

Skin as a route of exposure to protein allergens

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Summary

Protein contact with skin is associated with a number of clinical conditions, including protein contact dermatitis and immunologic contact urticaria. This article reviews the clinical and other selected evidence that proteinaceous materials penetrate skin. It is concluded that whilst penetration of intact proteins through normal skin is extremely low and without consequence, any damage to the skin barrier may allow penetration. As a result, risk assessment for contact of protein with skin must take into account potential barrier impairment and thus the possibility of both the induction and the elicitation of allergic skin reactions.

Introduction

The compact layer of keratin-rich corneocytes that comprises the outer horny layer (stratum corneum) acts as an important primary barrier to exogenous compounds. However, a normal healthy stratum corneum barrier is not totally impenetrable and many topically applied, low molecular mass, moderately lipophilic compounds have been demonstrated to penetrate the skin readily *in vivo* and *in vitro*.¹ In contrast, ionized compounds do not penetrate the predominantly lipophilic stratum corneum particularly easily. In addition, it is generally considered that there is a molecular mass cut-off for permeability of healthy skin. It has been suggested that a compound must be < 500 Da to penetrate the stratum corneum.² This viewpoint can be challenged however, as cutaneous reactions arise in humans following skin contact with proteinaceous substances. Although it remains unclear whether human skin contact with proteins can induce sensitization, predictive sensitization and allergenicity tests with proteinaceous test materials often yield positive results. To elicit this form of skin toxicity, the protein applied topically, or a smaller moiety derived from it, must enter the viable epidermis. Here, we discuss the evidence for

skin penetration of macromolecules > 500 Da and the potential for proteases, present within the stratum corneum or skin microflora, to break down proteins that come into contact with the skin, into smaller, possibly more easily penetrating, but still potentially antigenic moieties. Better understanding of this area is important for risk assessment of proteinaceous materials that come into contact with skin.

Skin conditions implying protein absorption in skin

The following discussion provides evidence for protein absorption into human skin, not an exhaustive literature review. Atopic dermatitis has been avoided to aid clarity as the contentious role of skin contact with proteins in this condition would complicate discussion without adding greatly to the scientific considerations herein.

The term 'protein contact dermatitis' was introduced by Hjorth and Roed-Pedersen³ to describe a distinct type of chronic recurrent occupational dermatitis (not urticaria) observed in Danish food handlers. The condition results from either simultaneous type I (IgE-mediated) immediate and type IV (T-cell mediated) delayed sensitivities or type I sensitivity alone, following contact with certain foods (Table 1). Contact urticaria is an immediate wheal-and-flare reaction resulting from either IgE-mediated (immunologic contact urticaria) or nonimmunologic mechanisms.⁴ Foods that can produce

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Accepted for publication 9 January 2002

Table 1 Examples of proteinaceous substances that can cause dermatitis and urticaria following human skin contact.

Substance	Molecular mass
Foods	
Egg (albumin)	≈ 45 kDa
Raw fish	–
Milk (casein)	≈ 25 kDa
Cheese	–
Peanut (lectin)	≈ 29 kDa
Meat	–
Wheat/flour (α -amylase)	≈ 45 kDa
Plant proteins	
Birch pollen allergen	18 kDa
Ragweed pollen	–
Natural rubber latex proteins	
Heveins	
Hev b1 (insoluble)	14.6 kDa
Hev b2 (soluble)	34–41.3 kDa
Hev b3 (insoluble)	23–27 kDa
Hev b4 (soluble)	50–75 kDa
Hev b5 (soluble)	16 kDa
Hev b6.01 (soluble)	20 kDa
Hev b6.02 (soluble)	4.7 kDa
Hev b7 (soluble)	46 kDa
Hev b8 (soluble)	14 kDa
Hev b9 (soluble)	51 kDa
Other classes/enzymes	
Cellulases	
Glucosylases	
Papains	
Subtilisins	
Xylanases	

either protein contact dermatitis or contact urticaria include raw fish (and other seafoods), meats, egg, peanut, flour, milk, cheese, fruits and vegetables.⁵ Contact with other substances [e.g. birch pollen allergen, natural rubber latex proteins and industrial enzymes, such as those used in the detergent (subtilisins and cellulases) and baking (amylases) industries]⁶ is also known to cause protein contact dermatitis or urticaria (Table 1). To cause these conditions, the allergen must penetrate or bypass the stratum corneum and enter the epidermis/dermis. Once the allergen has been absorbed into the skin, it is considered that IgE bound to the skin's unique antigen presenting cell, the Langerhans' cell, may mediate the development of protein contact dermatitis.^{7,8}

However, a vital question is by what route individuals diagnosed with protein contact dermatitis have absorbed proteinaceous material so as to cause production of specific IgE antibodies. They may come into frequent or prolonged skin contact with a proteinaceous substance, and it seems likely that a defective skin barrier function, present for example as a result of

atopy, an inflammatory skin condition, skin abrasion and/or irritative mechanisms may favour the development of protein contact dermatitis or contact urticaria. Evidence for this comes from studies on protein hydrolysates (derived from collagens, keratins, wheat, milk, silk, etc.) in hair conditioners. Patch tests to 22 different types of protein hydrolysates were negative in 11 hairdressers suffering from hand dermatitis.⁹ Only one, already suffering from hand dermatitis, had a positive prick test to Crotein Q (hydroxypropyl trimonium hydrolysed collagen) and suffered from contact urticaria. Of 2160 patients with respiratory symptoms (due to an undefined cause), only 10 individuals exhibited a positive prick/scratch test to Crotein Q and none of these suffered from any clinical skin symptoms.⁹ However, two adults, already suffering from chronic atopic dermatitis and hence probable reduced skin barrier function, also had contact urticaria resulting from hydrolysate contact. This suggests that pre-existing skin damage plays a role in protein contact dermatitis and contact urticaria in humans. Nevertheless, there is evidence that allergy to protein hydrolysates is very low, despite extensive exposure.¹⁰

Natural rubber latex proteins are present in latex gloves and other devices, and are an important cause of type I allergy and contact urticaria 'syndrome' in humans. The cornstarch powder on medical gloves adsorbs latex proteins and transfers them to the skin¹¹ and airways of the user.¹² Latex allergy has been reviewed extensively.^{13,14} The primary route of exposure to natural rubber latex proteins *via* which individuals become sensitized is not always clear. In healthcare workers, powdered gloves which generate aerosolized allergens have been linked with increased latex allergy¹⁵ although many afflicted individuals have had pre-existing skin lesions or dermatitis.¹⁶ Increased hydration and irritation of the occluded skin with excessive glove use and handwashing may also exacerbate skin conditions. Provocation tests, where dermatitis sufferers applied skin protection cream to only one hand, suggest that the use of skin protection creams may favour skin uptake of allergens from gloves, thus increasing allergic reactions and not decreasing them as intended by their use.¹⁷

Enzyme proteins have a low order of general toxicity, the main safety concern being the generation of IgE-mediated respiratory allergy. Historically, there is ample evidence of the capacity of enzymes to give rise to allergic asthma.^{18,19} Although these cases arose through excessive occupational exposure, the protease enzymes involved gave rise only to skin irritation, not skin allergy, even though, via diagnosis of the asthma, there was clear evidence of enzyme-specific IgE.²⁰

Incidences of allergic skin reactions to enzymes have been reported in certain susceptible individuals in an occupational setting.^{20–23} However, the vast majority of enzyme-related dermatoses are as a result of irritancy. Exposure to toxicologically relevant quantities of enzymes is mainly occupational; in final products, such as laundry products, the levels of enzymes are low, ranging from 0.1 to 2%. Indeed, one very detailed investigation of possible adverse skin reactions to laundry detergents in a large cohort of individuals failed to find any evidence of enzyme allergy.²⁴ Thus the evidence suggests that the ability of enzyme proteins to penetrate skin and induce or elicit allergy by this route is extremely low.

Stratum corneum structure and barrier disruption

Flynn stated in 1990 that for small molecule penetration: 'The intracellular protein [of the stratum corneum] is dense and presents such a thermodynamically and kinetically impossible passageway that, for all practical purposes, it is impenetrable. Current dogma says that diffusion occurs via the interstitial lipid'.²⁵ It remains likely that intercellular pathways are also the most favourable but still tortuous routes for the penetration of macromolecules through healthy human skin, hence penetration is normally low. Evidence for this comes from studies on the absorption of dextrans (4–10 kDa peptides) in the presence of the penetration enhancer *n*-octyl- β -D-thioglucoside (OTG).²⁶ In intact skin, dextran penetration was negligible (flux $J_s = 0.096 \mu\text{g}/\text{cm}^2/\text{h}$). In OTG-treated skin, penetration was greatly enhanced (flux $J_s = 27.67 \mu\text{g}/\text{cm}^2/\text{h}$). The levels of dextran penetration in OTG-treated skin were similar to those in tape-stripped skin (flux $J_s = 33.88 \mu\text{g}/\text{cm}^2/\text{h}$). Electron microscopy revealed that OTG exfoliated the cell membranes and dissociated adherent corneified cells in the stratum corneum, suggesting that the intercellular cohesive laminae and the barrier functions were destroyed. OTG also solubilized the stratum corneum proteins and extracellular ceramides.

Ceramides, an end product of keratinocyte differentiation, are the major lamellar phase-forming lipids of the stratum corneum and are secreted in the extracellular space, acting as a mantle around the corneified cells of the stratum corneum.²⁷ Atopic dermatitis patients show reduced levels of ceramides in their nonlesional and lesional skin, in comparison with healthy individuals.^{28,29} As a result their skin barrier is impaired and it is feasible that macromolecules may be absorbed into their skin. The levels of production of skin ceramides

have been attributable to the balance of enzyme activity involved in sphingolipid synthesis in the outer layers of the skin. One such cutaneous enzyme that is expressed highly in atopic dermatitis patients is sphingomyelin deacylase.³⁰ Ceramidase, which breaks down ceramides and is expressed by staphylococcal bacteria on the skin surface, has also been implicated in reduced ceramide levels in atopic individuals.³¹

The stratum corneum (granular cells and intercellular space) also possesses other active enzymes, including serine proteases, trypsin and chymotrypsin, which can cleave peptides and have been implicated in the desquamation process.^{32–34} Reduced trypsin activity has been seen in the skin of atopics in comparison with normal healthy skin.³³ It is interesting to note that ovalbumin is known to be a member of the serine protease inhibitor family (as a noninhibitory homologue) and is a substrate for serine protease.^{35,36} A 30-kDa trypsin-like protease in the plantar stratum corneum has also been shown to digest casein.³² It is possible that interactions with stratum corneum enzymes may play a role in ovalbumin- and casein-derived skin allergy.

An endogenous lectin, concanavalin A, has also been isolated from human stratum corneum and is localized exclusively to the membranes of the stratum corneum by immunohistochemistry of intact skin.³⁷ These authors suggest that this endogenous lectin may act as a major carbohydrate-binding protein that cross-links adjacent corneocytes together in the stratum corneum. Perhaps peanut lectin, upon contacting the skin, can disturb normal endogenous lectin functions during the manifestation of peanut-related allergy.

The roles of hair follicles, sebaceous glands and sweat glands in protein penetration have not been investigated extensively, but may provide an important route of entry. Also, the superficial film of sebum/sweat may act as a vehicle for proteins that contact the skin. However, the most likely event that allows penetration of levels of proteins significant enough to cause toxicity, is the presence of an already damaged/compromised stratum corneum which can be caused in a wide variety of ways (Table 2).

Table 2 Causes of reduced stratum corneum barrier integrity in normal skin.

Pre-existing dermatitis	e.g. irritant, allergic
Physical damage	e.g. burned, shaved, wounded
Chemical damage	e.g. detergents and other penetration enhancers
Increased hydration	e.g. excessive hand washing
Occluded skin	e.g. wearing of gloves

Conclusions

The available evidence leads us to conclude that penetration of proteins through intact stratum corneum of 'normal' individuals is very low and normally does not produce clinical effects. However, penetration through chemically/physically compromised skin (including where such damage is not visible to the naked eye) or ceramide-deficient skin in atopics is significant enough to cause contact urticaria or allergic contact dermatitis. Because compromised skin is not uncommon, an evaluation of potentially increased penetration of proteins in this population should be included in risk assessments for protein. It is our view that protein contact with absolutely normal skin is essentially without consequence, but induction and elicitation of allergic responses may arise via contact of proteinaceous materials with compromised skin.

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Key points

- Skin contact with proteinaceous materials can result in type 1 IgE-mediated allergies and urticaria.
- Healthy noncompromised human skin provides an effective barrier to protein (molecular weight $> \approx 1$ kDa) penetration.
- Compromised human skin, with reduced barrier properties, could allow sufficient penetration of protein allergens to cause clinical symptoms.
- Skin can be compromised in many different ways: physical damage, chemical insult or inherent abnormalities.
- Compromised skin is common in the human population.
- Enhanced skin penetration through compromised skin should be considered in risk assessments for proteins.