Enzymes and sensitization via skin exposure: A critical analysis

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ABSTRACT

Some proteins, including enzymes, can induce allergic sensitization of various types, including allergic sensitization of the respiratory tract. There is now an increased understanding of the role that the skin plays in the development of IgE-mediated allergy and this prompts the question whether topical exposure to enzymes used widely in consumer cleaning products could result in allergic sensitization. Here, the evidence that proteins can interact with the skin immune system and the way they do so is reviewed, together with a consideration of the experience gained over decades of the use of enzymes in laundry and cleaning products. The conclusion drawn is that although transcutaneous sensitization to proteins can occur (typically through compromised skin) resulting in IgE antibody-mediated allergy, in practice such skin contact with enzymes used in laundry and cleaning products does not appear to pose a significant risk of allergic disease. Further, the evidence summarized in this publication support the view that proteins do not pose a risk of allergic contact dermatitis.

1. General introduction

It is well established that in certain circumstances inhalation exposure to enzymes of bacterial and fungal origin, such as those used for many decades in cleaning and detergent products, have the potential to induce allergic sensitization of the respiratory tract and occupational respiratory allergy/asthma (Pepys et al., 1969; Flindt, 1969; Cullinan et al., 2000; Sarlo, 2002; Brant et al., 2006; Adisesh et al., 2011; Baur et al., 2013). It is for this reason that substantial efforts have been made into the identification and implementation of occupational airborne exposure limits (e.g. Sarlo, 2002; Basketter et al., 2010). Adherence to these limits has been shown to be successful in avoiding occupational allergic disease (Sarlo, 2002; Basketter et al., 2015, 2021). Furthermore, the application of careful safety evaluation continues to be promoted to ensure that allergy hazard does not translate to a risk in consumer products.

As will be considered in more detail below, there has in recent years been an increasing appreciation that skin exposure to certain proteins can induce immune and allergic responses (Kimber et al., 2014). It is therefore perhaps inevitable that the question has been asked whether topical exposure to enzymes might in some instances have the potential to cause allergic sensitization (e.g. Wuthrich, 1985; Basketter and Raulf, 2020). It goes without saying that exposure to foreign proteins can always be expected to initiate some form of immune response; however

the properties which confer upon a specific protein the ability to induce an allergic response is beyond the scope, having been reviewed recently elsewhere (Krutz et al., 2020). In this paper two key aspects are considered: skin sensitization resulting in allergic contact dermatitis (ACD), and also whether dermal contact can lead to sensitization of the respiratory tract associated with rhinitis and asthma. However, before exploring these issues it is necessary to consider the extent to which proteins experienced at skin surfaces have the potential to engage with the cutaneous immune system and induce sensitization.

2. Topical exposure to proteins and engagement with the immune system

Studies of the development and regulation of food allergy have been instrumental in informing an understanding of the initiation of immune responses to proteins encountered via skin surfaces. The 'dual allergen hypothesis', as first articulated by Gideon Lack, proposed that an important route of allergic sensitization to food proteins is via skin exposure, but that oral consumption of potentially allergenic food proteins (especially in infancy and ahead of exposure via the skin or another route) favours the induction of immunological tolerance (Lack, 2008). Consistent with this there is evidence that dietary exposure to food enzymes does not lead to allergic sequelae (Bindslev-Jensen et al., 2006). Clinical studies have provided support for the dual allergen hypothesis,

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showing the early dietary introduction of peanut or egg into the infant diet is associated with a significantly reduced risk of allergy to these foods (Du Toit et al., 2015; Perkin et al., 2016). In addition, it has been shown that, consistent with the skin being an important route of sensitization, high levels of environmental exposure to peanut during infancy promotes sensitization (Fox et al., 2009). Moreover, in a separate investigation it was found in peanut allergic subjects that T lymphocytes responding to peanut protein extracts were predominantly of the skin-homing subset expressing CLA (cutaneous lymphocyte antigen), indicative of initial priming and sensitization through the skin (Chan et al., 2012). It is now considered that the development of allergy to food proteins, especially during infancy, results primarily from skin contact (Van Splunter et al., 2020; Brough et al., 2020; Sahiner et al., 2021).

A detailed consideration of the immunological mechanisms through which transcutaneous exposure to food proteins results in the acquisition of allergic sensitization is beyond the scope of this manuscript. Briefly, however, a growing body of evidence indicates that the local responses which are triggered by contact with protein allergens in the skin, and that drive development of an allergic response, are characterized by the increased expression of thymic stromal lymphopoietin (TSLP), an epithelial cytokine, and the production of interleukins 25 and 33 (IL-25 and IL-33). Together these cytokines induce the activation of type 2 innate lymphoid cells (ILC2) and cutaneous dendritic cells (DC) that in turn stimulate Th2 responses and the production of IgE antibody (Van Splunter et al., 2020; Sahiner et al., 2021).

What is clear, however, is that the effective development of sensitization to food proteins resulting from skin exposure will be significantly facilitated by, or may even be entirely dependent upon, reduced barrier function (either acquired or heritable) (Paller et al., 2019). It is known, for instance, that loss-of-function mutations of the *FLG* gene that encodes the epidermal barrier protein filaggrin are associated with allergic sensitization to peanut proteins (Brown et al., 2011). Similarly, there is a correlation between barrier dysfunction associated with atopic dermatitis and sensitization to food proteins (Flohr et al., 2014; Brough et al., 2015). Of relevance also is a recent study which found that the regular application of moisturizers to the skin of infants enhanced the development of food allergy. The interpretation is that the use of moisturizers can enhance transcutaneous sensitization to food proteins (Perkin et al., 2021). These authors also speculated that the frequent moisturiser use might even damage the skin, consistent with the results of much earlier clinical studies (Held et al., 1999; Held, 2001).

Sensitization to proteins via skin contact has also been the subject of studies in mice. It was shown that compromised barrier function induced by disruption of the stratum corneum supported effective sensitization of mice to protein antigens (Strid et al., 2004). In fact, the same authors also reported that epicutaneous exposure to peanut protein not only stimulated allergic sensitization, but also prevented the subsequent induction of immunological tolerance by oral exposure (Strid et al., 2005). Similar results have been described by others (Noti et al., 2014). It has also been reported that repeated exposure of mice to peanut proteins via undamaged skin resulted in allergic sensitization (Tordesillas et al., 2014). In common with human data, loss-of-function mutations of the gene encoding filaggrin are associated with priming to protein allergens at skin surfaces (Fallon et al., 2009; Walker et al., 2018).

Taken together the available data indicate clearly that topical exposure to protein allergens can result in transcutaneous sensitization; the implication being that under certain circumstances at least some proteins are able to gain access to viable skin tissues. However, it is necessary to view that conclusion in the context of various considerations and caveats, as follows:

(a) The studies cited above have focused largely or exclusively on known protein allergens, and in particular on peanut allergens. There is good reason why this is the case given the importance of peanut allergy and the need to understand how sensitization to

peanuts is acquired. Nevertheless, the consequence is that the phenomenon of transcutaneous sensitization has been explored with only a very limited range of proteins.

(b) It could be argued that the effectiveness with which peanut allergens appear to induce transcutaneous sensitization might not be representative of all proteins. It has, for example, been reported that peanut extracts may, in addition to their allergenic properties, act as adjuvants providing a non-specific stimulus for the elicitation of immune or allergic responses (Tordesillas et al., 2014).

(c) It is important to appreciate that for proteins to induce sensitization following exposure to the skin (or indeed following exposure via any route) they must be inherently allergenic. There is no evidence that proteins that are non-allergenic will acquire the potential to induce sensitization simply as the result of skin contact.

(d) Finally, it is clear that effective induction of sensitization to proteins at skin surfaces is highly favoured by, and possibly completely dependent upon, an acquired or inherent defect of barrier function. This is certainly true in humans, and it has been pointed out that in mice virtually all demonstrations of transcutaneous sensitization to proteins have employed models with disrupted barrier function (Smith et al., 2017). The conclusion drawn is that under conditions of normal barrier function the access of proteins to viable skin tissue will be very limited, whereas when skin is damaged, access of topically encountered proteins may be facilitated.

It is against this background that the ability of enzymes to cause sensitization via skin contact (skin sensitization and/or sensitization of the respiratory tract) is examined here.

3. Skin sensitization

Skin sensitization describes the process through which an inherently susceptible subject acquires immunological responsiveness to a low molecular weight chemical allergen (a contact allergen). Exposure to the contact allergen at the skin surface provokes an immune response which, if of sufficient magnitude, results in sensitization. If the sensitized subject is then exposed subsequently to the same contact allergen (or to a structurally very similar contact allergen) then a stronger and accelerated secondary immune response will be elicited at the site of exposure. This in turn results in a cutaneous inflammatory reaction that is recognized clinically as ACD (Rustemeyer et al., 2011; Kimber et al., 2002a, 2011; Martin, 2015; Nassau and Fonacier, 2020).

The whole process of skin sensitization relies on the coordinated interaction between many different cell types and molecules, and is tightly regulated in time and space. The important events have been reviewed previously (Rustemeyer et al., 2011; Kimber and Dearman, 2002a; Kimber et al., 2011; Kaplan et al., 2012; Ainscough et al., 2013; Martin, 2015; Martin et al., 2018). The key elements can be summarized briefly as follows. For skin sensitization to be acquired the inducing low molecular weight chemical must gain access to the viable epidermis via the stratum corneum. Low molecular weight allergens are too small to be recognized in their own right by the immune system and are unable to stimulate an immune response. A critical event is consequently the need for such a chemical to form stable associations with host proteins in order to acquire immunogenic potential. For this reason skin sensitizing chemicals are naturally protein-reactive (normally electrophilic), or can be converted in the skin to electrophilic species. The resultant immunogenic chemical (hapten)-protein conjugate is recognized and internalized by dendritic cells (DC) in the skin. These DC migrate from the skin to draining lymph nodes and are responsible for processing the antigen and presenting it to responsive T lymphocytes (that is, those T lymphocytes that bear a complementary receptor for the antigen). Antigen-driven activation of responsive T lymphocytes induces cellular

division (clonal expansion) and differentiation, and this is the point at which sensitization is acquired. Following subsequent contact of a sensitized subject with the inducing contact allergen the expanded population of allergen-specific T lymphocytes are stimulated to mount a more vigorous secondary response resulting in the elicitation of ACD. The major components of the process that culminates in the acquisition of skin sensitization have been articulated in an Adverse Outcome Pathway (AOP; OECD, 2012; MacKay et al., 2013; Kimber et al., 2018), and this provides a useful notation for describing the Key Events (KE) on the pathway to the induction of sensitization.

The skin sensitization AOP is comprised of 4 kE that occur (more or less) in sequence. These are as follows. KE1 (the molecular initiating event) describes the formation of stable chemical-protein conjugates that provide an immunogenic stimulus. As mentioned above, contact allergens may be naturally electrophilic, or may require conversion to an electrophilic species in the skin (via enzymatic or oxidative mechanisms) (Aptula et al., 2007). KE2 describes the initiation of inflammatory responses in the skin mediated by keratinocytes and other cells. This response induces production and release of so-called danger signals (Matzinger, 1994; McFadden and Basketter, 2000; Kimber et al., 2002b). These signals collectively promote the initiation of fully effective adaptive immune responses. KE3 describes the activation, differentiation, mobilization and migration of epidermal Langerhans cells and other cutaneous DC, associated with the processing, delivery and presentation of antigen (Kashem et al., 2017). The final stage, KE4, is the activation, proliferation (clonal expansion) and differentiation of antigen responsive T lymphocytes resulting in the acquisition of skin sensitization.

All of the events summarized above, and the eventual acquisition of skin sensitization, are predicated on the low molecular weight chemical being able to gain access across the stratum corneum to the viable epidermis where engagement with the elements of the immune system first occurs. For some time it had been assumed that for effective access, and therefore for effective sensitization, there was a requirement that contact allergens had a molecular weight (MW) of less than 500 Da (Bos and Meinardi, 2000; Gerberick et al., 2004); the implication being that chemicals with a higher MW were unable to induce sensitization. However, it is now widely accepted that there is no such cut-off and that chemicals of higher MW can induce skin sensitization (Roberts et al., 2012; Fitzpatrick et al., 2017). Nevertheless, it is clear that proteins will have much greater difficulty in reaching the viable epidermis. For this reason, the ability of proteins to induce skin sensitization through intact skin has commonly been largely discounted (Basketter et al., 2008), albeit with the recognition that disruption of skin barrier function may make sensitization possible (Smith Pease et al., 2002). In this respect, it is worth mentioning that a mini-epidemic of IgE mediated allergy arose in Japan as a consequence of dermal exposure to poorly hydrolysed wheat proteins used in a facial soap (Yagami et al., 2017).

In line with the above, and as described earlier, there have emerged some persuasive lines of evidence indicating that, under certain circumstances, proteins might be able to gain sufficient access to epidermis and beyond, to interact with immune cells and molecules, and to induce an immune response (Strid and Strobel, 2005; Kimber et al., 2014; Smith et al., 2017; Van Splunter et al., 2020; Brough et al., 2020; Sahiner et al., 2021). It is relevant, therefore, to consider whether there may be opportunities for proteins encountered at skin surfaces, with either normal or disrupted barrier function, to induce skin sensitization and allergic contact dermatitis.

There have been sporadic reports that some enzymes can elicit positive responses in animal methods (usually guinea pig methods) for identification of skin sensitizers (Coenen et al., 1995; Bergman and Broadmeadow., 1997). However, it must be appreciated that such methods (the most commonly used being the guinea pig maximization test, the occluded patch test in guinea pigs, and the mouse local lymph node assay) were in each instance designed exclusively for the identification of the skin sensitization potential of low molecular weight

chemicals, and not for proteins: in such assays, allergenicity would be entirely confounded by immunogenicity (Basketter and Kimber, 2018). More recently, alternative non-animal skin sensitization methods have been validated for the identification of skin sensitizing chemicals, and these *in vitro* approaches are if anything even less well equipped to evaluate the skin sensitizing potential of proteins (Rossi and Ezendam, 2018; De Avila et al., 2019; Strickland et al., 2019).

The failure of detergent enzymes to cause skin sensitization is indicated by clinical studies (White et al., 1985; Belsito et al., 2002), and the general conclusion that has been drawn previously is that detergent enzymes lack the ability to cause skin sensitization and ACD (Basketter et al., 2008, 2012a; b). In addition to the clinical evidence, it is reassuring that the human repeated insult patch test (HRIPT) studies which have been conducted with enzymes yielded negative results (Griffith et al., 1969).

Although the interpretation has been previously that proteins are simply too large to penetrate the skin sufficiently to trigger an immune response (Smith Pease et al., 2002), it is now clear that this does not tell the whole story. Arguably the most important inference is that even if proteins are able to gain access to the viable epidermis and interact with the cutaneous immune system, they will not provoke the class of response necessary for the induction of skin sensitization or the subsequent elicitation of ACD.

It is necessary now to turn to a consideration of whether skin contact with enzymes has the potential to induce allergic sensitization of the respiratory tract, and the health risks that may, or may not, be associated with that.

4. Sensitization of the respiratory tract

Allergic sensitization of the respiratory tract to proteins is most commonly associated with the induction of an IgE antibody response. The process of IgE-mediated allergy can be summarized briefly as follows. Exposure to a protein allergen via a relevant route, and in sufficient quantity, results in elicitation of an IgE antibody response that is driven by the activation of Th2-type T helper cells and associated cells and cytokines (some of which were mentioned above). IgE antibodies distribute systemically and bind to tissue mast cells and blood basophils via specific membrane receptors. At this point sensitization has been acquired. If subsequently the sensitized subject is exposed again to the same protein allergen then an allergic reaction will be provoked. This results from the allergen cross-linking membrane-bound IgE antibody that then triggers the release of a variety of inflammatory mediators that act in concert to drive an acute inflammatory reaction that is recognized clinically as allergy. This allergic reaction is manifest at the site of contact with the allergen, or in some cases if the response to allergen is very vigorous then it can result in life-threatening systemic changes described as anaphylaxis.

As discussed above, it is known that in some instances exposure of workers to fungal or bacterial enzymes via inhalation can result in sensitization of the respiratory tract and respiratory allergy (Pepys et al., 1969; Flindt, 1969; Cullinan et al., 2000; Sarlo, 2002; Brant et al., 2006; Adisesh et al., 2011; Baur et al., 2013). It is likely that inhalation exposure to such enzymes represents the most common, and probably the most effective, route through which sensitization of the respiratory tract is induced. Nevertheless, the purpose here is to consider whether skin exposure of subjects to the same types of enzymes can result in sensitization of the respiratory tract, and whether this might result in adverse health effects.

There are two reasons why it is relevant to address this question. The first is, because as discussed earlier in this article, there is now evidence that topical exposure to at least some allergenic proteins can result in the acquisition of sensitization such that allergic reactions can be elicited in distant tissues. The best evidence for this is the acquisition of allergy to food proteins resulting from skin exposure (Van Splunter et al., 2020; Brough et al., 2020; Sahiner et al., 2021).

The second reason is because there exists a precedent for skin exposure to an allergen resulting in sensitization of the respiratory tract. This precedent is based on low molecular weight chemicals (rather than proteins) that are known to cause respiratory sensitization and occupational asthma, examples being acid anhydrides and diisocyanates. Although it was once assumed that sensitization of the respiratory tract to chemical allergens would result solely from inhalation exposure, there is now a body of evidence that skin exposure can be equally effective, or even more effective, at driving respiratory sensitization to chemicals (Karol et al., 1981; Rattray et al., 1994., Kimber and Dearman, 2002b; Tarlo and Malo, 2002; Bello et al., 2007; Redlich and Herrick, 2008; Redlich, 2010; Tsui et al., 2020). The point is that for sensitization of the respiratory tract the initial stimulus driving the acquisition of sensitization can in principle be delivered at a different anatomical site (the skin). It is important to bear in mind, however, that although sensitization of the respiratory tract resulting from skin exposure to low molecular weight chemical respiratory allergens can and does occur, as discussed above chemicals are far more effective than proteins in gaining access to the cutaneous immune system.

Given that there is evidence that some protein allergens can induce sensitization via skin contact, and that sensitization of the respiratory tract to certain chemical allergens can be acquired by skin exposure, it is relevant, therefore, to address whether the development of respiratory sensitization to industrial enzymes might result from skin contact.

Notwithstanding the above comments regarding a general inability to cause classical ACD, there is evidence that topical exposure to protein allergens can rarely cause local allergic reactions. Protein contact dermatitis has been recognized for more than four decades (Hjorth and Roed-Petersen, 1976; Barbaud et al., 2015; Barbaud, 2020). It is reported usually as an IgE-mediated occupational immunologic contact urticaria associated with exposure to food proteins. Lesser common causes that have been reported include latex and various animal proteins. It is important to emphasise, however, that most reports of contact urticaria associated with skin exposure to enzymes have taken the form of small or individual case studies (Kanerva and Tarvainen, 1990; Morren et al., 1993; Kanerva et al., 1997, 1998; Kanerva and Vanhanen, 1999, 2001; Soto-Mera et al., 2000). Recently, an overview concluded that while enzymes have some potential to cause immunologic contact urticaria, this remains an uncommon observation (Basketter and Raulf, 2020).

If it is accepted that under certain conditions skin exposure to bacterial and fungal enzymes will have some potential to provoke IgE antibody responses then an important question is how effective this route of exposure is in driving systemic sensitization, and specifically sensitization of the respiratory tract.

The available evidence suggests that extensive skin exposure to laundry and cleaning products containing microbial enzymes is not associated, or only very rarely associated, with allergic sensitization, or with the development of adverse effects on the skin (Cormier et al., 2004; Sarlo et al., 2010). In the study reported by Cormier et al. (2004), which was conducted in the Philippines, no evidence of IgE antibody to enzymes was found among a cohort of nearly 2000 atopic women who regularly used a variety of laundry products. It is significant that, in order to enhance the sensitivity of the study, these subjects were atopic, a term describing a predisposition to the development of IgE antibody responses.

It must be acknowledged that in the study cited above the consumer products studied contained only relatively low concentrations of enzymes (0.1% or less). Nevertheless, the evidence presented suggested that the risk of IgE-mediated allergic sensitization resulting from topical exposure to enzymes in laundry products is very low or absent, even following prolonged periods of repeated, daily exposure.

Consistent with these data is an absence of evidence that prolonged topical exposure to products containing microbial enzymes is associated with sensitization of the respiratory tract (see above section 3).

The conclusion drawn is that although in certain circumstances some proteins have the potential to induce allergic sensitization following skin exposure, the potential hazard does not appear to translate into a significant risk of sensitization to enzymes in laundry and cleaning products. Such a conclusion is consistent with some of the considerations discussed earlier: that proteins may differ in their potential to induce IgE antibody responses following skin contact, and that there may exist significant inter-individual differences in skin barrier function that will in turn influence the ability of proteins encountered at skin surfaces to gain access to the cutaneous immune system.

However, probably the most important factors in determining whether potentially allergenic proteins will have the potential to elicit IgE antibody responses in the skin are the levels of exposure and the duration of exposure. The evidence suggests that even prolonged skin exposure of atopic subjects to potentially allergenic proteins in commercial laundry and cleaning products results only extremely rarely in IgE antibody production or sensitization. The conclusion drawn is that when safety evaluation is properly applied, the risk of developing respiratory allergy to enzymes from skin exposure to laundry and cleaning products is negligible. This encouraging conclusion is perhaps strengthened by the knowledge, mentioned earlier, that where safety evaluation is inadequate, hazard does translate to allergic risk with consequent adverse health effects (Yagami et al., 2017).

5. Concluding comments

A variety of enzymes that have found use in a range of domestic laundry and cleaning products, are well known to possess an intrinsic respiratory allergenic potential, which may drive immediate-type allergic reactions via IgE mediated mechanisms. The implementation of airborne exposure limits and appropriate occupational hygiene have together effectively managed the risk to the workforce (eg Basketter et al., 2015, 2021). Alongside the occupational considerations, consumer risk also can be managed via careful, targeted, safety evaluation, for both of which guidance is readily available (ACGIH, 2001; ACD, 2019; AISE, 2018, 2020; AMFEP, 2013; AMFEP/CEPI, 2019; AMFEP/-FEDIMA, 2018; Basketter et al., 2010). Evidence of effective management of consumer risk is available (eg Sarlo et al., 2010).

There is a growing appreciation that in certain circumstances some proteins have the potential to gain access to the viable epidermis following skin contact and trigger an allergic response. It has been recognized, however, that for proteins to gain access to viable skin there may be a requirement for disrupted barrier function. Nevertheless, the ability of at least some proteins to induce transcutaneous sensitization prompts a re-evaluation of whether topical exposure to proteins can cause skin sensitization resulting in ACD, or IgE-mediated sensitization of the respiratory tract associated with occupational asthma. The data summarized above indicate clearly that proteins do not generally pose a risk of allergic contact dermatitis. As a corollary, it is concluded that the risk of skin exposure to enzymes used in laundry and cleaning products resulting in sensitization of the respiratory tract is negligible. More generally, provided that an thorough safety evaluation of the consumer use of such product has been made, it can be concluded that enzymes by any route of exposure do not represent a health issue for consumers. Such safety evaluation should be conducted according to best practice, e.g. following the recommendations given in "Guidance for the Risk Assessment of Enzyme-Containing Consumer Products" (ACI, 2019).

CRediT authorship contribution statement

David A. Basketter: Conceptualization, Investigation, writing original (minor), reviewing, editing, Writing – review & editing, Funding acquisition, Project administration. **Ian Kimber:** Conceptualization, Investigation, writing original, reviewing (major), editing, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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