Structure and function of the tooth–epithelial interface in health and disease

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Introduction

Three types of mucous membranes (masticatory, lining, and specialized) line the oral cavity and form the structural boundary between the body and the external environment. Although each type of mucosa protects against mechanical and microbial damage, the epithelia exhibit considerable differences in their histology, thickness and differentiation suitable for the functional demands of their location. Furthermore, the structure of different epithelia reflects their effectiveness as a barrier to the penetration of microbes and noxious agents into the deeper tissues (141,160). Mucosal epithelia are composed of continuously dividing and shedding populations of keratinocytes whose proliferation is confined to the basal layer. The teeth, passing through the gingival masticatory mucosa, create a unique environmental challenge to the protective continuity. At the interface where the healthy gingiva meets the tooth surface the structural continuity is secured by the junctional epithelium attached to the tooth surface by a distinct mechanism known as the epithelial attachment apparatus (143). As opposed to the constantly renewing epithelia, teeth are units of nonshedding surfaces, which provide a solid substratum not only for the attachment of the junctional epithelial cells but also for bacterial colonization and spreading in the oral cavity. At the dentogingival junction the bacterial colonies, exhibiting a variety of virulence factors (68), pose a potential threat to the epithelial attachment. The attachment may be affected directly by bacteria or indirectly through their ability to activate inflammatory and immune processes, which contribute to the composition of the gingival crevice fluid (GCF) and thus to the conditions under which the epithelial attachment apparatus is formed and/or maintained. In addition to the attachment, the renewal rate and reparative capacity of the junctional epithelial cells are equally important for the health of the dentogingival junction. Accordingly, any degenerative episodes that involve the cells responsible for the attachment may gradually lead to the detachment of the junctional epithelium from the tooth surface and compromise the protective mucosal continuity.

Periodontal diseases are associated with bacterial plaque infecting the dentogingival margin and causing inflammation in the underlying tissues (53,87). Logically, one of the main concerns of periodontal research has been to describe the inflammatory reaction in the adjacent tooth-supporting tissues and the consequences it may have at the alveolar bone margin (70,121,147). Less attention has been focused on the effects of factors released from bacteria and/or from the inflammatory reaction on the junctional epithelial cells. The precise mechanisms of epithelial degeneration and/or activation leading to the detachment and apical migration of the junctional epithelial cells and consequent conversion of the gingival sulcus into an infected periodontal pocket have not yet been discovered. This article deals with factors associated with periodontal tissue protection and destruction with special reference to the junctional epithelial cells.

Junctional epithelium

Schroeder and Listgarten first clarified the anatomy and histology of the dentogingival junction in their monograph: “Fine structure of developing epithelial
Fig. 1. Schematic illustration of the different epithelia at the dentogingival junction. The junctional epithelium (JE) exhibits a distinct phenotype that allows the tissue to attach to the tooth surface and participate in the host defense in a number of ways.

Second, it allows the access of GCF, inflammatory cells and components of the immunological host defense to the gingival margin. Third, junctional epithelial cells exhibit rapid turnover, which contributes to the host–parasite equilibrium and rapid repair of damaged tissue (48). Although the importance of the junctional epithelium in host defense is well recognized, an exact understanding of its role in the pathogenesis of periodontal diseases is largely missing (Fig. 3). A major part of the older literature on junctional epithelium describes its histological features at different stages of development or disease (113,141). More recent studies report on the distribution of cytoplasmic keratin filaments in the junctional epithelial cells, on different cell surface antigens and receptors, intercellular adhesion molecules and details of the nonepithelial cells within the junctional epithelium and its nerve supply [for a review see (142)]. These results consistently support the view that to be able to form a successful junction between two dissimilar tissues and to respond in a flexible manner to the external environment, the epithelial cells responsible must have a distinct undifferentiated phenotype (160) (Fig. 4). The phenotype of junctional epithelium, at least partly, reflects a self-instructed adaptation of oral mucosa to the

attachment of human teeth’ (143). Since then, knowledge of the junctional epithelium has been reviewed in numerous articles (90,142,144,161,168). It is commonly accepted that the junctional epithelium exhibits several unique structural and functional features that contribute to preventing pathogenic bacterial flora from colonizing the subgingival tooth surface. First, junctional epithelium is firmly

Fig. 2. Photomicrograph demonstrating the junctional epithelial DAT cells on the tooth surface (a). When the gingiva is displaced laterally the DAT cells are left on the enamel (E) surface. The degeneration of the DAT cells appears to be a prerequisite for the apical advancement of bacterial plaque (P). D = dentin. The junctional epithelium (JE) attached to tooth (to enamel or cementum (C) as in (b) forms a structural barrier against the bacterial plaque. Polymorphonuclear leukocytes that cover the plaque (P) have migrated through the junctional epithelium into the sulcus. At the apical part of the junctional epithelium, cells (arrow) are seen to grow/proliferate into the connective tissue (CT). A higher magnification (c) of the apical part of the junctional epithelium. Note the dark-stained DAT cells along the cementum surface, the epithelial ‘finger’ proliferating apically and the absence of inflammatory infiltrate. Gingival epithelial cells grown on decalcified dentin matrix (d) show apparent ability to extracellular collagenolysis (e).
existence of a solid tooth surface penetrating it (134,168). It is pertinent to suggest that the phenotype required for epithelial adaptability may also render the cells vulnerable to the action of various exogenous and endogenous agents. Since our understanding of the pathogenic mechanisms that lead to failure in junctional epithelium defense is limited, there is a clear need for fundamental research into the junctional epithelial cells and their functions under different clinical conditions.

Epithelial attachment apparatus

The attachment of the junctional epithelium to the tooth is mediated through an ultramicroscopic mechanism defined as the epithelial attachment apparatus (143). It consists of hemidesmosomes at the plasma membrane of the cells directly attached to the tooth (DAT cells) and a basal lamina-like extracellular matrix, termed the internal basal lamina, on the tooth surface [Fig. 5 (80)]. By morphological criteria the internal basal lamina between the junctional epithelial DAT cells and the enamel is quite similar to the basement membrane between the epithelium and the connective tissue. However, by biochemical criteria the internal basal lamina differs essentially from the established basement membrane composition and thus from the external basal lamina.

The components of the internal basal lamina are synthesized by the DAT cells in the absence of the immediate vicinity of connective tissue (80,142). Internal basal lamina proteins include laminin and type VIII collagen (128,130). Laminin, identified as type 5, is localized mainly to the optically electron-dense part of the internal basal lamina and it seems to be associated with hemidesmosomes (69,93,100). Characteristically, the internal basal lamina lacks laminin-1 and type IV collagen, which are components of true basement membranes (129,130). In addition, the internal basal lamina structure may involve other molecules that are unique to this structure (L. Häkkinen, unpublished results).

Fig. 3. The junctional epithelium (JE) is repeatedly or continually exposed to bacterial challenges, which may lead to the failure of the junctional epithelium and eventually to subgingival plaque formation (brown), conversion of the gingival sulcus into a periodontal pocket and increase in the size of the inflammatory focus in the connective tissue (gray).

Hemidesmosomes have a decisive role in the firm attachment of the cells to the internal basal lamina on the tooth surface. Recent data suggest that the hemidesmosomes may also act as specific sites of signal transduction and, thus, participate in regulation of gene expression, cell proliferation and cell differentiation (71). The intracellular part of hemidesmosomes consists of at least two distinct proteins, the 230kDa bullous pemphigoid antigen (BP230) and plectin, which is a high molecular weight cytomatrix protein (Fig. 5). These proteins mediate the attachment of the epithelial cell cytoplasmic keratin filaments to two transmembrane components of the hemidesmosome known as the 180kDa bullous pemphigoid antigen (BP180) and α6β4 integrin (71,174). The α6β4 integrin plays an important role in the interaction of epithelial cells with the extracellular matrix (101,127). This interaction utilizes the intracellular plectin connected through the β4-domain of the integrin to laminin-5 (ligand for the α6β4 integrin) in the internal basal lamina (66,69,169). In general, the interaction between the different components of the extracellular matrix and the cell surface molecules linked to the intracellular cytoskeleton is fundamental for cell adhesion, cell motility, synthetic capacity, tissue stability, regeneration and responses to external signals (175). Since the biochemical composition of internal basal lamina matrix differs from that of the true basement membrane, the behavior of the attached DAT cells cannot be directly deduced from the data reporting on the behavior of basal cells that grow adjacent to the external basal lamina (167).

Turnover of the junctional epithelial cells

The junctional epithelium is a stratified epithelium composed of two strata, the basal layer facing the connective tissue and the suprabasal layer extending
to the tooth surface (Fig. 2 and 4). Coronally, close to the sulcus, junctional epithelium is about 15 cell layers thick and narrows towards the apical part of the tissue. The turnover rate of junctional epithelium is exceptionally rapid. In nonhuman primates it is about 5 days and approximately twice the rate of the oral gingival epithelium (153). Previously it was thought that only epithelial cells facing the external basal lamina were rapidly dividing. However, recent evidence indicates that a significant number of the DAT cells are, like the basal cells along the connective tissue, capable of synthesizing DNA, which demonstrates their mitotic activity (133, 135). At the coronal part of the junctional epithelium, the DAT cells typically express a high density of transferrin receptors (131), which supports the idea of their active metabolism and high turnover (172). The findings suggest that the DAT cells have a more important role in tissue dynamics and reparative capacity of the junctional epithelium than has previously been thought (143). Based on these data, alternative models for the turnover of DAT cells can be proposed (Fig. 6). The existence of a dividing population of epithelial cells (DAT cells) in a suprabasal loca-

Fig. 4. Haematoxylin-eosin staining of a clinically healthy human dentogingival junction (a) shows an inflammatory infiltrate in the connective tissue (CT) lateral to the junctional epithelium (JE). The junctional epithelium exhibits wider intercellular spaces as compared to the sulcular epithelium (SE). The wide intracellular spaces allow the access of the components of the immunological defense into the sulcus. DAT cells directly attached to the tooth are seen as a string lateral to the enamel space (ES). Note the most coronal DAT cell (→) that appears to be supported only by the tooth and thus forms the medial wall of the sulcus (see also schematic illustration in Fig. 8). Reaction with a monoclonal antibody to keratin 19 (b) shows that all the suprabasal junctional epithelial cells express this protein, as do the undifferentiated basal cells. The reaction with the antibody to keratin 10 shows that terminal differentiation is a characteristic feature of the oral epithelium (OE) but not of the sulcular or junctional epithelia. The DAT cells along the tooth surface (d) are especially rich in K19.
Fig. 5. A schematic illustration of a DAT cell shows the structural and molecular composition of the epithelial attachment apparatus (EAA). N = nucleus of a DAT cell, IF = cytoplasmic keratin filaments (intermediate size filaments). The hemidesmosomes at the plasma membrane are associated with the \( \alpha_6\beta_4 \) integrin that communicates with Ln-5 = laminin 5 located mainly in the internal basal lamina, the extracellular domain (?) for BP180 is a collagenous protein (perhaps type VIII), that has not yet been definitely characterized. LL = lamina lucida, LD = lamina densa, SLL = sublamina lucida, IBL = internal basal lamina.

Fig. 6. The mechanism of DAT cell turnover is not fully understood. Considering the fact that the DAT cells are able to divide and migrate, three possible mechanisms can be proposed. These are (1) the daughter cells produced by dividing DAT cells replace degenerating cells on the tooth surface, (2) the daughter cells enter the exfoliation pathway and gradually migrate coronally between the basal cells and the DAT cells to eventually break off into the sulcus, or (3) epithelial cells move/migrate in the coronal direction along the tooth surface and are replaced by basal cells migrating round the apical termination of the junctional epithelium.
polysaccharides, lipoteichoic acids, short-chain fatty acids, and phospholipases, have been shown to affect adversely the metabolism of epithelial cells in cultures, leading to changes in proliferation and/or production of cytokines and matrix metalloproteinases (43,44,64,118–120,156). Understanding of the potential significance of these events in relation to the pathogenesis of periodontal pocket formation calls for further studies.

**Junctional epithelium in the antimicrobial defense**

Junctional epithelium consists of active populations of cells and antimicrobial functions, which together form the first line of defense against microbial invasion into tissue (Fig. 7). Even though junctional epithelial cell layers provide a barrier against bacteria many bacterial substances, such as lipopolysaccharide, pass easily through the epithelium but have only limited access through the external basal lamina into the connective tissue (146). Both the internal and external basal laminae act as barriers against infective agents.

Rapid turnover, as such, is an important factor in the microbial defense of junctional epithelium (see below). Also, because the area covered by the dividing cells in the junctional epithelium is at least 50 times larger than the area through which the epithelial cells desquamate into the gingival sulcus, there is a strong funnelling effect that contributes to the flow of epithelial cells (145). Rapid shedding and effective removal of bacteria adhering to epithelial cells is therefore an important part of the antimicrobial defense mechanisms at the dentogingival junction.

There is increasing evidence indicating that several specific antimicrobial defense systems exist in the oral mucosa. Many epithelial cell types, including junctional epithelium, have been found to contain enzyme-rich lysosomes. Their fusion with plasma membrane is triggered by elevation of the intracellular calcium concentration (122,145). In rats, the lysosomes have been demonstrated to contain cysteine proteinases (cathepsin B and H) active at acidic pH (190). Porcine epithelial cells of Malassez share several characteristics with junctional epithelial cells and are able to produce several neutral proteinases, including collagen-degrading enzymes and a chymotrypsin-like proteinase (44). The role of these enzymes in the antibacterial mechanism has not yet been studied. Recently, it has been found that the junctional epithelial cells lateral to DAT cells produce matrilysin (matrix metalloproteinase-7) (176). In Paneth cells of the mouse intestine this enzyme is able to activate the precursor peptide of α-defensin, an important antimicrobial agent of mucosal epithelium (184). It is possible that a similar active matrilysin/defensin system exists in junctional epithelium, as in other mucosa exposed to bacteria such as intestine, lungs and urogenital tissues (88). Another possible effect by which matrilysin contributes to the mucosal defense is the release of bioactive molecules from the cell surfaces which play a role in the inflammatory reaction. Such effects are currently being actively explored.

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**Fig. 7.** Several antimicrobial mechanisms exist in the junctional epithelium. In the coronal part of the junctional epithelium quick cell exfoliation (1) because of rapid cell division (2) and funnelling of junctional epithelial cells towards the sulcus hinder bacterial colonization (see text). Laterally, the (external) basement membrane forms an effective barrier against invading microbes (3). Active antimicrobial substances are produced in junctional epithelial cells. These include defensins and lysosomal enzymes (4). Epithelial cells activated by microbial substances secrete chemokines, e.g. interleukin-8 and cytokines, e.g. interleukins -1 and -6, and tumour necrosis factor-α that attract and activate professional defense cells, such as lymphocytes (LC) and polymorphonuclear leukocytes (PMN). Their secreted product, in turn, cause further activation of the junctional epithelial cells (5). CT = connective tissue.
Despite the selective barrier formed by the gingival basal laminas, components of the inflammatory and immunological defense pass easily through the basement membrane and epithelium into the sulcus. Here they play an important role in restricting bacterial access into the subgingival tissues (for a review see [81]) (Fig. 2a,b, 4a). Leukocytes, especially the polymorphonuclear leukocytes that migrate through the junctional epithelium, comprise probably the most important defense mechanism at the gingival margin (112). The cell surface carbohydrates (1) expressed by the junctional epithelial cells are thought to respond to extracellular molecular changes in a manner which allows the cells to communicate with their environment. The cells respond actively to bacterial infection by producing cell adhesion molecules (intercellular adhesion molecule-1) and chemotactic substances (chemokines such as C5a, leukotriene B4, lymphocyte function-associated antigen-3 and interleukin-8) that facilitate the migration of leukocytes through the junctional epithelium (16,28,99,171).

In the subsequent line of defense inflammatory mediators and antibodies produced by macrophages, lymphocytes and plasma cells in the gingival tissues restrict the spreading of bacterial infection into the connective tissue and systemic circulation. Significant amounts of lymphocytes may be present also within the junctional epithelium, thus contributing to the protective functions of the tissue (148,149). Recently, it has been suggested that supplementary to system-derived antibodies and antibodies produced locally by plasma cells, the junctional epithelial cells may also have a secretory function (77).

The detachment of the DAT cells from the tooth surface – (host vs. bacteria; battle for surface)

Role of the gingival crevice fluid

GCF is an exudate of varying composition found in the sulcus/periodontal pocket between the tooth and marginal gingiva. GCF contains components of serum, inflammatory cells, connective tissue, epithelium, and microbial flora inhabiting the gingival margin or the sulcus/pocket (26, 37, 41, and articles in this issue) (Fig. 8). In the healthy sulcus the amount of GCF is very small. However, its constituents participate in the normal maintenance of function of the junctional epithelium throughout its lateral and vertical dimensions, including the most coronal DAT cells. During inflammation the GCF flow increases and its composition starts to resemble that of an inflammatory exudate (26). The increased GCF flow contributes to host defense by flushing bacterial colonies and their metabolites away from the sulcus, thus restricting their penetration into the tissue.

The main route for GCF diffusion is through the (external) basement membrane and then through the relatively wide intercellular spaces of the variable thickness junctional epithelium into the sulcus. Although all the junctional epithelial cells are constantly exposed to the GCF and its various constituents, the nutritional and other vital conditions in the different parts of the junctional epithelium depend on a large number of local factors. Clearly, the changes in the composition of the GCF caused either by bacteria, bacterial metabolites/enzymes or other factors, or the inflammatory reaction is likely to be...
largest at the most coronal junctional epithelial cells. A considerable number of bacteria and host-derived products found in the GCF have been associated with the initiation and progression of periodontal disease. The bacterial agents include endotoxins, hydrogen sulfide, butyric and propionic acids, bacterial collagenases and other proteases (e.g. trypsin-like), and a variety of enzymes, such as hyaluronidase and neuraminidase (37). Host-derived agents include components associated with the inflammatory reaction, such as factors of the complement system, prostaglandins, different cytokines, intracellular enzymes, and products of tissue breakdown such as lactate dehydrogenase, aspartate aminotransferase, polyamines, and collagen peptides (37,41,82). Furthermore, antimicrobial agents and leukocyte-derived enzymes such as lysozyme, alkaline phosphatase, β-glucuronidase, cathepsin D, elastase, collagenase, and lactoferrin as well as osteonectin and fibronectin are also found in the GCF. Indeed, the GCF contains a wide variety of biologically active molecules with the potential capacity to affect the growth of junctional epithelium/DAT cells as well as oral bacteria, both competing for the tooth surface at the dentogingival interface (Fig. 8 and 9, Table 1).

Role of the polymorphonuclear leukocytes

Polymorphonuclear leukocytes form the most important line of defense against bacterial plaque at the gingival margin (112). When the polymorphonuclear leukocytes reach the bacteria, they release the contents of their granules and may adhere to individual bacteria and phagocytose them. The polymorphonuclear leukocytes do not, however, appear to have the capacity to remove dental plaque but rather form a protective wall against it. Therefore, polymorphonuclear leukocytes are a major contributor in the host–parasite equilibrium but have a limited capacity to reclaim any tooth surface once lost to the plaque bacteria. On the other hand, activated polymorphonuclear leukocytes can cause tissue damage as a result of a variety of enzymes, oxygen metabolites, and other components that are released from their granules during the battle against microbes (3,179,187). The polymorphonuclear leukocytes have two main types of granules that contain agents that are effective in killing the bacteria. The azurophilic (primary) granules contain myeloperoxidase, lysozyme, elastase, cathepsin G, urokinase, acid hydrolases, and defensins, whereas the specific (secondary) granules contain lactoferrin, elastase, and lysozyme (30). Activated polymorphonuclear leukocytes also generate hydrogen peroxide (H₂O₂) and highly reactive oxygen radicals with the potential to destroy bacteria and gingival cells (21,30). The polymorphonuclear leukocytes are most effective in aerobic conditions close to the gingival margin (30), suggesting a different role for them in anaerobic periodontal lesions. While directing their effects to the invading microbes the powerful polymorphonuclear leukocyte substances also potentially affect the structural cells and extracellular matrix. Polymorphonuclear leukocytes may also become activated in inflammatory lesions and release specifically their secondary granule contents during their chemotactic migration (63,189). This phenomenon may also take place in tissues such as the junctional epithelium. Therefore, the effects of the secondary granule contents on gingival epithelium are of special interest in research into the failure of the junctional epithelium and the formation of periodontal pockets.

Lactoferrin is an important antimicrobial protein present in the secondary granules of polymorphonuclear leukocytes. It has high affinity for iron (5,32,85) and it acts on bacteria by causing iron de-
Table 1. Dental plaque and gingival crevice fluid (GCF) components associated with periodontal diseases

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cultured plaque bacteria</th>
<th>Dental plaque in vivo</th>
<th>Human GCF/GCW</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Healthy</td>
<td>Periodontal disease</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonia</td>
<td>14–86nm (176)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butyric acid</td>
<td>9.1 mM (151)</td>
<td>0–0.2 mm (104,105)</td>
<td>0.08 mM (19)</td>
</tr>
<tr>
<td>Hydrogen sulfide</td>
<td>539 nm (115)</td>
<td>1–50 p.p.m.</td>
<td>(98)</td>
</tr>
<tr>
<td>Interleukin-1β</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Lactoferrin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipoteichoic acid</td>
<td>50 µg/ml (181)</td>
<td></td>
<td></td>
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<tr>
<td>Lipopolysaccharide</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Prostaglandin E2</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Propionic acid</td>
<td>113 µm (151)</td>
<td>0.8–0.9 µm (104,105)</td>
<td>9.5–44 µm (104,105)</td>
</tr>
<tr>
<td>Transforming growth factor-α</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Tumor necrosis factor-α</td>
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GCW, gingival crevicular washing.

pletion, and thus reduction in bacterial cell division rate, glucose metabolism and macromolecular synthesis (7,178). Besides bacteriostatic activity, lactoferrin also has bactericidal effects, at least in vitro. Binding of lactoferrin to bacteria and their subsequent lysis has been reported in a number of studies (7,8,12,36). Furthermore, lactoferrin may exert antimicrobial effects through interfering with bacterial attachment and colonization in the oral cavity (4,159,178). In addition to the effects described above, lactoferrin may also interfere with the host defense not only by blocking complement activation and inhibiting hydroxyl radical formation but also by enhancing it by stimulating polymorphonuclear leukocyte recruitment to the infected sites (12). In the GCF from sites exhibiting gingival inflammation or periodontal pockets the amount of lactoferrin is significantly elevated (1000–1500 µg/ml) as compared to the healthy sites (500 µg/ml) (2,46). While microbial multiplication is already significantly inhibited at low lactoferrin concentrations (50 µg/ml), even high amounts of lactoferrin (> 500 µg/ml) seem to have no effect on epithelial cell division in model systems simulating the junctional epithelium in vivo (118). High concentrations of lactoferrin do, however, hamper epithelial cell growth by interfering with their adhesion and spreading. The molecule may, thus, have a role in delaying the repair of the junctional epithelium/DAT cell population during severe inflammation.

Role of host proteinases and inflammatory mediators

Degradation of extracellular matrix during periodontal inflammation is a multistep process that involves several proteolytic enzymes. Different cell types of periodontal tissue produce matrix metalloproteinases (collagenases, stromelysins, gelatinases, membrane-type metalloproteinases), plasminogen activator, cathepsins and elastase (17,165). In response to the bacteria and inflammatory cytokines, fibroblasts, junctional epithelial cells, osteoblasts/osteoclasts, macrophages, and polymorphonuclear leukocytes release proteinases that are involved in the defense against microbes. At the same time they also contribute to periodontal tissue destruction by degrading extracellular matrix and basement mem-
bran components (17,60,132,158). In concert, matrix metalloproteinases are able to degrade all extracellular matrix proteins (17). Collagenases degrade interstitial type collagen fibrils (I, II, III), whereas gelatinases, stromelysins, and membrane-type metalloproteinases have the ability to degrade fibronectin and gelatin (denatured collagen), and basement membrane components including type IV collagen, entactin, nidogen, and laminin (17,72,102). Neutrophil elastase and cathepsin G are capable of degrading basement membrane type IV collagen and laminin, and also type VIII collagen, found in the internal basal lamina (61,78). Proteinases of host origin are thus capable of degrading all known extracellular components of connective tissue and epithelium including components of both the external basal lamina (basement membrane at the connective tissue–junctional epithelium interface) and the internal basal lamina at the epithelium–tooth interface. Therefore, these enzymes seem to have the potential to contribute to the lateral and apical proliferation of the junctional epithelium into the connective tissue (Fig.2) as well as to epithelial disintegration through degradation of the internal basal lamina and increase in epithelial permeability. However, electron microscopic studies on DAT cells attached to teeth extracted because of advanced periodontitis do not support the idea that enzymatic degradation of the epithelial attachment apparatus precedes the degeneration of DAT cells (111). On the contrary, the DAT cells in this material seemed to be more severely affected than the epithelial attachment apparatus once produced and maintained by the degenerating cells. This implies that it might be more rewarding to focus the studies of pocket formation on mechanisms that disturb the vital functions of the DAT cells rather than on the destruction of the matrix components of the epithelial attachment apparatus.

As described elsewhere in this issue, regulation of proteinase activities is a complex process involving activation of latent precursor molecules as well as inhibition of the active enzymes. Therefore, the actual damage caused by, for example, polymorphonuclear leukocyte proteinases may be limited in the presence of proteinase inhibitors, such as α2 macroglobulin, α1 antitrypsin and tissue inhibitors of metalloproteinases, found in the junctional epithelium and in the gingival crevice. In fact, a recent study has demonstrated that there is only a limited amount of active metalloproteinases in the pocket epithelium obtained from sites of periodontitis (140). Since the degradation of the extracellular matrix by metalloproteinases plays a major role in the inflamed connective tissue, it is pertinent to ask how significant is the failing support of the connective tissue to the integrity of the junctional epithelium. Obviously, a less effective connective tissue support predisposes the tissue to intraepithelial splits and contributes to the lateral and apical proliferation of the epithelium. Together with increased permeability of the junctional epithelium, this may alter the nutritional conditions of the DAT cells on the tooth surface and expose them to agents from the dental plaque. Another area of junctional epithelium biology that has not been addressed in depth is the composition of the epithelial interstitial matrix and how its degradation affects the behavior of junctional epithelial cells. Also, a limited proteolytic cleavage of matrix molecules, e.g. laminin, fibronectin and proteoglycan, may expose cryptic molecular sites with biological activity not possessed by the intact molecule (39). These active tissue fragments may regulate cell adhesion, migration and proliferation in inflamed tissue (175). For instance, while intact laminin-5 promotes formation of hemidesmosomes and inhibits cell migration, its fragment produced by a proteolytic cleavage promotes epithelial cell migration (49). Even though fragments of fibronectin and collagen have been demonstrated in GCF their effects on junctional epithelium/pocket epithelium are unknown (38). In future, extracellular matrix molecules and their fragments can be expected to provide useful tools for prevention and management of periodontal disease. For example, laminin-5 treatment of teeth or titanium dental implants may enhance long-term stability of epithelial attachment (35).

Cytokines, especially the interleukins -1 and -6, and tumor necrosis factor-α, and the arachidonic acid metabolite prostaglandin E₂, have been strongly associated with periodontal disease (for a review see (47)). These inflammatory mediators are secreted into the GCF by both leukocytes and activated junctional epithelium cells and their amounts have been shown to increase at sites exhibiting periodontal tissue destruction [Table I; (95,103,109,117)]. Interleukin-1, interleukin-6 and prostaglandin E₂ stimulate bone resorption and contribute to periodontal tissue destruction also by inducing matrix metalloproteinase (collagenase) production (47). Interleukin-1 has been shown to promote fibroblast proliferation and production of other cytokines by periodontal cells, and to activate the arachidonic acid pathway and thus production of prostaglandin E₂ (27,34,110). Prostaglandin E₂ in turn has an over-
all catabolic effect on gingival fibroblasts, as shown by suppressed DNA synthesis and collagen synthesis (6). Studies reporting the effects of inflammatory mediators on the junctional epithelium and its functions are not available today.

**Role of bacterial products**

It is plausible that several products released from bacteria during periodontal infection have junctional epithelium as their major target tissue. Even though there is ample evidence that bacterial substances have a multitude of effects on several cell types, ranging from activation of cell functions to cell death, the importance of specific substances in initiation and progression of periodontal diseases is not yet understood. We review here the literature of the main bacterial products and their potential effects on the junctional epithelium.

In the early phase of plaque formation gram-positive bacteria accumulate on the tooth surface close to the most coronal junctional epithelium/DAT cells which may thus be exposed to the cell wall components of these typically supragingival bacteria; namely the peptidoglycan matrix, surface antigens, teichoic and lipoteichoic acids. Lipoteichoic acids are genus- and species-specific molecules (45) that are synthesized especially abundantly from sucrose at neutral pH (79,124). The precise functions of lipoteichoic acids in bacteria have not yet been established. However, they have been implicated as carriers in cell wall synthesis, inhibitors of autolytic activity and as reservoirs of cations (45,125). In the oral cavity lipoteichoic acids are thought to mediate bacterial adhesion to human cells and teeth (13,67,123).

If the bacterial plaque is allowed to grow, the amount of gram-negative bacteria increases. The cell wall of these bacteria is composed of a thin peptidoglycan layer and a bilayered outer membrane, which contains the major surface antigens including the porin proteins, and lipopolysaccharides (lipopolysaccharides/endotoxins) (55). The variation in the lipopolysaccharides between bacterial genera, species, and even within species (54,180,181) may account for the reported differences in host responses to lipopolysaccharides derived from different bacterial populations.

From the periodontal point of view both lipoteichoic acids and lipopolysaccharides are interesting molecules. They are released from the bacteria into the extracellular environment during bacterial disruption, and also during normal cell wall turnover of living bacteria (42,45,55,56,68,150,173,181). Thus, varying amounts of lipoteichoic acids and lipopolysaccharides are present at the gingival margin where they may stimulate leukocyte function, increase cytokine and inflammatory mediator production and activate the complement system as shown in studies *in vitro* (15,86,89,96). In addition to their role as inflammation modulators lipoteichoic acids and lipopolysaccharides have been shown to affect periodontal tissues, e.g. by stimulating bone resorption (9,59,94). Furthermore, lipopolysaccharides appear to have the ability to increase epithelial permeability and penetrate healthy gingival sulcular epithelium (120,138,154). Lipopolysaccharides from *Salmonella enteritidis*, *Escherichia coli*, *Actinobacillus actinomyctemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *Porphyromonas asaccharolytica* have been shown to stimulate human gingival fibroblast proliferation at low (1 μg/mL) concentrations and suppress their proliferation at higher (>10 μg/mL) concentrations (11,31,65,74,83,84). High concentrations of lipopolysaccharides from non-oral bacteria (*E. coli*, 5000 μg/mL) have been shown to increase the proliferation of basal cells of the junctional epithelium in an animal model (167). In human epithelial cell and junctional epithelial tissue cultures lipopolysaccharides from oral pathogens show only slight or no effects on the growth and mitotic activity of the cells (120). This indicates that lipopolysaccharides, despite their established role as modulators of inflammation, may not significantly harm the epithelial cells at the concentrations found in dental plaque and GCF (below 50 μg/mL). Therefore, lipopolysaccharides do not appear to have a key role in DAT cell degeneration and detachment from the tooth surface.

When epithelial cells in different culture systems are exposed to lipoteichoic acids from gram-positive oral bacteria (*Streptococcus sanguis* and *Streptococcus mutans*, 10–50 μg/mL) their growth and mitotic activity are consistently reduced (120). According to these results, the lipoteichoic acids appear to have the potential to interfere with the renewal of various types of epithelial cells. As discussed before, a prolonged inhibition of the renewal of the coronal junctional epithelium/DAT cells would most likely lead to their degeneration and detachment, and eventually to subgingival colonization by gram-negative periodontal pathogens.

Periodontopathogenic bacteria release numerous proteolytic enzymes that are a prerequisite for the normal metabolism of amino acids and for the survival of these mainly asaccharolytic bacteria in the
oral environment (for a review see [68]). Bacterial collagenases, gelatinases, and trypsin- and chymotrypsin-like enzymes have been shown to degrade host extracellular matrix macromolecules and basement membrane components in vitro suggesting that also they have the potential to contribute to periodontal tissue destruction, as do the host-derived proteinases (17,116). This is especially true in the junctional epithelium, where the bacterial enzyme concentrations are much higher than in the connective tissue. Degradation of immunoglobulins and complement proteins by bacterial proteinases may result in an incomplete host defense and thus facilitate bacterial colonization and growth. Some bacterial proteinases are able to activate host matrix metalloproteinases and thereby increase the total proteolytic activity in the infected tissue (29,157). The complex interactions of bacterial and host-derived proteinases and their inhibitors, as well as the presence of bacteria capable of degrading the proteinase inhibitors (24,107,139) make the exact role of the bacterial proteolytic enzymes in periodontal tissue destruction difficult to study. It appears, however, that the role, if any, of the bacterial enzymes on epithelial detachment and DAT cell viability/degeneration is primarily indirect and because of changes in the living conditions of these cells rather than of direct effects on the cells or on the epithelial attachment apparatus (111).

The oral cavity provides bacteria with a large number of ecologic niches and substrates that can be metabolized to different end products depending on the bacterial population and the prevailing environmental conditions. Therefore, the composition of a bacterial population and its virulence, associated with specific pathogenic mechanisms, reflects the interaction of a large number of constantly changing variables determined not only by the oral microflora itself but also by the host. For example, the metabolism of carbohydrates in dental plaque depends on the pH, the oxygen gradient, the amount and quality of available substrates, and most importantly, the bacterial composition of the plaque (92,185). As a principal rule, cariogenic, supragingival plaques contain a high percentage of streptococci, whereas subgingival plaques growing in periodontal pockets contain high proportions of gram-negative anaerobes (22,23,185). The main metabolic end product of the streptococcal plaque is lactic acid, whereas the gram-negative bacteria produce butyric acid more abundantly (68,91,106,164,185) (Fig.10). It is noteworthy that certain bacteria can take advantage of the metabolic end products released by other bacteria and thereby contribute to the production of agents that are increasingly detrimental to the host tissues. Anaerobic plaque bacteria, especially the asaccharolytic bacteria, utilize amino acids and peptides, derived either from diet or tissue/cell breakdown, for their sources of energy. This requires the presence of proteolytic enzymes that first degrade the macromolecules (proteins) into small peptides or amino acids which are then further metabolized by the bacteria (68,185). When amino acids are utilized as an energy source, ammonia, sulfur-containing compounds (hydrogen sulfide) and short-chain fatty acids, especially butyric and propionic acids, are formed (23,68,164).

Butyric and propionic acids are short-chain fatty acids containing four and three carbon atoms, respectively. They are produced by periodontopathogenic bacteria, such as Porphyromonas, Fusobacterium, Prevotella and Treponema (18–20,22,51,97,164). As described above, the production of these acids depends on a variety of environmental factors including the proteolytic activity and the pH in the pocket. The maximal activity of proteolytic enzymes produced by periodontal pathogens is at pH 7.0–8.0. Some periodontal bacteria (e.g. Prevotella intermedia) are, however, capable of surviving and functioning over a much broader pH range and even of raising the pH by producing ammonia, and thereby making the environment more suitable for bacterial populations adapted to alkaline conditions (68). It is of particular interest that the concentrations of butyric and propionic acids found in human plaque and GCF correlate directly with the degree of gingival inflammation and periodontal pocket depth (Table 1[19,104,105]). Furthermore, the application of millimolar concentrations of these short-chain fatty acids onto the healthy gingiva of beagle dogs has been reported to produce a marked gingival inflammatory response (152). More recently, human studies have shown that both food, which supports bacterial generation of high levels of short-chain fatty acids (50 mm), and short-chain fatty acids (100 mm) applied directly to healthy human gingiva elicit an inflammatory response in the tissue. This response can be demonstrated by increased GCF flow, subgingival temperature, polymorphonuclear leukocyte emigration, and elevated levels of inflammatory cytokines (interleukin-8) in the GCF (75,106,191). Short-chain fatty acids have also been shown to activate leukocytes to release inflammatory cytokines and extend their lifetime (163,166). The activation of the inflammatory response and simultaneous inhibition of the polymorphonuclear leukocyte function...
(phagocytosis and degranulation) (106) can alter and prolong the inflammatory response and lead to more severe tissue destruction.

In cell cultures, butyric and propionic acids, applied in concentrations found in human plaque and GCF, have been shown to inhibit the proliferation of both fibroblasts and epithelial cells (33,119,151,156). Microbial populations producing these short-chain fatty acids may thus significantly impair the rapid renewal of the junctional epithelium/DAT cells and thereby counteract one of the tissue's main host protective functions. Taken together, the current literature suggests that butyric and propionic acids play a role in the initiation and progression of periodontal pocket formation by triggering and modifying the inflammatory response and by hampering the turnover and repair of the tissues at the dentogingival junction.

Utilization of amino acids by bacteria for their energy needs, results in the formation of short-chain fatty acids, hydrogen sulfide and ammonia as by-products. Like the short-chain fatty acids, ammonium and hydrogen sulfide have potentially detrimental effects on periodontal cells. Ammonium (20–80 mM) has been shown to cause cell vacuolization in chondrocytes and to inhibit (2–10 mM ammonium) collagen secretion by human gingival fibroblasts in vitro (62,177). Whether or not the ammonium levels produced by plaque bacteria significantly influence epithelial cells is not known.

Hydrogen sulfide is a highly toxic compound that causes adverse effects on human tissues (eyes and respiratory tract) at concentrations of 50 p.p.m. (50 μg/mL) and above (14). Hydrogen sulfide has been detected in the GCF collected from gingival sulcus/pocket and its amount has been shown to increase in gingival inflammation (155). In the concentrations found in dental plaque (1–50 ppm) hydrogen sulfide has been shown to be toxic to HeLa cells (98). Interestingly, oxidation of hydrogen sulfide results in sulfate formation and detoxification of the compound, whereas its toxicity is increased in the presence of short chain hydrocarbons, ethanol and/or proteins (14). Considering the protein-rich, short-chain fatty acid-containing and anaerobic conditions in the subgingival space, hydrogen sulfide appears to be a potential candidate to cause significant damage to the junctional epithelium/DAT cells on the tooth surface during periodontal disease progression.

**Role of risk factors for periodontal disease**

It is clear that periodontal diseases are primarily caused by bacterial infections and that a number of risk factors contribute to the susceptibility of indi-
viduals to these infections, and to the pathogenesis and severity of the disease. These factors include smoking, diabetes, immunosuppression, genetic factors, stress and age (136). Studies on how the risk factors influence disease progression have mainly been focused on the inflammatory reaction (10,52,57,58,76,108,114,137,162,186,188). The conclusion from these studies is that a sound inflammatory host response is needed for successful periodontal defense. Factors that modify this response may either cause an overwhelming reaction or an inadequate reaction, both of which may accelerate tissue destruction. It is interesting that periodontal risk factors, such as hyperglycaemia and chemical compounds released from tobacco, have harmful effects on the renewing capacity of both fibroblasts and bone cells (40,50,170) However, very little is known about the influences of any of the risk factors on oral gingival/sulcular or junctional epithelium (64). From the defense point of view, it would be important to examine whether these factors also impair the turnover or other defense mechanisms of the junctional epithelium and thus contribute to the degeneration of the dentoepithelial junction.

It is also quite possible that a specific response of junctional epithelial cells to bacterial or host substances is a key factor in the pathogenesis of certain forms of periodontal diseases. One example could be localized juvenile periodontitis, where a rapid apical growth of the junctional epithelium is associated with a typically distributed pocket formation. A local junctional epithelial cell defect might in this case result, for example, in a decreased ability of the DAT cells to form hemidesmosomes. In fact, in Kindler syndrome a defect in the formation of hemidesmosome-associated fibers consisting of type VII collagen leads to detachment of skin and gingival epithelium and to early onset periodontitis (183).

**Conclusions**

The precise mechanisms that lead to the degeneration and detachment of the junctional epithelium from the tooth surface and to the conversion of a gingival sulcus into an infected periodontal pocket have not been established. The destructive mechanisms may involve many of the modalities of both the host defense and microbial virulence. Studies of the degeneration process of the junctional epithelial cells themselves are, however, largely missing. The junctional epithelium consists of distinct populations of cells at different anatomical locations. All these cells have a responsive phenotype that allows them to exhibit specific functions in periodontal defense and to take or lose an active role during periodontal disease progression. The junctional epithelium is firmly attached to the tooth by the suprabasal DAT cell/internal basal lamina structure, and to the connective tissue by the basal cell/external basal lamina complex. Environmental conditions differ essentially in these two locations and even similar pathogenic challenges may become modified and cause considerably different cellular responses. While the coronal DAT cells grow close to the bacterial plaque the apical and lateral parts of the junctional epithelium are likely to be influenced by the connective tissue and the inflammatory reaction. The GCF provides the most coronal DAT cells with the necessary conditions to survive but it also contains a variety of biologically active molecules with the potential capacity to affect their vital functions and behavior. Although the significance of the various components of the GCF to the failure of the junctional epithelium and its DAT cells calls for further studies there are a few candidates that are of particular interest today. Among these are the polymorphonuclear leukocyte products that may have direct influences on DAT cell survival and/or their adhesion and bacterial lipoteichoic acids and metabolic end products, such as propionic and butyric acids that may interfere with cell division and thus the turnover and reparative capacity of the junctional epithelium. Similarly, inflammatory factors and components associated with periodontal risk factors may reach high concentrations in the GCF and severely interfere with the junctional epithelium's normally protective functions.

When the physiology of the junctional epithelium and its molecular reactions during different external challenges are known in more detail the tissue's role in the failure of the healthy dentogingival junction and the reasons that make some individuals susceptible to periodontal diseases will be better understood.

**References**


109. Overman DO, Salonen JI. Characterization of the human


