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Discoid Lupus Erythematosus Exacerbated by Radiation Therapy
（角質細胞接着における正常と疾患）

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Order and disorder in corneocyte adhesion

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ABSTRACT

Epidermal cornified cells are attached to each other with modified desmosomes, namely corneodesmosomes. Changes in corneodesmosome degradation process influence the total thickness of stratum corneum and the surface appearance of the skin. The major extracellular constituents of corneodesmosomes are desmoglein 1, desmocollin 1 and corneodesmosin. The intracellular part of corneodesmosomes is cross-linked into cornified cell envelopes. Corneodesmosomes are degraded from the central surface area of each cell. Peripheral corneodesmosomes retain structural integrity up to the skin surface. A hypothesis where tight junctions in the stratum corneum play a role in this spatial difference in corneodesmosome degradation has recently been proposed. Genetic defects in corneodesmosin and inhibitors for proteases involved in corneodesmosome degradation result in accelerated desquamation and severe barrier impairment, presenting as the inflammatory type of peeling skin syndrome and Netherton syndrome, respectively. Abnormal corneodesmosome degradation is also found in more common skin diseases including ichthyosis vulgaris, atopic dermatitis, psoriasis vulgaris, lichen planus and soap-induced xerosis.

INTRODUCTION

Skin is the largest organ in the body and its main function is to cover and protect the body. The protective barrier functions reside largely in the stratum corneum of the epidermis, the most superficial layer of the skin.[1-4] Structural integrity of the stratum corneum is maintained by cornified cells interconnected with corneodesmosomes, which are modified desmosomes found in the stratum corneum. The relatively steady thickness of the stratum corneum is maintained by strictly controlled degradation of corneodesmosomes.[5] Accelerated corneodesmosome degradation leads to severe barrier defects such as those seen in Netherton syndrome (NS) and peeling skin syndrome (PSS), as well as delayed degradation results in hyperkeratosis seen in conditions such as ichthyosis vulgaris and soap-induced xerosis. In this review, we give a synopsis of the current research findings regarding the formation and degradation of corneodesmosomes, as well as the pathological mechanisms of some diseases characterized by abnormal corneocyte adhesion and/or dissociation.

NORMAL CORNEOCYTE ADHESION AND DESQUAMATION

What holds corneocytes together, lipids or corneodesmosomes?

For a long time, a two-component model of the stratum corneum, the ‘bricks and mortar’ model proposed by Elias[6] has been accepted as the basic theory behind the structure and function of the stratum corneum. Here, the bricks are the corneocytes and the mortar is the intercellular lipid. The intercellular lipid is purported to provide both barrier and cohesive functions. There is little doubt regarding its importance in forming the water permeability barrier,[7] but its significance as a cohesive component is rather controversial.

As a corneocyte adhesion structure, the corneodesmosome has drawn attention, because its role in cell adhesion has been established by Chapman *et al*. who examined the relative contribution of three factors: (1) extracellular lipids; (2) corneodesmosomes; (3) lipid envelopes to corneocyte cohesion.[8] Cohesion strength of the outer stratum corneum was measured directly by cohesometry. Removing intercellular lipid component did not decrease adhesiveness of stratum corneum, but increased it.[8,9] Corneodesmosomes seem to be the major determinant of stratum corneum cohesiveness. Furthermore, recent corneodesmosin knockout studies on mice and in humans clearly demonstrated critical roles of corneodesmosomes in cell adhesion (see below).[10-12]

Distribution and structures of corneodesmosomes

In normal human skin, the stratum corneum displays a basket-weave like appearance all over the body except for the palms and soles. The cells there are attached exclusively at the cell periphery by corneodesmosomes (Figs 1, 2 and 3).[13-15] Corneodesmosomes are found only at the cell edges in the skin's surface, while they are diffusely distributed around the cell surface in the deep layers. Loss of corneodesmosomal components in the central areas of the cell surface starts in the deep cornified layers.

A corneodesmosome is a modified form of desmosome which differs ultrastructurally (Fig. 4).[13,16-18] There is an electron dense mid-line structure in the extracellular parts (desmoglea) of desmosomes. When desmosomes are transformed into corneodesmosomes between the stratum granulosum and the stratum corneum, desmoglea loses its tri-lamellar structure and becomes a homogeneously electron dense 'plug'. On the cytoplasmic side, components of the attachment plaques (desmosomal plaques) are cross-linked into and become parts of cornified cell envelopes.[19]

Components of corneodesmosomes

Desmosomes are composed of several cytoplasmic and transmembrane proteins.[20,21] The latter are members of the cadherin families known as desmogleins and desmocollins. Desmoglein 1 and desmocollin 1 constitute extracellular parts of corneodesmosomes as well.[22-25] Corneodesmosomes have an additional unique extracellular component known as corneodesmosin. Corneodesmosin is a 52-56 kDa glycoprotein produced by keratinocytes.[23,26] It is stored and secreted by lamellar granules. After the secretion from the apical cell surface of granular cells, corneodesmosin is localized in the extracellular structures of corneodesmosomes and covalently crosslinked to the cornified cell envelopes. This coincides with the morphological transformation of desmosomes into corneodesmosomes (Fig. 4). Expression of a chimeric protein consisting of the N-terminal domain of corneodesmosin and the transmembrane domain of E-cadherin promotes cell-cell aggregation in transfection experiments.[27] This suggests that corneodesmosin mediates homophilic binding to counterparts on adjacent corneocytes. During corneocyte maturation, corneodesmosin is progressively proteolyzed. Its actual function as an adhesive molecule has recently been revealed by severe skin phenotypes found in corneodesmosin knockout in mice and humans (see below).

What is known about corneodesmosome degradation?

Both exogenous and endogenous proteases are implicated in cleavage of the corneodesmosome junctions. Among the endogenous proteases are kallikrein-related peptidases (KLKs) and cathepsins, both produced by keratinocytes. KLKs constitute a

family of 15 (chymo) trypsin-like serine proteases (KLK1-15) and function through proteolytic cascades.[28-31] In the skin, at least eight KLKs, including KLK5 (stratum corneum tryptic enzyme) and KLK7 (stratum corneum chymotryptic enzyme) are expressed and secreted into the extracellular space at the transition point between the granular and cornified layers.[32] KLK14 is unique in its high expression in the plantar epidermis.[33] Because KLK5 can activate itself as well as other KLKs, KLK5 was considered to be the initiator of KLK activation cascades.[28-34] However, this explanation for KLK5 auto-activation has been questined. It has been shown recently that KLK5 needs to be activated by the membrane protease matriptase to gain stronger activation.[35] Activated KLK5, KLK7, and KLK14 then degrade corneodesmosomal components.[31]

Two cysteine proteases, cathepsin V (also called cathepsin L2 or stratum corneum thiol protease) and cathepsin L-like enzyme,[36] as well as one aspartic acid protease, cathepsin D are all involved in corneodesmosomal degradation.[37,38]

A *Staphylococcus aureus* exogenous protease known as V8 has been implicated in corneodesmosomal degradation.[39] Topical application of V8 protease was shown to cause loss of corneodesmosome integrity, corneocyte cohesion, and increased epidermal permeability. This may be relevant in the pathological mechanisms of atopic dermatitis (AD), because skin lesions are often colonized by *Staphylococcus aureus.*

Inhibitors of desquamation enzymes

Several protease inhibitors are implicated in the regulation of desquamation-associated proteolysis. Among them is polyprotein lympho-epithelial Kazal-type-related inhibitor (LEKTI) encoded by *serine specific inhibitor Kazal type 5 (Spink5).* LEKTI consists of 15 Kazal-type serine protease inhibitory domains.[40] It is expressed in the granular layer

of the epidermis and secreted into the extracellular space.[41] Its fragments inhibit epidermal KLK5, -7, and -14 forming a tight binding complex.[42] A model in which pH controls KLK activities by regulating their interaction with LEKTI has been proposed.[42] Here, in the deep stratum corneum, neutral pH allows strong interaction between LEKTI and its KLK targets, thus preventing corneodesmosomal cleavage. As the pH acidifies moving upward, LEKTI and KLK dissociate, allowing proteases to progressively degrade its corneodesmosomal targets.

LEKTI2, encoded by *Spink9,* has recently been identified as a KLK5-specific inhibitor highly expressed in the palmo-planter epidermis.[43,44] Still another KLK inhibitor found very recently is Spink6.[45] While it is most prominently expressed at the palmoplantar sites Spink6 is expressed in the epidermal granular cells at the different body sites including the face, arms, trunk, and legs while inhibiting KLK5, KLK7 and KLK14. Recombinant Spink6 inhibits desquamation of plantar skin *ex vivo* as well.

Two other serine protease inhibitors implicated in desquamation control are skin-derived anti-leukoproteases and secretory leukocyte protease inhibitor (SLPI).[46] Both inhibitors have the ability to effectively reduce desquamation *in vitro*. In particular, SLPI is a potent inhibitor of KLK7. A cysteine protease inhibitor, cystatin M/E inhibits cathepsin V.[47,48] Cystatin M/E is highly expressed in the epidermis and co-localizes with cathepsin V on (corneo) desmosomes after secretion. Alpha-2 macroglobulin-like 1 (A2ML1) is a novel epidermal pan-protease inhibitor expressed and secreted in the granular layer.[49] It can bind KLK7 and may also bind cathepsin L2 and cathepsin L-like enzyme. This suggests that A2ML1 may play a role in controlling the desquamation process. Cholesterol sulphate acts as a potent inhibitor of serine proteases.[50] Zn^{2+} is also a very potent inhibitor of different KLKs, including KLK5 and KLK7.[51,52]

Activators of desquamation enzymes

Matriptase is a transmembrane serine protease expressed in various epithelial tissues including the epidermis and hair which undergoes efficient autoactivation. It has been shown recently that matriptase is an efficient activator of epidermal KLKs and initiates the proteolytic cascade reaction.[35]

KLKs involved in desquamation have optimum activity at slightly alkaline pH levels, but healthy skin has an acidic pH. Use of soap and detergents on the skin can activate epidermal proteases by increasing skin pH.[53]

Spatially different degradation of corneodesmosomes

The reason corneodesmosomes are present only at the cell periphery in the upper stratum corneum remains unknown. However, we have recently proposed a model suggesting that tight junctions may play a role in the spatially different degradation process of corneodesmosomes (Fig. 5).[15] The presence of tight junctions in the epidermis has generally been appreciated in the stratum granulosum.[54] We found tight junction-like structures and immunoreactivities against occludin and claudin-1 in the cell periphery of cornified cells (Figs 6 and 7). These tight junction-like structures were sandwiching peripheral (corneo) desmosomes. A tracer perfusion assay and distribution analysis of KLK 7 suggested that tight junction-like structures may protect peripheral corneodesmosomes from premature degradation.

ABNORMAL CORNEOCYTE ADHESION AND DESQUAMATION

LEKTI and Netherton syndrome

NS (MIM 256500) is a rare autosomal recessive disorder characterized by severe ichthyosis, hair-shaft defects (bamboo hair) and atopic features caused by mutations in the *SPINK5* gene encoding LEKTI (Fig. 8a).[55] Insufficient LEKTI activity results in increased proteolytic activity of KLKs and premature desquamation.[25,56] Recently, matriptase was shown to initiate disease onset in a mouse model of NS by activating epidermal KLKs.[35] Ablation of matriptase from NS-model mice prevented detachment of stratum corneum and improved the barrier function of the epidermis. This adds new insights into development of effective treatment for NS.

Corneodesmosin and peeling skin syndrome

Importance of corneodesmosin in the skin barrier function was first highlighted by lethal phenotypes of corneodesmosin knockout mice.[10,11] In the skin of these mice, corneodesmosomes were structurally abnormal and the stratum corneum was detached from the granular layer.[10] Next, in human skin, Oji *et al*. found that the inflammatory type of PSS (MIM 270300) is caused by a homozygous nonsense mutation in the corneodesmosin gene.[12]

PSS is a rare autosomal recessive genodermatoses characterized by superficial exfoliation of the outer epidermis.[57,58] Several types of PSS have been described.[58-60] Two main subtypes are noninflammatory type A and inflammatory type B.[58] Type A is characterized by noninflammatory and asymptomatic peeling, with general health remaining intact and its causative genetic defects remain unknown. In type B, there are erythematous migratory patches with a peeling border; pruritus or burning may be conspicuous (Fig. 8b). Type B may also be associated with several noncutaneous anomalies and various abnormal laboratory findings. Overlapping features between PSS

type B and NS have been reported in the literature.[58,60] Those include autosomal recessive inheritance, lifelong skin shedding with erythema, pruritus, epidermal hyperplasia, and increased serum IgE levels. Although characteristic hair abnormalities and double-edged scales of NS are missing in PSS, differential diagnosis can sometimes be difficult. Some argued that PSS type B and NS might be the same disease.[58] This is perhaps not surprising since LEKTI is a serine protease inhibitor involved in the degradation of corneodesmosin.[42]

Matriptase and ichthyosis with hypotrichosis

Missense mutations in the *ST14* gene encoding matriptase, underlie autosomal recessive ichthyosis with hypotrichosis syndrome (ARIH, MIM 610765) characterized by congenital ichthyosis, abnormal hair and corneal involvement.[61,62] *ST14* hypomorphic mice with minimal expression of epidermal matriptase mRNA, demonstrated a phenotype resembling that of ARIH.[63] Because matriptase initiates KLK-activation cascades involved in desquamation as described above, ichthyosis in ARIH is likely to be caused by decreased corneodesmosome degradation. This hypothesis is supported in a study in which we observed of ARIH skin which had an increased number of corneodesmosomes.[61] Still others emphasize the importance of decreased profilaggrin processing in the pathogenesis of ichthyosis.[63]

More recently, it has been elucidated that congenital ichthyosis, follicular atrophoderma, hypotrichosis, and hypohidrosis (IFAH, MIM 602400) are caused by loss-of-function mutations in *ST14*.[64] This is an autosomal recessive syndrome characterized by diffuse congenital ichthyosis, patchy follicular atrophoderma, generalized and diffuse nonscarring hypotrichosis, marked hypohidrosis, and woolly

hair. There are some overlapping features between ARIH and IFAH. *ST14* knockout mice died shortly after birth.[65,66] Although the skin showed malformation of the stratum corneum, an epidermal barrier defect, and generalized follicular hypoplasia reminiscent of IFAH, the phenotypes of matriptase knockout are much more severe in mice than in humans.

Other skin diseases

Abnormal corneocyte desquamation occurs in more common diseases as well. Increased stratum corneum serine protease activities were detected in AD acute eczematous lesions.[67] Although loss-of-function mutation in the filaggrin gene was found to be the most significant genetic factor predisposing patients to AD, not all patients carry the filaggrin gene mutations.[68] Since NS shares several features with AD, involvement of LEKTI in pathogenesis has been speculated. Association with Glu420→Lys polymorphism of the *SPINK5* gene was reported in patients with AD from different ethnic groups.[69-71] Decreased expression of LEKTI was found in keratinocytes from AD patients carrying the *SPINK5* polymorphism as well.[72] A 4-bp insertion in the 3' untranslated region of the *KLK7* gene which may lead to increased proteolytic activities in the stratum corneum is significantly associated with AD.[73] *Staphylococcus aureus* extracellular protease may also be involved in corneodesmosomal degradation in AD skin.[39]

In ichthyosis vulgaris skin, strong corneodesmosin staining was detected at the skin surface.[74] Serine protease activity of the stratum corneum was lower in ichthyosis vulgaris.[75] Reduced corneodesmosome degradation and increased expression levels of desmoglein 1 was reported in the stratum corneum of soap-induced xerosis.[76] A reduced

degradation of corneodesmosomal proteins was also seen in psoriatic lesions.[77] In the hyperkeratotic skin lesions of psoriasis and lichen planus, abnormal distribution patterns of desmoglein 1 were detected on the tape-stripped cornified cells.[78]

Among experimental animal conditions where abnormalities in corneodesmosomes might be involved in pathogenesis are as follows. A null mutation in the *cystatin M/E* gene in mice results in abnormalities in cornification and desquamation as well as neonatal lethality.[48,79] Transgenic mice which express excessive KLK7 developed a scaly skin phenotype with epidermal hyperplasia, dermal inflammation, and severe pruritus.[80] Mice deficient in cathepsin D showed impaired stratum corneum morphology as well.[81]

CONCLUSIONS

We have reviewed factors involved in the formation and degradation of corneodesmosomes. We also reviewed corneodesmosome abnormalities found in skin diseases. We hope that this knowledge can help us to develop effective strategies to correct abnormal desquamation processes in various skin diseases.

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Figure legends.

Figure 1. Corneodesmosin immunofluorescent staining of normal human skin. Cytoplasm of the granular cells (G) and cell attachment sites in the stratum corneum (C) are positively labeled. Paraffin-embedded sections were deparaffinized and warmed in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 9.0) for 18 min at 95°C and incubated with polyclonal antibodies against a central domain of human corneodesmosin (green). The sections were stained with highly cross-absorbed Alexa-Fluor 488 goat anti-rabbit IgG (Molecular Probes, Eugene, OR). Nuclei were stained with DAPI (blue).

Figure 2. Desmoglein 1 immunofluorescent microscopy of tape stripped cornified cells. Note that only cell periphery is positively stained in the corneocytes from the cell surface (a), but the whole cell surface is positive in the cell from a deeper layer (b). Stained as described by Naoe et al.[14]

Figure 3. Corneodesmosin immunoelectron microscopy. (a and b) In the cellular border between the fourth and fifth cornified cells, corneodesmosomes with positive immunolabels are only detected at the periphery close to the edge of the fifth cell (red arrow). No corneodesmosomes are seen in the central area (red bracket). In figure b, the fifth cell is colored pink and corneodesmosomes in green, in order to better visualize the special relationship. (c) A higher magnification view of the marked rectangular areas shown in a and b. C1 to C6, the first to the sixth layer of the stratum corneum. G, granular layer. Stained with the method described in a previous study.[15]

Figure 4. Structural differences between desmosomes (a) and corneodesmosomes (b).

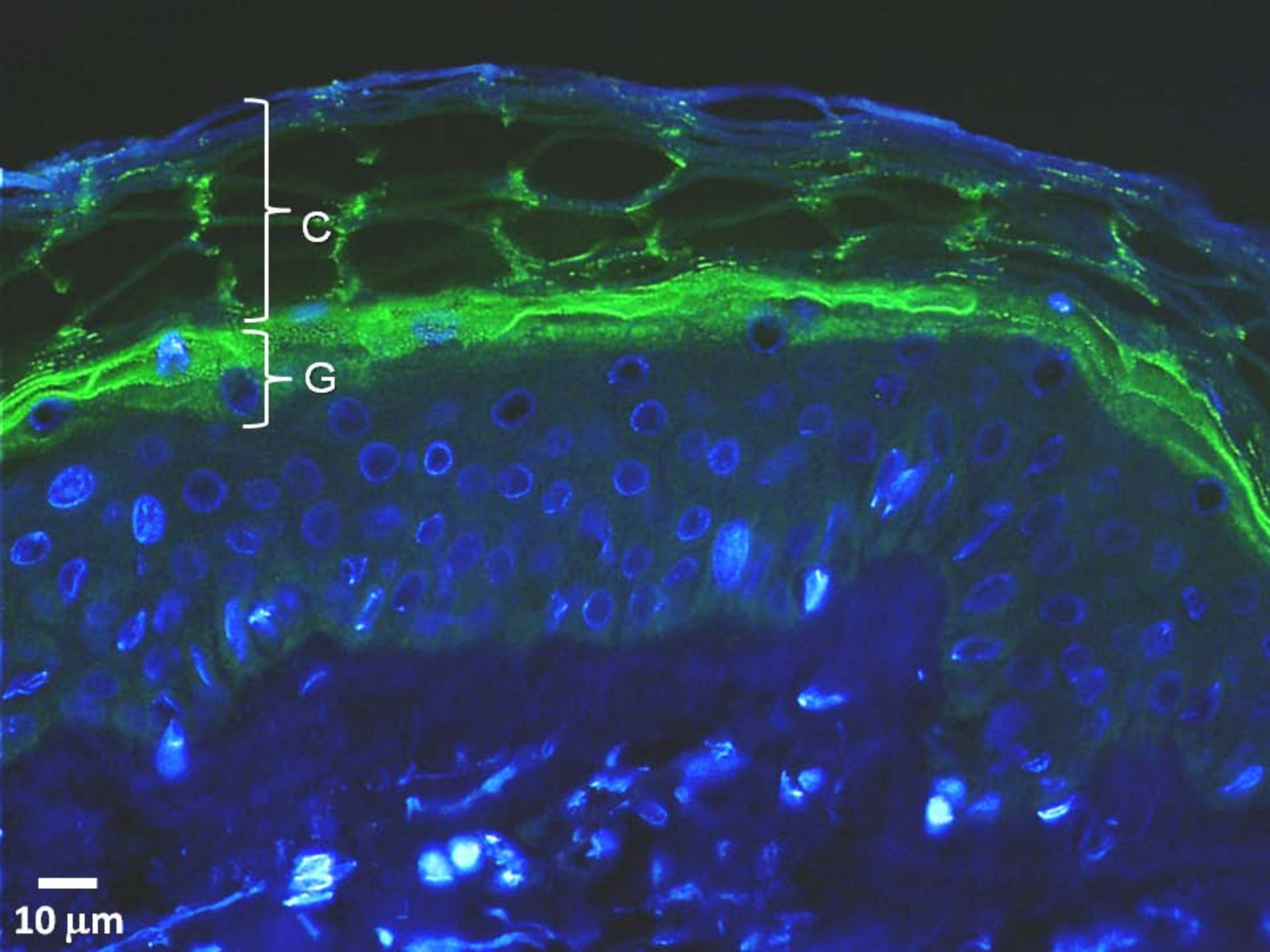
Note tri-lamellar structure of the desmoglea (arrowheads) of desmosomes (D) and homogeneously electron dense desmoglea of corneodesmosome (CD). Transmission electron microscopy.

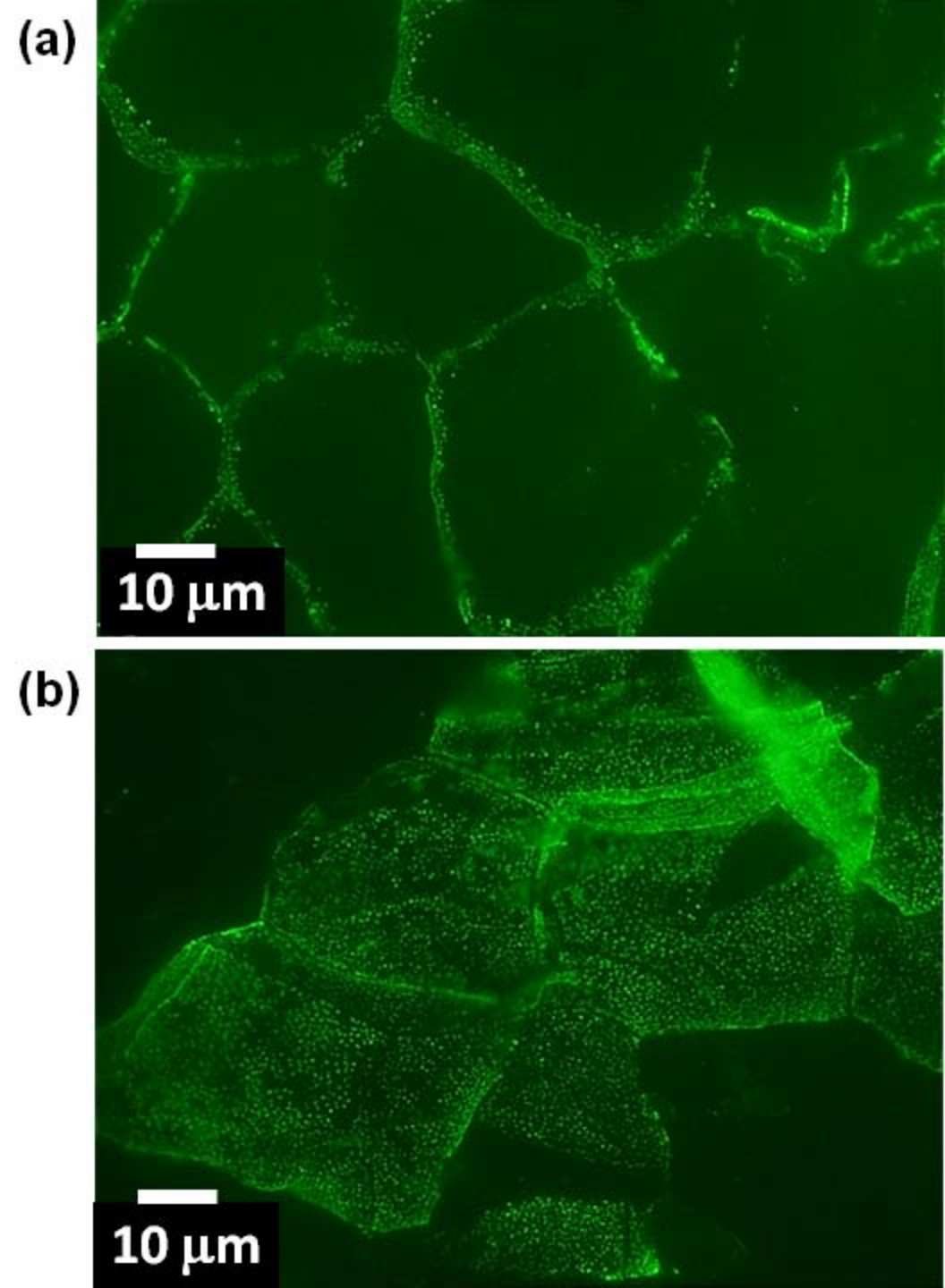
Figure 5. A hypothetical model to explain special differences in the desquamation process. Proteases involved in desquamation (yellow) and their inhibitors (green) are secreted from the apical surface of granular cells (G). Desquamation enzymes released from inhibition by their inhibitors degrade corneodesmosomes (black) in the stratum corneum (C). However, peripheral corneodesmosomes shielded by tight junctions (magenta) are not degraded up to the skin surface. T, transitional cell.

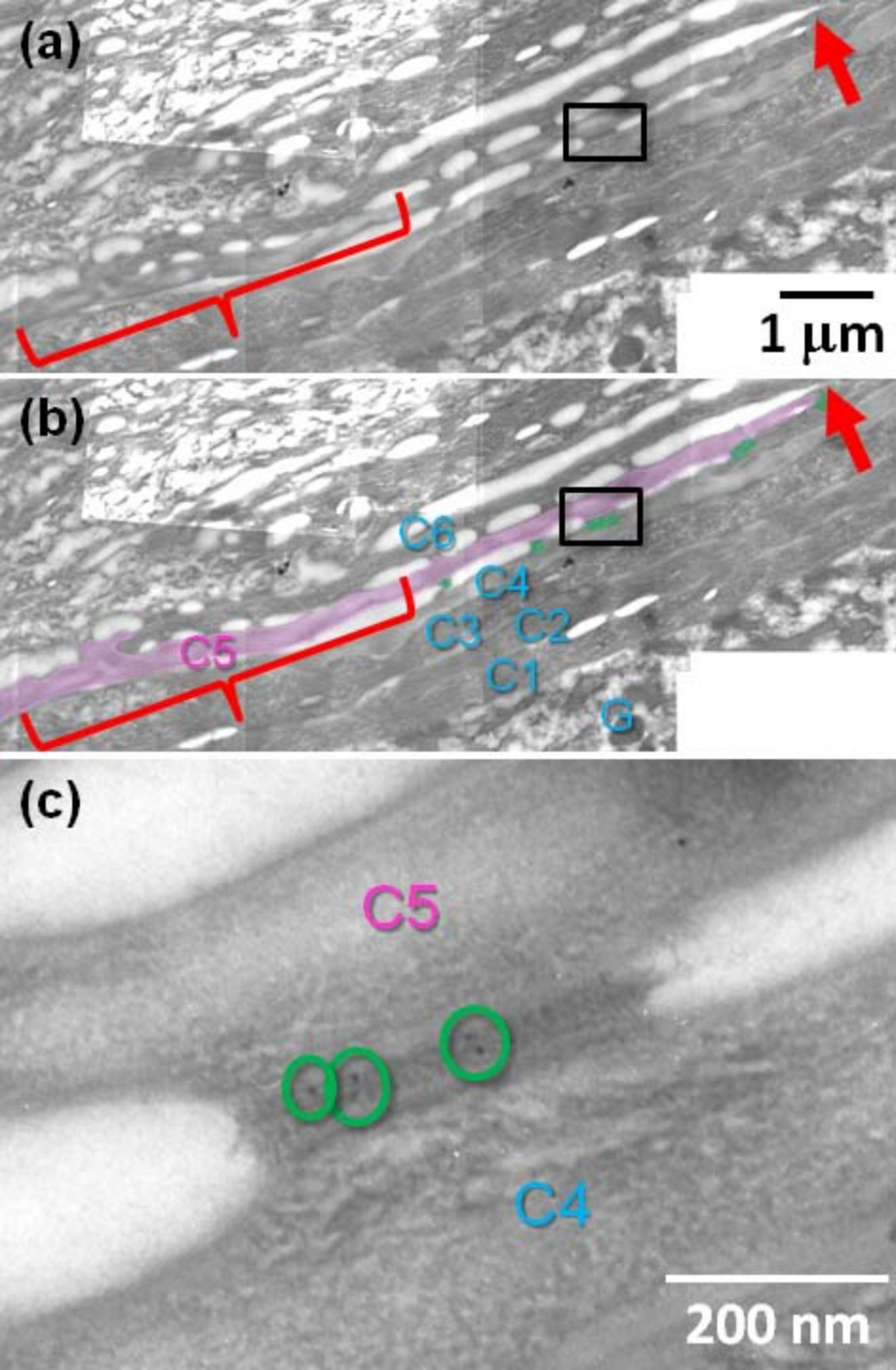
Figure 6. Tight junctions (red) in granular cells (a) and cornified cells (b). Transmission electron microscopy of normal human skin. C, cornified cell, CD, corneodesmosome. D, desmosome. G, granular cell.

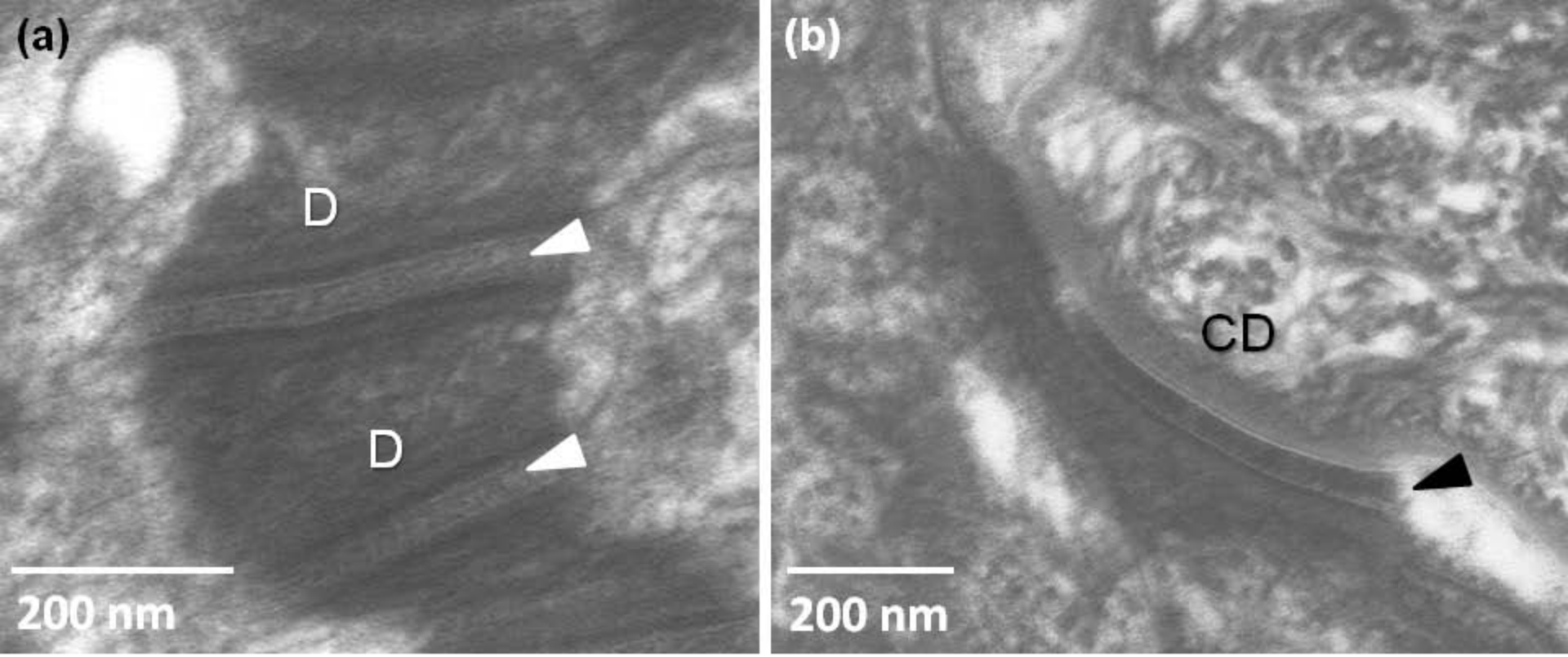
Figure 7. Occludin immunostaining of normal human skin. (a) Occluding positive staining is seen in the granular layer (arrowheads) and possible cornified cells (arrows). Immunofluorescent microscopy. Nuclei are stained with propidium iodide (orange). (b – d) Occludin immunoelectron microscopy. (b) In the granular cells, occluding labels (arrows) are detected at the cell membrane. (c and d) In the stratum corneum (C), occluding labels are seen along the cornified cell envelopes at the periphery. Panel d is a higher magnification view of the marked rectangular areas shown in panel c. The staining method was described in a previous study.[15] D, desmosomes. G, granular layer.

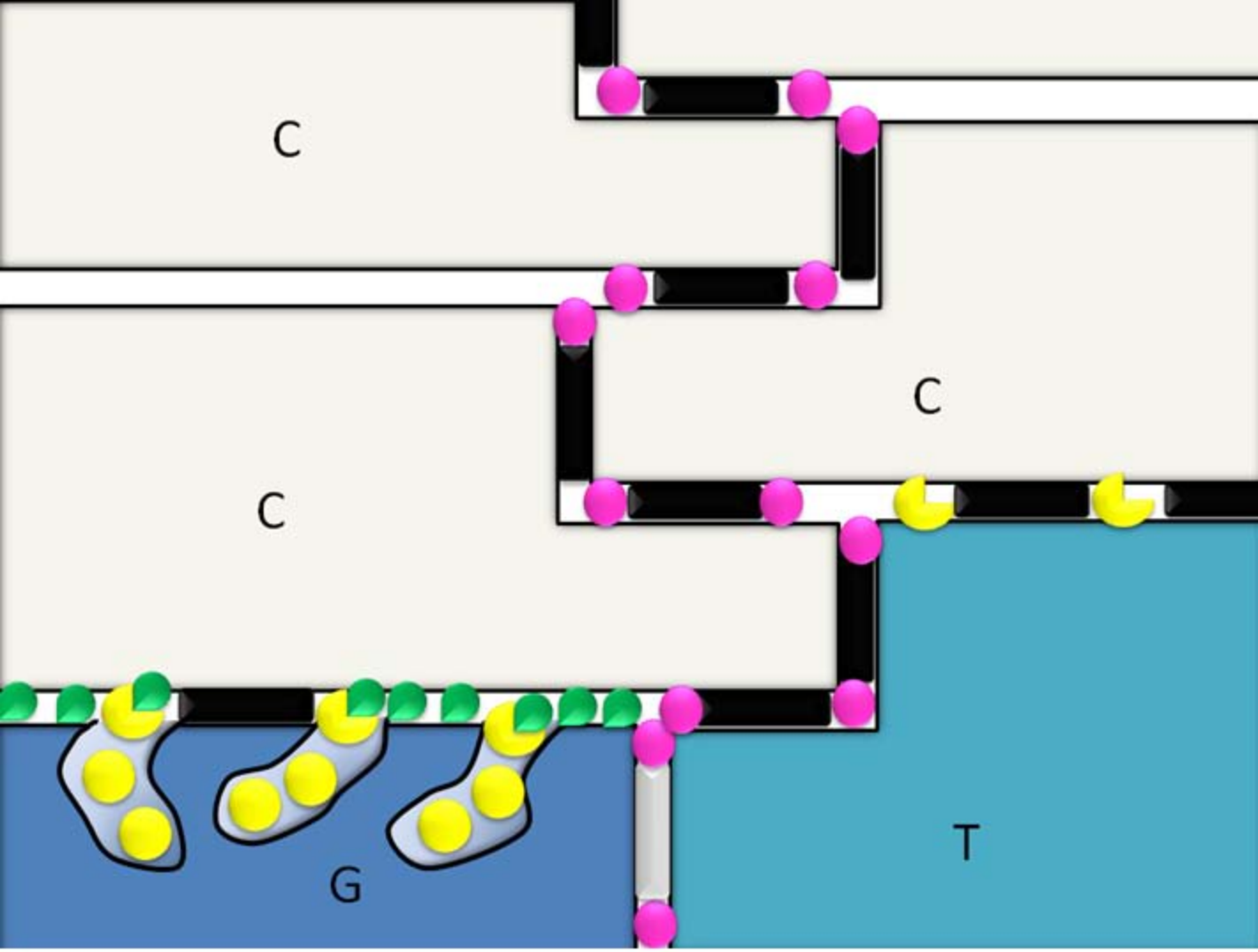
Figure 8. Clinical pictures of patients with Netherton syndrome (a) and the inflammatory type of peeling skin syndrome (b). Lamellar scaling is evident in both diseases.


C
G
10 µm


(a)
10 μm
(b)
10 μm


(a)
1 μm
(b)
C6
C4
C3
C2
C5
C1
G
(c)
C5
C4
200 nm


(a)
D
D
200 nm
(b)
CD
200 nm



Inactive proteases

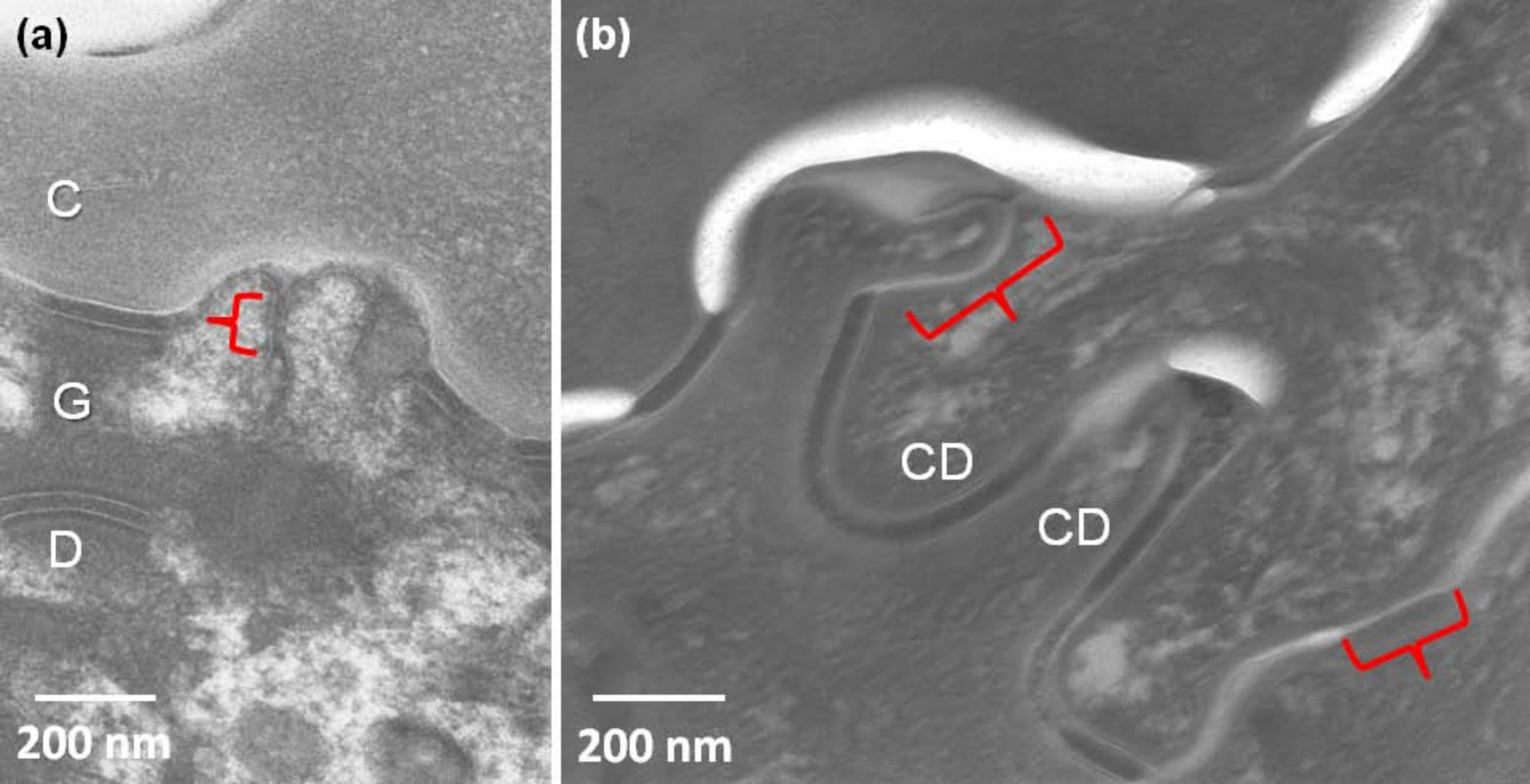
Activated proteases

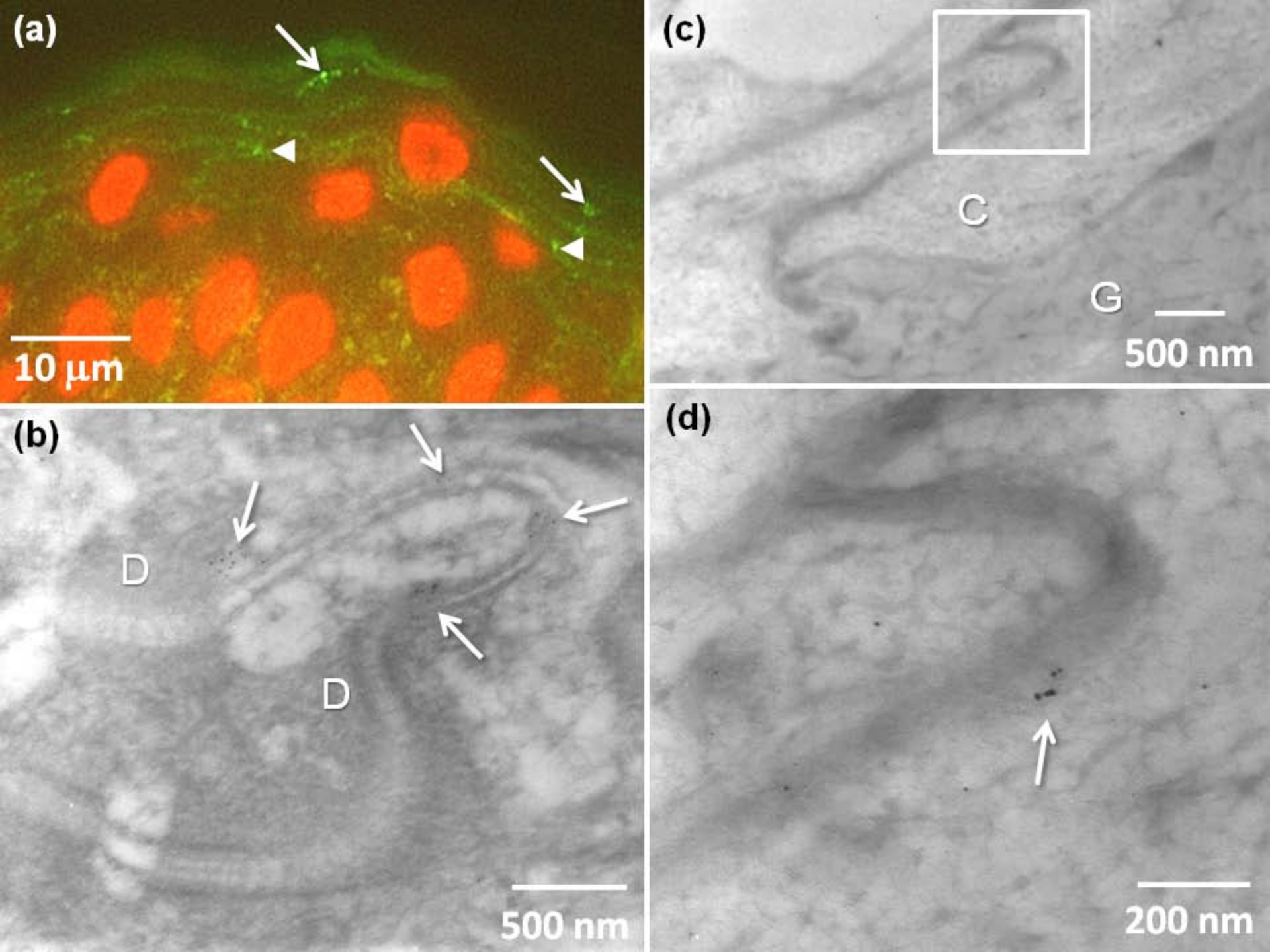
Protease inhibitors

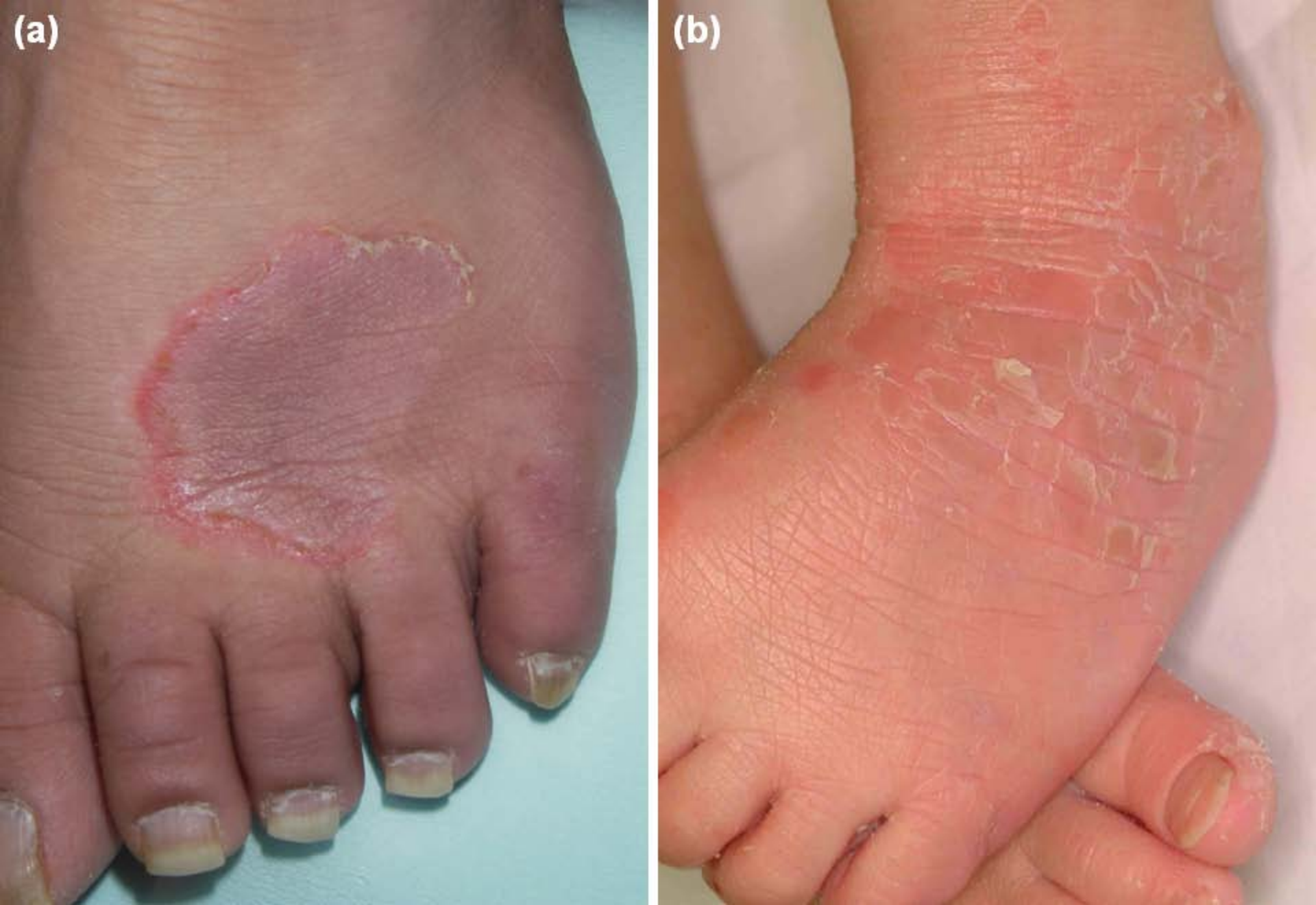
Tight junctions

Desmosomes

Corneodesmosomes


(a)
C
G
D
200 nm
(b)
CD
CD
200 nm


(a)
10 µm
(b)
D
D
500 nm
(c)
C
G
500 nm
(d)
200 nm


(a)
(b)