HUMAN SKIN IS AS AN EXTRA-PULMONARY SITE OF SECRETION OF SURFACTANT PROTEINS

İnsan derisi: bir ekstra pulmoner sürfaktan salınım bölgesi

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Abstract

Surfactant is a surface active material composed of both lipids and proteins which is produced by alveolar type II pneumocytes in the lung. Surfactant enhances pathogen clearence and regulates adaptive and innate immune-cell functions. Recently, the presence of surfactant proteins in human skin, being found in variable amounts in epidermis, dermis, hair follicles, sweat and sebum was reported. These surfactant proteins show characteristics that may be critical to local barrier and defence functions of the skin. Review data demonstrates that human skin expresses surfactant proteins constitutively in skin tissue. This editorial describes the surfactant proteins and their possible function within the skin

Key Words: *Skin; Surface - Acitve Agents.*

Özet

Surfaktant yüzey aktif materyal olup lipit ve proteinlerden oluşur ve akciğerlerde alveolar tip II pnömositler tarafından üretilir. Surfaktant, patojenlerin arındırılması ile adaptif ve immün hücre fonksiyonlarının düzenlenmesini artırır. Surfaktant proteinlerinin, insan deri dokusunda ve farklı oranlarda, epidermis, dermis, kıl folikülleri, ter ve sebumda bulunduğu yakın zamanda gösterilmiştir. Derinin bölgesel bariyer ve savunma mekanizmasında surfaktant proteinlerinin kritik moleküller olduğu gösterilmiştir. Derlemede sunulan veriler insan deri dokusunun surfaktant proteinlerinin, derinin bir bileşeni olarak salgılandığını göstermektedir. Bu derleme, surfaktant proteinlerinin derideki olası fonksiyonlarını tanımlamaktadır.

Anahtar kelimeler: *Deri; Yüzey aktif maddeler.*

Introduction

Surfactant is a surface active substance which is synthesized by alveolar epithelial type II cells of lungs. Surfactant is composed of 80 % phospholipids, 10 % proteins and 10 % neutral lipids. At present four surfactant proteins have been identified and characterized in the lung, surfactant protein-A (SP-A) (1), surfactant protein-B (SP-B) (2) , surfactant protein-C (SP-C) (3) and surfactant protein D (SP-D) (4). SP-A, SP-D and other collectins have C-type (calcium dependent) lectin activity. Collectins have collagen like amino (N) terminal regions and C-type carbohydrate recognition domains (CRDs). SP-A is the major surfactant protein. It is a hydrophilic glycoprotein with a molecular mass of 28-36 kDa and isoelectric points ranging from 4.8 to 5.2 (5) . SP-D (43 kDa reduced) consists of at least four distinct structural domains: a short, N terminus domain, a relatively long collagenous domain, a short thyroxine amphipathic peptide or coiled-coil neck domain, trimeric subunits (3x43 kDa) which associate at their N-terminus (6). Collectins bind Gram-positive and Gram-negative bacteria, viruses, fungi and allergens (7,8,9). Also, SP-A and SP-D play a major role within the immune system, participating in the initiation of and having further roles in non-antibody-mediated immune responses. Many studies have indicated that SP-A and SP-D can bind or agglutinate various bacteria and viruses usually through their CRD (Carbohydrate Recognition Domain)(10) . Basically, SP-A and SP-D enhance the affinity of pathogens to phagocytic cells, which results in elimination and later clearance of the pathogen.

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Geliş tarihi:17 Aralık 2003

Protein-carbohydrate interactions play an important role in two phases of the immune response, namely pathogen recognition and those cellular interactions that lead to pathogen neutralization. SP-A and SP-D are now considered to be "host defense molecules" exerting this effect within the lung by binding foreign bodies through their CRD regions) (11,12).

SP-B is a small (Mr: 8.7 kDa) lipid-associated hydrophobic protein presents in mammalian lung surfactant (amino acid residues 1-79) (13). SP-C is a lipoprotein, consisting of 35 amino acids with alfa-helical domains between the residues 9-14 (14). SP-B and SP-C are small hydrophobic and promote very rapid adsorption of lipids to the air liquid interface (15). SP-B and SP-C are derived from the proteolytic processing of much larger primary translation products encoded on human chromosomes 2 and 8 respectively (16,17). Both SP-B and SP-C significantly increase the ability of surfactant phospholipids to adsorb to the air-liquid interface and facilitate the formation of surfactant monolayer by accelerating the spreading of phospholipids and stabilizing the pulmonary alveoli (18).

Tissue distribution of SP-A and SP-D

Surfactant proteins are predominantly localized in the lungs. However, there is growing evidence that the presence of both SP-A and SP-D are not restricted to the lungs. The localization of the surfactant protein particularly in mucosal tissue and their presumed roles in controlling immune responses lead to their recognition as mucosal-associated collectins. Sites of extrapulmonary expression have also been descried in small mammals. Expression of the SP-A protein and its mRNA has been demonstrated in the rat small and large intestines (19) and intestinal lumen (20). Chailey-Heu et al. (21) reported the expression of both SP-A and SP-D in mesenteric cells. Motwani et al. (22) demonstrated the presence of SP-D gene product in the lung, heart, stomach, and kidney of rats by Northern blot and RT-PCR. Also, Fisher and Mason (23) demonstrated the location of both mRNA and SP-D protein in mucus –secreting cells in the gastric mucosa of rats, although not in the duodenum. Expression of SP-A and SP-D in porcine Eustachian tube has been detected (24). RT-PCR and Northern hybridisation of both SP-A and SP-D cDNA sequences showed 100 % homology between these proteins in lung and Eustachian tube. Madsen et al. (25) showed the tissue expression of SP-D in the lung and the kidney, brain, testis, pancreas, salivary gland, heart, prostate and small intestine as well as the placenta which also produced clear signals. Fisher and Mason (23) reported the SP-D message in RNA extracted from skin blood vessel in rats. Therefore, both SP-A and SP-D molecules can be considered to play a general role in primary host defence, in addition to their surface-tension lowering properties. SP-A, SP-B and SP-D have now been found to be widely distributed in a variety of no-pulmonary tissues including human skin (26). Kankavi and Roberts (27) showed that SP-A and SP-D are present in synovial fluid of normal horses. Another study of Kankavi (28) reported that human Eustachian tube expresses significant amount of SP-A, SP-B, SP-D and human organ of Corti SP-B and kidney SP-A and SP-D. Recent studies of Leth-Larsen et al. (29) have provided that SP-D is expressed in the female genital tract, the placenta and in amniotic fluid and suggested that endometrial SP-D may prevent intrauterine infection at the time of implantation and during pregnancy. MacNeill et al. (30) reported that SP-A is an innate immune factor, which is expressed in the vaginal mucosa and is present in vaginal lavage fluid. Recent studies of Condon et al. (31) showed that SP-A acts as mediator of parturition by signaling initiation of parturition.

Epidermal surfactant proteins

The skin is the body's largest organ (comprising more than 10 % of the body weight). Besides serving as the physical boundary of the body, it has many functions including thermoregulation, physical protection and integrity against dangers from the environment and contact with other objects, synthesis of vitamin D (requiring UV radiation), protection of inner tissues from dehydration and it also acts as a barrier preventing systemic infection from invading surface microorganisms, viruses and allergens (32).

The development in molecular genetic or recombinant DNA techniques has yield much information about skin structural proteins. Some of these skin structural proteins are specific for the epidermis. Epidermal structural skin proteins include actin, α-actinin, adducin, filaggrin, integrins, involucrin, keratins (skin specific), locicrin spectrin and trichohyalin. In the dermis, collagens, elastin, fibrillin, plectin and vimentin are the major proteins (33).

The skin develops from surface ectoderm and from mesoderm of the dermatome. The lateral plate mesenchyme develops gradually and progressively over the first six months, and end of the second trimester. Particularly, during the third trimester of human gestation there is a significant increase in the turbidity of the amniotic fluid, which surrounds the foetus in this period (34). The amniotic fluid turbidity may be related to an increase in lung-derived phospholipid and additionally to the presence of lamellar bodies derived from type II cells within lung tissue (35). Alternatively, this turbidity may arise primarily from detachment of the vernix caseosa away from the foetal skin surface (34-36). The mechanism underlying vernix detachment from the skin surface is still obscure. The vernix caseosa is a biologic film, consisting of lipids and proteins, with both hydrophilic and hydrophobic domains (37). This biofilm is probably formed through an increase in sebaceous gland activity, synchronized with the desquamation of foetal corneocytes during the last trimester of foetal life (38). The proteolipid film slowly increases to eventually fully cover the foetal skin surface during the critical period of adaptation before birth .

It is possible that the surfactant system evolved initially in the skin and was then subsequently utilized and modified in the lung (39). Table 1 summaries the common features that shared by lung and skin. In the skin, lipid is present within intracellular lamellar bodies localised in stratum granulosum layer, when cell moves into the stratum corneum this lipid within lamellar bodies is extruded into the intercellular spaces to form lipid bilayers around and between corneocytes (40). This process shares some of the common features of pulmonary surfactant production by type II alveolar cells. The main difference of lipid composition in lung and stratum corneum is the lipid composition; lipid in the stratum corneum is a mixture of cholesterol, ceramides and free fatty acids (41).

Table 1 Lung and skin: shared features (26).

Function	Epidermis	Serves as a topical barrier at environmental interface; skin permeability barrier.
	Lung	Forms a topical barrier at environmentalinterface; gas exchange surfaces.
Lipid secretion	Epidermis	Keratinocytes
	Lung	From type II alveolar cell
Primary cell structure at air-gas interface	Epidermis	Corneocyte
	Lung	Type I alveolar cell
Delivery of barrier structural lipids	Epidermis	Lamellar bodies
	Lung	Lamellar bodies
Lamellar body content (protein)	Epidermis	Acid phosphatases, glycosidases, proteases, lipases
	Lung	Acid phosphatases, glycosidases, proteases, lipases and surfactant proteins and lipids
Lamellar body content (lipid)	Epidermis	~ 40 % phospholipids, 20 % glycolipids, 20 % free sterols, 20 % other neutral lipids
	Lung	85 % phospholipids, 10 % free sterols, 5 % neutral lipids
Hormonal stimulus	Epidermis	Epidermal growth factor, glucocorticosteroids, T3 (Thyroxine)
	Lung	Epidermal growth factor, glucocorticosteroids, T3

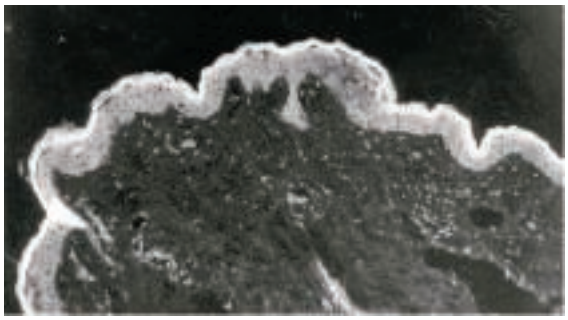

Figure 1. Epifluorescence appearance of skin (x 200) (26).

The most important function of the epidermis is maintaining the barrier which protects the organism against damage from the environment and desiccation. This barrier is located in the upper layer of the epidermal stratum corneum (SC) (Figure 1) (26) and consists of at least two components: the proteinaceous, highly cross-linked, cornified cell envelope and the extracellular lipid lamellae (42). Barrier properties of the SC are largely dependent on the intactness of the lipid lamellae that surround corneocytes. The epidermis contains keratinized stratified squamous epithelial cells that are continously replaced from the subdermal keratinocytes. These are stem cells in the basal epithelial layer, which differentiate as they move upwards. The epidermis is a highly differentiated tissue, covered by corneocytes derived from matrix cells at the border of dermis. The cornification is a process which starts roughly 120 μm below the corneocyte layer (43).

SC protects the underlying tissue and restrains keratinocyte proliferation to maintain the required levels of homeostasis of the epidermal barrier. This barrier controls and regulates transcutaneous water loss and the penetration of topically applied substances into the epidermis. Therefore, it is very important in determining the transdermal delivery of either exogenous environmental agents or deliberately applied pharmaceuticals (so-called 'transdermal' or 'topical' drugs). The stratum corneum contains only 15 % water, but 70 % protein and 15 % lipids. This composition is quite different from that of the epidermis, dermis or any other viable subdermal tissue.

The epidermal permeability barrier is enriched in ceramides, free fatty acids and cholesterol (44). Intercellular lipids are mainly cholesterol, ceramides, and free fatty acids synthesized in the epidermis (45). The organization of the stratum corneum has been likened to that of a "brick wall" or "bricks and mortar structure". The corneocytes can be represented as bricks and the intercellular lipids as the mortar (46). The barrier function of a normal epidermis depends very much on the quality of its bricks and mortar. The building blocks of the epidermal barrier are formed during the complex terminal differentiation program from inner living and dividing basal keratinocytes. Culminating in the formation of flattened cornified cells (corneocytes) which, as they are moved towards the surface, are naturally sloughed by abrasion. Corneocytes are anucleate cells composed of an insoluble filamentous peripheral envelope. The high water insolubility of this cornified cell envelope is attributed the presence of highly cross linked proteins through the formation of disulfide bonds (47) and N (γ-glutamyl) lysine isodipeptide bonds (48). Additionally, various cysteine-rich proteins such as loricrin (49), involucrin and cysteine-rich envelope proteins also suggested as precursor proteins for the cell envelope (50). The viable epidermis, localized under the stratum corneum, is stratified and contains 10-20 layers of keratinizing epithelial cells. Its major role is to synthesize new cells and to replace the stratum corneum. Melanocytes, present within this layer, are responsible for the skin pigmentation (51). Langerhans cells are also present in viable epidermis and participate in immunologic responses and antigen presentation (52). The Merkel cells play a major role in sensory reception (52). In pathological situations such as psoriasis vulgaris, T-cells and neutrophils may infiltrate into the epidermis.

Lipids are an essential component of the epidermis, being involved in maintaining cell structure, controlling growth and differentiation, determining cohesion and desquamation and in formation and function of the permeability barrier. The structure and composition of the epidermal lipids are very important for normal skin function. The epidermal lipids are phospholipids, monohexosylceramides, ceramides, cholesterol and its acyl esters, cholesterol sulfate, triglycerides, and free fatty acids (53).

Many reports have suggested a role for the lamellar bodies in secreting and maintaining the permeability barrier of the skin (54,55,56). In the non-keratinising epithelia, at least two types of lamellar bodies may contribute to this barrier function. The expression of surfactant proteins by human skin tissues has been showed by Kankavi using Western blotting, immunohistochemical techniques, RT-PCR, and souther blotting (27). There are no reports as yet in the literature concerning the function of the surfactant proteins in the skin except the presence of SP-D mRNA in rat skin (23). Surfactant proteins A, B, C and D are present in variable amounts in epidermis, dermis, hair follicles, sweat and sebum, and surfactant proteins may be critical to local barrier and defence functions of the skin (26). Human keratinocytes contains mRNA sequences specific for surfactant proteins A, B, C and D (Figure 2) (26). Initially, examination of fresh abdominal human skin samples indicated the presence of SP-A, B, C and D by SDS-PAGE and Western blotting with specific labelled antibodies and having same moleculer weight in lung and skin samples (Figure 4). Histological distribution of surfactant proteins are within the structures of the skin indicated that surfactant proteins mainly localize on the epidermis layer of skin (Figure 3). Figure 3 clearly shows that the SP-A, B and D specific antibodies are bound to proteins in the epidermis (A, B and D), dermis (B) and around the hair follicle (A, B and D) structures of the skin (26). All skin surfactant proteins showed cDNA similarity with those found in the lung (Figure 2) (26). SP-A and SP-D also bind D-mannose (Figure 5). The carbohydrate-binding properties of SP-A and SP-D are quite similar that of the SP-A and SP-D in lung. When a carbohydrate-binding collectin such as SP-A and SP-D attaches to the microbial surface, various host defence mechanisms are initiated including neutralization, opsonization and phagocytosis of the microorganism. Consequently, their surface tension lowering and carbohydrate-binding properties, surfactant proteins contribute to the immune surveillance of the skin and add to active barrier mechanism.

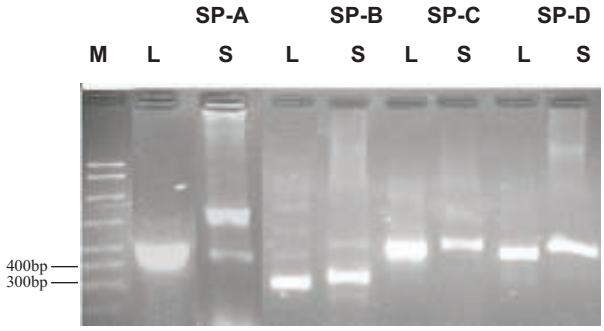

Figure 2 RT-PCR amplification of surfactant proteins from human skin. The PCR reactions were resolved in a 2 % agarose gel, containing ethidium bromide (0.25 µg/ml) and using a 0.5 x TBE (Tris-borate-saline) running buffer (1 in 20 dilution of 10 x TBE: 0.89 M Tris base, 1.12 M boric acid, 0.02 M EDTA, pH 8.0). (M: molecular weight marker, L: lung, S: skin) (26).

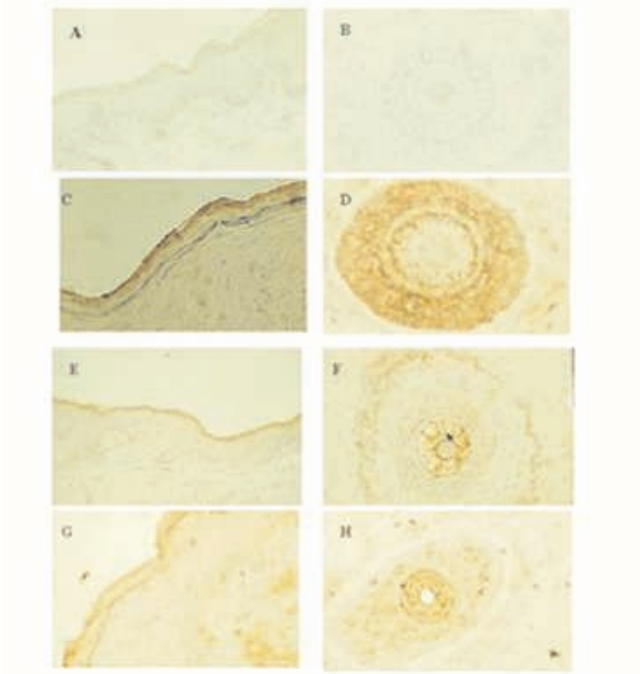

Figure 3 Immunohistochemical localization of surfactant proteins in normal human skin and hair shaft. The tissues were stained using the peroxidase-labelled secondary antibodies technique and

counterstained with Mayer's hematoxylin. Original magnification for each section was: A: skin control without primary antibody (25 x), B: hair shaft control without primary antibodies (40 x), C: SP-A in human skin (40 x), D: SP-A in hair shaft (40 x), E: SP-B in human skin (25 x), F: SP-B in hairshaft (40 x), G: SP-D in human epidermis (40 x), and H: SP-D in hair shaft (40 x). Original magnification for each section was x 250. Control sections without primary antibody A: skin and B: sebaceous gland and respective antibody stained sections and D (26).

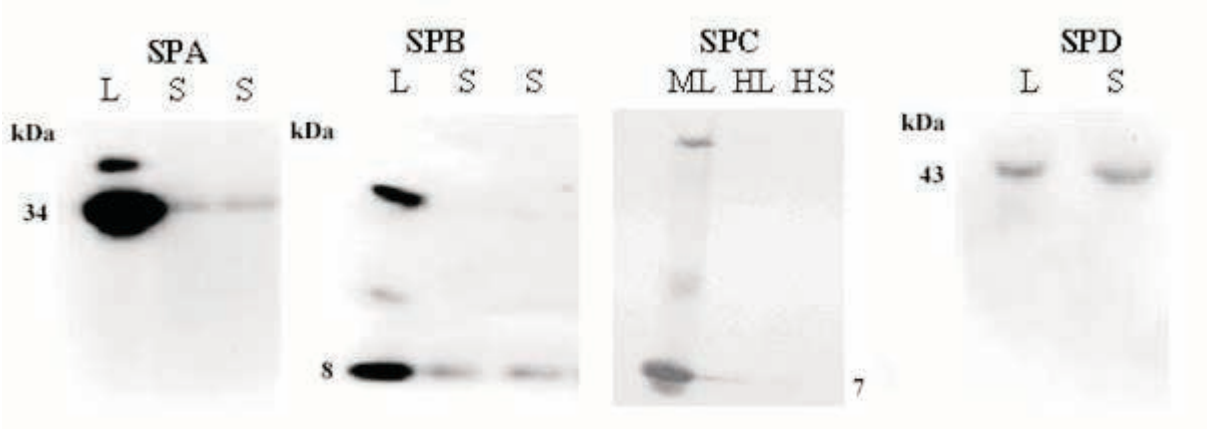

Figure 4. Western blotting of skin extract for SP-A, B, C and D using lung extract as a positive control. Immunodetection was performed with specific anti-surfactant protein antibodies (26).

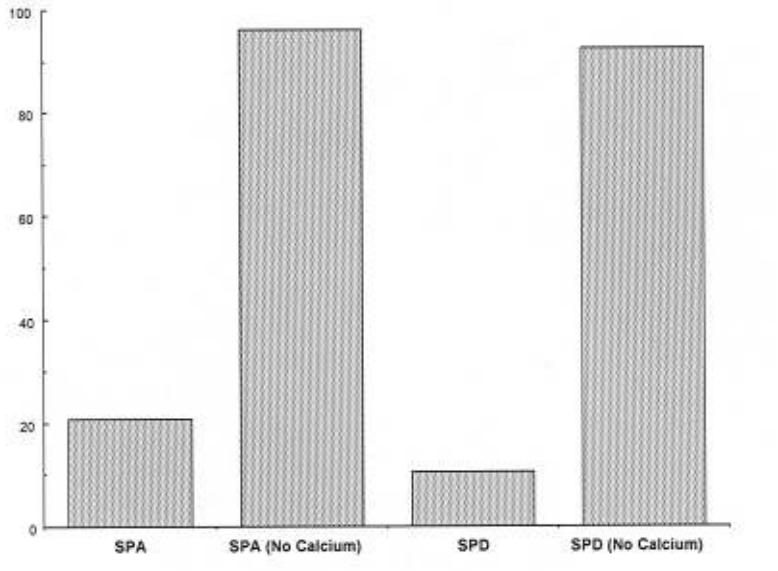

Figure 5. Binding of mannose by skin SPA and SPD in the presence and absence of calcium ions (33uM), data shown is the average of duplicates (26).

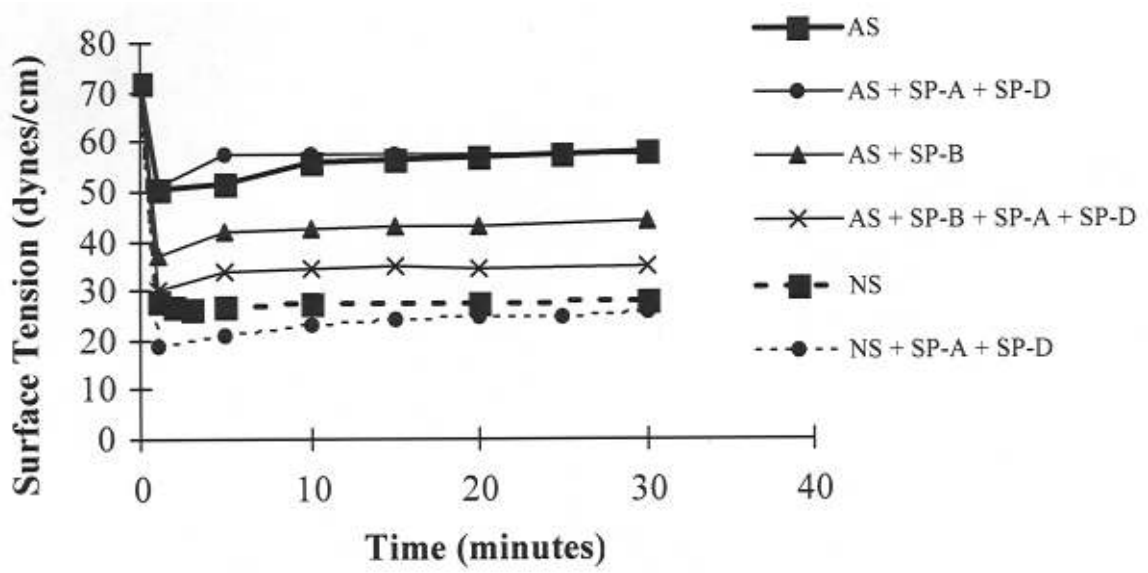

Figure 6 Surface tension reducing effects of surfactant proteins (1% A, B and D) from skin measured by the Wilhemy balance. NS: normal sebum, AS: artificial sebum. (Insufficient SP-C was available for inclusion in this analysis) (26).

The antibacterial activity of skin surface lipids is primarily due to lipids secreted by sebaceous glands and particularly their content of free fatty acids (57,58,59). Human SC lipids, predominantly derived from keratinocytes, also have a significant antistaphylococcal activity (60). Sebaceous glands are distributed all over the surface of skin with the exception of the palmar and plantar regions (61). They are abundant i.e. 300 to 900 per square meter in the head neck and shoulder region (62,63,64). The forehead is one of the sebum-rich areas (65). Androgenic hormones secreted from testes, ovaries and adrenal glands stimulate sebum secretion (66). Age and gender are other determinants of sebaceous gland activity. Sebaceous gland activity was found to be high in utero, decreasing shortly after birth (67). From one year after birth to puberty its level remains low(66,67). Following an increase in circulating androgens, these glands start to secrete increased amounts of sebum until the late teens (66). Sebum has only limited barrier properties as polar and non-polar materials can easily penetrate through this layer (67,68). While the epidermal barrier function largely depends on intercellular lipids in the stratum corneum, skin surface lipids are mainly derived from sebum. Native sebum spreads on the skin instead of making droplets . Following its secretion, sebum becomes mixed with lipids from the keratinizing epithelium and this forms the skin surface lipid film. A major function of sebum is contributing to the defense function of skin against microorganisms. The most abundant free fatty acids in sebum is palmitoleic (70). It was subsequently shown that the most active antimicrobial fatty acid within the sebum is palmitoleic acid (71).

Ultrastructural and in vivo studies have shown that surfactant is produced by extrusion of the lamellar bodies, which occurs in all serosal cells including those in the synovium and peritoneum. Surfactant has been shown to play a role in suppressing some functions of immune cells within the lung and outside the lung.

In-vitro studies showed that the surface tension of SP deficient artificial sebum is lowered by skin SP-B and SP-A and SP-D. A lower surface tension may enable not only sebum to spread more efficiently over the skin but also lower the interfacial tension allowing better "wetting" of allergens and other extrinsic (non-self) proteins (Figure 6). Consequently, as in the lung, microorganisms and other foreign bodies on and in the skin may be able to be more readily assessed and recognized by host defense mechanisms. Studies on lung surfactant proteins have shown that SP-A and SP-D are the major immune reactive/defensive proteins in this surfactant family. Mannose-binding and the surface tension-lowering ability of skin SP-A and SP-D were found similar to lung surfactant proteins, indicating that they might bind to certain carbohydrate domains on the external surfaces of many bacteria and microorganisms with the presence of Ca^{++}. Thus it can be hypothesized that surfactant proteins in the skin have some putative functional role which is similar to that exerted in other tissues.

Additionally, Narendran et al (40) have been suggested that the increase in amniotic fluid turbidity with advancing gestational age was secondary to an interaction between pulmonary surfactant and vernix caseosa. Therefore, roles of surfactant proteins in human gestation must not be omitted.

This review describes recent observation of Kankavi (26) and Mo et al. (2006) (72) regarding the detection of surfactant proteins in skin and its appendages (Figures 2,3,4). It can be postulated that surfactant proteins present in these sites may contribute to host defence function, although a role in surface tension lowering (Figure 6) may also be possible.

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