Acinar epithelial cell laminin-receptors in labial salivary glands in Sjögren's syndrome

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Abstract

Objective

To analyze the epithelial cell-basement membrane attachment, in particular in the secretory end pieces (responsible for secretion of saliva) and in Sjögren's syndrome (SS) characterized by acinar cell failure.

Method

Immunohistochemistry with laminin receptor chain-specific monoclonal antibodies to integrin (Int) subunits, Lutheran blood group antigen and α-dystroglycan.

Results

Only acinar cells contained Int α1 and α2 subunits. This staining was interrupted but strong in controls, but very weak in SS. Both acinar and ductal cells contained Int α3, α6, β1 and β4 and Lutheran blood group antigen and ductal cells also contained α-dystroglycan. These staining patterns were similar in SS and controls.

Conclusions

Binding of the acinar and ductal cells to the basement membrane laminins seems to be mediated by Int α3β1, α6β1 and α6β4 integrin-receptors and Lutheran blood group antigen and α-dystroglycan non-integrin receptors. This structure-supporting system is intact in SS, compatible with the maintenance of the tubuloalveolar architecture of the SS glands. The irregular staining pattern of the acinus-specific Int α1β1 and α2β1 was compatible with a regulated signaling role, which was apparently impaired in SS. Indeed, their laminin counterparts (Lm -1/111 and -2/211) are also aberrant in SS revealing this as the central cell-matrix defect in the syndrome.

Key words

Salivary gland, acinar cells, basement membrane, laminin, integrin, Sjögren's syndrome.

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Introduction

Basement membrane (BM) is specialized extracellular matrix supporting all epithelial tissues as well as blood vessel endothelium and muscle, fat and Schwann cells (1). Tissue and cell specific BMs regulate morphogenesis of glandular tissues and are essential for their proper maintenance in adulthood (1, 2). BM is formed by two independent networks consisting of laminins and type IV collagens, which are linked to each other by nidogen-1 (3-5).

These BM structures are recognized by integrins, which form transmembrane αβ heterodimers. Their cytoplasmic part is connected to the cytoskeleton of the cell, but as mentioned, their extracellular part binds various components of the extracellular matrix (6, 7). In addition to the anchorage function important for tissue integrity, some integrins transmit signals from the extracellular ligands to the cell, which regulate migration, growth and differentiation of the cell. Integrins use multiple intracellular signaling routes, which in part overlap with those utilized by growth factor receptors (8). Currently 24 distinct heterodimeric αβ integrins composed of 18 different α-chains and 8 β-chains have been recognized (9). Lately, it has been found that laminins are also recognized by some non-integrin receptors, such as α-dystroglycan and the Lutheran blood group antigen so also these were analyzed in the present study (10, 11).

Sjögren's syndrome (SS) is a common autoimmune disease affecting tubuloalveolar epithelial cells of the exocrine glands (12) clinically characterized by dry eyes (keratoconjunctivitis sicca) and dry mouth (xerostomia) (13). Salivary duct and acinar epithelial cells are separated from the interstitial connective tissue stroma by an intervening BM (14). The glandular architecture and function are in healthy glands maintained by BM. According to our hypothesis, this important regulatory element is deranged in SS and might well explain the acinar cell atrophy, ductal cell hyperplasia and secretory failure, which are typical features of SS.

Immunolocalization studies have disclosed a rather complex molecular composition of the morphologically relatively simple and homogenous BM of the salivary glands. Very little has been published, however, on the BM composition of the human minor labial salivary glands, which are of primary importance for the moistening of the oral mucosal membrane between meals. Matrix metalloproteinases capable of degrading type IV collagen of BM are altered in patients with SS (15-17) and might explain some of the aberrant features of the acini and ducts in SS (13). On the other hand, laminin expression has been reported to increase very early in SS (18, 19). Our earlier studies on the content of laminin α, β and γ chains showed that BM of both acinar and ductal labial salivary gland contain laminin-5/332, -6/311 and -10/511 trimers, whereas BM of only acini contain laminin-1/111, -2/211 and -8/411 trimers (14). In particular, Lm-α1 chain/Lm-1/111 was suggested to play a functional rather than structural (supporting) role as its expression was heterogeneous (patchy) in healthy glands and altered and very weak in SS. Indeed, it has been described that human submandibular HSG intercalated duct progenitor cells differentiate into acinar cells, when cultured on laminin-1/111 containing substrates (20). Laminin-1/111 is also involved in the generation of synapses between postganglionic parasympathetic nerve terminal and its target cells (21), which is important for the neural control and proper functioning of the exocrine glands.

The current evidence suggests that the laminin composition of and distribution in BM are important for the normal structure and function of labial salivary glands and pathologically altered in the autoimmune exocrinopathy known as Sjögren's syndrome. We hypothesized that to be able (or unable) to get adequate mechanical BM support and to receive regulatory laminin signals, also the integrin and non-integrin laminin receptors (6) should show segment (ducts *vs.* acini) specific distribution on the surface of healthy glandular epithelial cells. This regulated and topologically organized integrin expression might be disturbed in SS. This hypothesis was tested using a relatively extensive set of

integrin α and β chain specific antibodies to profile the salivary gland epithelial cells for their laminin receptors.

Materials and methods

Patients and tissue samples

The ethics committee of the Joint Authority for the Hospital District of Helsinki and Uusimaa, HUS, Finland, approved the study. All subjects gave their informed consent. The diagnosis of SS was based on the modified European criteria for SS (22).

Labial salivary glands were obtained from 5 patients with primary SS and from 6 healthy controls. Five to ten labial salivary glands were removed under local anesthesia and processed for histopathology (23). The tissues were snap frozen in liquid nitrogen. Frozen sections were cut at 6 μm and fixed in acetone at -20°C. Some sections were stained with hematoxylin-eosin to evaluate their histology.

Indirect immunofluorescence staining

The following monoclonal antibodies (MAbs) were used for indirect immunofluorescence staining of the laminin receptors: TS2/7 against Int α1 (24), 10G11 against Int α2 (25), J143 against Int α3 (26), GoH3 against Int α6 (27), 9.1 Int α7 (a kind gift from Dr R.H. Kramer) (28), LM 142.69 against Int αV (29), 102DF5 against Int β1 (30), 90BB10 against Int β3 (31), AA3 against Int β4 (32), IIH6 against α-dystroglycan (33) and BRIC221 against Lutheran blood group antigen (34). Sections were washed in 10 mM of phosphate buffered 0.15 mM saline (PBS, pH7.4), containing 0.1% Triton X-100. Normal goat normal serum (X0907, Dako, Glostrup, Denmark) was used to diminish non-specific background labeling. After incubation with the above mentioned specific primary antibodies sections were washed in PBS and the bound antibodies were visualized using fluorescein isothiocyanate-conjugated secondary antibodies, goat anti-mouse IgG for monoclonal mouse antibodies (Alexa Fluoro 488, Molecular Probes, Eugene, OR), goat anti-rat IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) for monoclonal rat anti-human GoH3 and goat anti-mouse IgM (Jackson) for monoclonal mouse anti-human IIH6 IgM. After washes in PBS, the specimens were embedded in fluorescent mounting medium (Dako) and examined under an Olympus AX70 (Hamburg, Germany) microscope. Staining intensity was graded to – = negative, ± = very weakly positive, + = positive, ++ = relatively strongly positive or +++ = very strongly positive and the staining pattern to continuous and interrupted staining. Control immunostainings were performed using irrelevant primary monoclonal antibodies at the same concentration as and instead of the primary specific antibodies or using conjugated secondary antibodies alone. Staining controls were negative confirming the specificity of the staining with the monoclonal antibodies used in the present study.

Results

Distribution of integrin α subunits in healthy labial salivary glands

In healthy human labial salivary glands, Int α3 and α6 subunits formed continuous lines on the basal domains (*i.e.*, on the cellular surface facing the BM) of the acinar and ductal cells (Fig. 1B and 1C). Immunoreactive Int α1 and α2 subunits were found only on the acinar cell membrane. Their staining pattern was often interrupted and irregular rather than continuous. Salivary ducts lacked these integrin subunits (Figs. 1A and 2A). Int α7 and αV subunits were not found in human labial salivary glands.

Distribution of integrin β subunits in healthy labial salivary glands

Int β1 and β4 subunits were expressed as continuous and strongly positive lines on acinar and ductal epithelial cells (Figs. 3A and 3B), but Int β3 subunit was not found at all in the human labial salivary glands.

Distribution of non-integrin receptors in healthy labial salivary glands

α-dystroglycan was weakly present around the ducts, but absent around acini (data not show). Lutheran blood group antigen formed strong and uninterrupted (continuous) lines around the acini and ducts (Fig. 3C).

Comparison of healthy controls and SS

Int α3, α6, β1 and β4 subunits were expressed on the acinar and ductal epithelial cells in the labial salivary glands. All these subunits formed continuous, uninterrupted lines around the acini and ducts. There were no differences in their staining intensity or their distribution between the healthy controls and the patients with SS.

Int α1 and α2 subunits were found almost exclusively on the acinar cells, where they formed clear lines on the basal aspect of the cell membrane in the healthy controls. The corresponding staining was weak in patients with SS (Figs. 1A, 2A and 2B *vs.* 1D, 2C and 2D).

Immunoreactivity for Int α7, αV and β3 were not present in human labial salivary glands, whereas α-dystroglycan stained weakly on the ductal, but not on the acinar cells, in a similar fashion in the healthy controls and Sjögren's syndrome. Lutheran blood group antigen was strongly present around the acini and the ducts both in the healthy controls and in SS (Figs. 3C and 3F).

Discussion

Integrin laminin receptors play important roles in the developing salivary glands (35). As the laminin receptors have overlapping and co-operative functions, extensive laminin receptor profiling was performed in the present study. As the combinatorial rules are known, profiling enables conclusions to be drawn on the various possible heterodimeric integrin receptors formed. This is necessary as the complex and variable expression of laminin receptors in the human labial salivary glands suggests that they play variable and multiple roles in adult labial salivary glands. Based on the present findings on the strong and continuous expression of Ints α3β1, α6β1 and α6β4 on the basal cell aspect of both acinar and ductal epithelial cells, it is concluded that they play important structure maintaining functions. They probably firmly attach the epithelial cells to the underlying BM. Indeed, these integrins form the receptor set for Lm-5/332 and Lm-10/511 (5), which were in our

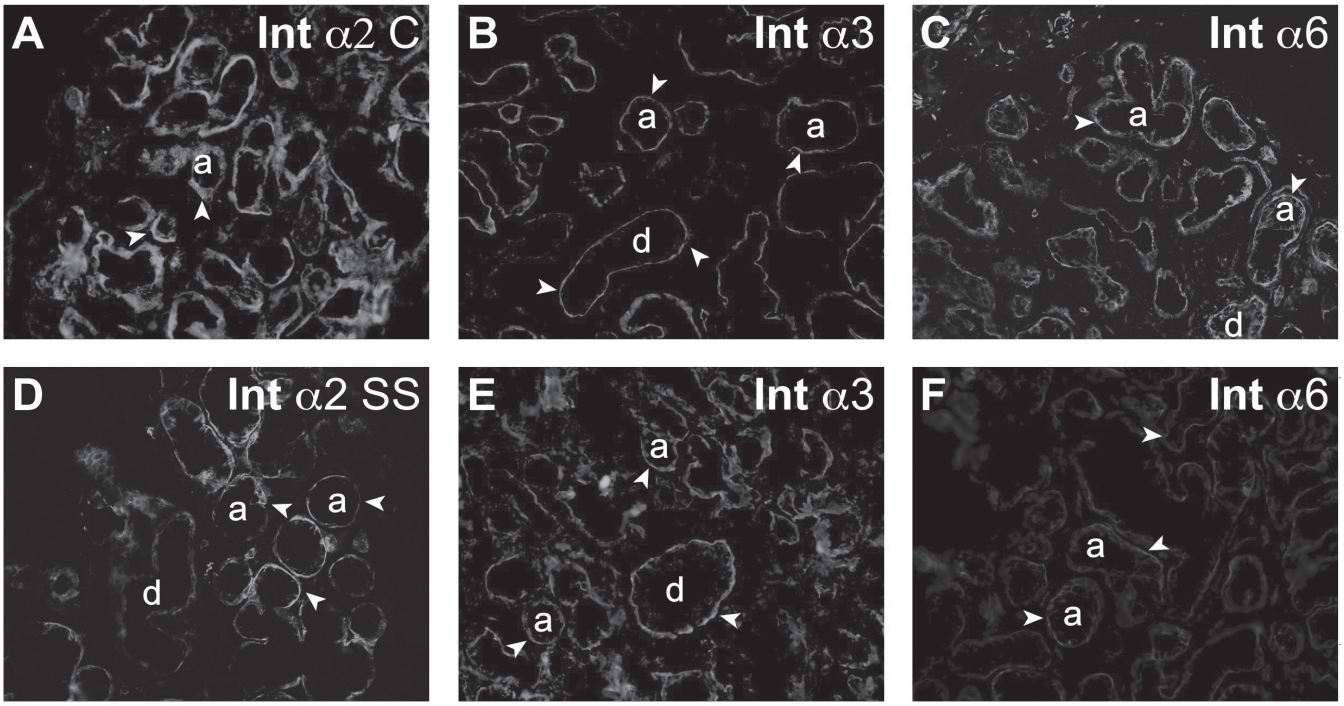

Fig. 1. Distribution of Int α2, α3 and α6 subunits in indirect immunofluorescence in human labial salivary glands of normal healthy controls (upper C row) and of Sjögren's syndrome patients (lower SS row). A, immunoreactivity for Int-α2 chain was confined to acini. B, Int-α3 subunit, and C, Int-α6 showed continuous lines of reactivity around the acini and ducts. D, immunoreactivity for Int-α2 subunit was weak and occasionally interrupted in Sjögren's syndrome. E, Int-α3, and, F, Int-α6 subunit reactivities on the basal acinar and ductal cell membranes formed in Sjögren's syndrome similar continuous lines around the epithelial cells as in healthy controls. **a** = acinus, **d** = duct. Magnification, x 200.

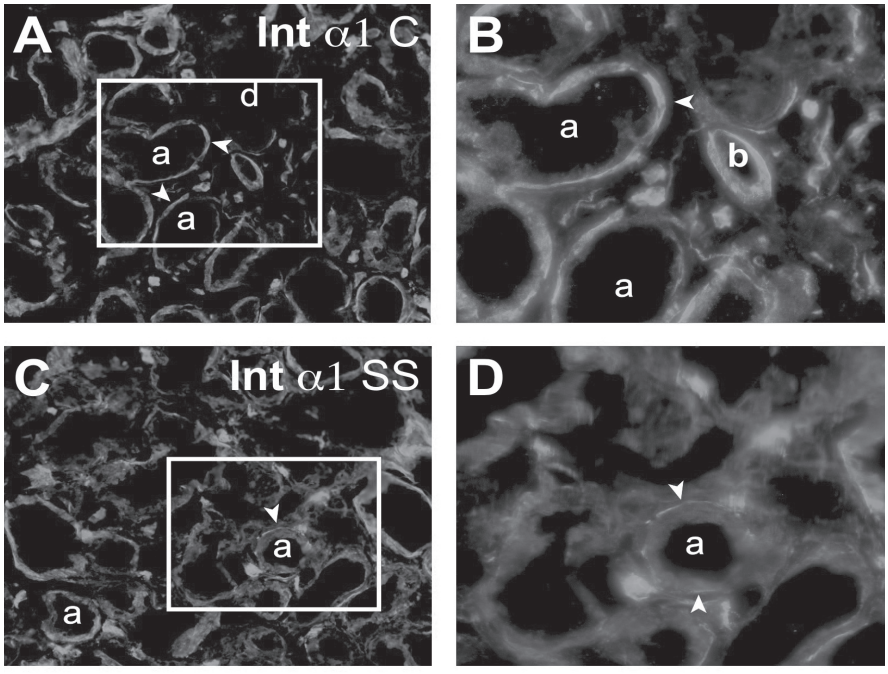

Fig. 2. Distribution of Int α1 subunit in indirect immunofluorescence in human labial salivary glands of normal healthy controls (panels A and B) and of Sjögren's syndrome (panels C and D). Immunoreactivity for Int-α1 subunit was restricted to the basal aspects of the acinar cell membranes (**a** = acinus), where it had an interrupted and irregular appearance, whereas cells of the ducts (**d** = duct) lacked Int-α1 immunoreactivity (panels A and B). The acinar Int-α1 subunit stained weakly in the labial salivary glands in Sjögren's syndrome (panels C and D). Staining of the basement membrane of the blood vessels (**b** = blood vessel) served as an internal sample control. The boxed areas in the left panel column (A and C) are shown at greater magnification in the right panel column (B and D), respectively. Magnification x 200 in panels A and C, x 400 in panels B and D.

previous work shown to be present and to form continuous and uninterrupted reciprocal lines in the acinar and ductal epithelial cell BMs (14). The perfect match between the topographical localization and pattern of expression of these receptor and their ligand counter parts further supports the conclusion about their role in the maintenance of the glandular architecture.

Lutheran blood group antigen formed strong and continuous lines along the basal aspects of the acinar and ductal epithelial cell membrane. Lutheran blood group antigen has been recently discovered to form a non-integrin receptor for Lm-10/511 to which it binds with a high affinity (11). This receptor-ligand interaction might help the Int α3β1, α6β1 and α6β4 receptor to Lm-511 and -332 interactions to prevent shedding of the salivary gland epithelial cells into saliva as a result of myoepithelial cell contractions and shear forces exerted by the flow of saliva.

Some integrin receptors may have multiple laminin ligands, with the above mentioned Int α6β4 being one of them. Apart from its avid binding to universal

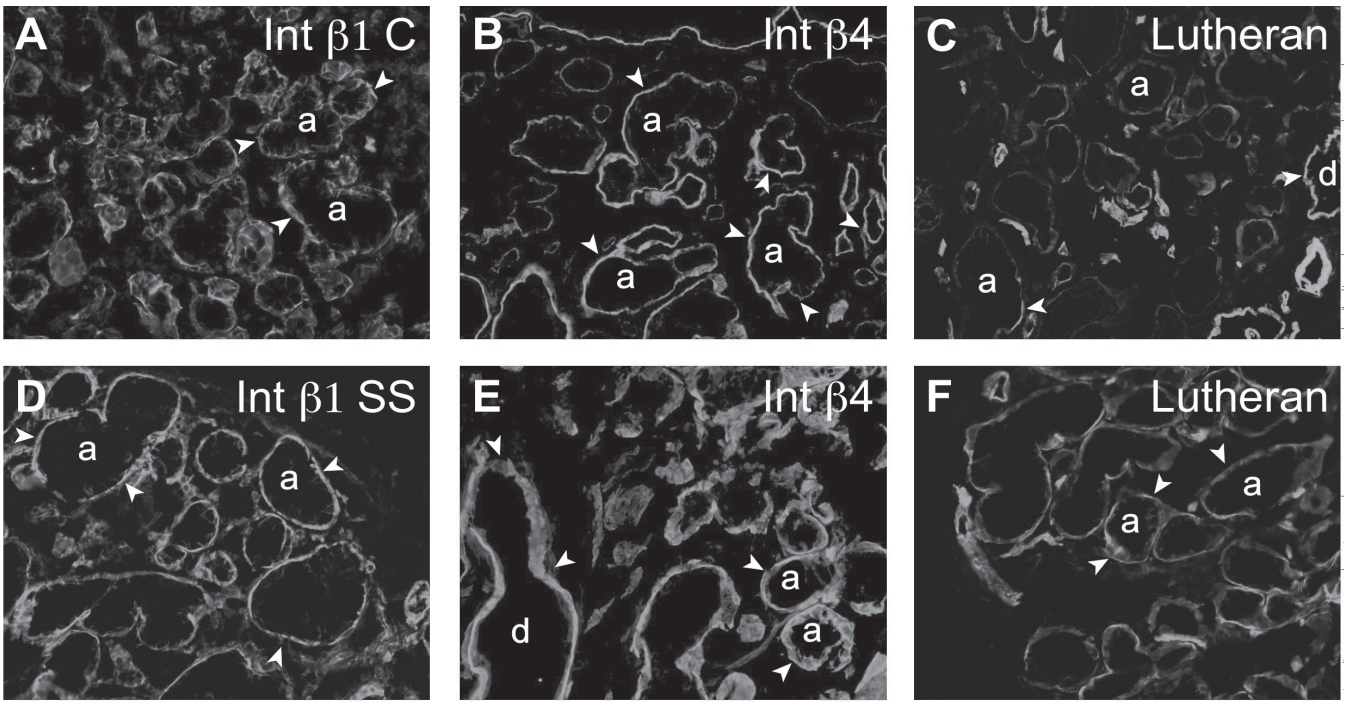

Fig. 3. Distribution and staining patterns of Int β1 and β4 subunits and Lutheran blood group antigen in labial salivary glands of healthy controls (A-C in the upper C row) and of patients with Sjögren's syndrome (D-F in the lower SS row) were similar. A, Int β1 subunit; B, Int β4 subunit; C, Lutheran blood group antigen; D, immunoreactivity for Int β1 subunit in Sjögren's syndrome; E, Int β4 subunit in Sjögren's syndrome; F, Lutheran blood group antigen in Sjögren's syndrome. **a** = acinus, **d** = duct. Magnification x 200.

salivary BM components Lm-5/332 and Lm-10/511 (expressed both around the acini and ducts), it also binds Lm-1/111 and Lm-2/211 (1). This seems to be of interest in this context for several reasons. First, in contrast to Lm-332 and -511, Lm-111 and -211 have been earlier reported to only surround salivary acini (14) so if reasons for the acinar cell/secretory failure in SS are looked for, these two last mentioned laminins and their integrin binding seem to be of particular interest. Second, in contrast to Lm-332 and -511, which were reported to display a strong and continuous staining pattern indicating some structural role, Lms -111 and -211 had an interrupted and apparently haphazard staining pattern suggesting a dynamic functional role. Finally, the staining pattern of Lms -111 and -211 in patients with SS was reported to differ from that of the healthy controls. Laminin α1 chain was almost totally lacking in SS and laminin α2 chain staining was weak (14). Function-related ligand-receptor interactions may, apart from structural cell-to-substrate binding, affect multiple different signal transduction pathways. They can have diverse effects on their target cells. For example, Int α6β4 stimulates the growth of normal epithelial cells and promotes differentiation of breast and prostate cells (36, 37). The weak expression of Lms -111 and -211 in SS would seem to impair acinar cell regulation via these laminins in diseased salivary gland acini. Apart from their structure supporting role, integrin and non-integrin laminin receptors may also regulate acinar cell function.

In contrast to the universal, all-around integrin receptors as those mentioned above, integrins α1β1 and α2β1 were found almost exclusively around the acini suggesting some acinar cell compartment-specific or -dominant function. It is probably relevant in this regard that these two integrin receptors form the major integrin receptors for the acinar compartment specific laminins Lm-111 and -211 (38) although they in some cells may also bind type IV collagen (39). In healthy control glands Int α1β1 and α2β1 were expressed in an interrupted and irregular pattern. Although double staining experiments were not performed, this interrupted and irregular pattern may match with their similarly distributed basement membrane ligand counterparts Lm-111 and Lm-211 (14). This indicates that Int α1β1 and α2β1 also in salivary glands bind to their Lm-111 and Lm-211 ligands to mediate cell-to-BM interactions.

Perhaps the most interesting finding of the present work was that the staining for Int α1 and α2 chains was very weak in the minor salivary gland cells in SS. As the corresponding α1β1 and α2β1 integrin receptors bind to Lm-1 and -2, which are also weakly expressed in SS (14), it seems that the Lm-1 and -2-to-Int α1β1 and α2β1 mediated signalling is defective in SS. This may relate to two central events in the acinar cell failure in SS. Integrin β1 did not differ between healthy controls and patients with SS, probably as it is also able to form heterodimers with alpha integrin chains other than α1 and α2.

The first set of observations, which seems to tie defective expression of Lm-111 and -211, on one hand, and of Int α1β1 and α2β1, on the other hand, to SS, is the tentative roles of Lm-111 and Lm-211 in synaptogenesis (40-42).

It has been known for some time that in some patients with SS of relatively short duration the impaired secretory function (diminished resting salivary flow) can not be explained by loss of the secretory glandular parenchyma. Several suggestions have been made to explain this impaired secretory function, such as the putative acetylcholine muscarinic 3 receptor blocking autoantibodies (43) and false translocation of the aquaporin-5 water channels (44). There is also some earlier evidence for a defective coupling between the postganglionic parasympathetic nerve terminals and acinar cells in heavily inflamed salivary gland tissue in the SS patients. This change, however, was focal and topologically limited to those glandular areas infiltrated by inflammatory lymphocytes (45, 46). It can hardly explain a global impairment of the glandular function. In contrast, the diminished expression of Lms -111 and -211 (14) together with that of Int $\alpha1\beta1$ and $\alpha2\beta1$ was seen in all acini. Lm -111 and -211 and their integrin receptors have been implicated in target recognition, differentiation and/or stabilization of synapses (47, 48). Defects in the synapses between the postganglionic acetylcholine containing parasympathetic nerve terminals and postsynaptic muscarinic receptor carrying acinar cell membrane could lead to impaired stimulus-response coupling and contribute to the secretory impairment.

Second, acini are subjected to continuous remodelling, which based on observations made in mice and rats may be quite rapid: their salivary glands are renewed approximately twice every year (49). This continuous remodelling means that when an acinar cell dies, it leaves an empty lot on the acinar BM. Although the acinar cell mass of the minor salivary glands diminishes somewhat upon aging (50), in a shorter perspective it is quite well maintained. This means that the lost apoptotic acinar cells are replaced. Recent evidence suggests that this occurs via recruitment from the progenitor pool located in the intercalated ducts. This short tubular segment of the salivary duct is located between the secretory acinus and saliva modulating striated duct. The simple and undifferentiated phenotype of the intercalated duct epithelial cells is not suggestive of any specialized function. Instead, it has been concluded that they form an undifferentiated progenitor cell pool. When such a cell divides, it is supposed that one of the two daughter cells migrates to the acinus into the now empty lot. In the acinus, it receives a differentiation signal, which in part derives from the acinar basement membrane and which induces maturation of the undifferentiated intercalated duct progenitor cell into a mature secretory acinar cell. This view has been supported by findings, which indicate that cloned intercalated duct cells have such a potential, i.e. that they can differentiate into acinar cells (51). Second, cells belonging to an immortalized human salivary HSG cell line, which have an intercalated duct epithelial cell phenotype, are induced to differentiate into acinar cells upon culture on Lm-111 containing substrate (20). They aggregate to clusters, in which the central cells not in contact with Lm-111 undergo apoptosis. A lumen is formed at the same time as the surviving cells differentiate and start to express acinar cell markers, such as salivary amylase and cystatin C not expressed by undifferentiated, intercalated HSG cells (52). Thus, the lack of Lms -111 and -211-to-Int $\alpha1\beta1$ and $\alpha2\beta1$ signaling might lead to an inability to maintain the acinar cells and acini, which is compatible with the well established but hitherto unexplained acinar cell atrophy and ductal epithelial cell hyperplasia in SS.

In conclusion, the acinar cell compartment specific Lms -111 and -211-to-Int $\alpha1\beta1$ and $\alpha2\beta1$ signalling may play an essential role for the maintenance of the structure and function of the healthy human labial salivary gland. Failure of this system may contribute to loss of function, e.g. via impaired synaptogenesis and acinar cell renewal. In SS the hyperplastic ducts might represent former acini surrounded by BM unable to stimulate intercalated duct-to-acinar cell transdifferentiation.

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