REVIEW

Factors Influencing the Induction Phase of Skin Sensitization

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The primary purpose of this review is to consider the factors that confer on chemicals the ability to induce skin sensitization and allergic contact dermatitis. It is clear that a number of requirements must be met if a chemical is to cause skin sensitization. Among the most important of these are access to the viable epidermis, protein reactivity (or conversion in the skin to a protein-reactive metabolite) and hence the ability to form stable conjugates with proteins, elicitation of cytokine production by skin cells, and the initiation of T-lymphocyte responses. In addition, qualitative aspects of induced immune responses will influence the form that allergic sensitization will take, and the conditions of exposure to the allergen may also result in the acquisition of specific immunologic tolerance rather than active sensitization. It is anticipated that an increasingly sophisticated understanding of the requirements for the development of skin sensitization and other forms of chemical-induced allergy will provide exciting new opportunities for toxicologic investigation and clinical management.

THE PURPOSE OF this article is not to review in detail the mechanisms of allergic contact dermatitis; recently detailed accounts are available elsewhere.[1–3] Rather the focus here is on the requirements for the acquisition of skin sensitization (the induction phase of contact allergy). Although not considered here, the elicitation of allergic contact dermatitis in a previously sensitized subject has rather different requirements.[1–7] In immunologic terms, the induction phase of skin sensitization can be summarized as follows. Several important changes in the skin are provoked following topical exposure to a contact allergen, including the induction or altered expression of various chemokines and cytokines that together orchestrate the development of cutaneous immune responses. A proportion of epidermal Langerhans' cells (LCs), some of which bear antigen, is induced to migrate, via afferent lymphatics, to draining lymph nodes, where they accumulate as mature dendritic cells (DCs). Antigen is presented to responsive contact allergen-specific T lymphocytes,[8,9] resulting in activation and selective clonal expansion of this population. The individual is now sensitized and is able to respond in a more aggressive and accelerated manner to the same chemical if encountered subsequently at the same or a different skin site.

The question is why is it that some chemicals have the potential to cause skin sensitization whereas others do not. To cause contact sensitization, a chemical must negotiate a number of potential obstacles; failure to overcome any one of these will prevent the successful induction of an appropriate cutaneous immune response. The properties that are required for a chemical to be a contact sensitizer are illustrated in Figure 1 and are considered below.

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Skin Penetration and Access to the Viable Epidermis

The first potential obstacle to a contact-sensitizing chemical is the stratum corneum. For a chemical to initiate an immune response in the skin, it must negotiate this barrier to access the viable epidermis where the cellular sentinels of the adaptive immune system (LCs) reside. Under normal circumstances, unless the barrier function of the skin has been compromised by trauma or disease, skin penetration will be determined by the physicochemical properties of the chemical. One parameter that is of some importance is the octanol/water partition coefficient (P or log P), a measure of the lipophilicity of the chemical. The higher the log P value, the more lipophilic the material, and, in general, lipophilic compounds penetrate the skin more easily than do hydrophilic materials. For various homologous series of chemicals, including phenols and alcohols, a good correlation exists between log P and absorption through skin. For structurally unrelated chemicals, however, permeability does not necessarily correlate directly with log P because other properties, such as molecular weight and melting point, may also play a role.[10] There is also a relationship between log P and skin sensitization for

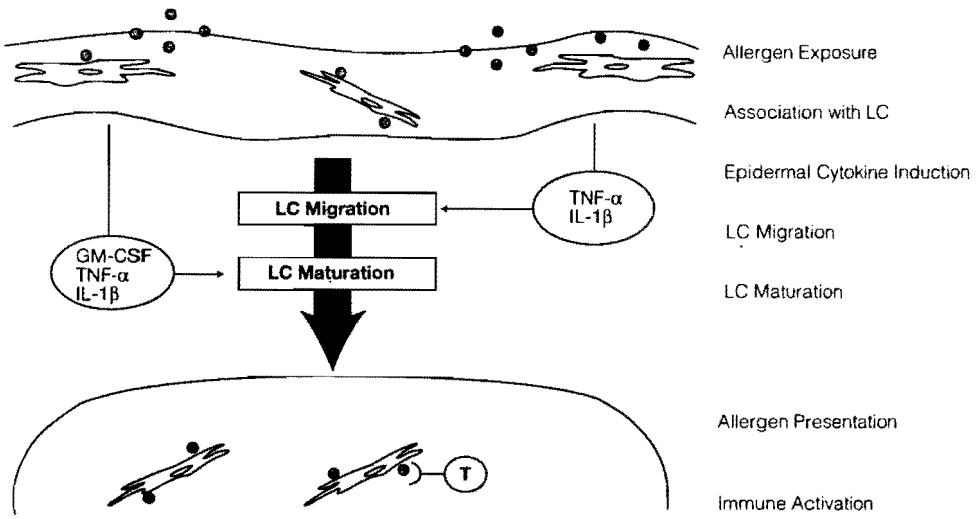

Figure 1. Factors known to influence the ability of a chemical to induce skin sensitization. The chemical allergen (●) must have the ability to penetrate the stratum corneum to access the viable epidermis. It must be protein reactive (or metabolized to a protein-reactive species) such that it forms stable complexes with host proteins. The hapten-protein complex must be recognized, internalized, and processed by epidermal Langerhans' cells (LCs). Sufficient levels of dermal trauma must be induced to provoke proinflammatory cytokine production by skin cells and thus stimulate the mobilization and directed migration of Langerhans' cells (some of which will carry allergen) via the afferent lymphatics and appropriate localization within the paracortical regions of the draining lymph node. Allergen expressed on the surface of lymph node dendritic cells must be recognized by specific T lymphocytes and the appropriate quality and quantity of T-lymphocyte differentiation and division stimulated.
GM-CSF = granulocyte-macrophage colony-stimulating factor; IL = interleukin; TNF = tumor necrosis factor.

structurally related chemicals, with, for example, the relatively lipophilic alkylated aldehydes (high log P) exhibiting marked contact sensitization potential whereas the hydrophilic hydroxyaldehyde oxidation products displayed lower log P values and reduced contact allergenic potential.[11] The importance of skin penetration is further illustrated by comparisons of the sensitizing activity of the alkylating agent streptozotocin (STZ) with its structural analogue *N*-methyl-*N*-nitrosourea (MNU).[12,13] The former was shown to lack skin sensitization potential, measured in mice as a function of induced proliferation in the lymph nodes draining the site of application, whereas the latter provoked vigorous responses.[12,13] The sugar substituent on STZ reduces lipid solubility (log P is lower by 2 units compared with MNU) and therefore presumably inhibits the passage of the chemical across the stratum corneum. Bypassing the stratum corneum by intradermal administration of STZ resulted in a dose-dependent activation of lymphocyte proliferation in draining lymph nodes, confirming that this chemical is inherently allergenic if it can access the viable epidermis.[13]

It has also been postulated that the molecular size of chemicals is an important determinant of contact sensitization potential (the so-called 500 D rule). The suggestion is that there is an upper size limit of approximately 500 D for molecules that can pass through the stratum corneum, based on the observation that most topically applied pharmaceuticals (both those used for dermatotherapy and in transdermal drug delivery systems) have a molecular mass of less than 500 D.[14] Furthermore, all components of the routine patch test series advised by the International Contact Dermatitis Research Group for the diagnosis of contact allergy, which comprises the most common skin sensitizers, are less than 463 D.[14] The only exception to this rule is neomycin sulfate, which has a molecular weight of 712 D. However, this molecule is a dimer of two neamine molecules (each with a molecular weight of 322 D), and it may be that the monomer is the sensitizing agent.

Protein Reactivity or Metabolism to a Protein-Reactive Species

A further key property of contact-sensitizing chemicals is protein reactivity or metabolism to a protein-reactive species. In their native state, low-molecular-weight chemicals (or "haptens") are unable to induce immune responses. Immune recognition requires the formation of a larger complex between the chemical and a protein. This theory of covalent interaction between skin proteins and

chemical sensitizers (the so-called electrophilic theory) was first postulated by Landsteiner and Jacobs in 1936[15] and has since been extended and refined by others.[16–18] However, the relationship between electrophilic activity and skin sensitization potential is not absolute. Some materials ("prohaptens") may be converted chemically, often by oxidation, to protein-reactive haptens; examples include limonene and colophony.[3,19,20] In addition, xenobiotic metabolizing enzymes in the skin, which is now recognized as an important site of extrahepatic metabolism, can convert prohaptens to electrophilic species as a result of detoxification of the parent molecule.[3,21,22] An example of the role that metabolism can play in the development of contact sensitization is the activation of cinnamic alcohol to the presumed allergen cinnamic aldehyde. Cinnamic alcohol is not itself protein reactive, and yet it is a known human allergen and a constituent of the European Standard Test Allergen fragrance mix. The presence of protein-bound cinnamaldehyde has been detected in skin treated with cinnamic alcohol, using immunohistochemical techniques; this moiety is a potent contact allergen and is presumably formed by the action of cutaneous alcohol dehydrogenase, providing for a potential mechanism for sensitization to cinnamic aldehyde.[21,22]

For effective sensitization, a chemical must therefore be inherently protein reactive or must be converted in the skin to a protein-reactive metabolite. Chemicals that are unable to associate effectively with proteins will fail to stimulate a cutaneous immune response. For those chemical contact allergens that require metabolism to a protein-reactive species, it is possible that genetic differences in metabolism may play a role in the differential susceptibility of individuals to the development of contact hypersensitivity responses to these materials.

Cutaneous Inflammation and Local Trauma

It is assumed that the hapten-protein conjugates formed as described above will be internalized and processed by epidermal LCs. Following activation, these cells are stimulated to leave the epidermis and migrate to draining lymph nodes, providing a mechanism for transporting antigenic signals from the skin to the regional lymph nodes. As part of the process of migration, LCs undergo functional maturation such that they lose the ability to process antigen and acquire instead the characteristics of antigen-presenting DCs. The mobilization and maturation of LCs are orchestrated by epidermal cytokines and chemokines, the downstream effects of which (eg, changes in adhesion molecule expression) facilitate the movement of LCs from the epidermis, their migration across the basement membrane, and their later localization within lymph nodes.[7–9] The response of LCs to contact allergens is impaired or inhibited and the development of skin sensitization is compromised if the necessary cytokine signals are unavailable.[8,9,23,24] Cytokines known to influence LC function and to be required for optimal contact sensitization include interleukin (IL)-1β, tumor necrosis factor α (TNF-α), and granulocyte-macrophage colony-stimulating factor. These three cytokines act in concert on LCs during their transit to the local lymph node to effect their functional maturation into immunostimulatory DCs.[25,26] In addition, IL-1β and TNF-α provide mandatory signals for the mobilization of LCs.[23,24,27,28] These cytokines must therefore be available locally at relevant concentrations for the normal development of skin sensitization. Other cytokines, such as IL-10, play a down-regulatory role in the skin in both the induction and elicitation phases of contact sensitization by inhibiting LC migration and accessory function and the production of inflammatory cytokines, including interferon-γ (IFN-γ).[29–31]

In many instances, it appears that topical administration of a contact allergen alone is sufficient to trigger the induction or up-regulation of those cytokines necessary for the effective acquisition of sensitization. Under these conditions of exposure, the chemical allergen itself causes sufficient cutaneous inflammation and irritation and hence the production of proinflammatory cytokines by skin cells. Chemical allergens that do not provoke the level of trauma necessary to provoke proinflammatory changes may fail to induce cytokine responses. The ability of physical inflammation to augment contact sensitization responses in human subjects was documented as long ago as 1966, prior to any understanding of the relevant biologic mechanisms.[32] More recent studies have demonstrated that individuals who are patch test positive (in this case, to colophony) have a lower threshold of sensitivity to the skin irritant sodium lauryl sulfate (SLS) than patch-test-negative matched controls, suggesting increased susceptibility to allergy in these subjects.[33] Further evidence of this relationship between irritation and sensitization derives from studies performed in mice with 2,4-dinitrochlorobenzene (DNCB), a potent contact allergen that is also a skin irritant at high concentrations. The ability of DNCB to induce draining lymph node activation following topical application was measured with or without the coadministration of SLS. At high (irritant) doses of DNCB, SLS did not impact on the levels of immune activation induced by the allergen. However, at lower (nonirritant) concentrations of DNCB, responses were augmented by

SLS. The interpretation is that topical exposure to comparatively high levels of DNCB provides both a sensitizing signal and sufficient trauma to provoke optimal proinflammatory cytokine production. Following exposure to lower (and less irritant) levels of the allergen, insufficient levels of inflammation are provoked, and optimal immune activation requires the provision of an exogenous inflammatory stimulus (supplied in this instance by SLS).[34]

In summary, therefore, it is our view that for the optimal acquisition of skin sensitization, a certain level of skin irritation or trauma will be required and that chemicals that fail to trigger sufficient local cytokine production may (in the absence of an additional exogenous proinflammatory stimulus) be unable to realize their full potential as allergens. Given that the chemical matrix in which a chemical is experienced on the skin can impact on both penetration and proinflammatory activity, it comes as no surprise that the vehicle in which a contact allergen is delivered to the skin can have important influences on sensitizing activity.[35–37] For example, it has been demonstrated that the sensitizing potential of the skin-sensitizing fluorochrome fluorescein isothiocyanate (FITC), measured as a function of induced proliferative responses in the draining lymph node, was augmented substantially by the addition of dibutyl phthalate (DBP).[35] In vitro skin absorption studies indicated that DBP was associated with a small increase in percutaneous absorption of FITC, but more importantly, DBP treatment resulted in a marked increase in the frequency of lymph node DCs bearing detectable antigen. In other experiments, coadministration of the skin irritant SLS with suboptimal concentrations of the contact allergen DNCB augmented proliferative responses as described above by provision of danger signals, under conditions in which no impact on the efficiency of skin absorption was observed.[34,36] Although it is clear that the vehicle matrix can have important effects on skin-sensitizing potency by a variety of mechanisms, not all allergens are affected similarly; thus, it is not possible at present to predict the likely impact of formulation without recourse to direct testing.[37–39]

Immune Recognition

The induction of skin sensitization and the subsequent elicitation of allergic contact dermatitis are dependent on the development of hapten-specific T lymphocytes. The inducing hapten is presented to responsive T lymphocytes in skin-draining lymph nodes by antigen-presenting cells. The T lymphocytes recognize the hapten as a structural entity attached to self-peptides anchored within the binding grooves of major histocompatibility complex determinants displayed by the antigen-presenting cells.[40,41] These cells either derive directly from antigen-bearing LCs that have migrated from the skin or are resident DCs that have acquired antigen from LCs arriving in the lymph nodes. The draining lymph nodes become activated, characterized by an increase in node weight and total cellularity, T-cell activation and proliferation, and the production of various cytokines. The importance of peripheral lymph nodes for the acquisition of contact sensitization has been confirmed recently by the observation that lymphotoxin-α–deficient mice that lack lymph nodes fail to develop skin sensitization.[42]

It appears that the vigor of the T-lymphocyte response to contact allergen is determined by a series of quantitative interdependent biologic relationships. The effectiveness of LC migration from the epidermis (and thus the amount of antigen reaching the node) correlates with the dose of chemical experienced. Further, the vigor of T-lymphocyte proliferation is dependent on the extent of DC accumulation in draining lymph nodes.[43] Finally, there is evidence also that the magnitude of the T-lymphocyte proliferative response (equivalent to the degree of clonal expansion) that occurs in the induction phase of contact sensitization in turn correlates with the vigor of the reactions provoked in the elicitation phase.[44] The assumption is that cell turnover in the induction phase will control the frequency of various specific effector cells that are responsible for eliciting allergic contact reactions following subsequent encounters with the inducing allergen.[45] Given these relationships, it is perhaps not unexpected that the threshold concentration of chemical that is necessary to provoke lymphocyte proliferation in mice has been shown to correlate with relative sensitizing potency. Thus, the dose of chemical required to stimulate a threshold (threefold increase compared with concurrent controls) level of thymidine incorporation in the murine local lymph node assay relates to what is known of the differential ability of agents to cause allergic contact dermatitis among humans.[46,47]

Quality of Immune Response

In considering the properties that confer on chemicals the ability to induce allergy, it is important to recognize that there is a qualitative element. In addition to causing skin sensitization, chemical allergens may also result in sensitization of the respiratory tract. A supplementary question is, therefore, why some chemical sensitizers (the majority) are associated usually, or exclusively, with allergic contact dermatitis whereas others are implicated as causes of occupational asthma. Such differences are not simply a result of the most

frequent routes of exposure. Studies in mice have revealed that chemical contact and respiratory allergens induce qualitatively divergent immune responses with respect to the activation of functional subpopulations of T lymphocytes.

The development of adaptive immune responses (including allergic responses) is orchestrated by the activity of $CD4^+$ T helper (Th) cells and $CD8^+$ T cytotoxic (Tc) cells and their cytokine products. For some years now, it has been known that during the course of the evolution of immune responses, discrete subpopulations of Th and Tc cells (designated Th1 and Th2 and Tc1 and Tc2 cells) differentiate from common precursors.[48] These subsets differ with respect to their cytokine secretion profiles, with type 1 (Th1 and Tc1) cells expressing, among other cytokines, IFN-γ and IL-2 whereas type 2 (Th2 and Tc2) cells preferentially secrete IL-4, IL-5, IL-10, and IL-13. The relevance of these subpopulations to the development of allergic reactions is that type 1 cells broadly favor cell-mediated immune responses (including delayed-type hypersensitivity reactions such as contact sensitization) whereas type 2 cells favor immunoglobulin E (IgE) antibody responses and immediate-onset allergic reactions.[49] We and others have shown that topical exposure of rodents to contact allergens such as DNCB or 2,4-dinitrofluorobenzene (DNFB), which are known or suspected not to cause sensitization of the respiratory tract, induce a preferential type 1 cytokine secretion profile.[50–53] In contrast, those chemicals such as trimellitic anhydride that have been shown in humans to cause allergic sensitization of the respiratory tract elicit instead selective type 2 responses.[51–53] From experiments conducted in Brown Norway strain rats and BALB/c strain mice, it appears that the contact sensitizer DNCB and the respiratory allergen trimellitic anhydride exhibit a species-independent innate ability to provoke type 1 and type 2 cytokine production patterns, respectively.[52] However, the longevity of exposure to a chemical allergen appears to influence markedly the induced cytokine milieu, with repeated exposure, particularly under occlusion or through damaged (tape-stripped) skin, resulting in a shift in cutaneous cytokine expression away from a Th1- (in which IFN-γ and IL-2 production predominates) to a Th2 (IL-4 and IL-10)-dominated response for some contact allergens. It seems likely that the expression of IL-4 (and possibly other type 2 cytokines), particularly at sites of dermal challenge, regulates what is considered to be a largely Th1- or Tc1-dependent immune process although the factors governing whether such is up- or down-regulated are still unclear.[54–56]

On this basis, it would appear therefore that although various factors described above will determine whether and to what extent allergic sensitization is induced, other (as yet undefined) factors related to the nature of the chemical allergen itself will govern the form that sensitization may take.

Sensitization versus Tolerance

As described above, there are a number of hurdles that a chemical allergen must overcome to have the intrinsic ability to cause skin sensitization when applied topically. However, this intrinsic potential to elicit contact allergy is not manifested in all exposed individuals. There has been some controversy regarding the genetic susceptibility of individuals to mount contact allergic responses, with some studies reporting positive findings such as increased familial risk of contact allergy[57] or the association of a particular metabolic phenotype with contact allergic patients.[58] Other studies, including those of monozygotic twins with nickel allergy, have failed to demonstrate a significant increase in susceptibility above the background population.[59,60] It is more likely that interindividual differences in the development of contact sensitization to known allergens are due to variations in the way in which an allergen is first experienced. In experimental animals it has been demonstrated that the route of initial exposure to an allergen is of critical importance in determining whether sensitization or immune tolerance occurs. Thus, prior oral exposure of mice to the potent skin sensitizer DNFB inhibited the development of allergic reactions to subsequent topical application of the same chemical.[61] Similarly, the development of contact hypersensitivity responses to nickel in guinea pigs could be markedly suppressed by prior oral exposure to the compound[62]; however, if the animals first experienced even low doses on the skin, tolerance did not develop.[63] Interestingly, this phenomenon appears to be relevant to the development of contact sensitization to nickel in humans, with the route (and age) of first exposure to nickel having marked effects on the incidence of allergic contact dermatitis.[64] In these studies, it was demonstrated that ear piercing strongly favored development of nickel contact hypersensitivity. However, patients having had oral contact with nickel-releasing appliances (dental braces) at an early age, but only if prior to ear piercing, showed a reduced frequency of nickel hypersensitivity. Frequencies of other hypersensitivities, in particular to fragrances, were not affected.

Conclusions

A number of characteristics can be identified that confer on chemicals the ability to induce skin sensitization and

allergic contact dermatitis. These include the capacity to gain access to the viable epidermis across the stratum corneum, to associate stably with host proteins, to provoke a certain degree of proinflammatory cytokine production by skin cells, and to be recognized by specific T lymphocytes. In addition, the quality of immune response (with respect to the activation of discrete T-lymphocyte subpopulations) provoked by a chemical allergen will influence the form that allergic disease will take. The effectiveness with which these requirements are met, and possibly other properties of the chemical that influence the vigor of induced immune responses, together with the extent of exposure, will dictate the degree to which sensitization is achieved. Furthermore, the conditions of exposure, particularly the age at which first exposure occurs and the route by which an allergen is first experienced (oral versus topical), will determine whether sensitization or tolerance ensues.

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