate proteoglycans are thought to be involved in the binding of extracellular matrix such as fibronectin and collagen (Uitto, 1991).

All collagens are composed of three polypeptide chains that are coiled around one another to form a triple helix structure (van der Rest and Garrone, 1991). The helical regions are

formed of repeating Gly-X-Y sequences where X and Y represent any amino acid. Most commonly, X is proline and Y is hydroxyproline. This pattern of amino acids is important in maintaining the tertiary conformation of the helix. Substitution of a single base pair can result in changing a critical amino acid to another (glycine to arginine) or create a premature stop codon. Mutations in collagen have been shown to be involved in heritable diseases such as dystrophic epidermolysis bullosa, Ehlers-Danlos syndrome, and osteogenesis imperfecta. Nonhelical sequences are also found; both at the ends of the collagen molecule and in the main body. These regions allow special attachment functions of the molecule as well as allow flexibility for the molecule to bend.

Type IV collagen of the lamina densa is composed of α -chains but rather than forming fibrils like collagen types I, II and III it forms a multilayered and highly insoluble network. It has 26 nonhelical sequences that gives it considerable flexibility but also make it susceptible to proteolytic enzymes. A "chicken wire" model has been proposed where four molecules join at their terminal ends to form a flattened parallelogram. Type IV collagen is encoded by six distinct genes and deletions have been shown in patients with glomerulonephritis (Zhang *et al.*, 1996) but not in vesiculobullous diseases at the present time.

Type VII collagen molecules are the major component the anchoring fibrils that align laterally and insert into the hemidesmosomal plaques and anchor the basement membrane to the underlying connective tissue stroma (Uitto *et al.*, 1992b). Type VII collagen is limited to the basement membrane of the stratified squamous epithelium of the skin, mucous membranes and the cornea of the eye. The type VII collagen gene (COL7A1) has been sequenced and localized to chromosome 3 (locus 3p21.1) (Parente *et al.*, 1991). The gene is very complex and at least 116 exons are present. Type VII collagen contains three identical $\alpha 1$ chains that have various noncollagenous interruptions in the Gly-X-Y pattern.

These interruptions are thought to create flexibility in the molecule. In particular, a 39 amino acid noncollagenous segment in the center of the helix is thought to correspond to the "hinge region" predicted by biochemical studies (Bachinger *et al.*, 1990). It also has terminal noncollagenous globular domains. The amino-terminal end is an approximately 140kD noncollagenous domain (NC-1) and the carboxy-terminal end is an approximately 20-kD noncollagenous domain (NC-2). Type VII collagen molecules align in the extracellular space and overlap their NC-2 ends. The antiparallel dimer is stabilized by disulfide bonds. The NC-2 portions are then cleaved off proteolytically and a stable type VII collagen dimer is the result. Numerous dimers align laterally to form anchoring fibrils that have multiple NC-1 domains at both ends. It has been theorized that it is the NC-1 domains that interact with the lamina densa and anchoring plaques as it contains segments that are similar to the adhesive areas of fibronectin and von Willebrand factor. Anchoring fibrils extend from the lamina densa and either 1) bend and reinsert in the lamina densa, thereby trapping interstitial collagen types I and III which are linked to the rest of the dermis or 2) insert into electron-dense anchoring plaques in the papillary dermis (Uitto, 1992a). The anchoring plaques are accumulations of basement membrane-like material deep to the basal keratinocytes in the dermis.

In normal skin these arrangements securely bind the cytoskeleton through the basement membrane components all the way to the underlying dermis and create a complex barrier that can withstand great stresses and shearing forces. Various disorders can upset these stable adhesions and result in vesiculation, ulceration and desquamation of the epidermis or mucosa following even very minor trauma.

2.5 Pathobiology of Epidermolysis Bullosa

Disease classification based on clinical findings of epidermolysis bullosa has produced many problems as there is so much overlap in the signs of the disease. For example most affected children have indistinguishable cutaneous features during the newborn period. An editorial by Fine (1988) reports cases that presented clinically as junctional EB but ultrastructural and immunofluorescence findings revealed they were actually unusual presentations of EB simplex. Fine points out that patients with inherited EB cannot be accurately subclassified on the basis of history and clinical findings and that routine light microscopy is of no use unless it is to rule out an acquired autoimmune blistering disease. Transmission electron microscopy and immunofluorescence mapping are the standard for diagnosing EB subtypes. Classification of EB based on the molecular defect will soon be possible.

Target genes for genetic dermatological disorders can be determined by unusual presentations of immunohistochemical staining or by identifying genetic linkages in families. Table 3 lists the known components of the basement membrane zone and includes the neighboring epithelium and lamina propria according to the simplex, junctional and dystrophic classification. Candidate genes that, if mutated or null, could explain blistering in these areas are listed along with references for those that have been investigated or at least suggested as being involved in epidermolysis bullosa. Unfortunately, the incidence and severity for periodontal disease in epidermolysis patients has not been documented as it has for oral blistering, salivary function and caries (Wright *et al.*, 1991, 1994). A predicted influence on the periodontium if these candidate genes were affected is presented. The relative range is from (-) to (++) with (-) predicting no effect on the periodontium and (++) indicating a severe phenotype for periodontal disease would be expressed. Those components, if missing or defective, that will result in a lack of integrity between the

junctional epithelium and the tooth are suspected of causing in the most severe form of periodontal disease in epidermolysis patients. Periodontal disease is predicted to be most severe in junctional epidermolysis bullosa if defective hemidesmosomal proteins or laminin-5 of the lamina lucida exist. These would be the most likely defects to prevent a proper seal between the tooth and junctional epithelial cells and allow bacteria to migrate into the connective tissue. In this way, bacteria would not require special virulence factors (Fives-Taylor *et al.*, 1996) or a host immune defect to get through or past the epithelial attachment. If laminin-6 and -7 are involved in the function of laminin-5 as discussed later (Champlaud *et al.*, 1996) then they also could result in a predisposition to periodontal disease if defective. Heparan sulfate proteoglycans are involved in the binding of extracellular matrix molecules such as fibronectin and collagen and laminin-1 is an important regulatory molecule of epithelial cell attachment (Uitto and Larjava, 1991) and could result in decreased attachment strength of the internal basement membrane. Type IV collagen has been shown to be fragmented in the basement membrane of the junctional epithelium and gingival pocket epithelium of patients with periodontal disease (Peng *et al.*, 1986) but both Sawada *et al.* (1990) and Hormia *et al.* (1992) have found that type IV collagen is present only in the external basement membrane of the junctional epithelium and not in the internal basement membrane where contact to the tooth is made. It is therefore unlikely that a defect in type IV collagen will have a major affect on the periodontal health.

Keratins 5 and 14 are present in the junctional epithelium along with keratins 13 and 19 (Schroeder and Listgarten, 1997). Defects in epithelial cell adhesion molecules $\alpha 2\beta 1$ and $\alpha 3\beta 1$ or keratins 5 and 14 would result in decreased cell-cell attachment and could result in sloughing of the surface epithelium. The resulting ulcerations could provide easier access for bacteria into the underlying tissue by travelling between epithelial cells that are not well connected to each other or to the basal cells. A similar situation could arise with

dystrophic blistering following a type VII collagen defect where sloughing of the epithelium allows bacteria direct access to the connective tissue attachment of the tooth.

Table 3 -Candidate Genes Encoding the Basement Membrane Zone in Different Forms of Epidermolysis Bullosa

Type of EB	Candidate Proteins	Reference	Predicted Influence on Periodontium
Simplex	Keratins 5 and 14	Vassar <i>et al.</i> , 1991	+
	Cell-cell adhesion molecules	Not known	+
Junctional	Hemidesmosomal proteins	Pohla-Gubo <i>et al.</i> , 1995	++
	BPA1, BPA2 and $\alpha 6\beta 4$ integrin	Gil <i>et al.</i> , 1994	
	Laminin-5	Pohla-Gubo <i>et al.</i> , 1995 Domloge-Hultsch <i>et al.</i> , 1992	++
	Laminin-6 and -7	Not known	+
	$\beta 1$ integrins	Hodival-Dilke <i>et al.</i> , 1996	+
	Type IV collagen	Not known	-/+
	Laminin-1	Not known	+
	Heparan sulfate proteoglycan	Not known	+
Dystrophic	Anchoring fibrils protein type VII collagen	Ryynänen <i>et al.</i> , 1991	+

Epidermolysis bullosa simplex is usually inherited in an autosomal dominant manner with complete penetrance although recessive inheritance has also been reported (see Table 1). In the case of EBS it was suspected that the defect could be in the keratin intermediate filaments of the epidermal cytoskeleton because the Dowling-Meara variant shows clumping of these filaments under electron microscopy. Rather than try to isolate proteins from the skin of diseased individuals and compare them to healthy controls, Bonifas *et al.* (1991) used genetic linkage analysis to assess whether the gene causing EBS is located at the same chromosomal sites as the genes encoding for keratins. Linkage analysis is the technique of looking for the chromosomal location of an unknown gene by comparing its inheritance pattern with that of a marker of known chromosomal location. The more cases

in whom the association between the disease and the marker exists, the greater the statistical probability that the unknown disease gene is close to the marker because the closer the two genes are to each other, the less likely they are to be separated by random recombination. Genetic linkage is expressed as a lod score which is defined as the log 10 of the odds that the disease and marker loci are linked rather than unlinked. A lod score of +3 (1000:1 odds) is taken as proof of linkage. In an autosomal dominant EBS, a child would inherit one normal chromosome and one mutant chromosome. Since large regions of chromosomes are passed as segments, but yet no two chromosomes are identical, it is possible to clone and sequence sections of the chromosome and identify polymorphisms in the genes. This led to the discovery of a mutation of amino acid 384 (leucine to proline) in the acidic keratin 14 gene on chromosome 12q in a family with Koebner EBS (Bonifas *et al.*, 1991 and Coulombe *et al.*, 1991) and a mutation of amino acid 475 (glutamic acid to glycine) in the basic keratin 5 gene on chromosome 17q in a family with Dowling-Meara EBS (Lane *et al.*, 1992). These and other studies have suggested that the severity of EBS is related to the type of keratin gene mutation and that the milder Weber-Cockayne and the more severe Dowling-Meara phenotypes can result from mutations on either the keratin 5 or keratin 14 gene (Ryynänen *et al.*, 1991, Hovnanian *et al.*, 1993). Combined lod scores of 12.98 has been reported for the keratin 5 gene and 3.0 for the keratin 14 gene (Uitto and Christiano, 1993). These mutations all affect the α -helical domain of keratin and probably inhibit keratin filament assembly through negative interactions the dimeric molecules. A resemblance to EBS was found in transgenic mice expressing mutant keratin in the basal layer of the stratified squamous epithelium. Blistering occurred easily in these mice (many of whom die prematurely) and basal cell cytolysis was observed at the light and electron microscopy level (Vassar *et al.*, 1991). The EBS phenotype could also result from mutations in nonkeratin genes such as those that encode proteins involved in insertion of intermediate filaments into desmosomes.

Junctional epidermolysis bullosa is the least well understood subgroup of EB diseases at the molecular level. Mutations have been mapped in some families in both EBS and DEB a number of years ago but have only recently been reported in JEB. Verrando *et al.* (1991) have suggested laminin-5 as a candidate protein responsible based on the level of tissue separation and immunofluorescence analysis. Pohla-Gubo *et al.* (1995) used monoclonal antibodies P1E1 against epiligrin and GB3 against laminin-5 (L-5) in patients with generalized atrophic benign epidermolysis bullosa (GABEB), a nonlethal form of JEB, and found no differences in expression from normal control skin. They are reported similar results for integrin subunits $\alpha 2$, $\alpha 3$, $\alpha 6$, $\beta 1$ and $\beta 4$. Of interest though is that monoclonal antibodies HD18 showed no reactivity and 233 showed reduced staining against BPA2 (type XVII collagen). Laminin-5 consists of 3 peptide chains ($\alpha 3\beta 3\gamma 2$) that are products of 3 distinct genes (LAMA3, LAMB3 and LAMC2 respectively) (Burgeson *et al.*, 1994). Mutations in the LAMC2 gene have been identified in three individuals with generalized (Herlitz) JEB (Uitto *et al.*, 1994). Herlitz-JEB presents with widespread blistering that involves tissue separation at the level of the lamina lucida. Linkage analysis supports the relationship of a defective LAMC2 gene in these families but these authors have also examined another four families which have been excluded from linkage to the LAMC2 locus. This suggests that H-JEB is a heterogeneous group of diseases and mutations in genes encoding for other proteins also produce the JEB phenotype (Uitto *et al.*, 1994). Mutations in the LAMB3 and LAMA3 genes have since been reported in H-JEB patients (Vidal *et al.*, 1995). Vidal *et al.* (1995) have cloned LAMA3 and shown that a 1-base pair deletion results in a frameshift and a premature stop codon in a H-JEB patient. Champlaud *et al.* (1996) report the existence of laminin-6 ($\alpha 3\beta 1\gamma 1$) and a previously unknown laminin-7 ($\alpha 3\beta 2\gamma 1$) that are found in association with laminin-5 and may help to stabilize the bond between the epithelium and the underlying stroma. It possible that mutations in laminin-6 or -7 may affect the performance of normal laminin-5.

Integrin $\alpha 6\beta 4$ is involved in the attachment of basal cells to laminin-5 through hemidesmosomes. Gil *et al.* (1994) have reported a variant of JEB with severely reduced $\beta 4$ expression but normal laminin-5 expression. This differs from JEB gravis which is considered to have normal $\alpha 6\beta 4$ expression but reduced laminin-5 expression (Gil *et al.*, 1994). Vidal *et al.* (1995) have found a deletion mutation in the LAMC2 gene of laminin-5 in H-JEB patients but that it only causes a clinical effect in those patients that are homozygous for the deletion.

Dominantly inherited forms are often more mild and localized than the recessively inherited forms of dystrophic epidermolysis bullosa. Ultrastructural findings include reduced numbers or absence, in the case of the most severe forms of recessively inherited DEB, of anchoring fibrils and sub-lamina densa blistering. Type VII collagen is the major component of the anchoring fibrils. In dystrophic epidermolysis bullosa a strong genetic linkage to the type VII collagen gene locus (COL7A1) has been demonstrated. Combined lod scores for dominant dystrophic EB is 26.97 and for recessive dystrophic EB is 3.97 (Uitto *et al.*, 1993). The close genetic linkage of the dystrophic EB phenotype to the COL7A1 gene suggests that type VII collagen is the candidate gene for the mutations responsible for the clinical presentation of the disease. Uitto *et al.*, (1993) reviews the possible genetic combinations between chromosomes and the severity demonstrated in the phenotype from a normal gene/null allele (an allele that is not expressed) situation where the phenotype is normal to a dominantly inherited mutation resulting in mild disease characteristics to severe disease being expressed as a result of a recessively inherited mutated gene, compound heterozygote or compound null allele. That is, a spectrum of different kinds of mutations could lead to altered expression of the type VII collagen gene which could explain the low abundance of anchoring fibrils in dominantly inherited DEB and the complete absence of anchoring fibrils in some recessively inherited forms.

Elucidation of precise molecular defects will allow the development of a rational treatment approach. Prenatal diagnosis of epidermolysis bullosa using DNA samples taken from chorionic villus biopsies, periumbilical blood samples or fetal cells from amniocentesis is presently available and it appears that there are no incidences of false-positive or false-negative diagnoses (Uitto *et al.*, 1992c). Possible areas for intervention in EB could be at the level of cell proliferation, matrix protein synthesis (collagens, proteoglycans, other basement membrane proteins), matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs). Future treatment of EB is aimed at gene replacement therapy. Cultured keratinocytes could be transfected with DNA that codes for the protein that are either missing or nonfunctional. This would be particularly useful in patients with a null allele who are altogether lacking anchoring fibrils or in patients with a dominant-negative mutation where the gene product actually interferes with the function of the normal protein (Uitto *et al.*, 1992a). Hovnanian *et al.* (1993) have hypothesized that mutations in the collagenous domain could result in the degradation of the normal molecules that participate in the formation of collagen type VII homotrimers. Gene replacement could also be used to upregulate the production of normal cytokines that stimulate gene expression of proteins that are at too low of levels. For example, transforming growth factor- β (TGF- β) is a powerful upregulator of type VII collagen gene expression so co-transfecting human keratinocytes with the genes for type VII collagen and TGF- β could be clinically significant in DEB patients with decreased anchoring fibrils (Uitto *et al.*, 1993). EB is a rare disease making it difficult to find funding to support research to benefit only a very small group of people.

2.6 Pathobiology of Kindler Syndrome

Forman *et al.* (1989) published histopathologic and immunofluorescence studies from a Kindler syndrome patient. They found atrophy of the epidermis with flattening of the rete ridges and basal cell vacuolization. Pigmentary incontinence, capillary dilatation and a mild lymphocytic infiltrate was found in the upper dermis. They reported a poor expression of bullous pemphigoid antigen (not stated as 1 or 2), laminin and type IV collagen along the basement membrane zone. Laminin and type IV collagen that was present in cleft areas appeared on the floor suggesting cleavage at the level of the lamina lucida. Electron microscopy revealed duplication of the lamina densa and attributed this to continued remodelling of the basement membrane zone. They also found focal clefts beneath degenerating basal cells. Fibroblasts were noted to have penetrated the basement membrane and reached the level of the basal keratinocytes. Focal areas of aggregated tonofilaments was noted in ruptured basal cells. Basal keratinocytes that remained attached to the basement membrane appeared normal in terms of desmosomes, hemidesmosomes, tonofilaments, anchoring filaments and anchoring fibrils. Laboratory investigations appeared normal including complete blood count, differential white cell count, blood urea nitrogen, serum creatine, electrolytes, serum calcium, blood glucose, liver function tests, urinalysis, chest radiograph and electrocardiogram.

Many questions remain about Kindler syndrome. It is suggested that Kindler syndrome and Weary's Hereditary Acrokeratotic Poikiloderma may be variants of the same disorder but the mode of inheritance is unclear. Most cases have been sporadic but kindreds that fit with both autosomal recessive and autosomal dominance inheritance patterns have been described. It is unusual, but not unique, that a gene can underlie both a dominant and a recessive disease. An example of this is osteogenesis imperfecta (OI). Type I and II OI are dominantly inherited diseases that result from a null allele or a structurally defective

collagen molecule respectively (Prockop, 1992). Type II lethal and type III OI is a recessively inherited type I collagen defect where a homozygous deletion creates a splice acceptor site and splicing of a critical exon results in the disease state (Bonadio *et al.*, 1990).

Chapter 3 - Aims of the Study

1) To determine if oral problems are associated with Kindler Syndrome.

It is hypothesized that Kindler patients are more susceptible to periodontal disease. Oral findings including poor oral hygiene, caries, overjet malocclusion, high palatal vault, limited jaw opening, ankyloglossia, atrophy of the buccal mucosa with widespread white macules and easily bleeding gingiva have been reported. Many case reports of Kindler syndrome make no mention of any dental findings.

2) To determine the pathobiological alterations of the components of the basement membrane zone in Kindler Syndrome.

It is hypothesized that Kindler patients have missing or altered distribution of basement membrane zone components. Kindler syndrome shares clinical features in common with epidermolysis bullosa and many types of EB have pathological changes to the basement membrane zone. For this reason, the basement membrane zone in a Kindler syndrome patient will be examined for pathobiological alterations.

3) To determine an effective mode of oral treatment and reconstruction in a patient with Kindler Syndrome.

It is hypothesized that a Kindler syndrome patient can be treated as a normal periodontal disease patient for dental treatment. A Kindler patient with an advanced periodontal condition and numerous missing teeth will be treated and the results evaluated as to their effectiveness in dealing with the oral problems.

Chapter 4 - Clinical Presentation of Kindler Syndrome; Case Report.

4.1 Case History

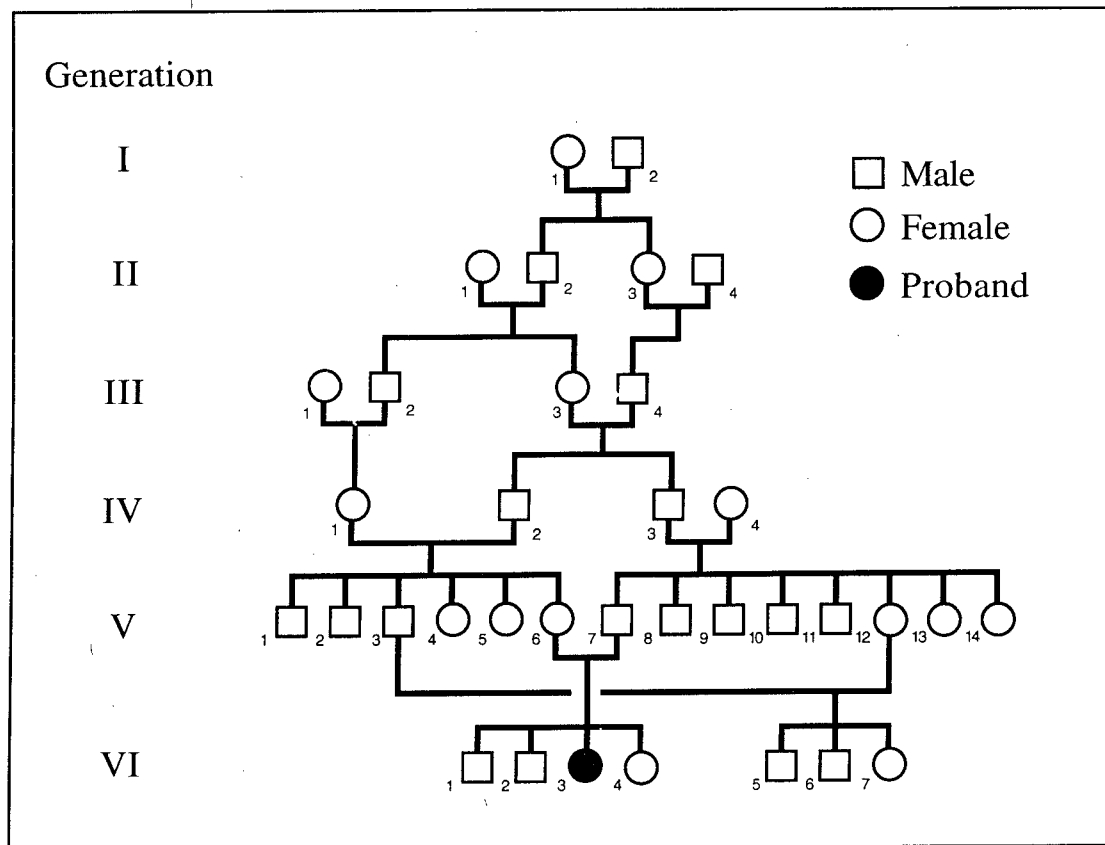
A 16-year-old female was seen at the University of British Columbia graduate periodontics clinic upon referral from the dermatology clinic of a local children's hospital. The patient was a refugee who had left Afghanistan five months previously and arrived in Canada three months after that via Pakistan. She was very mature for her age, appeared to be adjusting well to life in Canada and was reasonably fluent in English. She had a history of blistering on her hands and feet in infancy and early childhood although this had become less of a problem with age. The patient still occasionally developed trauma-induced blistering and skin fragility.

There was a history of photosensitivity dating from early childhood, exposure to sunlight resulting in facial erythema, swelling and headaches. Physicians in Afghanistan and India treated her with antibiotics for at least two years as a young child but apparently no specific investigations had been undertaken nor a diagnosis established. She had a history of an adverse reaction to penicillin at age ten and was not using any medications at the time of examination.

Her parents, who were thought to still be in Afghanistan but whose location was unknown to the patient, are first cousins. There is a long history of consanguinity in the patient's family including her great-grandparents on her father's side were first cousins, her two grandfathers were brothers, and her grandmother on her mother's side is a first cousin of her grandfather. Her parents are first cousins as are her aunt and uncle, with whom she presently lives. The patient also lives with one female and two male cousins. She has two

sisters aged twelve and eight and a brother aged seven who are thought to reside with their parents in Afghanistan. Other than herself, however, she was unaware of family members being affected.

Figure 2 - Unusual Pedigree of Patient with Kindler Syndrome. Relatives were either unavailable or unwilling to submit to examination or give blood samples. Proband believes she is the only affected family member.



4.2 Clinical Finding and Diagnosis

The examination report from a pediatric dermatologist had listed her skin as generally dry and atrophic with poikilodermatous changes (Fig. 3A and B). There was freckling on her neck and abdomen and hypopigmented lesions associated with poikiloderma on her arms and legs. Marked atrophy of the skin on her hands and feet with webbing of the proximal web spaces was noted. There were also one or two hyperkeratotic lesions on the plantar surfaces of her feet although none were present on the palms of her hand. There was a tiny erosion on the palmar aspect of her right wrist. Her hair was normal. All of her nails showed some mild ridging and yellowish discoloration of the great toe nails and dystrophy of the fifth toe nails were noted.

Figure 3 - Dorsal Surface of the Hand. A and B) Atrophic changes including wrinkling of the skin and xeroderma.



Dental findings included an anatomically small mouth with limited opening, seven missing mandibular teeth, partially impacted maxillary wisdom teeth, no caries or history of caries, generalized tooth mobility ranging from slight to extreme. Little plaque was found upon probing except in the mandibular premolar region and in furcation areas. Minor amounts of supragingival calculus were found. Gingival bleeding occurred spontaneously in many sites (Fig. 4B) and was generalized upon probing with Grade III and IV papillary bleeding index (Muhlemann, 1977). The medical report for our present case stated the patient had gum disease and dental caries for approximately five years. Our oral examination and history, taken later, revealed extreme tooth mobility for much longer than five years but no caries or history of caries.

Figure 4 - Clinical Presentation Upon Initial Examination.

A) Maxillary incisors, facial view. Note inflamed swollen gingiva. Recession around the central incisors.



B) Facial surfaces of teeth in maxillary right quadrant. Fragile gingival tissue that bleeds spontaneously and lifts from the tooth surface when an air blast is applied.



C) Anterior palate with edematous, inflamed soft tissue.

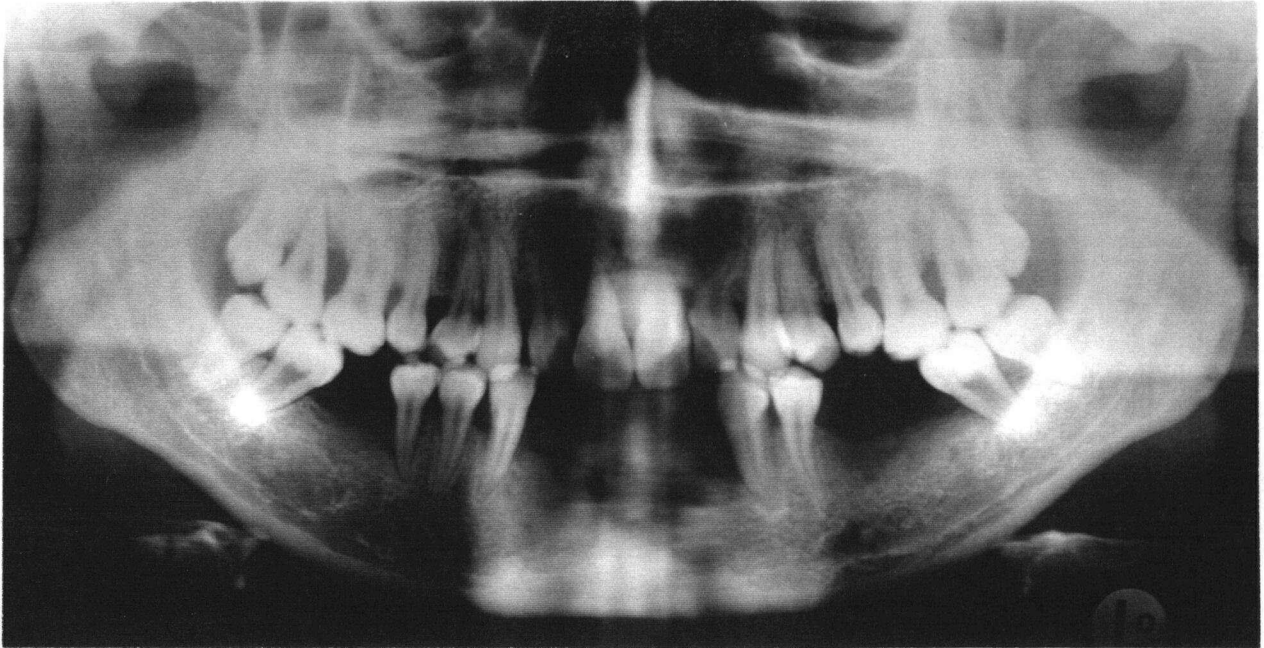


The gingiva was very thin and fragile and appeared transparent over the necks of some teeth. In some areas of attached gingiva a thin slough of epithelial surface could be removed by rubbing with the side of a periodontal probe. There was soft tissue cratering and rolled margins at a number of sites with the gingiva lifting away from several teeth with a gentle blast of air (Fig. 4). Probing depths were 5-11 mm around the maxillary anterior teeth and 4-6 mm around all posterior teeth. Localized areas of 1-3 mm gingival recession were present.

Radiographically, bone loss extended to the apical third of the roots around the maxillary central incisors and to the middle third of the roots around many posterior teeth although a number of these latter teeth had isolated surfaces with more extensive loss of bone. Many of the roots were shorter in length than average and tapered markedly toward the apex (Fig. 5). The maxillary anterior teeth also have large pulp chambers (Fig. 5B).

Figure 5 - Radiographic Survey.

A) Panoramic radiographic view exposed May, 1995. Right mandibular central incisor exfoliated at age 10 and other missing teeth were highly mobile and were extracted 6 months prior to radiograph.



B) Severe bone loss indicated in periapical view of the maxillary incisors.



C) Bone loss associated with lateral incisor and molars. No caries detected.



The patient reported brushing twice per day and flossing in the evening. Her hands did not hurt and did not impede her from brushing. Her gingiva bled excessively when she brushed and while she slept. She had never visited a dentist while living in Afghanistan and there was no suggestion from the patient's history that any of her dental visits since then had included professional cleaning of teeth.

All her deciduous teeth exfoliated between four and seven years of age and the permanent right mandibular central incisor exfoliated spontaneously when she was ten years old. While in Pakistan awaiting immigration, she saw a dentist who extracted six loose mandibular teeth and made her a cast lower partial denture. Later, in the United States while awaiting permission to enter Canada she experienced a periodontal abscess and visited a dental office where she had full-mouth radiographs taken and was placed on antibiotics. The patient could not remember a time when her teeth did not hurt and

doubted that any dental treatment short of full-mouth extractions and complete dentures would help her.

After an oral examination was carried out, diagnoses of periodontitis associated with systemic disease, gingivitis and partial edentulousness was assigned (Proceedings of the World Workshop in Clinical Periodontics, 1989). From the patient's dental history it is likely that she had generalized prepubertal periodontitis which has continued on to affect the permanent dentition in a manner similar to generalized juvenile periodontitis. A prognosis was hard to determine as the oral complications of her genetic disorder are not well documented or understood. Based upon advanced loss of periodontal attachment, several teeth were considered to have a poor or even hopeless prognosis. No teeth were extracted prior to initial mechanical therapy, however, as we were uncertain of the effects that periodontal treatment would yield in this case.

Prior to initiating therapy, a number of diagnostic tests were undertaken. These included a DNA probe test for the presence of periodontal pathogens (DMDx Plus Test, OmniGene®, Cambridge, MA), an AST test for the crevicular fluid activity of aspartate aminotransferase as a marker for tissue destruction (Colgate Periogard®), a BANA test for the presence of the anaerobic bacteria *Porphyromonas gingivalis*, *Bacteroides forsythus* and *Trepomena denticola* which have peptidases that can hydrolyze N-benzoyl-DL-arginine-2-naphthylamide (Knowell Periodontal Technologies®, Toronto, ON), and a noncommercial elastase test (Uitto *et al.*, 1996) The bacterial tests were carried out to determine if certain commonly accepted periodontopathogens were present at high threshold levels and to provide information on possible antibiotic selection. Enzyme tests were used as possible indicators of disease activity.

The DNA probe test results were negative for the presence of *Actinobacillus actinomycetemcomitans* (<0.1% of total or fewer than 10^3 cells) and showed low levels (0.1-0.9% of total or 10^3 cells) for *Porphyromonas gingivalis*, *Prevotella intermedia*, *Eikenella corrodens*, *Fusobacterium nucleatum*, *Campylobacter rectus*, *Bacteroides forsythus* and *Treponema denticola*. The BANA test results were negative for all teeth tested, the AST test was positive for five of twelve sites tested. Elastase activity was high, typical of untreated periodontal disease (Uitto *et al.*, 1996). Test sites were selected on the basis of disease severity. These test results are presented in Table 4.

A medical consultation was obtained prior to initiating any dental treatment and no special recommendations were made by the patient's dermatologist. Selected pulp testing of teeth with severe bone loss to near the apex was carried out and the right maxillary central incisor was found to be nonvital.

Table 4 - Pre- and Post-treatment Diagnostic Data

	Pretreatment	After Treatment	Two Year Follow-up
1) DNA probe test	Aa(-), Pg(±), Bf(±), Ec(±), Fn(±), Cr(±), Pi(±), Td(±)	not tested	not tested
2) BANA test	negative	negative	negative
3) AST test	42% of test sites positive	negative	8%
4) Elastase test*	0.867	0.051	not tested
5) Probing pocket depths >4mm	42	8	10
6) Spontaneous bleeding and bleeding on probing sites	198	10	40
7) Tooth mobility (Miller, 1938)			
Physiologic	6 teeth	12	19
Class I	7	4	2
Class II	8	5	1
Class III	2	1	0

(-) indicates pathogens are less than 0.1% of total or fewer than 10^3 cells, (±) indicates pathogens range from 0.1 to 0.9% of total or 10^3 cells. Aa, *Actinobacillus actinomycetemcomitans*; Pg, *Porphyromonas gingivalis*; Bf, *Bacteroides forsythus*; Ec, *Eikenella corrodens*; Fn, *Fusobacterium nucleatum*; Cr, *Campylobacter rectus*; Pi, *Prevotella intermedia*; Td, *Treponema denticola*.

* 100 µl of an oral rinse sample was incubated with 1mM of suc-ala-ala-val-p-nitroanilide for 2 hours at room temperature. The enzyme activity is expressed as optical density at 405 nm after subtracting the 0-hour value (Uitto *et al.*, 1996).

4.3 Periodontal Treatment

Oral hygiene instruction for toothbrushing and flossing was given and the patient was advised to use a toothpaste that did not contain sodium lauryl sulfate, a detergent which might have adverse effects on her fragile gingival epithelium (Herlofson and Barkvoll, 1993). Scaling and root planing was carried out under local anesthetic. Based on BANA and DNA probe test results, supportive antimicrobial therapy was considered unnecessary.

Six weeks after completion of initial therapy the gingiva appeared less inflamed and the patient reported her mouth was more comfortable although there were still numerous areas with bleeding on probing. Further instrumentation resulted in greatly decreased spontaneous bleeding, decreased bleeding on probing, less clinical inflammation (Fig. 6A and B), decreased probing depths and decreased tooth mobilities. This response suggested an improved prognosis. Significantly enlarged gingival tissue on the palatal aspects of the maxillary anterior teeth was removed surgically to improve access for oral hygiene. The area healed without complications (Fig 6C). Due to continuing extreme mobility, however, the maxillary right central incisor was extracted and a composite splint from canine to canine retained the crown in place following resection of the root (Fig 6C and D).

Supportive maintenance visits since that time have included further diagnostic tests (see Table 4), localized instrumentation and gentle polishing of the teeth. Aspartate aminotransferase (AST) and elastase tests have been negative or comparable to healthy controls. Localized areas of tissue inflammation and destruction have been noted although most have been associated with the presence of the partial denture. These areas have been treated with debridement and denture adjustments. A new partial lower denture was treatment planned to be constructed in conjunction with the permanent maxillary fixed bridge. No further periodontal surgeries have been treatment planned, even in maxillary

molar regions where areas of localized pocketing exist, because of the very fragile and unmanageable gingival tissue, small opening of the mouth, and the good response to nonsurgical therapy.

Figure 6 - Clinical Presentation Following Mechanical Root Instrumentation.

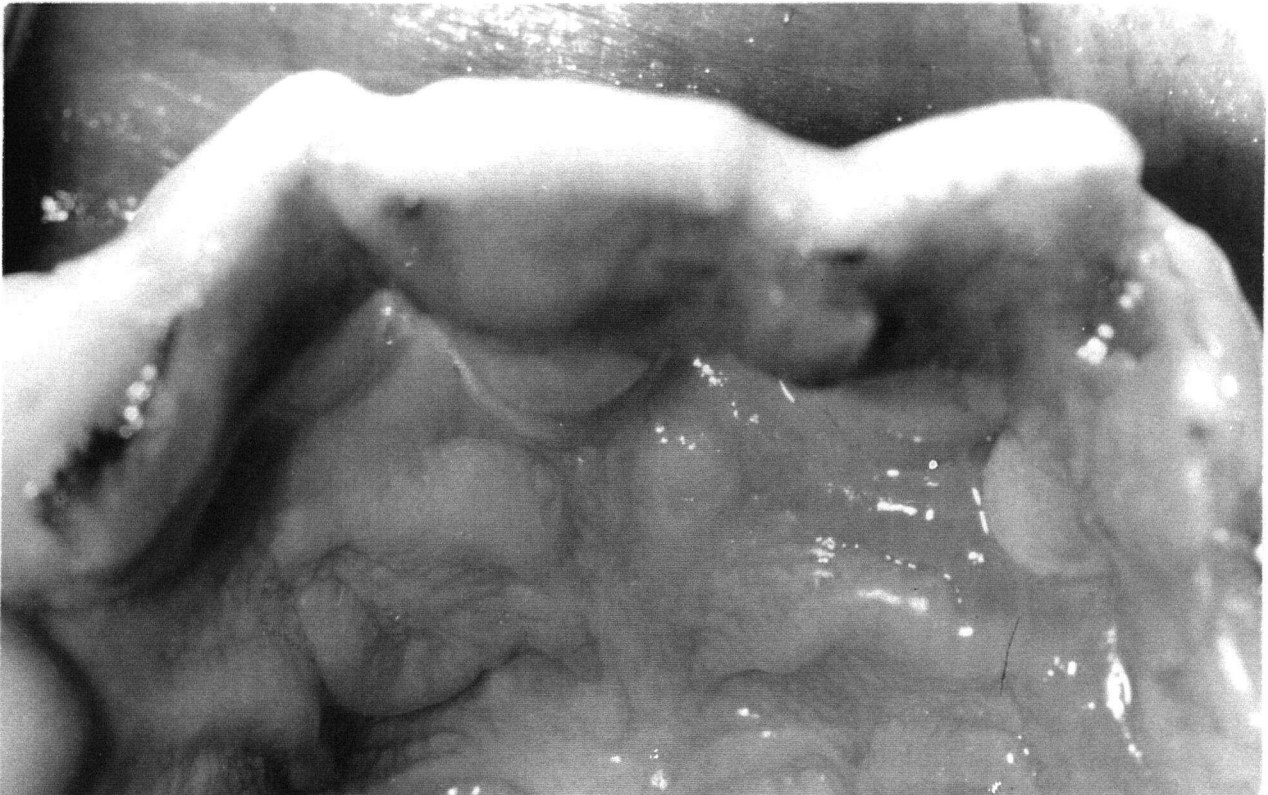
A) Areas of bleeding and inflammation still exist but to a lesser degree and mainly associated with denture abutment teeth.



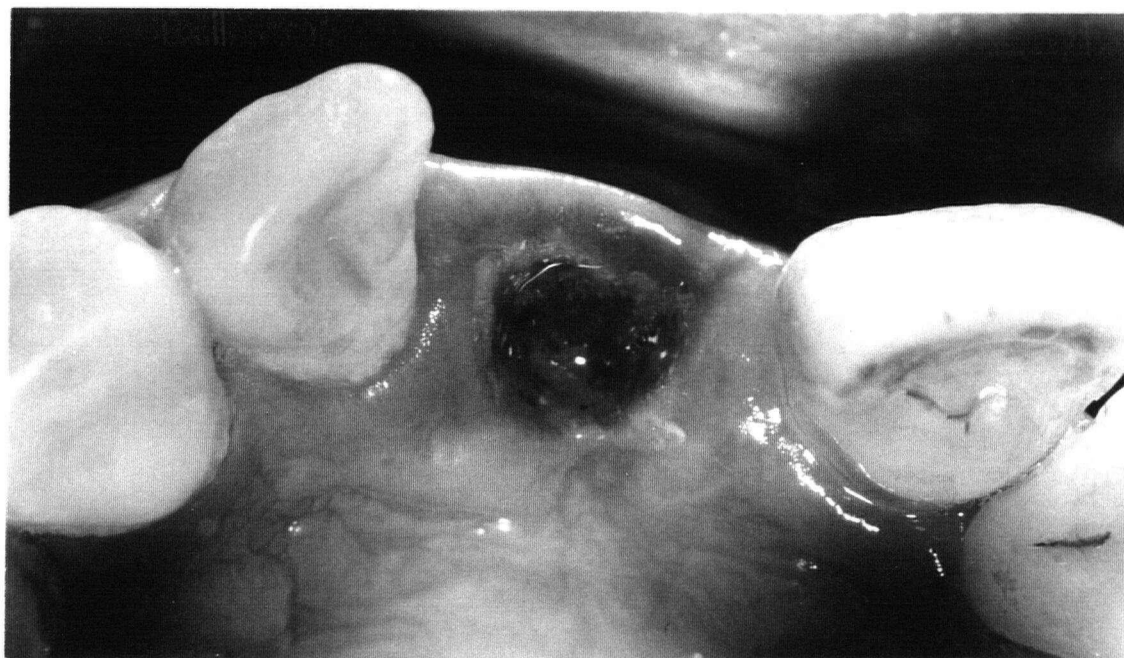
B) Facial gingiva showing less signs of inflammation (compare with Fig. 4B).



C) Anterior palatal tissue following root instrumentation and gingivectomy. The maxillary right central incisor was extracted and its crown incorporated into a mesh and composite splint (compare to Fig. 4C).



D) Following extraction, an attempt was made to preserve the facial profile of the alveolar ridge by contouring the pontic tooth to support the tissue.



Considering the good oral hygiene of our patient and that her periodontal condition was so severe at such a young age, it is very likely related to her genetic condition. Supporting this view is the past history of prepubertal periodontal disease which has been shown to have a familial distribution in many cases (reviewed by Michalowicz, 1993). It is interesting to note that she did not have high counts of bacteria considered to be periodontopathogenic in the areas sampled. For example, many patients with early-onset periodontal disease do harbor *Actinobacillus actinomycetemcomitans* (Slots *et al.*, 1980, Zambon, 1985). In patients with advanced periodontal disease, systemic antibiotic therapy without mechanical debridement may change the subgingival microflora and favor the occurrence of periodontal abscesses (Topoll *et al.*, 1990). Our patient was using systemic antibiotics continuously for years in her childhood. This may have altered her oral flora but it seems unlikely that any influence on the oral flora would still be present a decade after

cessation of taking systemic antibiotics. In addition, most of the periodontal destruction occurred after this episode.

Tissue destruction appeared active during the initial examination based on AST and elastase tests. AST is a cytoplasmic enzyme whose presence extracellularly indicates cell death. Chambers *et al.* (1991) have concluded that elevated AST can predict short term probing attachment loss. Neutrophil elastase has shown to have a sensitivity of 84% and a specificity of 66% with future probing attachment loss (Palcanis *et al.*, 1992). It is possible that periodontal cases with defective host defense are susceptible to normal oral flora. Test results in cases such as these should be interpreted carefully because of possible disturbing influences of the underlying host defect.

A remarkable improvement was seen in the condition of the gingiva following conservative periodontal therapy. Spontaneous bleeding and bleeding on probing greatly decreased with repeated instrumentation. While gingival bleeding is not considered a particularly reliable indicator of periodontal disease activity (Haffajee *et al.*, 1987) we speculate that congenitally fragile gingival tissue may give positive bleeding signs even without underlying inflammatory changes.

Host susceptibility factors may play a significant role in periodontitis and this risk may be under genetic influence (Sofaer, 1990). It is important to note, however, that not all genetic predispositions to periodontitis involve defects of the immune system. Structural defects in the periodontal tissues can act as elements of significant risk as is seen in the severe forms of early onset periodontitis associated with defects in collagen synthesis and cross-linking in Ehlers-Danlos syndromes (Linch *et al.*, 1979).

The patient has been followed on periodontal recalls for over two years since initial therapy was completed. Probing pocket depths have generally remained constant over this time period. Bleeding sites are always present but change in location, number and severity between recall visits. The most common bleeding sites are around abutment teeth supporting the lower partial denture.

4.4 Orthodontic and Prosthodontic Treatment

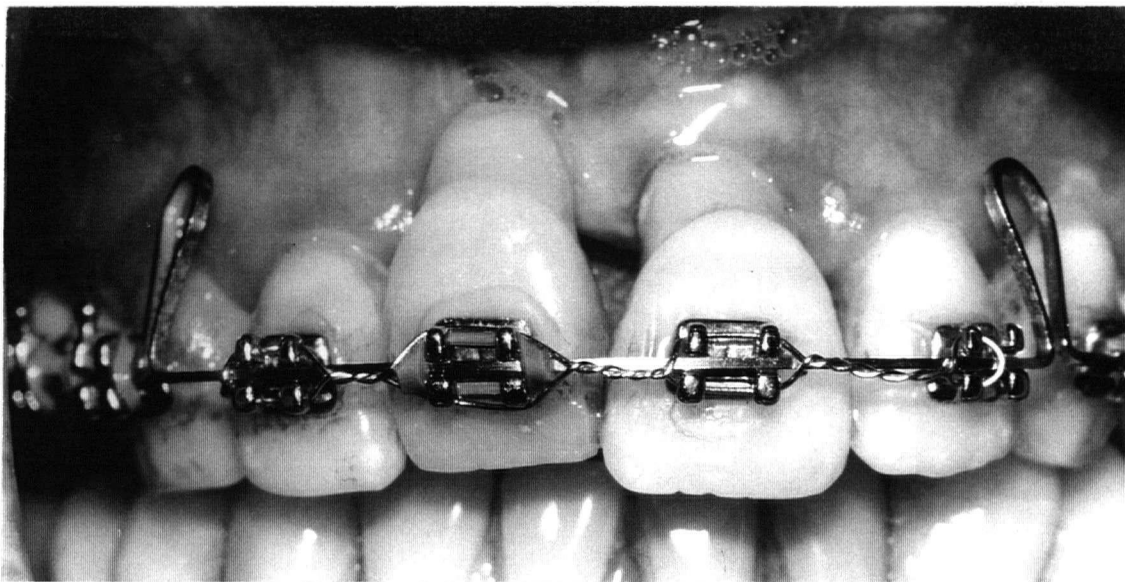
The patient has been maintained on periodontal debridement and polishings every three months and is practicing excellent oral hygiene. There are still sites that bleed on probing and even spontaneously. Many of these areas appear to be related to the presence and design of the lower partial denture. Most probing depths are three millimeters or less. The alveolar ridge has collapsed in where the right maxillary central incisor was extracted. This was not unexpected considering the degree of bone loss but it urged us to avoid further extractions as long as possible. The treatment plan involved placing a six-unit fixed bridge from maxillary canine to canine with five abutments. To do this, orthodontic therapy was required as the malalignment of the incisors precludes draw of the bridge without severe over-reduction of the preparations (Fig. 7A-D). As the bone level is in the apical third of the maxillary anterior incisors, and the patient's physiological response to moving the teeth was unknown, the risk of losing teeth due to orthodontic treatment was explained to the patient. The patient accepted this risk of potential further bone loss and loss of the incisors. Alternatively, if incisors were lost during the orthodontic treatment the plan was to continue with the canine to canine fixed bridge but place it on less abutments.

Figure 7 - Orthodontic Treatment

A Maxillary anterior teeth prior to orthodontic banding.



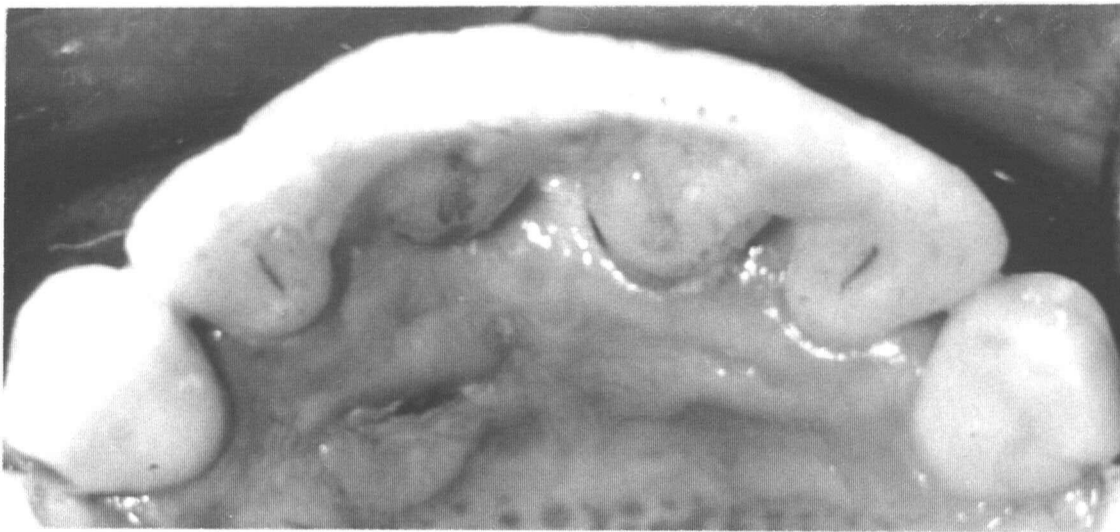
B Missing right maxillary central incisor was ligatured in place during orthodontic treatment.



C Removal of orthodontic bands.



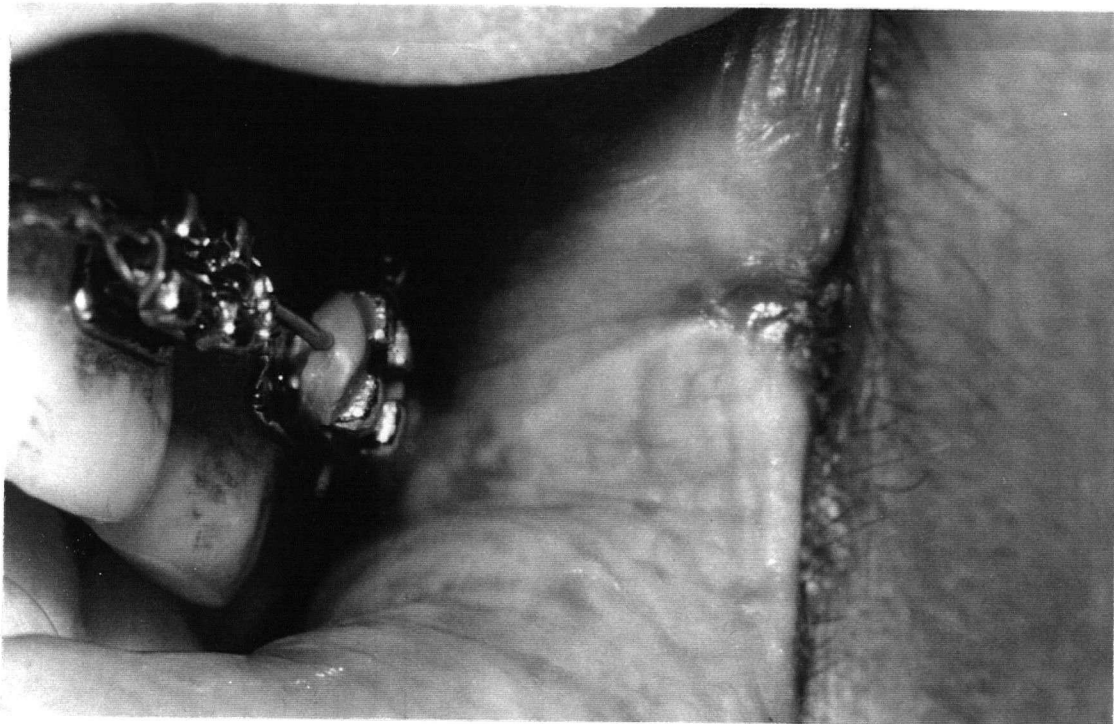
D Temporization with acrylic splint.



The orthodontic treatment involved bonding teeth all the maxillary teeth except the second molars and placing a .014 inch nickel titanium wire to align teeth the remaining incisors. The patient required constant use of orthodontic wax over the bonds and wires as the friction caused erythema and occasional areas of ulceration on the mucosa of the upper lip. Over a period of six months the teeth were derotated and then brought palatally with a rectangular wire to reduce the overjet. During the orthodontic treatment, the crown of the extracted maxillary right central incisor was held in place with a figure eight .010 inch ligature wire to maintain the appearance of all teeth being present. The orthodontic bands were left on the teeth for one month following completion of active tooth movement. Upon removal of the bands tooth maxillary left central incisor had class II mobility and the maxillary lateral incisors had class I mobility. The canines had physiologically normal mobility. Prosthodontic treatment commenced soon after removal of the orthodontic brackets as the temporary and permanent bridges could provide proper retention of the recently-aligned teeth.

During the orthodontic treatment the patient developed painful patches on the buccal mucosa and at the corners of the mouth (Fig. 8). There was the appearance of a white sheen that resembled leukedema. There were also erosions along the margin where the lower lip contacts the upper lip. The patient was given a 0.12% chlorhexidine mouthrinse and the problem had improved five days later at a follow-up visit. These painful white areas reoccurred two weeks later and were treated with kenolog in orabase by a private practice periodontist as this is the accepted first line of treatment for management of desquamative gingivitis (Nisengard and Rodgers, 1987). These lesions did not recur again during the period of orthodontic treatment. Hovnanian *et al.* (1989), in a review of the Kindler syndrome literature, describes three cases where white spots or leukokeratosis was observed on the oral or labial mucosa.

Figure 8 - Appearance of Leukedema that Appeared at the Angle of the Lip and on the Buccal Mucosa During Orthodontic Treatment.

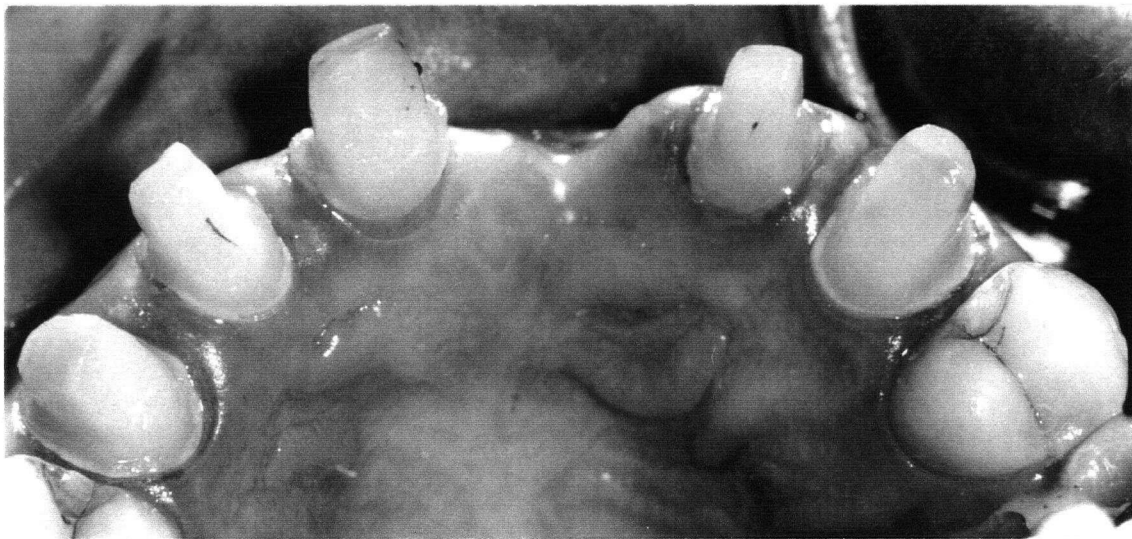


Prosthodontic treatment involved the construction of a maxillary canine-to-canine six unit fixed bridge with all teeth except the right maxillary central incisor present as abutments and a new cast partial mandibular denture replacing the first molars and all incisors. The maxillary anterior teeth had very large pulp chambers and pinpoint pulp exposures occurred on the left central and lateral incisor's mesial-incisal edges during reduction of the teeth. All the reduced surfaces were acid etched with 10% phosphoric acid and bonded (Scotchbond 3M Dental Products, St. Paul MN 55144) (Fig. 9A). Later, the right lateral incisor abscessed and was endodontically treated. The lower canines were too long esthetically and so were reduced incisally approximately two millimeters. The opposing canines were built up palatally to provide occlusion with the lower canines. When the patient originally presented to the graduate periodontics clinic she was wearing a lower partial denture that clasped numerous abutment teeth. Many of these cast clasps were

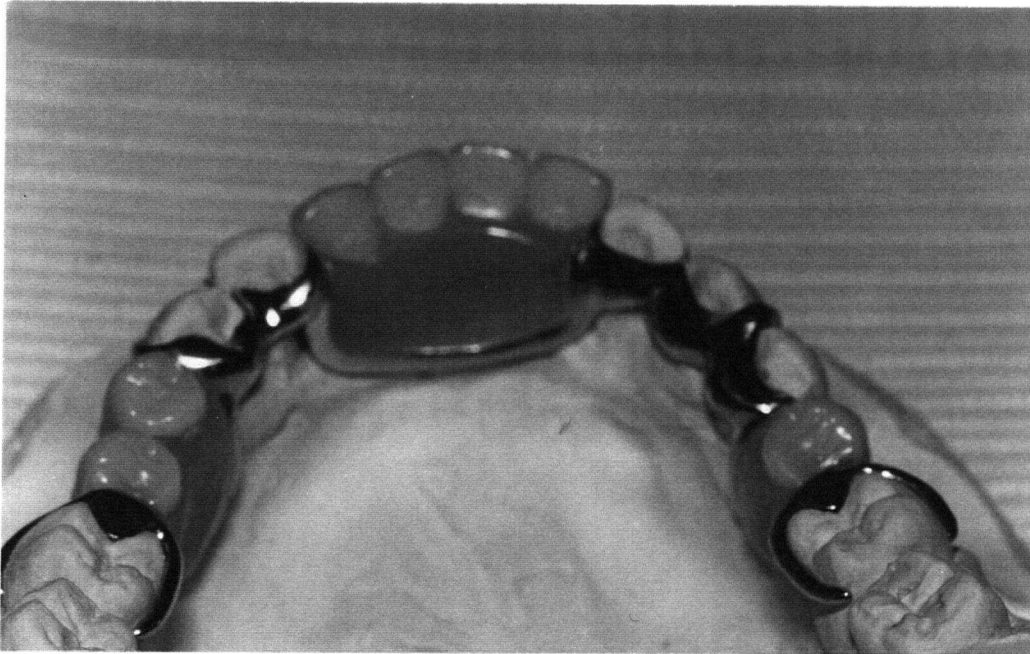
broken in addition to the major connector being distorted so the clasps did not sit bilaterally into the rest seats. Areas with inflammation and spontaneous bleeding or bleeding on probing that were hardest to resolve were around teeth that had denture clasps on them.

Figure 9 - Prosthodontic Treatment.

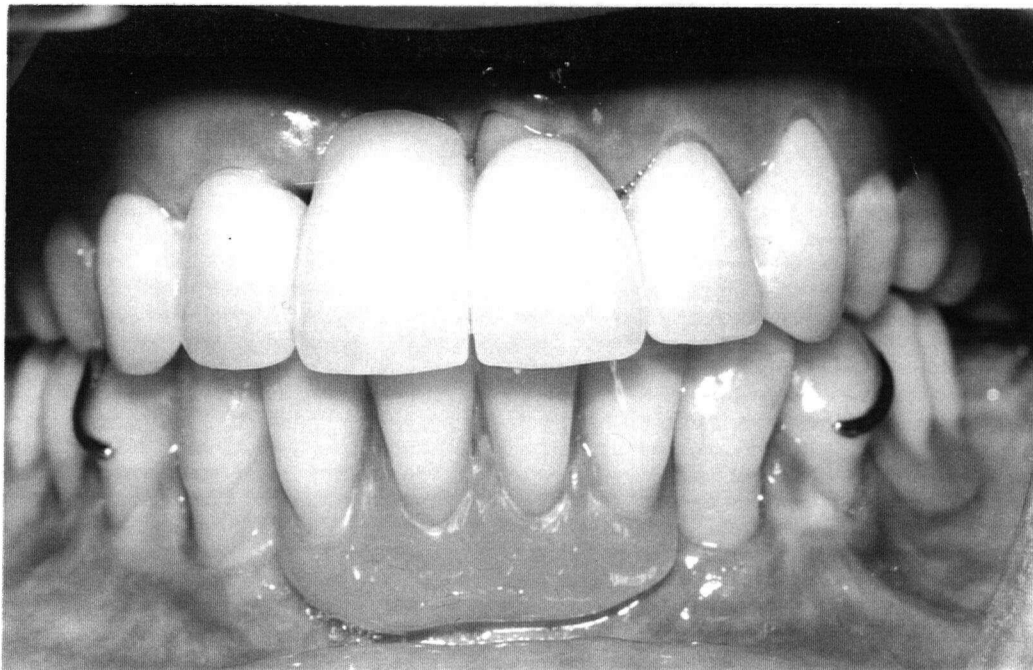
A Prepared maxillary abutment teeth for fixed bridge. Note that the facial aspect of the alveolar ridge has collapsed in following extraction of the right maxillary central incisor.



B Mandibular removable partial denture design to remain clear of the gingival margin.



C Maxillary fixed bridge and mandibular removable partial denture in place.



Chapter 5 - Pathology

5.1 Materials and Methods

Biopsies of noninflamed oral mucosa and marginal gingival tissue with and without characteristic vesiculobullous lesions were taken from a 16-year-old female patient diagnosed with Kindler syndrome. Separate samples were prepared for hematoxylin and eosin staining, immunofluorescence staining and electron microscopy.

The biopsy material for immunofluorescence microscopy was immediately embedded in Histo-Prep (Fisher Scientific, Fair Lawn, NJ), frozen in liquid nitrogen, and stored at -80 °C. Frozen sections were later cut at six μm , placed on TESPA (3-Aminopropyl-triethoxy-silane, Sigma Chemical Co., St. Louis, MO) treated slides, fixed in -20 °C acetone for five minutes, allowed to air dry, and rinsed in one mg/ml bovine serum albumin (BSA) (Sigma) in Dulbecco's phosphate-buffered saline (PBS) (Gibco, BRL). Immunofluorescence staining was carried out as described previously (Larjava *et al.*, 1993). Briefly, sections were incubated at room temperature with antibodies diluted in phosphate buffered saline (PBS) containing one mg/ml bovine serum albumin (BSA) for one hour according to the concentrations listed in Table 5. The sections were then rinsed five times for 10 minutes each with the PBS/BSA solution and stained with a 1:50 dilution of affinity-purified rhodamine-conjugated secondary antibodies (Boehringer-Mannheim Biochemicals, Indianapolis, IN) for one hour. Sections were rinsed twice in double distilled water and mounted using cyanoacrylate adhesive (Chemoco). Samples were examined using a Zeiss Axioskop 20 fluorescence microscope, and photographed using an MC 80 Zeiss microscope camera. Antibodies against the following integrin subunits and their ligands were used: $\beta 4$, $\beta 1$, αv , $\alpha v\beta 6$, $\alpha 3$, type IV and VII collagens, laminin-1 and -5, and bullous pemphigoid antigens 180 and 230 (Table 5). Approximately 40 sections

were stained with each immunofluorescence antibody. Control stainings for nonspecific binding were carried out using rabbit and mouse serum, and by omitting the primary antibody, all of which were negative.

Healthy human gingiva and periodontal pocket tissues were also prepared and stained as above for comparison to the three Kindler tissues (noninflamed oral mucosa, inflamed gingiva and blistered gingiva). The results of healthy gingiva and periodontal pocket stainings were comparable to similar stainings done previously in our lab (Haapasalmi *et al.*, 1995).

Table 5 - Antibody Types and Concentrations for Immunofluorescence.

<u>Integrin/Ligand</u>	<u>Dilution</u>	<u>Antibody</u>	<u>Reference</u>
Integrin $\alpha 3$	1:75	MAb J143	Kantor <i>et al.</i> , 1987
Integrin $\beta 1$	1:500	Antiserum, 3847	Roberts <i>et al.</i> , 1988
Integrin $\beta 4$	1:250	MAb 345-11A	Kennel <i>et al.</i> , 1989
Integrin $\alpha v \beta 6$	1:20	MAb E7P6	Weinacker <i>et al.</i> , 1994
Type IV collagen	1:40	MAb 68-124-1	ICN Biomedicals (Costa Mesa, CA)
Type VII collagen	1:100	MAb 1345	Chemicon (Temecula, CA)
Laminin-1	1:250	Serum	Risteli and Timpl, 1981
Laminin-5	1:30	MAb GB3	Verrando <i>et al.</i> , 1987
Bullous pemphigoid 1 (BP 230)	1:100	Ascites fluid, MAb 10C5	Hopkinson <i>et al.</i> , 1994
Bullous pemphigoid 2 (BP 180)	1:100	PAb J17	Hopkinson <i>et al.</i> , 1992
Rabbit Serum	1:500	Chemicon	
Mouse Serum	1:500	Chemicon	

Representative sections of inflamed and noninflamed oral mucosa were stained with hematoxylin and eosin for histopathological analysis. Skin biopsies from normal and

abnormal areas were also taken from skin of the left foot by a dermatologist. This material was processed for both light and electron microscopy investigation.

Sections were also viewed under a confocal laser microscope to further examine the abnormalities observed under immunofluorescent microscopy. Separate biopsy sections were prepared for electron microscopy by fixation in 2.5% glutaraldehyde for one hour, rinsed twice PBS according to the standard protocol of Dawes (1981) and viewed at 14,000x and 24,000x magnification.

DNA was extracted from a blood sample from the patient and was used to carry out direct mutation analysis of collagen type VII in the laboratory of Dr. Jouni Uitto, Jefferson Institute of Molecular Medicine, Philadelphia.

Oral rinse samples were taken and the number of epithelial cells and polymorphonuclear cells in the saliva were compared to control patients who were orally healthy or had periodontal disease (Pauletto *et al.*, unpublished data).

5.2 Results:

Investigation of three oral biopsy types was carried out:

- 1) Noninflamed buccal mucosa - light microscopy
 - immunofluorescence microscopy
 - electron microscopy
- 2) Inflamed palatal mucosa
 - light microscopy
 - immunofluorescence microscopy
 - electron microscopy
- 3) Blister in inflamed palatal mucosa following scaling and root planing
 - immunofluorescence microscopy

In addition, previous light and electron microscopy carried out by a dermatologist on skin samples from this same patient were reviewed.

Light Microscopy

Skin punch biopsies from "normal" and "rubbed" areas of the left foot were taken by a dermatologist at a local hospital. The biopsies are similar and showed features of poikiloderma congenitale as seen in adults and adolescents. There is no blistering or other suggestion of epidermolysis bullosa. There is a thick stratum corneum but thin epidermis proper with flattened epidermal ridges. Occasional melanophages are present in the papillary dermis and the papillary dermis is fibrosed. In the "normal" skin, the capillaries are prominent and there is some mild lymphangiectasis. This is no basal hydropic change (edema) or degeneration. There is no inflammatory infiltrate in the dermis and no suggestion of epidermal clefts although the stratum corneum has separated from the stratum lucidum. (Data not shown.)

Hematoxylin and eosin stains were carried out on the palatal mucosa that was removed during a gingivectomy procedure. No inflammation was noted in the epithelium. A heavy mixed infiltrate of plasma and lymphohistiocytic cells were found in the edematous lamina propria as well as signs of melanin incontinence and increased vascularization. These

findings are consistent with longstanding inflammation. Atrophy also existed beneath the epithelium. There were no gross vesicular changes and the basement membrane zone appeared intact.

The previous skin biopsy material was reviewed at the time of the oral biopsy and the majority of basal cells were considered to be well paved. In some areas the basilar cells became almost round suggesting the tissue is friable and easily separated. There was no inflammatory cells in the epithelium but there was in the connective tissue. A separation occurs at a lower level in the basement membrane zone and higher level in the epithelium. There was also the possibility of microbasilar vesiculation although this may have been artifact. Liquifaction necrosis also appeared to be present.

Blood Samples for Mutations in Type VII Collagen

DNA was extracted from a blood sample from the patient and was used to carry out direct mutation analysis of type VII collagen. It would have been expedient to start with linkage analysis but this was not possible without blood samples from her parents whose location has been unknown since the patient left Afghanistan. No mutations were found by comparing known sequences DNA coding for type VII collagen and more defects at the protein level need to be determined to give clues as to which candidate genes to examine. The patient's only relatives with whom she has contact, her Aunt, Uncle and cousins, have refused to donate blood samples for research purposes.

Immunofluorescence Microscopy

No basement membrane zone components or integrins studied appeared to be absent (Figs. 10, 11 and 12). The results of these panels will be discussed below under subtitles according to where the biopsy tissue was taken from. Control stainings were performed

omitting the primary antibody or by using rabbit serum staining. No specific staining was obtained with either of these control staining procedures (not shown).

Figure 10 - Immunolocalization of Epithelial Anchoring Molecules in Frozen Sections of Oral Mucosa. **A)** Laminin-5 (LM-5) staining of inflamed gingiva in Kindler patient. **B)** LM-5 staining suggesting split in lamina lucida. **C)** BPA 1 in Kindler patient appears normal. **D)** LM-5 staining in inflamed Kindler gingival tissue. **E)** Noninflamed oral mucosa from Kindler patient. LM-5 staining reaches into the connective tissue. **F)** BPA 2 in Kindler patient appears normal.

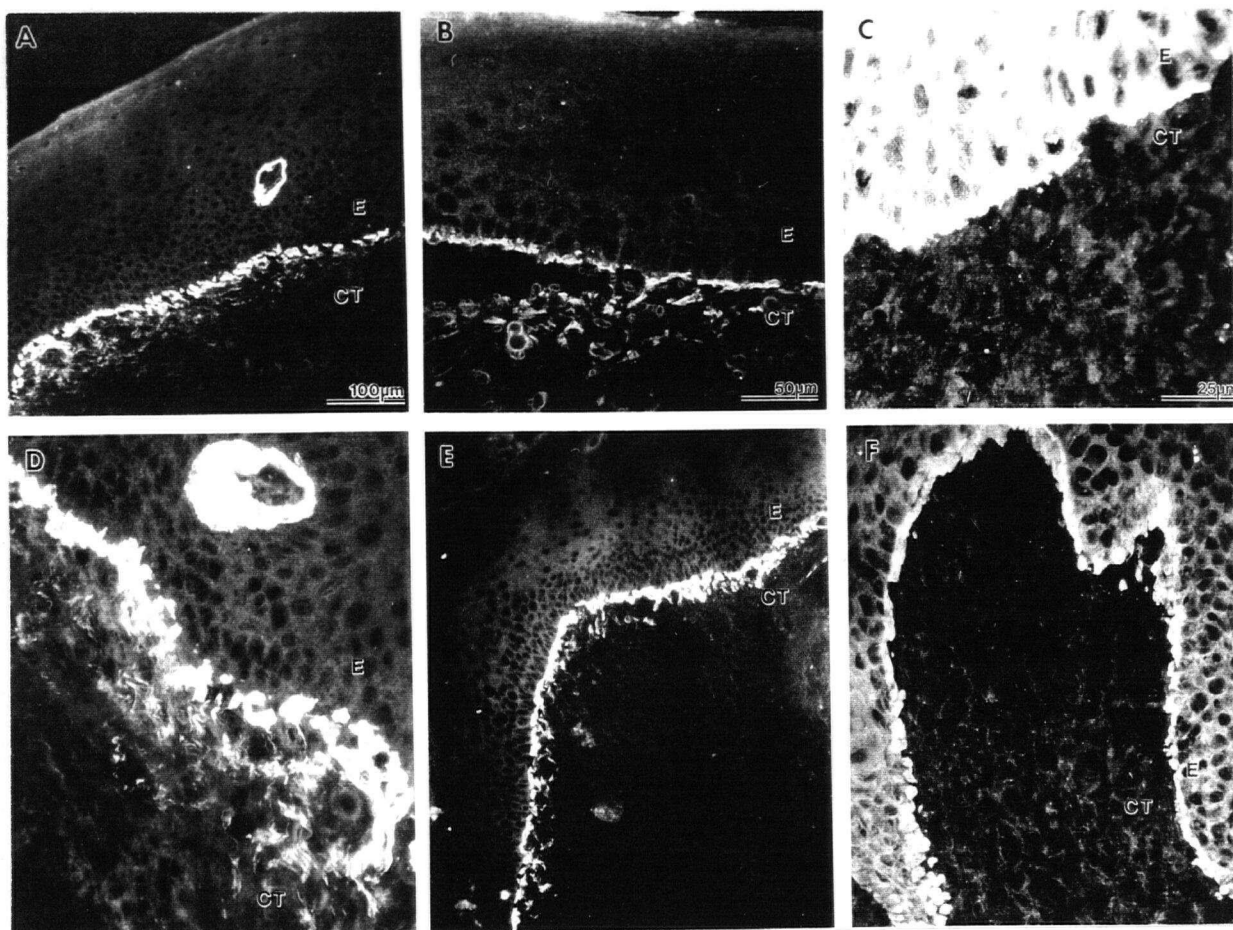


Figure 11 - Immunolocalization of Type VII Collagen. **A)** Normal staining at basement membrane zone in healthy gingiva. **B)** Staining extending into connective tissue in Kindler patient. **C)** Staining as seen in severe periodontal inflammation of an adult periodontitis patient. **D)** Most normal appearing staining pattern found in Kindler patient. **E)** Biopsy tissue of noninflamed buccal oral mucosa in Kindler patient.

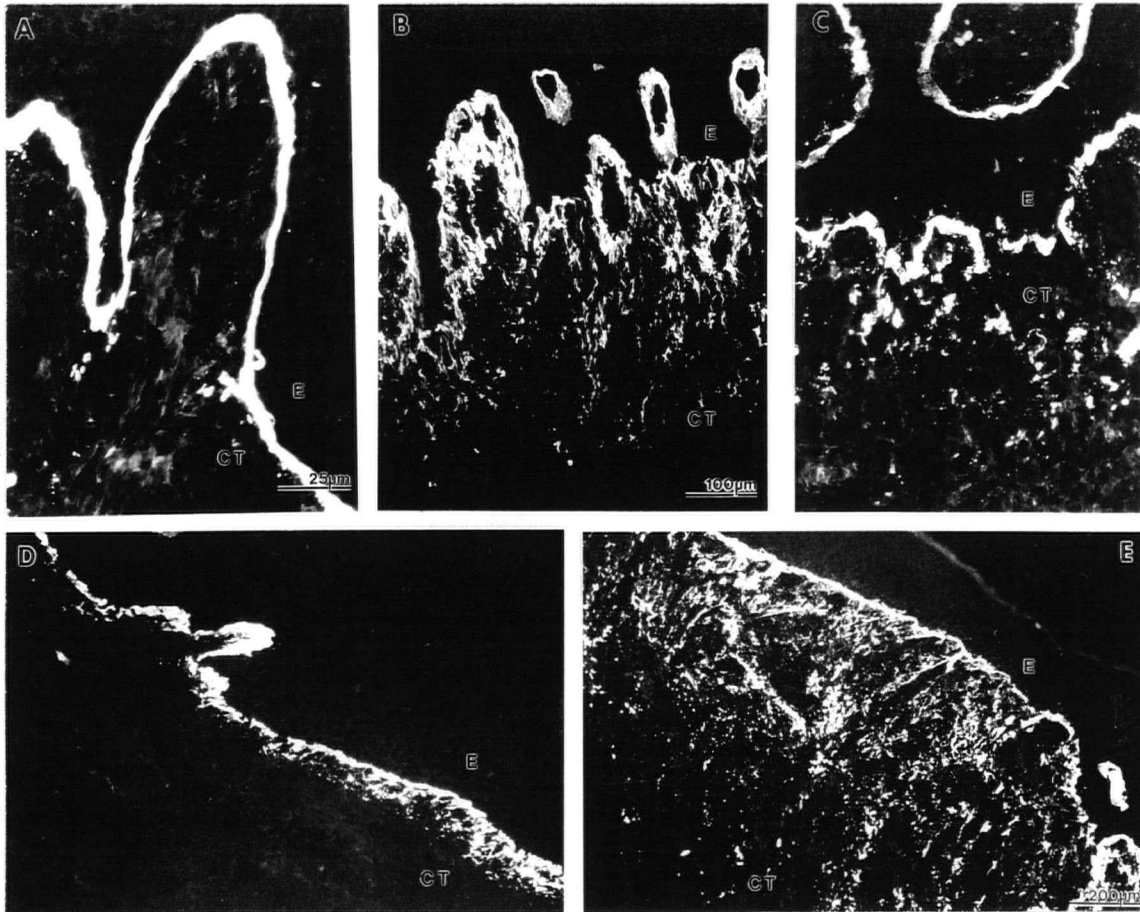
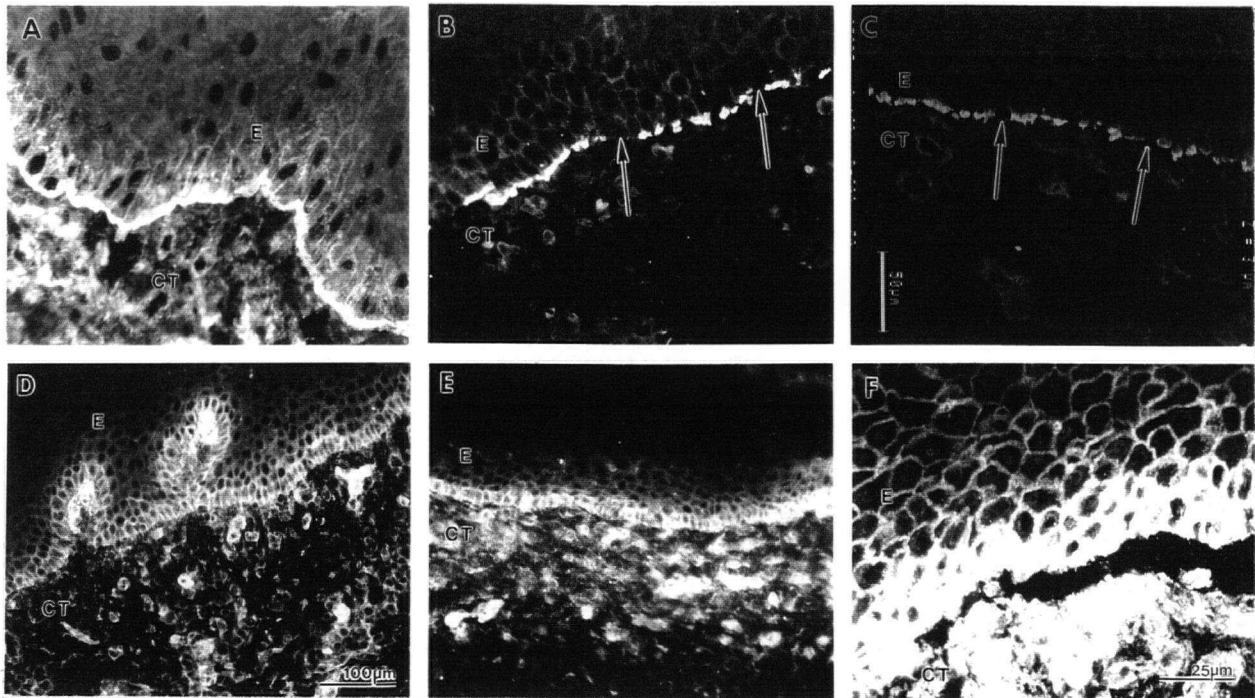


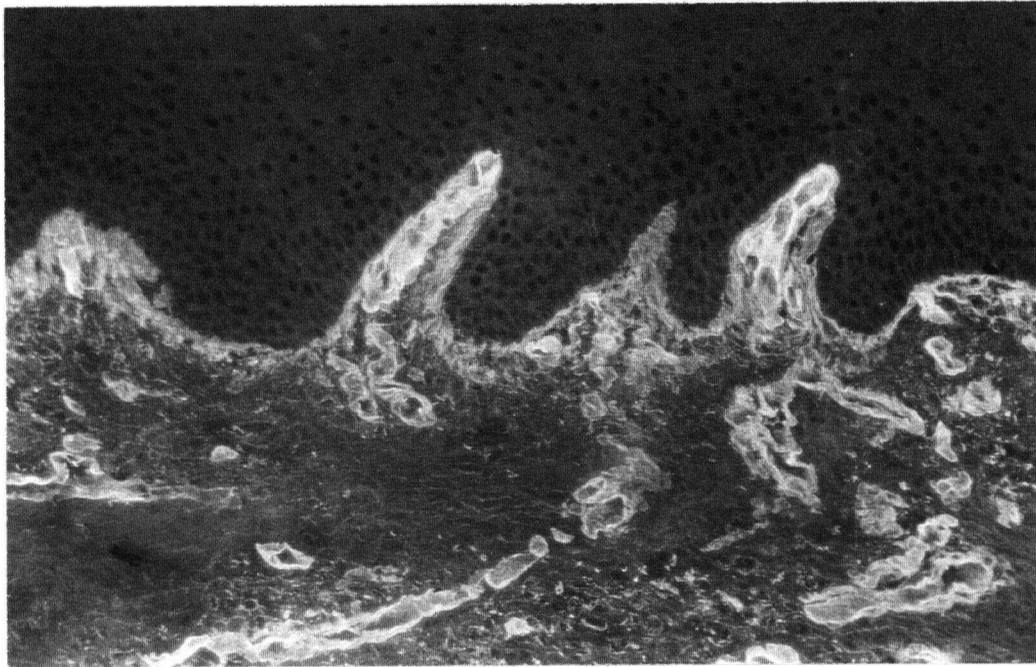
Figure 12 - Immunolocalization of $\beta 1$ and $\beta 4$ Integrins. **A-C)** $\beta 4$ integrin staining at basement membrane (BM). **A)** Normal staining in adult healthy gingiva. **B)** Kindler patient demonstrating apparent discontinuities (arrows) in the BM. **C)** Confocal laser microscope image also demonstrates discontinuities (arrows) in the BM. **D-F)** $\beta 1$ integrin staining. **D)** Inflamed gingiva in Kindler patient. **E)** Noninflamed buccal mucosa from Kindler patient. **F)** Blister in Kindler patient shows $\beta 1$ in several cell layers.



Basement Membrane Components in Inflamed Gingiva

Type IV collagen is a product of epithelial and endothelial cells and is a constituent of the lamina densa of the basement membrane (Uitto and Larjava, 1991). The localization of type IV collagen was similar to control tissues (not shown). Normal staining was observed in a linear pattern along the basement membrane zone under the basal epithelial cells and around the blood vessels (Fig. 13). Numerous blood vessels were present in the inflamed tissue.

Figure 13 - Immunofluorescence Staining of Type IV Collagen in Inflamed Kindler Gingival Tissue.

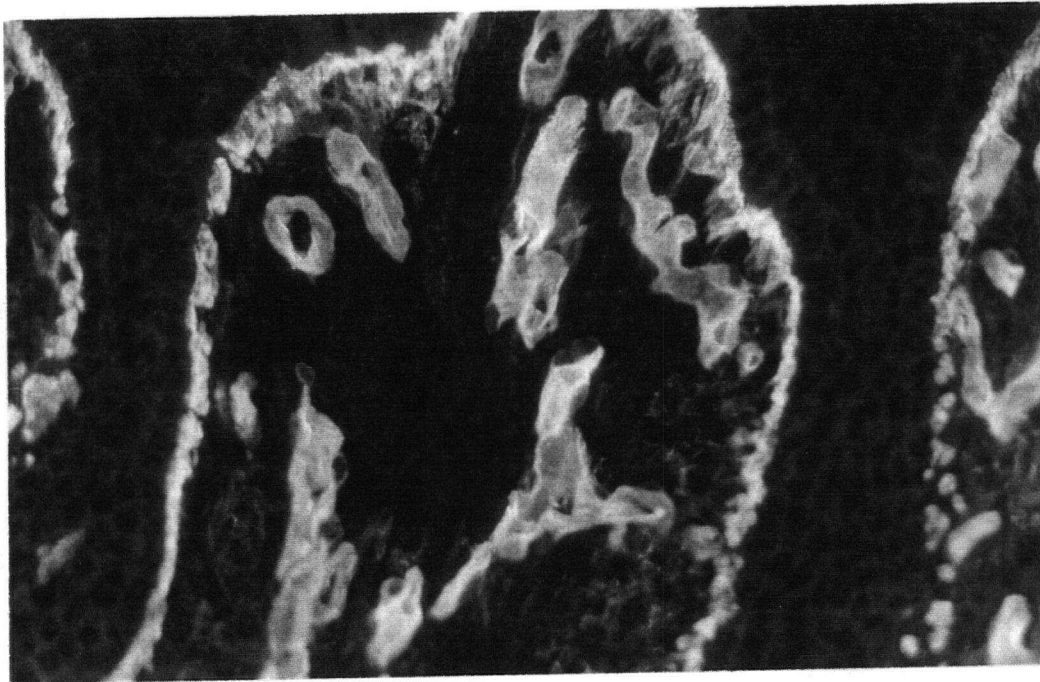


Type VII collagen is the major component of the anchoring fibrils that are thought to connect hemidesmosomes to the connective tissue. The normal staining is a linear distribution along the basement membrane zone as was seen in the healthy control tissue (Fig 11A). In the inflamed Kindler gingiva type VII collagen did stain along the basement membrane but in addition, large amounts of type VII collagen was seen streaking into in the connective tissue stroma (Fig. 11B). No epithelial cells were seen invading the underlying stroma that would explain this finding. Confocal laser microscopy was used to determine that the streaks staining into the connective tissue was actually continuous with the staining at the basement membrane zone (not shown). Periodontal pocket control tissue (Fig. 11C) showed discontinuous staining of type VII collagen and some staining in the connective tissue but the appearance was considerably different from that observed in all Kindler tissues.

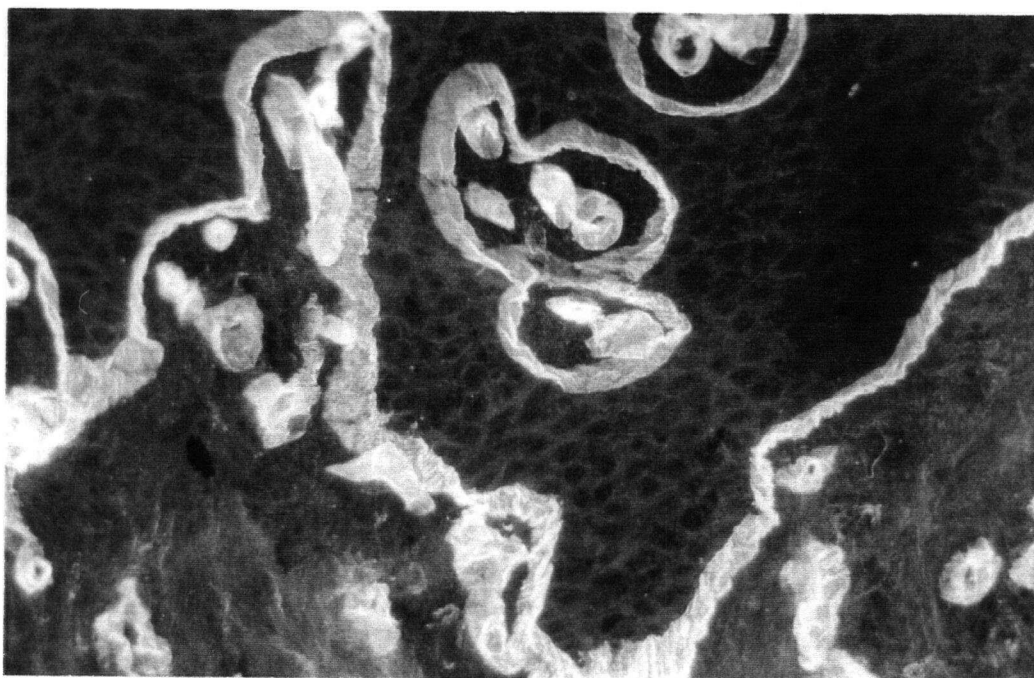
Laminin-1 is a component of the lamina densa along with type IV collagen. Similar to type IV collagen, laminin-1 was located in the epithelial and vascular basement membranes except that the staining was not a continuous line but rather had irregular, nonstaining breaks between the positively staining globular structures (Fig. 14A). Linear continuous staining along epithelial and vascular basement membranes was observed in the healthy (Fig. 14B) controls and the periodontal pocket controls.

Figure 14 - Immunofluorescence Staining of Laminin-1 in the Basement Membrane Zone.

A) Inflamed Kindler syndrome gingiva.



B) Healthy control gingiva.



Laminin-5 is a component of the anchoring filaments of the lamina lucida that normally stains linearly along the basement membrane zone. In the inflamed Kindler gingiva laminin-5 was mainly limited to the basement membrane zone but was not a continuous line as was seen in periodontal pocket tissue from an otherwise healthy individual (Fig 10A). There were fairly regularly-distributed breaks in the staining that gave the appearance of globular structures with spaces between them similar, but not identical because of the spacing, to that observed in the laminin-1 staining. In addition, laminin-5 reaches out a short distance into the connective tissue in both the inflamed and noninflamed Kindler tissues (Figs. 10D and E).

$\beta 1$ Integrins normally localize in cell-cell contact areas between basal keratinocytes and are thought to mediate cell-cell adhesion (Larjava *et al.*, 1992). $\beta 1$ integrin stained around the periphery of basal epithelial cells but with greater intensity between neighboring cells and occasionally in the adjacent connective tissue separate from the basement membrane zone (Fig. 12D-F). This same staining pattern was seen in healthy tissue and tissue from periodontal pockets (not shown).

Integrin $\alpha 6\beta 4$ is a component of the hemidesmosome that attaches basal keratinocytes to the basement membrane, possibly to laminin-5 and/or collagen type VII. $\beta 4$ integrin stained along the basement membrane zone but demonstrated focal losses leaving gaps that did not stain (Fig 12B). This appearance was similar under confocal laser microscopy, demonstrating that no staining was present in these gaps at different levels of focusing through the tissue section (Fig. 12C). This pattern was similar to type IV collagen staining except the gaps were larger and the stained areas did not have a globular appearance but were more linear in shape. In healthy controls the staining was continuous and also extended part way up the sides of the basal keratinocytes rather than just on the basement membrane surface (Fig 12A). Some focal loss of $\beta 4$ integrin staining was seen in

periodontal pocket controls but the gaps were much smaller than those seen in the inflamed Kindler gingiva.

Bullous pemphigoid antigens 1 (BPA 230) and 2 (BPA 180) are components of the hemidesmosome. BPA 2 stained as a continuous line along the basement membrane zone (Fig 10F). BPA 1 was similar except again there were breaks in the continuity similar to the $\beta 4$ integrin stain.

Basement Membrane Components in Oral Mucosa

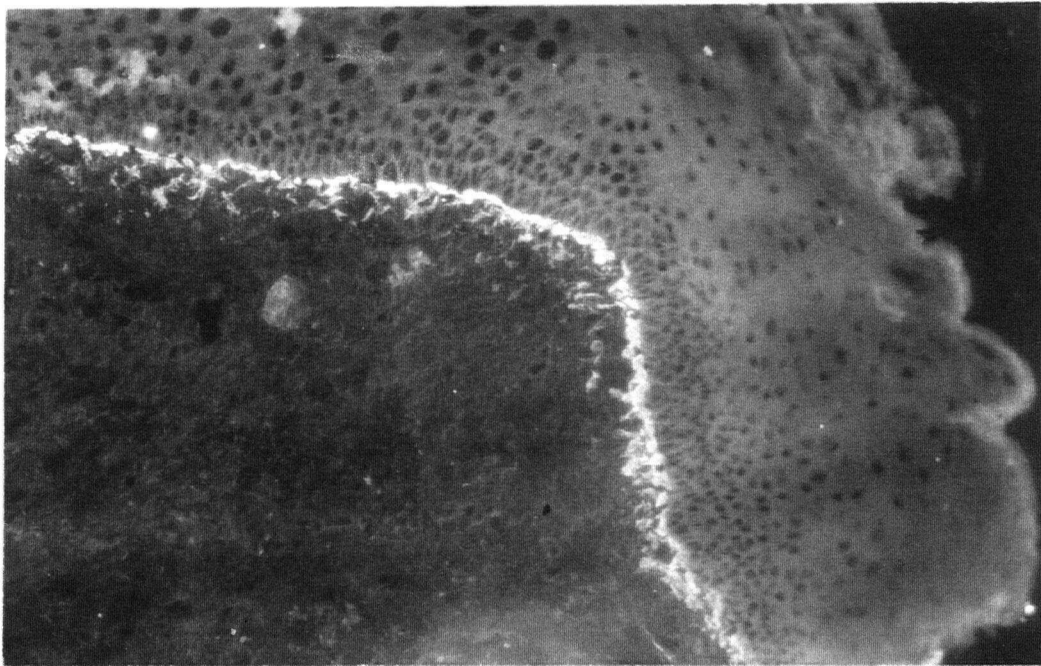
Type IV collagen presented a normal staining pattern along the basement membrane and circumferentially around blood vessels in the connective tissue (not shown).

In many areas the type VII collagen appeared similar to that found in the inflamed gingiva; extending deep into the connective tissue. Some areas did not streak far into the connective tissue but the staining was still a much wider band than in normal healthy tissue (Fig 11D).

Laminin-1 staining was similar in the noninflamed mucosa as described above for the inflamed gingiva.

Laminin-5 staining in the noninflamed mucosa was limited to the basement membrane zone and was discontinuous but in a different manner than was reported for the inflamed gingiva (Fig. 15). The globular appearance observed in the inflamed tissue (Fig 10D) was not seen but rather a line that contained irregular breaks giving the appearance that this part of the basement membrane had actually been cut out and removed.

Figure 15 - Irregular Breaks in Staining of Laminin-5 in the Basement Membrane Zone of Noninflamed Kindler Syndrome Oral Mucosa.

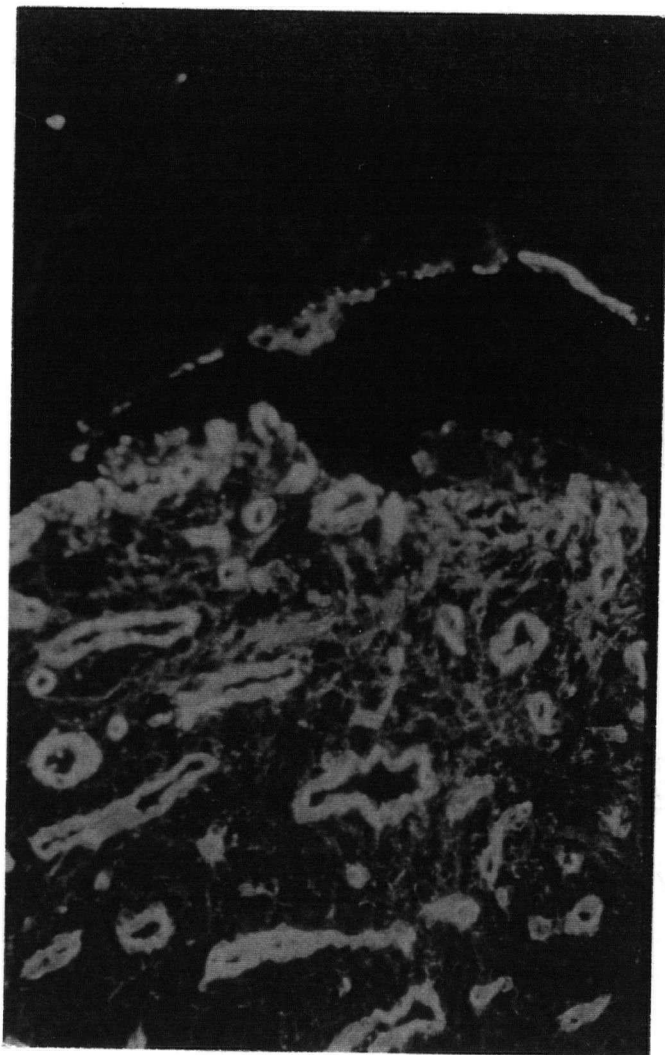


Integrin $\beta 1$ was present in the basal epithelial layer and stained similarly to that reported for the inflamed Kindler mucosa, healthy tissue and tissue from periodontal pockets (Fig. 12E).

Basement Membrane Components in the Blister

Type IV collagen presented a normal linear staining pattern along the basement membrane zone and was usually located along the floor of the blister. Surprisingly though, there were rare areas of weaker staining where the type IV collagen stained on the roof of the blister (Fig. 16). Based on stainings of other protein components suggesting a split at the level of the lamina lucida, it would be expected that type IV collagen would stain on the floor of the blister as the lamina densa is internal to the location of the lamina lucida.

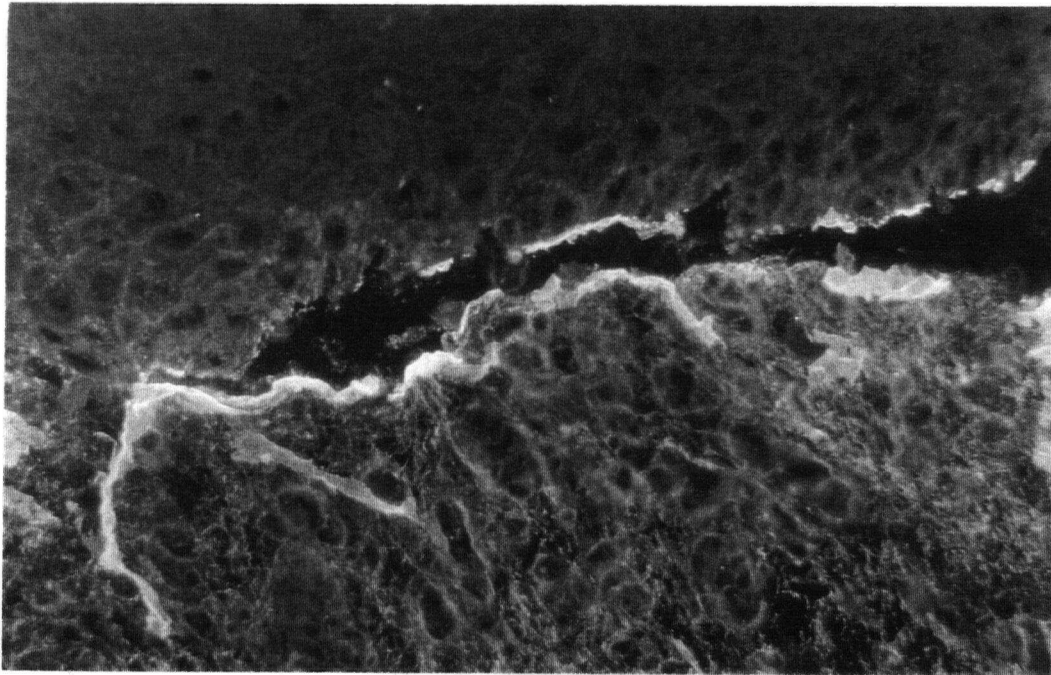
Figure 16 - Type IV Collagen Staining in Blister.



Type VII collagen stained deep into the connective tissue as described in the inflamed gingiva section although the basement membrane zone staining was found only on the floor of the blister (not shown).

Laminin-5 was usually fairly continuous in the blister areas but was located either on the floor or the roof in the split (Fig 10B). In some areas the staining started on the roof but then ended and started again on the floor all within the same blister (Fig 17).

Figure 17 - Laminin-5 Staining on Both the Floor and Roof of a Blister in Inflamed Kindler Syndrome Gingiva.



Integrin $\beta 1$ was present in the blistering areas and stained the periphery of basal epithelial cells. It was present only on the roof of the blister and extended suprabasally for up to eight layers (Fig 12F).

$\beta 4$ integrin staining surrounded the basal keratinocytes and was localized to the blister roof. Occasional gaps in the staining were noted (not shown).

A blister area was not available to stain for laminin-1 and bullous pemphigoid antigens 1 and 2.

Table 6 - Summary of Immunofluorescence Staining in Kindler Syndrome.

	<u>Molecule</u>	<u>Location</u>	<u>Comment</u>
1) Inflamed gingiva			
	Collagen IV	Lamina densa	Normal location and pattern
	Collagen VII	Anchoring fibrils	Streaking deep into the lamina propria
	Laminin-1	Lamina densa	Normal location but discontinuous
	Laminin-5	Lamina lucida	Normal location but discontinuous
	BPA 1	Hemidesmosome	Normal location but discontinuous
	BPA 2	Hemidesmosome	Normal
	$\beta 1$ integrin	Basal keratinocytes	Normal location
	$\beta 4$ integrin	Hemidesmosome	Normal location but discontinuous
2) Noninflamed mucosa			
	Collagen IV	Lamina densa	Normal location and pattern
	Collagen VII	Anchoring fibrils	Streaking into the lamina propria or widened band on BMZ
	Laminin-1	Lamina densa	Normal location but discontinuous
	Laminin-5	Lamina lucida	Normal location but discontinuous
	BPA 1	Hemidesmosome	Not tested
	BPA 2	Hemidesmosome	Not tested
	$\beta 1$ integrin	Basal keratinocytes	Normal location
	$\beta 4$ integrin	Hemidesmosome	Normal location but discontinuous
3) Blister			
	Collagen IV	Lamina densa	Mainly floor, occasionally roof
	Collagen VII	Anchoring fibrils	Floor and deep into lamina propria
	Laminin-1	Lamina densa	Not tested
	Laminin-5	Lamina lucida	Roof and floor of blister
	BPA 1	Hemidesmosome	Not tested
	BPA 2	Hemidesmosome	Not tested
	$\beta 1$ integrin	Basal keratinocyte	Roof, up to 8 layers suprabasally
	$\beta 4$ integrin	Hemidesmosome	Roof

Integrin $\alpha v \beta 6$ was not routinely observed in either healthy or inflamed biopsy tissue.

There were small patches of positive staining for integrin $\alpha v \beta 6$ in the inflamed Kindler gingiva that extended for a distance of about seven basal epithelial cells and suprabasally for three to four layers (Fig. 18). This was a fairly rare finding. Integrins of the αv -family are rarely expressed in normal epithelial but rather are present in wound keratinocytes.

Integrin $\alpha 3 \beta 1$ was present in visually normal amounts and location in the area of the basal epithelial cells but also extended into one or two cell layers above the basal layer (Fig. 19). This is not unusual in inflamed tissue.

Figure 18 - Small Patches of Positive Staining for Integrin $\alpha v \beta 6$ in the Inflamed Kindler Gingiva.

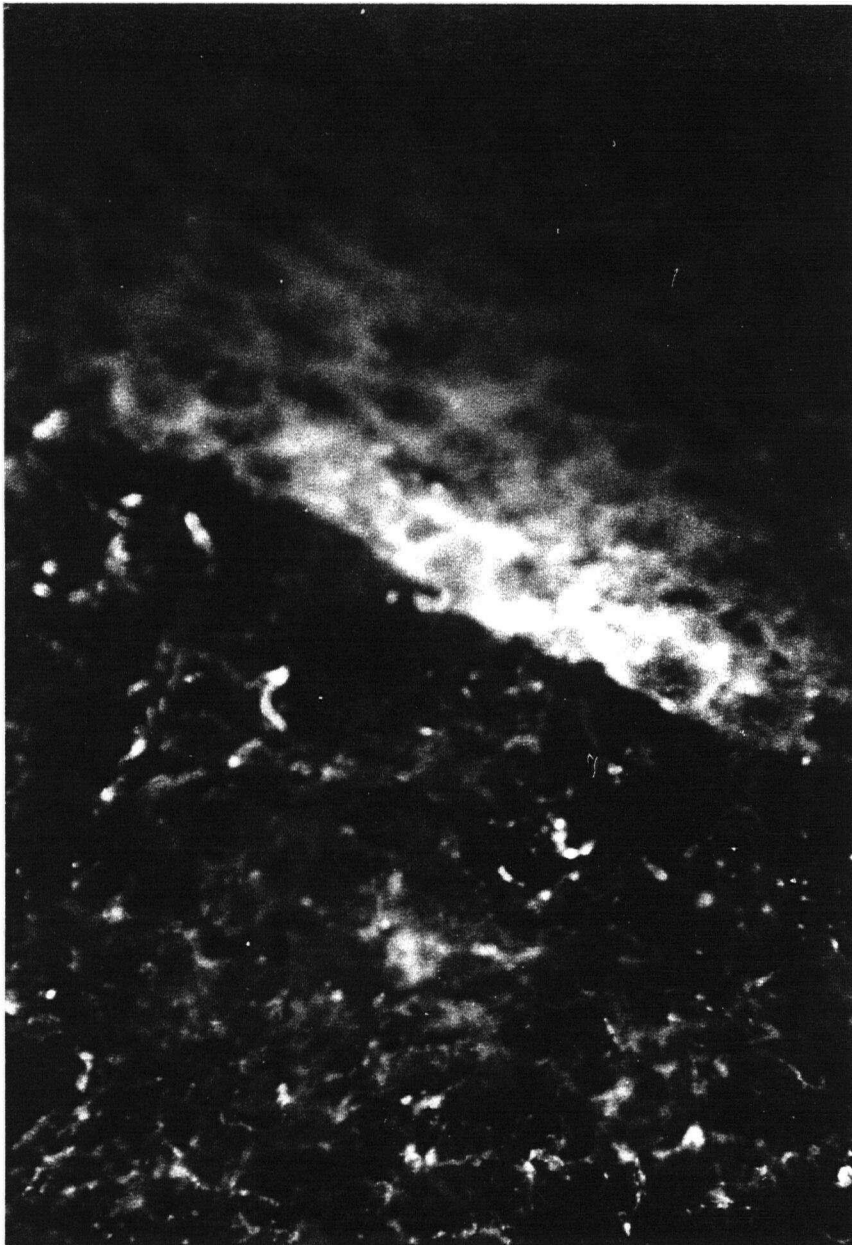
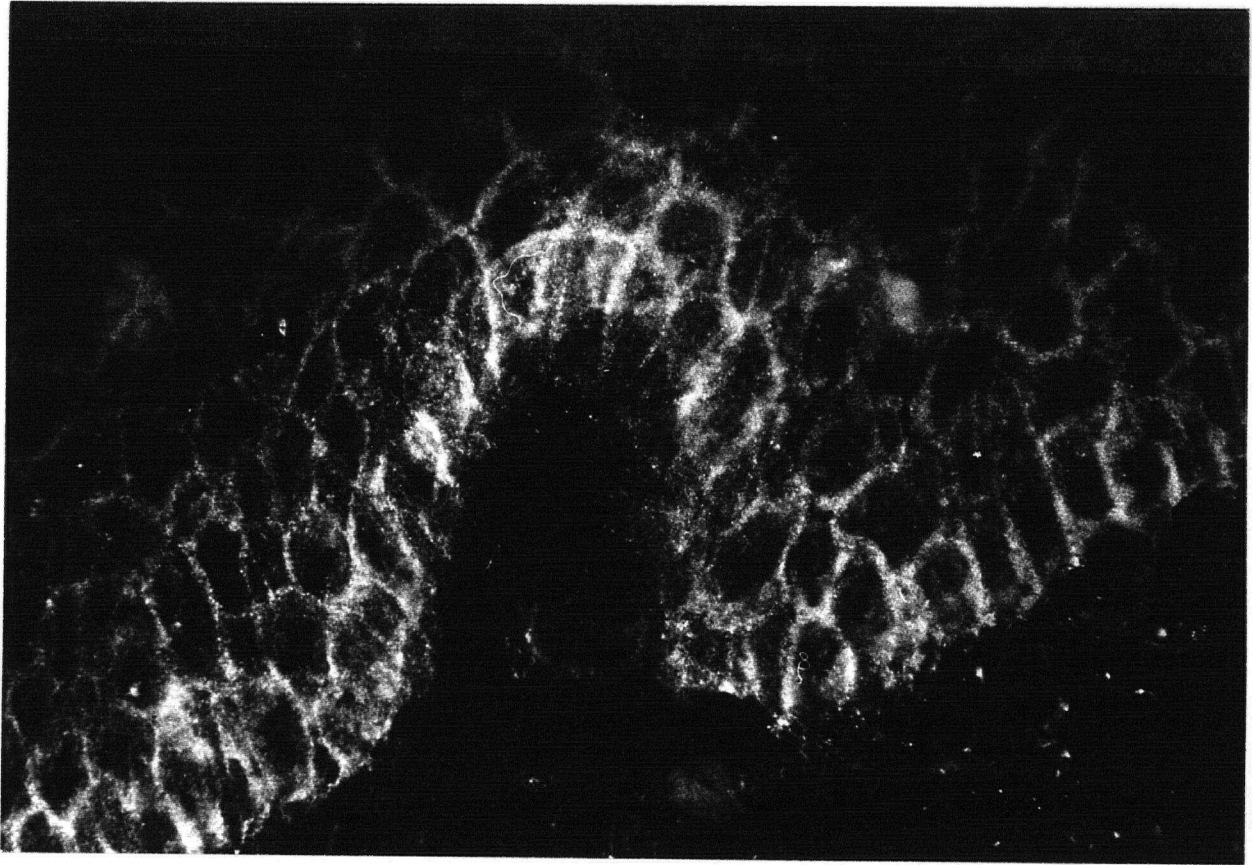


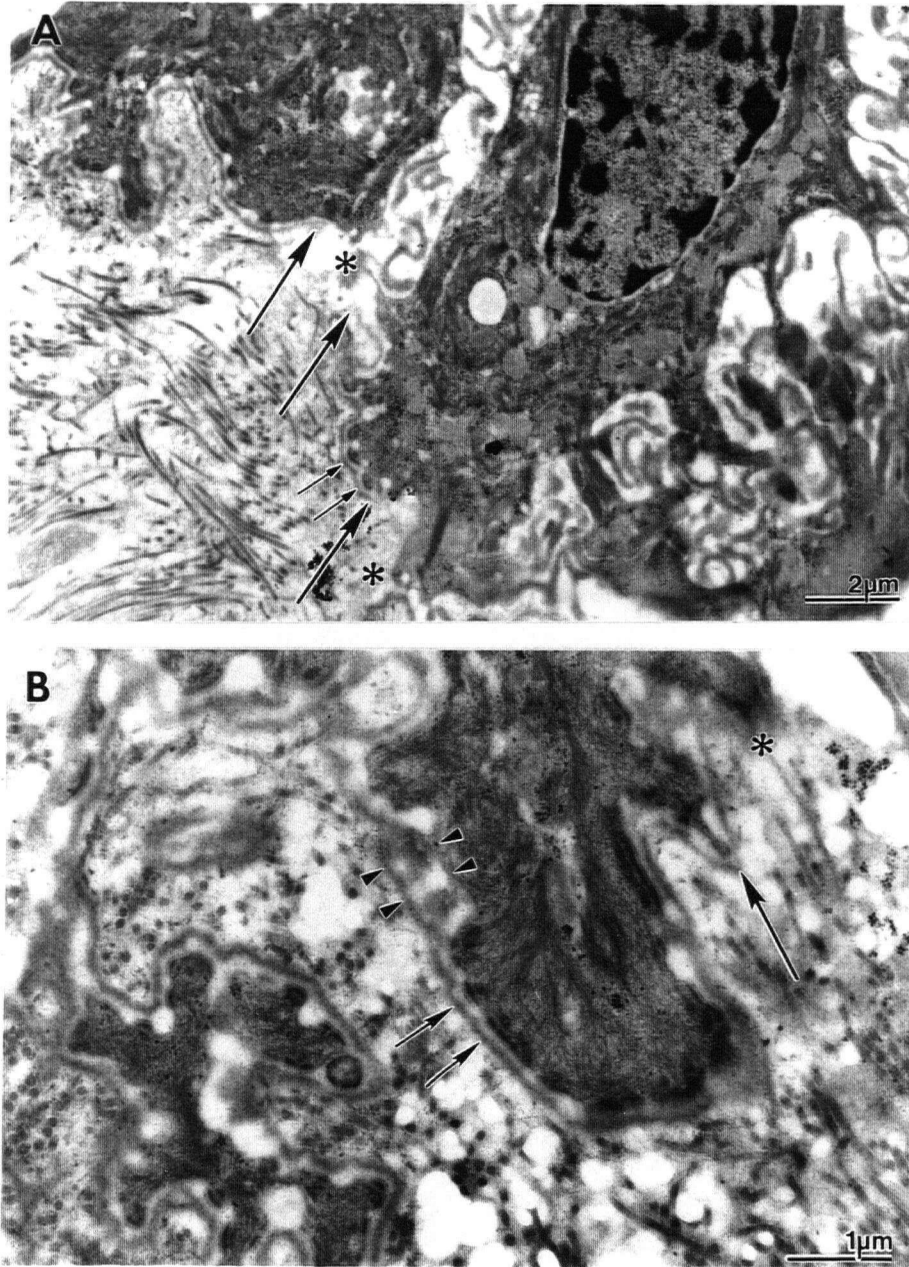
Figure 19 - Integrin $\alpha 3 \beta 1$ Immunofluorescence Staining of the Basal Epithelial Cells and Extending Suprabasally in Inflamed Kindler Gingiva.



Electron Microscopy

In the normal basement membrane zone under electron microscopy basal epithelial cells can be visualized in proximity to lamina densa and lamina lucida and hemidesmosomes are present. Areas of a normal appearance of the basement membrane zone with hemidesmosomes present are common in the Kindler noninflamed mucosa biopsy tissue. At various places though, the basement membrane appears to end abruptly. Other areas demonstrate the basement membrane separating from the basal epithelial cells and a lack of hemidesmosomal attachment (Fig. 20).

Figure 20 - Electron Micrographs Showing Irregularities in Basement Membrane (BM). **A)** Small arrows - normal hemidesmosomes, large arrows - BM appears to end, star - discontinuities in BM. **B)** Small arrows - hemidesmosomes, arrowheads - BM separates from basal epithelial cell and no hemidesmosomes present, large arrows - BM loses its organization, star - BM appears to be missing.



Epithelial cells and polymorphonuclear cells per milliliter of saliva were calculated and compared with counts for 30 orally healthy patients and 50 patients with periodontal disease. The Kindler syndrome patient had three- and five-times the epithelial and PMN cell counts in saliva respectively of the orally healthy patients and two- and three-times the counts of the periodontal disease patients. The standard deviations of these cell counts were extremely high (approximately 100% of average values) and the oral rinse volume was constant at three milliliters rather than adjusted according to the size of the mouth.

Chapter 6 - Discussion

Clinical Findings

As little is known about the dental findings or the oral treatment of Kindler syndrome it is interesting to consider another rare syndrome with some features in common. Papillon-LeFèvre syndrome is an autosomal-recessive trait characterized by diffuse palmar plantar keratosis and premature loss of both the deciduous and permanent dentitions (Papillon and LeFèvre, 1924). Approximately 200 cases have been reported with an incidence of one in four million (Gorlin *et al.*, 1964). The etiology is unclear presently and numerous contributing factors have been put forth including a defect in vitamin A metabolism (Gorlin *et al.*, 1964), cementum acting as an irritant (Smith and Rosenzweig, 1967), an imbalance of collagenolytic activity (Shoshan *et al.*, 1970), a lymphocyte immune defect (Haneke *et al.*, 1975), impaired PMN chemotaxis and phagocytosis function (van Dyke *et al.*, 1984) and bacterial involvement (Clerehugh *et al.*, 1996). Historically, Papillon-LeFèvre syndrome was thought to lead to the inevitable loss of both the deciduous and permanent dentitions but more recently cases have been published showing good response to radical dental treatment (French *et al.*, 1995, Tinanoff *et al.*, 1995, Kressin *et al.*, 1995). These cases involved extraction of the deciduous teeth at age three years and a period of edulism prior to eruption of the permanent dentition, antibiotic coverage during eruption of the permanent teeth and in some cases systemic treatment with retinoid therapy.

This Kindler syndrome patient presented with early onset, localized periodontal disease, severe gingival bleeding and gingival fragility. The gingival tissues responded well to nonsurgical scaling and root planing. The palatal gingiva appeared to heal normally following a gingivectomy that spanned from right maxillary cuspid to left maxillary cuspid. Healing also appeared clinically normal following extraction of the maxillary right central

incisor although soon after the extraction the facial gingiva collapsed in, creating a problem for esthetic prosthodontic reconstruction. Orthodontic treatment did not appear to create any unusual complications in this patient except for the recurrent leukedema type appearance along the buccal mucosa and at the angle of the lip. Because of the greatly reduced bone support around the maxillary incisors the teeth were derotated slowly with a soft orthodontic wire. The design of the cast partial lower denture aimed at keeping the major connector as far coronal from the gingival margin as possible to minimize plaque accumulation and irritation in this area. In the previous lower denture the tooth surfaces with the most bleeding after treatment were those that contacted a denture clasp.

Supportive periodontal therapy has been carried out every three months for over two years. The probing depths, bleeding on probing and tooth mobility are considerably improved over pretreatment levels (Table 1) but the patient does go through cycles of gingival inflammation and pain even with good home oral hygiene. While gingival bleeding is not a particularly reliable indicator of periodontal disease activity (Haffajee *et al.*, 1987) it is still one of the best clinical indicators presently available. Both Lang *et al.* (1986) and Badersten *et al.* (1990) have found that the maximal positive predictive value of bleeding scores is approximately 30% which is not particularly sensitive but that the lack of bleeding on probing has a fairly high specificity in indicating periodontal stability. Unfortunately these studies, carried out in individuals with adult periodontitis but who are otherwise healthy, are not necessarily applicable to patients with structural defects in the periodontium. We speculate that congenitally fragile gingival tissue may give positive bleeding signs even without underlying inflammatory changes.

Little can be determined from the epithelial and PMN cell counts other than PMN cells are present in the saliva of the Kindler syndrome patient and that defects in PMN are not likely the cause of the advanced periodontal disease observed in this patient.

Histology

No histological examinations of the oral mucosa in a Kindler syndrome patient have been reported. Hovnanian *et al.* (1989) report on their light microscopy findings on skin and review those of others in cases where Kindler syndrome was diagnosed. The features that were noted were all common to a poikilodermic state and include epidermal atrophy, vacuolization of the basal layer, vascular ectasia, lymphohistiocytic infiltrate including melanophages in the upper dermis, cystoid bodies in the basal layer and papillary dermis, dissociation of the basement membrane and destruction of connective tissue in the dermis. The level of cleft formation is not clear from light microscopy. This is consistent with the present findings.

Histopathology

In normal oral mucosa, type VII collagen was localized using immunofluorescence and was found to be restricted to a linear distribution along the basement membrane zone. This is the expected result as type VII collagen is the protein that forms the major component of the anchoring fibrils (Keene *et al.*, 1987). In the Kindler syndrome inflamed gingival biopsy tissue, the localization of type VII collagen was vastly different in that it was not only localized to the basement membrane zone but also streaked deep into the connective tissue of the lamina propria. In some sections the staining was found throughout the height of the connective tissue. Confocal laser microscopy imaging was used to confirm that the streaking is continuous from the area of the basement membrane into the connective tissue rather than separate from the type VII collagen in the basement membrane zone. As a control, to determine this staining pattern was not simply a result of inflammation, staining of type VII collagen in chronic adult periodontitis gingival tissue was also carried out. An unusual presentation of type VII collagen where the staining was also present at a distance from the basement membrane was found but to a much lesser degree than in the Kindler

gingiva. There were areas in the inflamed Kindler gingiva that displayed a less streaked presentation of type VII collagen staining but it was still not the presentation following the basement membrane zone expected in normal, clinically healthy tissue. Noninflamed Kindler oral mucosa also displayed abnormal streaking of type VII collagen into the connective tissue in a similar manner to the inflamed Kindler gingiva.

Type VII collagen was found in abnormal locations deep in the connective tissue in both inflamed blister areas and in nonblister, noninflamed tissue in a similar fashion to that reported by Haapalainen *et al.* (1995) in dermal lichen planus and to a lesser degree in occasional cases of chronic inflammatory periodontal disease. Type VII collagen, the major component of anchoring fibrils, was found in large amounts penetrating deep into the connective tissue unlike in normal oral mucosa where it was found only along the basement membrane zone. Mauviel *et al.*, (1994) examined the effects of various cytokines on the expression of type I and VII collagen genes in human dermal fibroblasts in culture. Pro-inflammatory cytokines interleukin-1 α (IL-1 α), interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), and leukoregulin (LR) all elevated type VII collagen mRNA levels 5-9 fold and had additive effects with transforming growth factor- β . A similar experiment by Konig and Brucker-Tuderman (1992a) also concluded that the synthesis of type VII collagen can be upregulated in both keratinocyte and fibroblast cultures by the cytokine transforming growth factor- β (TGF- β). Konig *et al.*, (1992b) found that TGF- β increases collagen type VII production by normal keratinocytes but not by keratinocytes from patients with mutilating dystrophic epidermolysis bullosa. They conclude that skin fragility and blistering is a result of insufficient type VII collagen. Coimbra *et al.*, (1991) have found evidence in a rabbit antglomerular basement membrane disease model that increased TGF- β secretion can explain the increased renal cortical collagen synthesis that results in severe fibrosis during immune-induced renal injury. They conclude that this model is consistent with TGF- β having a role in directing fibrinogenesis

in the kidney. Lianos *et al.*, (1994) attributed the increased TGF- β levels in rats with anti-glomerular basement membrane disease to originate from macrophages that infiltrated into the glomeruli. Similarly, patients with systemic sclerosis, a disease with tissue fibrosis, has been shown to have a concomitant increase in the expression of type VII collagen and elevated expression of TGF- β 1 and TGF- β 2 (Rudnicka *et al.*, 1994). Of interest is that the excess type VII collagen was present in the systemic sclerosis patient's dermis. The authors state that the aberrant type VII collagen expression may be related to the TGF- β present in the same location and contribute to the tightly bound appearance of the skin. It is possible that the increase in type VII collagen extending into the mucosal lamina propria in the Kindler syndrome biopsy may be due to an attempt at wound healing by the body. This increase of type VII collagen may be directed in part by TGF- β that is at increased levels due to the inflammatory cells (macrophages) present in the periodontal tissues. This would also explain why no mutations were found in the sequencing of the type VII collagen.

High expression of type VII collagen in noninflamed mucosa is more difficult to explain. Epithelial tissue is a plentiful source of various cytokines including interleukins, tumor necrosis factors, chemokines, colony-stimulating factor, interferons and growth factors (Feliciani *et al.*, 1996). It is possible therefore, that the elevated expression of type VII collagen is due to an alteration of the epithelial cells which release cytokines (TGF- β for example) that are able to upregulate their own and/or fibroblast type VII collagen production. If this is the case then altered distribution of type VII collagen is a consequence rather than a cause for alterations at the basement membrane zone.

Discontinuities of the basement membrane zone were seen in immunostaining of the adhesion molecules. This was especially apparent in α 6 β 4 integrin staining where discontinuities were observed throughout the basement membrane zone area and also in

laminin-5. Considerably more of the length of the basement membrane zone stained positive than there were areas that were negative to these immunofluorescence stains. Confocal laser microscope imaging was used to demonstrate the discontinuities in the basement membrane with numerous stains. Focusing through the basement membrane areas that did not stain with immunofluorescence confirmed the presence of gaps and suggests this is not due to artifact.

The distribution of basement membrane components on the floor and roof of the trauma induced blisters was not entirely consistent but overall appears to suggest that the split is at the level of the lamina lucida. Laminin-5, a component of the lamina lucida, was found both on the floor and roof of the blister whereas $\beta 4$ integrin, a component of the hemidesmosome was always limited to the roof of the blister and collagen type VII, the major component of the anchoring fibrils was always limited to the floor of the blister. Type IV collagen, a component of the lamina densa that is located deep to the lamina lucida was usually located on the floor of the blister but there were exceptions when it was found on the roof. Integrin $\beta 1$, found on basal keratinocytes, was only found on the roof of the blister. This suggests that the blistering in Kindler syndrome is junctional but that splits may occur in the lamina densa in addition to the lamina lucida.

Of the major known proteins of the basement membrane zone, all were found to be present. From the clinical presentation of the syndrome it is apparent that a mutation exists that affects this region. It is possible that at least one of the basement membrane proteins that is present is defective or not fully functional. Another possibility is that there are other proteins present that were not examined (for example, laminins-6 and -7) or there are other, yet unknown components that are missing or nonfunctional. The mutation may not relate directly to the protein structure but rather through a secondary messenger such as a missing or improper response to a cytokine.

Integrin $\alpha v \beta 6$ was not routinely observed in either healthy or inflamed biopsy tissue but was present in rare, small patches in the inflamed Kindler gingival tissue (Fig. 13). Integrins of the αv -family are rarely expressed in normal epithelial but rather are present in wound keratinocytes Larjava *et al.*, 1993, Haapasalmi *et al.*, 1996). Integrin $\alpha v \beta 6$ appears to be present in the unusual epithelium of leukoplakias (about 25% of cases), lichen planus (about 90% of cases) and squamous cell carcinoma about 90% of cases) (Hamidi *et al.*, unpublished). Patients with certain forms of epidermolysis bullosa appear to be more susceptible to developing squamous cell carcinoma (McGrath *et al.*, 1992 and Newman *et al.*, 1992). The expression of integrin $\alpha v \beta 6$ in Kindler syndrome may be associated with previous blistering. Alternatively, its presence may be associated with a premalignant epithelial phenotype such as leukoplakia or lichen planus.

Mice deficient in $\alpha 3$ integrin develop microscopic blisters at the epidermal-dermal interface (Hodivala-Dilke *et al.*, 1996). Therefore, we investigated whether the distribution of $\alpha 3$ integrin is normal in oral epithelium in Kindler syndrome. Integrin $\alpha 3 \beta 1$ was present in visually normal amounts and location in the area of the basal epithelial cells but also extended into one or two cell layers above the basal layer (Fig. 19). This is common in inflamed periodontal tissue (Haapasalmi *et al.*, 1995).

It was noted that while most areas of the basement membrane zone examined under electron microscopy appeared normal with the basal lamina in close proximity to basal epithelial cells and hemidesmosomes present, there were areas where the basal lamina appeared to be separated from the basal epithelial cells or altogether missing. It is possible that these areas have been damaged by blistering or trauma or that these are areas that are susceptible to developing blisters.

Age Affect on Blistering.

It is interesting to consider why blistering has decreased as the patient has reached her teenage years. Decreased blistering at puberty, especially in females, has also been reported in EBS-K and EBS-DM (Pearson, 1988). Blistering may begin along the small discontinuities of the basement membrane observed in the immunofluorescence stainings. The decrease in blistering with age may be related to compensation on the part of the connective tissue. In part, type VII collagen increases and spread into the dermis may strengthen the dermo-epidermal junction and decrease blistering. It would be of interest to examine the skin of a younger Kindler patient who is at the active blistering stage and determine if this streaming of type VII collagen into the dermis is present.

Dr. M.P. Marinkovich, Director of the Blistering Disease Clinic at the Stanford University School of Medicine has suggested that as people with epidermolysis bullosa come of age they tend to be more careful with their skin and oral mucosa but that this alone does not explain the decreased incidence of blistering (personal communication).

It is conceivable that blistering decreases not as a result of increased strength of the dermoepidermal junction with age but rather due to a decreased immune response and therefore less leukocytes infiltrating into an area. There has not been an increased rate of infections reported in epidermolysis bullosa simplex or Kindler syndrome patients in their teenage years though so this theory seems unlikely.

A more likely theory relates to the rate of tissue remodelling and epithelial turnover. Through birth to puberty the individual is growing rapidly. It is possible a high epithelial cell turnover associated with rapid growth makes the epidermis more vulnerable to blistering and that this blistering would decrease as growth slows down. The gingiva has an epithelial cell turnover rate of 10-12 days whereas the junctional epithelium turns over

every four-to-six days (Skougaard, 1970). It is possible that the junctional epithelium continues to be more affected than the gingiva where blistering also decreases with age. An actual mechanism (hormonal for example?) has not been determined.

Severity of Periodontal Disease

The gingiva is composed of two different types of stratified epithelia, the junctional and oral epithelia, and an underlying layer of dense connective tissue called the lamina propria that contains supra-alveolar fibers, blood and lymphatic vessels and nerves. The transcutaneous perforation of the tooth through the gingival soft tissues is a unique situation in the body, and the clinically normal gingiva is under constant microbial and antigenic challenge. Extrinsic and intrinsic defense systems exist to protect against gingival and periodontal infection. Extrinsic protection is from the mechanical resistance of the keratinized gingival epithelium supported by the supra-alveolar fiber apparatus and also from salivary gland immunoglobulin A (IgA) secretions that counteract bacterial colonization (Crawford *et al.*, 1975). Intrinsic protection operates within and beneath the dentogingival junction and the specialized junctional epithelium plays a key role.

The junctional epithelium of the free marginal gingiva forms the epithelial attachment of gingiva around the neck of each tooth. The specializations that allow the junctional epithelium to attach to the tooth also make it more susceptible both to mechanical trauma and bacterial invasion. The epithelial attachment consists of a basement membrane (internal basal lamina) and hemidesmosomes that are structural analogs of their counterparts that comprise the junction between epithelium and subadjacent connective tissue. The junctional epithelium consists of only two layers, basal and suprabasal, and does not keratinize. Basal cells are cuboidal and suprabasal cells are flattened, elongated and oriented parallel to the tooth surface. The basal cells interface with the connective tissue via hemidesmosomes. This attachment is continuously renewed throughout life

(Schroeder and Listgarten, 1977). The junctional epithelium is surrounded laterally by the gingival plexus, a thin vascular network consisting mainly of postcapillary venules, supplies nutrients and inflammatory cells to the junctional epithelium. The junctional epithelium has a low density of intracellular junctions (desmosomes and gap junctions) and contains many intracellular spaces (Hashimoto *et al.*, 1986). This allows for transmigration of cells that make up the internal protection against gingival and periodontal infection. Neutrophils from the gingival plexus actively migrate through the junctional epithelium in an apical to coronal direction into the sulcus following a chemotactic gradient from bacteria (Attström, 1975). These neutrophils phagocytose bacteria encountered along this intraepithelial pathway (Saito *et al.*, 1987) or kill bacteria through the complement (C3b) or Fc immunoglobulin G receptors they carry (Charon *et al.*, 1982). Junctional epithelial cells can also kill bacteria with enzymes contained in lysosomes (Lange *et al.*, 1972). Macrophages and T-lymphocytes also migrate out of the gingival plexus venules into the connective tissue and in adult chronic inflammatory lesions there is a predominance of B-lymphocytes and plasma cells (Page and Schroeder, 1976).

Intrinsic gingival defense systems can be interfered with at many levels; failure of neutrophils to resist bacterial colonization and to allow invasion of the tissue, failure of the neutrophil-assisted antibody killing of bacteria, or failure of the lymphocyte lines of defense. This can be as a result of functional defects or decreased cell numbers that is under genetic control resulting in decreased host resistance.

The Kindler patient did not have abnormal levels of common periodontal pathogens as determined by DNA probe and BANA test sampling which raises the question whether the weak link was then in the host immune cells that are responsible for killing bacteria or due to weakened structural defenses such as the dentogingival junction to allow severe periodontal disease to develop at a young age. The high level of neutrophils in oral rinses

and the differential cell count taken from the patient suggest that there is not a neutropenia problem. Other than dental abscesses, which cannot be considered outside the range of normal considering the local findings of advanced periodontal disease, the patient has not been subject to recurrent infections and this makes it less likely that the periodontal condition is due to defective PMNs. It is then logical to suspect that a defect exists in the structural defense at the periodontal interface. It is possible that a "normal oral flora" may be enough to produce a periodontal lesion whereas it would only produce an established gingivitis where an intact basement membrane zone exists.

It is possible that defects in basement membrane proteins contribute to our patient's periodontal condition. Forman *et al.* (1989) reported poor expression of bullous pemphigoid antigen, and type IV collagen along the dermoepidermal junction. This has not been confirmed by other studies and it is possible that these and other defects exist. Basement membrane proteins could either be absent or possibly damaged by proteolytic enzymes. Alexander and Damoulis (1994) suggest that collagenase-positive cells are localized at the interface between the epithelium and the connective tissue in gingival tissue and this type of process could also explain breakdown at the basement membrane level.

All cells of the junctional epithelium are similar ultrastructurally, and unlike gingival epithelium, contain only a few cytoplasmic filaments. These filaments are composed of keratins 5, 13, 14 and 19 (Schroeder and Listgarten, 1997). Keratins 5 and 14 have been found to be mutated in the skin in some forms of EBS and this may weaken the structure of the junctional epithelium. A similar mechanism may exist in Kindler syndrome although the blistering is at the level of the lamina lucida suggesting a defect in laminin-5. If laminin-5, or a related protein such as laminin-6 or -7 that are suggested to stabilize laminin-5, is dysfunctional then separation of the internal basal lamina could allow normal

flora to invade into the connective tissue compartment and cause loss of collagen and eventually attachment loss.

Kindler Syndrome as a Unique Entity

Starting with Teresa Kindler in 1954 the question has been asked whether Kindler syndrome is simply a combination of epidermolysis bullosa and poikiloderma congenitale in the same individual. Kindler herself suggested it would be a remarkable coincidence for these two rare diseases to manifest themselves in the same person. Similarities between epidermolysis bullosa and Kindler syndrome include congenital blistering that tends to decrease with age into the teenage years, possible mucosal involvement but to a lesser degree than may present in EB (oral mucosa, urethral and subglottic stenosis), webbing between digits and ultrastructural cleavage has been reported at epidermal, junctional and dystrophic level. (Hovnanian *et al.*, 1989, but not other authors).

Relationship to Epidermolysis Bullosa

Kindler (1954) originally considered the possibility that her patient had the coincident occurrence of dystrophic epidermolysis bullosa and congenital poikiloderma. It has been suggested that HAP/Kindler syndrome could be related to epidermolysis bullosa simplex (McKusick, 1994) although histological features do not necessarily agree with this. The various epidermolysis bullosa syndromes are classified into epidermolytic, junctional and dermolytic (dystrophic) types and numerous autosomal dominant and autosomal recessive types of varying severity exist. Weary *et al.* discussed the possibility that HAP was a variant of epidermolysis bullosa but concluded this was not the case based on 1) trauma or friction did not appear to be the cause of blistering and 2) blister formation was unlikely to be limited to the acral locations in a condition as generalized as epidermolysis bullosa.

Weary versus Kindler Disorders and Patterns of Inheritance

An ongoing debate exists in the dermatology literature on whether Weary's HAP and Kindler Syndrome are variants of the same disorder or different diseases. Both disorders have in common early progressive poikiloderma that resolves slowly with age.

Photosensitivity appears only to occur in Kindler patients, but not in all cases, and is never reported in HAP patients. The medical histories of Weary's kindred suggest that trauma and sunlight do not contribute to blister formation whereas these appear to be important factors in Kindler patients. Eczematous dermatitis and acrokeratoses have been described in HAP patients but not in Kindler patients although, again, not all HAP patients have these clinical features. Our patient appears to fit better into the classification of Kindler rather than HAP as she has a history of photosensitivity and blister formation on the palatal mucosa following scaling.

Numerous other features are inconsistently associated with either HAP or Kindler syndrome including palmoplantar keratoderma, ectropion, gingival fragility, webbing of digits and urethral stenosis. Weary described a family tree with 10 affected members and concluded the disorder was autosomal dominant with incomplete penetrance and variable expression. Hacham-Zadeh and Garfunkel (1985) reported on two related families with affected siblings who had a common great-grandfather and the parents in one family were first cousins. They suggested an autosomal recessive mode of inheritance for Kindler syndrome. In discussing another disorder, loosely termed congenital ichthyosis, Passwell *et al.* (1973) reasoned it was autosomal recessive based on 1) it did not show a wide variability in expressivity, 2) parental consanguinity with neither parent expressing the disorder, and 3) all affected members are in one sibship. Points 2) and 3) appear to fit well for cases described as Kindler syndrome and for our case in particular.

Table 7 - Associated Findings With Epidermolysis Bullosa, Kindler Syndrome and Hereditary Acrokeratotic Poikiloderma (Weary).

	EB	Kindler Syndrome	HAP
Blistering	+ (congenital)	+ (congenital)	+ (rarely congenital)
Poikiloderma	-	+	+
Onset	-	Infancy- starts on face	Infancy (spares face)
Acral keratoses	-	+/-	+
Eczema	-	+/-	+
Nail dystrophy	+/-	+/-	-
Mucosal involvement	+/-	+	-
Mode of inheritance	AD/AR/XR	AR (?)	AD
Ultrastructural cleavage	E/J/D	J (E/D?)	E
Photosensitivity	-	+/-	-

AD = autosomal dominant; AR = autosomal recessive; XR = X-linked recessive;
E = epidermal; J = junctional; D = dystrophic (dermal)

Considerable variance in expressivity has been reported but this is difficult to determine with so few cases to study. Alper *et al.* (1978) and Bordas *et al.* (1982) consider Kindler syndrome to be a distinct entity whereas Draznin *et al.* (1978) view HAP as a variant of Kindler syndrome. If Weary-Kindler is to be accepted as one disorder, then isolated cases must either be viewed as mutations or there is both a dominant and recessive mode of inheritance. As Kindler syndrome and HAP cannot be easily separated on clinical grounds or genetic background data, it will be useful to look for mutations in components at the dermal-epidermal junction and classify these diseases according to mutations.

Chapter 7 - Conclusions

Kindler Syndrome is an extremely rare genetic disorder whose oral findings and treatment have not been reported. Kindler syndrome is most likely a separate disorder from epidermolysis bullosa and hereditary acrokeratotic poikiloderma as discussed previously and summarized in Table 7. Features of the pathobiology determined in this study include 1) blister formation at the level of the lamina lucida, 2) no components of the basement membrane examined were found to be missing, 3) collagen type VII was found to display streaking deep into the connective tissue of the lamina propria rather than the normal appearance following linear to the basement membrane zone, 4) sequencing of the type VII collagen DNA from a blood sample of a Kindler syndrome patient did not turn up any mutations, 5) breaks in the continuity of the basement membrane zone components were seen both in immunofluorescent staining and electron microscopy examinations.

The clinical presentation of a female 16-year-old patient with Kindler syndrome was examined. She presented with localized early onset periodontal disease, severe gingival bleeding and gingival fragility. Dental caries (Forman *et al.*, 1989) and dystrophic teeth (Alper *et al.*, 1978) have been reported as clinical findings in some cases but this does not appear to be a common occurrence and the patient reported here had clinically normal, caries free teeth. Although high caries rates have been reported in cases of epidermolysis bullosa (Wright *et al.*, 1994) there is no reason to believe this is also the case in Kindler syndrome.

Medical treatment for Kindler syndrome is presently only palliative and includes skin emollients and sunscreens. Dental treatment has not been previously published. In this case a Kindler patient has received dental care in the disciplines of periodontics, orthodontics, removable prosthodontics and fixed prosthodontics.

This patient appears to have responded favorably to conservative periodontal therapy without chemotherapeutics although her long-term periodontal prognosis is unknown. The cyclic nature of gingival inflammation may result in further loss of periodontal support but further follow-up is necessary to determine this. The patient is as comfortable with her oral condition as she ever has been since age five years so there is certainly benefit to providing periodontal care even if it does not halt the progression of attachment loss.

References

- Alexander MB, Damoulis PD. The role of cytokines in the pathogenesis of periodontal disease. *Current Opinion in Periodontol* 1994; 39-53.
- Alper JC, Baden HP, Goldsmith LA. Short reports. Kindler's Syndrome. *Arch Dermatol* 1978; 114:457.
- Attström R. The roles of gingival epithelium and phagocytosing leukocytes in gingival defense. *J Clin Periodont* 1975;2:25-30.
- Bachinger HP, Morris NP, Lunstrum GP, Keene DR, Rosenbaum LM, Compton LA, Burgeson RE. The relationship of the biophysical and biochemical characteristics of type VII collagen to the function of anchoring fibrils. *J Biol Chem*. 1990;265:10095-10101.
- Badersten A, Nilveus R, Egelberg J. Scores of plaque, bleeding, suppuration and probing depth to predict probing attachment loss. 5 years of observation following nonsurgical periodontal therapy. *J Clin Periodontol* 1990;17:102-107.
- Battaglia C, Mayer U, Aumailley M, Timpl R. Basement-membrane heparan sulfate proteoglycan binds to laminin by its heparan sulfate chains and to nidogen by sites in the protein core. *European J Biochem* 1992;208:359-66.
- Bonadio J, Ramirez F, Barr M. An intron mutation in the human $\alpha 1(I)$ collagen gene alters the efficiency of pre-mRNA splicing and is associated with osteogenesis imperfecta type II. *J Biol Chem*. 1990;265:2262-2268.
- Bonifas JM, Rothman AL, Epstein EH Jr. Epidermolysis bullosa simplex. Evidence in two families for keratin gene abnormalities. *Science*. 1991;254:1202-1205.
- Bordas X, Palou J, Capdevila JM, Mascaro JM. Gross and microscopic symposium. Kindler's syndrome. Report of a case. *J Am Acad Dermatol* 1982; 6:263-265.
- Bottomley WK, Box JM. Dental anomalies in the Rothmund-Thomson syndrome. *Oral Surg*. 1976;41:321-326.
- Burgeson RE, Chiquet M, Deutzmann R, Ekblom P, Engel J, Kleinman H, Martin GR, Meneguzzi G, Paulsson M, Sanes J, Timpl R, Tryggvason K, Yamada Y, Yurchenko PD. *Matrix Biol*. 1994;14:209-211.
- 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.

Chambers DA, Imrey PB, Cohen RL, Crawford JM, Alves MEAF, McSwiggen TA. A longitudinal study of aspartate aminotransferase in human gingival fluid. *J Periodontal Res* 1991;26:65-74.

Champlaud M-F, Lunstrum GP, Rousselle P, Nishiyama T, Keene DR, Burgeson RE. Human amnion contains a novel laminin variant, laminin 7, which like laminin 6, covalently associates with laminin 5 to promote stable epithelial-stromal attachment. *J Cell Biol*. 1996;132:1189-1198.

Charon JA, Metzger Z, Hoffeld JT, Oliver C, Gallin JI, Mergenhagen SE. An *in vitro* study of neutrophils obtained from the normal gingival sulcus. *J Periodont Res* 1982;17:614-625.

Clerehugh V, Drucker DB, Seymour GJ, Bird PS. Microbiological and serological investigations of oral lesions in Papillon-LeFèvre syndrome. *J Clin Pathol* 1996;49:255-257.

Coimbra T, Wiggins R, Noh JW, Merritt S, Phan SH. Transforming growth factor-beta production in anti-glomerular basement membrane disease in the rabbit. *Am J Pathol* 1991;138:223-34.

Coulombe PA, Hutton ME, Letai A, Hebert A, Fuchs E. Point mutations in human keratin 14 genes of epidermolysis bullosa simplex patients: genetic and functional analysis. *Cell*. 1991;66:1301-1311.

Crawford JM, Taubman MA, Smith DJ. Minor salivary glands as a major source of secretory immunoglobulin A in the human oral cavity. *Science* 1975;190:1206-1209.

Crawford EG, Burkes EJ, Briggaman RA. Hereditary epidermolysis bullosa: oral manifestations and dental therapy. *Oral Surg Oral Med Oral Pathol* 1976;42:490-500.

Dawes CJ. *Biological techniques for transmission and scanning electron microscopy*. Ladd Research Industries, Inc., Burlington VT, 1981.

Domloge-Hultsch N, Gammon WR, 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.

Dowling GB, Meara RH. Epidermolysis bullosa resembling juvenile dermatitis herpetiformis. *Br J Dermatol*. 1954;66:139-143.

Draznin MB, Esterly NB, Fretzin DF. Congenital Poikiloderma with features of hereditary acrokeratotic poikiloderma. *Arch Dermatol* 1978; 114:1207-1210.

- Engvall E. Laminin variants: why, where and when. *Kidney International* 1993;43:2-6.
- Engvall E. Structure and function of basement membranes. *Int J Devel Biol* 1995;39:781-787.
- Fässler R, Georges-Labouesse E, Hirsch E. Genetic analysis of integrin function in mice. *Curr Opin Cell Biol* 1996;8:641-646.
- Feliciani C, Gupta AK, Sauder DN. Keratinocytes and cytokine/growth factors. *Crit Rev Oral Biol Med* 1996;7:300-318.
- Fine JD. Changing clinical and laboratory concepts in inherited epidermolysis bullosa. *Arch Dermatol*. 1988;124:523-526.
- Fine JD, Bauer EA, Briggaman RA, *et al*. Revised clinical and laboratory criteria for subtypes of inherited epidermolysis bullosa. *J Am Acad Dermatol*. 1991;24:119-135.
- Fives-Taylor P, Meyer D, Mintz K. Virulence factors of the periodontopathogen *actinobacillus actinomycetemcomitans*. 1996;67:291-297.
- Forman AB, Prendiville JS, Esterly NB *et al*. Kindler syndrome: report of two cases and review of the literature. *Pediatric Dermatol* 1989; 6:91-101.
- French D, Scott H, Overall CM. Papillon-LeFèvre syndrome associated early onset periodontitis: a review and case study. *J Can Dent Assoc* 1995;61:432-438.
- Fuchs E. Epidermal differentiation. *Curr Opin Cell Biol*. 1990;2:1028-1035.
- Gil SG, Brown TA, Ryan M, Carter WG. Junctional epidermolysis bullosis: defects in expression of epiligrin/nicein/kalinin and integrin $\beta 4$ that inhibit hemidesmosome formation. *J Invest Dermatol*. 1994;103:31S-38S.
- Gipson IK, Spurr-Michaud SJ, Tisdale AS. Hemidesmosomes and anchoring fibril collagen appear synchronously during development and wound healing. *Dev Biol*. 1988;126:253-262.
- Gorlin RJ, Sedano H, Anderson VE. The syndrome of palmar-plantar hyperkeratosis and premature periodontal destruction of the teeth. *J Pediatric* 1964;65:895-908.
- Haapalainen T, Oksala O, Kallioinen M, Oikarinen, Larjava H, Salo T. Destruction of the epithelial anchoring system in lichen planus. *J Invest Dermatol* 1995;105:100-103.
- Haapasalmi K, Mäkelä M, Oksala O, Heino J, Yamada KM, Uitto V-J, Larjava H. Expression of epithelial adhesion proteins and integrins in chronic inflammation. *Am J Pathol* 1995;147:193-206.

Hacham-Zadeh S, Garfunkel AA. Kindler syndrome in two related Kurdish families. *Am J Med Genet* 1985; 20:43-48.

Haffajee AD, Socransky SS, Goodson JM. Clinical parameters as predictors of destructive periodontal disease activity. *J Clin Periodontol* 1987; 10:257-265.

Haneke E, Hornstein OP, Lex C. Increased susceptibility to infection in the Papillon-LeFèvre syndrome. *Dermatologia* 1975;150:283.

Hashimoto S, Yamamura T, Shimono M. Morphometric analysis of intercellular space and desmosomes of rat junctional epithelium. *J Periodont Res* 1986;21:510-520.

Herlofson BB, Barkvoll P. Desquamative effect of sodium lauryl sulfate on oral mucosa. A preliminary study. *Acta Odontologica Scand* 1993;51:39-43.

Hodivala-Dilke K, DiPersio CM, Kreidberg J, Hynes R. Alpha-3 integrin deficient mouse keratinocytes: in vivo and in vitro investigation. *Abstract Keystone Symposia Molecular Cell Biol* 1996:316

Holbrook KA. Extracutaneous epithelial involvement in inherited epidermolysis bullosa. *Arch Dermatol* 1988;124:726-731.

Hopkinson SB, Riddelle KS, Jones, JC. Cytoplasmic domain of the 180-kD bullous pemphigoid antigen, a hemidesmosomal component: molecular and cell biologic characterization. *J Invest Dermatol.* 1992;99:264-270.

Hopkinson SB, Jones, JC. Identification of a second protein product of the gene encoding a human epidermal autoantigen. *Biochem J* 1994;300:851-857.

Hopkinson SB, Baker SE, Jones JC. Molecular genetic studies of a human epidermal autoantigen (the 180-kd bullous pemphigoid antigen/BP180): identification of functionally important sequences within the BP 180 molecule and evidence for an interaction between BP 180 and $\alpha 6$ 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.

Hovnanian A, Christiano AM, Uitto J. The molecular genetics of dystrophic epidermolysis bullosa. *Arch Dermatol.* 1993;129:1566-1570.

Hovnanian A, Pollack E, Hilal L, Rochat A, Prost C, Barrandon Y, Goossens M. A missense mutation in the rod domain of keratin 14 associated with recessive epidermolysis bullosa simplex. *Nature Genet.* 1993;3:371-332.

Hovnanian A, Blanchet-Bardon C, de Prost Y. Poikiloderma of Theresa Kindler: report of a case with ultrastructural study, and review of the literature. *Pediatric Dermatol* 1989;6:82-90.

Jedrychowski JR, Duperon D. Childhood hypophosphatasia with oral manifestations. *J Oral Med* 1979;34:18-22.

Jones JC, Kurpakus MA, Cooper HM. A function for the integrin $\alpha 6 \beta 4$ in the hemidesmosome. *Cell Regul* 1991;2:427-438.

Kapasi AY, Khopkar U, Raj S, Wadhwa S. Weary-Kindler syndrome with multiple seborrheic keratoses. *Int J Dermatol* 1993;32:444-445.

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.

Keene DR, Sakai LY, Lunstrum GP, Morris NP, Burgeson RE. Type VII collagen forms an extended network of anchoring fibrils. *J Cell Biol* 1987;104:611-621.

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.

Kindler T. Congenital poikiloderma with traumatic bulla formation and progressive cutaneous atrophy. *Br J Dermatol* 1954; 66:104-111.

Konig A, Brucker-Tuderman L. Transforming growth factor- β stimulates collagen VII expression by cutaneous cells in vitro. *J Cell Biol* 1992a;117:679-680.

Konig A, Lauharanta J, Brucker-Tuderman L. Keratinocytes and fibroblasts from a patient with mutilating dystrophic epidermolysis bullosa synthesize drastically reduced amounts of collagen VII: lack of effect of transforming growth factor-beta. *J Invest Dermatol* 1992b;99:808-812.

Koshiba H, Kimura O, Nakata M. A clinical and histologic observation of enamel hypoplasia in a case of epidermolysis bullosa hereditaria. *Oral Surg* 1977;43:585-589.

Kressin S, Herforth A, Preis S, Wahn V, Lenard HG. Papillon-LeFèvre syndrome - successful treatment with a combination of retinoid and concurrent systematic periodontal therapy: case reports. *Quintessence International* 1995;26:795-803.

Kurpakus MA, Quaranta V, Jones JCR. Surface relocation of $\alpha 6 \beta 4$ integrins and assembly of hemidesmosomes in an *in vitro* model of wound healing. *J Cell Biol* 1991;115:1737-1750.

Lane EB, Rugg EL, Navasria H, Leigh IM, Heagerty AH, Ishida-Yamamoto A, Eady RA. A mutation in the conserved helix termination peptide of keratin 5 in hereditary skin blistering. *Nature*. 1992;356:244-246.

Lang NP, Joss A, Orsanic T, Gusberti FA, Siegrist HE. Bleeding on probing. A predictor for the progression of periodontal disease. *J Clin Periodontol* 1986;13:590-596.

Lange D, Schroeder HE. Structural localization of lysosomal enzymes in gingival sulcus cells. *J Dent Res* 1972;51:272-278.

Larjava H, Zhou C, Larjava I, Rahemtulla F. Immunolocalization of $\beta 1$ integrins in human gingival epithelium and cultured keratinocytes. *Scand J Dent Res* 1992;100:266-273.

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 Diseases*. 1996;2:77-86.

Leigh IM, Lane EB. Mutations in the genes for epidermal keratins in epidermolysis bullosa and epidermolytic hyperkeratosis. *Arch Dermatol*. 1993;129:1571-1577.

Li K, Sawamura D, Giudice GJ, Diaz LA, Mattei MG, Chu ML, Uitto J. Genomic organization of collagenous domains and chromosomal assignment of human 180-kD bullous pemphigoid antigen (BPAG2), a novel collagen of stratified squamous epithelium. *J Biol Chem*. 1991;266:24064-24069.

Li K, Giudice GJ, Tamai K, Do HL, Sawamura D, Diaz LA, Uitto J. Cloning of partial cDNAs for mouse 180-kD bullous pemphigoid antigen (BPAG2), a highly conserved collagenous protein of the cutaneous basement membrane zone. *J Invest Dermatol*. 1992;99:258-263.

Lianos EA, Orphanos V, Cattell V, Cook T, Anagnou N. Glomerular expression and cell origin of transforming growth factor-beta 1 in anti-glomerular basement membrane disease. *Am J Med Sci* 1994;307:1-5.

Lin AN, Carter DM. Epidermolysis bullosa. *Annual Rev Med*. 1993;44:189-199.

Linch DC, Acton CHC. Ehlers-Danlos syndrome presenting with juvenile destructive periodontitis. *Br Dent J* 1979; 147:95-96.

- Marinkovich MP. The molecular genetics of basement membrane diseases. *Arch Dermatol* 1993;129:1557-1565.
- Mauviel A, Lapiere JC, Halcin C, Evans CH, Uitto J. Differential cytokine regulation of type I and type VII collagen gene expression in cultured human dermal fibroblasts. *J Biol Chem* 1994;268:25-28.
- McGrath JA, Schofield OM, Mayou BJ, McKee PH, Eady RA. Epidermolysis bullosa complicated by squamous cell carcinoma: report of 10 cases. *J Cutaneous Path* 1992;19:116-123.
- McKusick VA. *Mendelian inheritance in man. A catalogue of human genes and genetic disorders*. Baltimore and London: The John Hopkins University Press; 1994:173650.
- Michalowicz BS. Genetic and inheritance considerations in periodontal disease. *Current Opin Periodontol* 1993;11-17.
- Miller SC. *Textbook of Periodontia*. 1st ed., Philadelphia, Blakiston, 1938.
- Mühlemann HR. Psychological and chemical mediators of gingival health. *J Prev Dent* 1977; 4:6-11.
- Newman C, Wagner RF Jr., Tying SK, Spigel GT. Squamous cell carcinoma secondary to recessive dystrophic epidermolysis bullosa. A report of 4 patients with 17 primary cutaneous malignancies. *J Dermatol Surg Oncolog* 1992;18:301-305.
- Nisengard RJ, Rodgers RS. The treatment of desquamative gingival lesions. *J Periodontol* 1987;58:167-172.
- Nowak AJ. Oropharyngeal lesions and their management in epidermolysis bullosa. *Arch Dermatol* 1988;124:742-745.
- Page RC, Schroeder HE. Pathogenesis of inflammatory periodontal disease. A summary of current work. *Lab Invest* 1976;34:235-249.
- Palcanis KG, Larjara IK, Wells BR, Suggs KA, Landis JR, Chadwick DE, Jeffcoat MK. Elastase as an indicator of periodontal disease progression. *J Periodontol* 1992;63:237-242.
- Papillon MM, LeFèvre P. Deux cas de keratodermie palmaire et plantaire symétrique familiale (maladie de Meteda) chez le frere et la soeur. Coexistence dan les deux cas d'alterations dentaire graves. *Bull Soc Fran Dermatol Syphil* 1924;31:82-87.

Parente MG, Chung LC, Ryyänen J, *et al.*. Human type VII collagen: cDNA cloning and chromosomal mapping of the gene. *Proc Natl Acad Sci USA* 1991;88:6931-6935.

Passwell J, Zipperkowski L, Katznelson D, *et al.* A syndrome characterized by congenital ichthyosis with atrophy, mental retardation, dwarfism, and generalized aminoaciduria. *J Pediatr* 1973; 82:466-471.

Paulsson M, Fujiwara S, Dziadek M, Timpl R, Pejler G, Backstrom G, Lindahl U, Engle J. Structure and function of basement membrane proteoglycans. *Ciba Foundation Symposium* 1986;124:189-203.

Paulsson M. Basement membrane proteins: structure, assembly, and cellular interactions. *Crit Rev Biochem Mol Biol.* 1992; 27:93-127.

Pearson RW. Clinicopathologic types of epidermolysis bullosa and their nondermatological complications. *Arch Dermatol* 1988;124:718-725.

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

Pohla-Gubo G, Lazarova Z, Giudice GJ, Liebert M, Grassegger A, Hinter H, Yancey KB. Diminished expression of the extracellular domain of bullous pemphigoid antigen 2 (BPAG2) in the epidermal basement membrane of patients with generalized atrophic benign epidermolysis bullosa. *Exp Dermatol.* 1995;4:199-206.

Pratilas V, Bieznanski A. Epidermolysis bullosa manifested and treated during anesthesia. *Anesthesiology* 1975;43:581-583.

Preus HR. Treatment of rapidly destructive periodontitis in Papillon-Lefevre syndrome. Laboratory and clinical observations. *J Clin Periodontol* 1988;15:639-643.

Proceedings of the world workshop in clinical periodontics. The American academy of periodontology. Chicago, Illinois, 1989.

Prockop DJ, Colige A, Helminen H, Khillan JS, Pereira R, Vandenberg P. Mutations in type 1 procollagen that cause osteogenesis imperfecta: effects of the mutations on the assembly of collagen into fibrils, the basis of phenotypic variations, and potential antisense therapies. *J Bone Mineral Res* 1993;8:S489-492.

Reuland-Bosma W, van den Barselaar MT, van de Gevel JS, Leijh PC, Vries-Huiges H, The HT. Nonspecific and specific immune responses in a child with Down's syndrome and her sibling. *J Periodontol* 1988a;59:249-253.

- Reuland-Bosma W, Liem RSB, Jansen HWB, van Dijk LJ, van der Weele LT. Morphological aspects of the gingiva in children with Down's syndrome during experimental gingivitis. *J Clin Periodontol* 1988b;15:293-302.
- Reuland-Bosma W, Liem RSB, Jansen HWB, van Dijk LJ, van der Weele LT. Cellular aspects of and effects on the gingiva in children with Down's syndrome during experimental gingivitis. *J Clin Periodontol* 1988c;15:303-311.
- Risteli L, Timpl R. Isolation and characterization 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 β 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.
- Rothmund A. Über kataract in Verbindung mit einer eigentümlichen Hautdegeneration. [Cataracts in association with a peculiar degeneration of the skin.] *Arch Ophthalmol*. 1887;4:159.
- Rudnicka L, Varga J, Christiano AM, Iozzo RV, Jimenez SA, Uitto J. Elevated expression of type VII collagen in the skin of patients with systemic sclerosis. Regulation by transforming growth factor-beta. *J Clin Invest* 1994;93:1709-1715.
- Ryynänen M, Knowlton RG, Parente MG, Chung LC, Chu M-L, Uitto J. Human type VII collagen: genetic linkage of the gene (COL7A1) on chromosome 3 to dominant dystrophic epidermolysis bullosa. *Am J Hum Genet*. 1991;49:797-803.
- Ryynänen M, Knowlton RG, Uitto J. Mapping of epidermolysis bullosa simplex mutation to chromosome 12. *Am J Hum Genet*. 1991;49:978-984.
- Saito I, Komiyama K, Moro I, Akachi K, Shiomi N, Ito K, Murai S, Umemura S. Ultrastructural and immunocytochemical characterization of polymorphonuclear leukocytes from gingival crevice in man. *J Periodontol* 1987;58:493-497.
- Sawada T, Yamamoto T, Yanagisawa T, Takuma S, Hasegawa H, Watanabe K. Electron-immunocytochemistry of laminin and type-IV collagen in the junctional epithelium of rat molar gingiva. *J Periodontol Res* 1990;25:372-376.
- Sawamura D, Nomura K, Sugita Y, *et al*. Bullous pemphigoid antigen: cDNA cloning and mapping of the gene to the short arm of human chromosome 6. *Genomics*. 1990;8:722-726.
- Schroeder HE, Listgarten MA. *Fine structure of the developing epithelial attachment of human teeth*. 2nd ed. Karger, Basel 1977.

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

Sedano HO, Gorlin RJ. Epidermolysis bullosa. *Oral Surg Oral Med Oral Path* 1989;67:555-563.

Seymour RA, Heasman PA. *Drugs, diseases, and the periodontium*. Oxford Medical Publications, Oxford 1992.

Shimizu H, McDonald J, Kennedy A, Eady R. Demonstration of intra- and extracellular localization of bullous pemphigoid antigen using cryofixation and freeze substitution for postembedding immunoelectron microscopy. *Arch Dermatol Res*. 1989;281:443-448.

Shoshan S, Finkelstein S, Rosenzweig KA. Disc electrophoretic pattern of gingival collagen isolated from a patient with palmoplantar hyperkeratosis. *J Perio Res* 1970;5:255-258.

Skougaard MR. Cell renewal, with special reference to the gingival epithelium. *Adv Oral Biol* 1970;4:261-288.

Slots J, Reynolds HS, Genco RJ. Actinobacillus actinomycetemcomitans in human periodontal disease: a cross-sectional microbiological investigation. *Infect Immun* 1980;29:1013-1020.

Smith P, Rosenzweig KA. Seven cases of Papillon-LeFèvre syndrome. *Periodont* 1967;5:42-56.

Smith BE, Bradshaw AD, Choi ES, Rouselle P, Wayner EA, Clegg DO. Human SY5Y neuroblastoma cell interactions with laminin isoforms on neurite outgrowth on laminin-5 is mediated by integrin alpha 3 beta 1. *Cell Adhesion and Communication* 1996;3:451-462.

Sofaer JA. Genetic approaches in the study of periodontal diseases. *J Clin Periodontol* 1990; 17:401-408.

Sonnenberg A, Calafat J, Janssen H, Daams H, van der Raaij-Helmer LM, Falcioni R, Kennel SJ. Integrin $\alpha 6 \beta 4$ complex is located in hemidesmosomes, suggesting a major role in epidermal cell-basement membrane adhesion. *J Cell Biol* 1991;113:907-917

Stepp MA, Spurr-Michaud S, Tisdale A, Elwell J, Gipson IK. $\alpha 6 \beta 4$ integrin heterodimer is a component of hemidesmosomes. *Proc Natl Acad Sci USA*. 1990;87:8970-8974.

Sugiyama S, Utani A, Yamada S, Kozak CA, Yamada Y. Cloning and expression of the mouse laminin $\gamma 2$ (B2t) chain, a subunit of epithelial cell laminin. *Eur J Biochem*. 1995;228:120-128.

Timpl R, Aumailley M. Biochemistry of basement membranes. *Advances in Nephrology From the Necker Hospital* 1989;18:59-76.

Tinanoff N, Temprow P, Maderazo EG. Dental treatment of Papillon-LeFèvre syndrome: 15-year follow-up. *J Clin Periodontol* 1995;22:609-612.

Thomson MS. A hitherto undescribed familial disease. *Br J Dermatol*. 1923;35:455-462.

Topoll HH, Lange DE, Muller RF. Multiple periodontal abscesses after systemic antibiotic therapy. *J Clin Periodontol* 1990; 17:268-272.

Uitto J. Molecular pathology of collagen in cutaneous diseases. *Advances Dermatol* 1991;6:265-286.

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

Uitto J, Chung-Honet LC, Christiano AM. Molecular biology and pathology of type VII collagen. *Exp Dermatol* 1992b;1:2-11.

Uitto J, Bauer EA, Moshell AN. Symposium on epidermolysis bullosa: molecular biology and pathology of the cutaneous basement membrane zone. *J Invest Dermatol*. 1992c;98:391-395.

Uitto J, Christiano AM. Inherited epidermolysis bullosa. Clinical features, molecular genetics, and pathoetiologic mechanisms. *Dermatologic Clinics* 1993; 11:549-563.

Uitto J, Pulkkinen L, Christiano AM. Molecular basis of the dystrophic and junctional forms of epidermolysis bullosa: mutations in the type VII collagen and kalinin (Laminin 5) genes. *J Invest Dermatol*. 1994;103:39S-46S.

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

Uitto V-J, Nieminen A, Coil J, Hurttia H, Larjava H. Oral fluid elastase as an indicator of periodontal health. *J Clin Periodontol* 1996;23:30-37.

van der Rest M, Garrone R. Collagen family of proteins. *FASEB J* 1991;5:2814-2823.

Van Dyke TE, Taubman MA, Ebersole JL, Haffagee AD, Socransky SS, Smith DJ, Genco RJ. The Papillon-LeFèvre syndrome. Neutrophil dysfunction with severe periodontal disease. *Clin Immunol Immunopathol* 1984;31:419-429.

Vassar R, Coulombe PA, Degenstein L, Albers K, Fuchs E. Mutant keratin expression in transgenic mice causes marked abnormalities resembling a human genetic skin disease. *Cell* 1991;64:365-380.

Vennos EM, Collins M, James WD. Rothmund-Thomson syndrome: review of the world literature. *J Am Acad Dermatol*. 1992;27:750-762.

Vennos EM, James WD. Rothmund-Thomson syndrome. *Dermatol Clinics*. 1995;13:143-150.

Verrado 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.

Verrando P, Blanchet-Bardon C, Pisani A, Thomas L, Cambazard I, Eady RA, Ortonne JP. Monoclonal antibody GB3 defines a widespread defect of several basement membranes and a keratinocyte dysfunction in patients with lethal junctional epidermolysis bullosa. *Lab Invest*. 1991;64:85-91.

Vidal F, Baudoin C, Miquel C, Galliano M-F, Christiano AM, Uitto J, Ortonne J-P, Meneguzzi G. Cloning of the laminin $\alpha 3$ chain gene (LAMA3) and identification of a homozygous deletion in a patient with Herlitz junctional epidermolysis bullosa. *Genomics*. 1995;30:273-280.

Weary PE, Hsu YT, Richardson DR, Caravati CM, Wood BT. Hereditary sclerosing poikiloderma. *Arch Dermatol* 1969;100:413-422.

Weary PE, Manley WF, Graham GF. Hereditary acrokeratotic poikiloderma. *Arch Dermatol* 1971; 103:409-422.

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.

Wright JT. Comprehensive dental care and general anesthetic management of hereditary epidermolysis bullosa. A review of fourteen cases. *Oral Surg Oral Med Oral Path*. 1990;70:573-578.

Wright JT, Fine J-D, Johnson LB. Oral soft tissue in hereditary epidermolysis bullosa. *Oral Surg Oral Med Oral Path*. 1991a;71:440-446.

Wright JT, Childers NK, Evans KL, Johnson LB, Fine J-D. Salivary function of persons with hereditary epidermolysis bullosa. *Oral Surg Oral Med Oral Path*. 1991b;71:553-559.

Wright JT, Fine J-D, Johnson LB. Dental caries risk in hereditary epidermolysis bullosa. *Pediatric Dent.* 1994;16:427-432.

Zambon JJ. Actinobacillus actinomycetemcomitans in human periodontal disease. *J Clin Periodontol* 1985; 12:1-20.

Zhang X, Zhou J, Reeders ST, Tryggvason K. Structure of the human type IV collagen COL4A6 gene, which is mutated in Alport syndrome-associated leiomyomatosis. *Genomics* 1996;33:473-479.