Allergic contact dermatitis: understanding the immune response and potential for targeted therapy using cytokines

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Allergic contact dermatitis is the most common job-related disease of the western world. The only available treatments are avoidance of contact with the allergen and the use of potent corticosteroids. Recently, the role of cytokines in the pathogenesis of this disease has been studied and, besides defining the key molecules and basic cellular immune responses responsible for disease development, these studies might help to develop new therapeutic strategies to target cytokines and thereby try to alter or abrogate ongoing immune reactions.

Allergic contact dermatitis (ACD) is one of the most common dermatoses, and its socioeconomic impact as an acquired, job-related disease is enormous. It is estimated that the percentage of ACD among all job-related diseases is 40–60%; its prevalence in the general population is 1.5–3.0%; and its incidence reaches 5–10/1000 people per year[1]. Often, the sensitizing allergens are common and difficult to avoid.

The distribution of ACD in the population is, however, rather heterogeneous. Whereas the prevalence of sensitization against the most common allergen, nickel, in the general population is 7%, this frequency rises to 20% in young women[2]. The reason for this increase is the frequent use of nickel-containing jewelry and body piercing, which facilitate sensitization in this group of people[1]. Even more pronounced are the differences in the distribution of ACD among various professions. Again, circumstances that facilitate penetration of potential contact allergens into skin, for example by disrupting the skin barrier (by frequent hand washing or wearing gloves), enhance the risk of sensitization. Hairdressers, metal workers, people working in the food industry and healthcare professionals are the most frequently affected individuals.

Clinical features of ACD

The clinical picture of ACD is that of an acute dermatitis. Lesions usually appear within 24–72 h of contact with the sensitizing agent and start with an erythematous reaction, often depicting the area where the allergen was applied. This erythema can then become vesiculous or exudative and show scales and crusts. The lesions are typically very itchy, and eczematous lesions can spread all over the body (hematogenous spread). Repeated contact with (low doses of) the sensitizing agent can promote the development of a more chronic and less inflammatory form of ACD.

Diagnosis of ACD is usually confirmed by epicutaneous patch testing; here, the history of the patient can give valuable hints as to the identity of the allergen. The potential allergens are then applied to the skin of the patient in subtoxic concentrations.

Besides avoiding contact with the allergen, application of topical steroids is the first choice for treatment. Very resistant and extensive cases require systemic steroids.

Immune reactions in ACD

Binding of haptens

ACD is a delayed-type hypersensitivity reaction mediated by T cells. ACD can be induced easily and reproducibly, and there are animal models in the mouse and guinea pig; for these reasons, ACD is one of the favorite model diseases used by immunologists to study primary T-cell responses. The allergens that induce ACD are mostly haptens. Haptens are small, chemically reactive substances that are only recognized by the immune system when bound to a protein or a peptide structure. Because these haptens are

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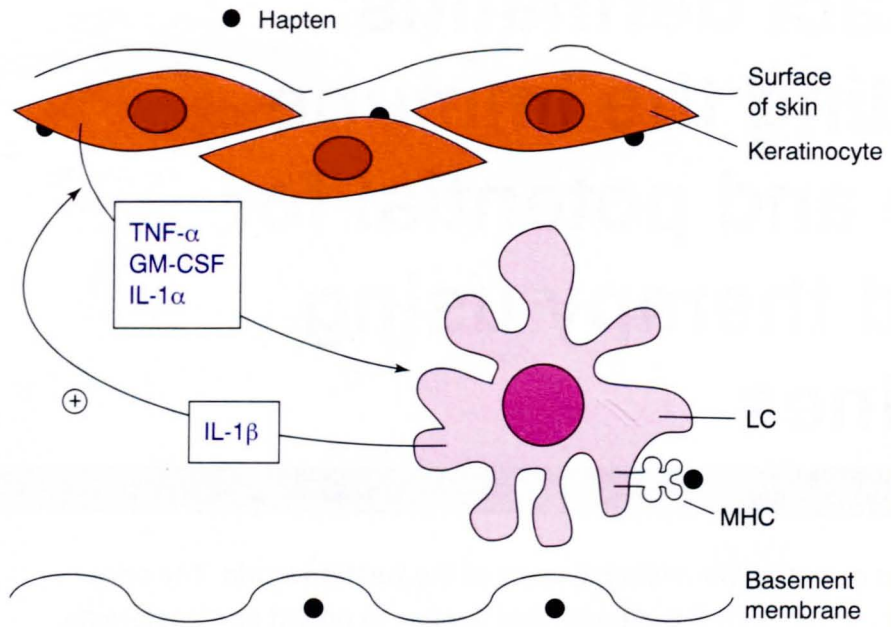

Figure 1. Cytokine cascade in the induction phase of contact sensitivity. Langerhans-cell (LC)-derived interleukin 1β (IL-1β) activates a cytokine cascade in epidermal keratinocytes after contact with hapten. Following binding of haptens, LCs start to release IL-1β, which is necessary for the induction of a cytokine cascade [tumor nectosis factor α (TNF-α), granulocyte–macrophage colony-stimulating factor (GM-CSF) and interleukin 1α (IL-1α)] in surrounding epidermal keratinocytes. This cytokine cascade then induces expression of major histocompatibility complex (MHC) molecules with bound haptens on the LC.

very chemically reactive, they bind rather non-specifically to a multitude of structures when applied to the skin. However, binding to epidermal Langerhans cells (LCs) is considered to be the critical interaction. LCs belong to the family of dendritic cells (DCs), which are potent antigen-presenting cells (APCs)[3]. Studies have shown that the number of LCs present in the skin directly correlates with the ease with which the animal can be sensitized to an allergen. (Similar studies have been performed with human skin that has been depleted of LCs by UV light.) It is virtually impossible to sensitize the animal through LC-depleted skin. It is unclear whether the haptens need to bind to LCs to activate them, or whether the haptens need to be processed by LCs. However, recent studies have revealed that haptens bind to certain amino acid residues on peptides that are subsequently presented to T cells by the major histocompatibility complex (MHC) molecules of the LC[4].

LC activation

Binding of haptens that act as allergens activates LCs *in situ*. Although the exact mechanism of activation remains unclear, recent studies have shown that tyrosine kinases of the Src family are involved in the early signal transduction events[5]; only 15 min after the binding of the allergen to the LC, the cell starts to upregulate interleukin 1β (IL-1β) mRNA and protein production[6] (Fig. 1). IL-1β is a 'primary' cytokine that can induce other proinflammatory cytokines. By releasing IL-1β, the LC therefore induces other epidermal cells such as keratinocytes to become activated and produce other cytokines. The release of IL-1β by LC seems to be essential for this process, because neutralization of IL-1β with a specific antibody prevents the induction of all other cytokines and also epicutaneous sensitization[7].

Cytokine cascade

The production of IL-1β by LCs induces a cascade of various other cytokines, mostly from keratinocytes. Among them are tumor necrosis factor α (TNF-α), the interleukins IL-1α, IL-6, IL-10, granulocyte–macrophage colony-stimulating factor (GM-CSF), macrophage inflammatory protein 2 (MIP-2) and interferon-induced protein 10 (IP-10); MHC class II molecules are also upregulated by IL-1β (Fig. 1). This mixture of cytokines contains factors such as TNF-α, IL-1, and GM-CSF that enhance the maturation status of LCs. As a result, LCs upregulate MHC class II molecules, adhesion molecules and co-stimulatory factors, and mature from potent antigen-processing cells into potent antigen-presenting cells[8]. Similar studies have been performed in the human system, with identical results. In mice, TNF-α and IL-1β are essential factors for this induction of contact sensitivity, because knockout mice that do not produce TNF-α or IL-1β are impeded in their ability to become sensitized to antigen[9,10].

LC migration

Besides inducing the maturation of LCs *in situ*, the cytokine arsenal of the epidermis also induces the emigration of LCs, via lymphatics, into the regional lymph node, where the initial contact between a LC and a T cell takes place. On their way to the regional lymph node, LCs continue to mature: they develop long dendrites, synthesize co-stimulatory factors and upregulate the production of MHC molecules. In human studies, LCs prepared from draining lymph vessels were much more potent stimulators of T-cell responses than LCs prepared directly from epidermis, suggesting that LCs emigrating from skin have functionally matured[11].

T-cell priming in the lymph node

Once the LC enters the regional lymph node, T cells that possess T-cell receptors (TCRs) complementary to the MHC molecule–allergen complex on the surface of the LC are activated. Both $CD4^+$ (MHC class II-restricted) and $CD8^+$ (MHC class I-restricted) T cells are stimulated – a finding that has important implications for the outcome of the T-cell response[12] (Fig. 2).

T cells can be further subdivided functionally into Th1 or Th2 cells (for $CD4^+$ T cells) and Tc1 or Tc2 cells (for $CD8^+$ T cells)[13]. Th1 and Tc1 cells produce IFN-γ and mediate cellular effector functions such as ACD, whereas Th2 and Tc2 cells produce IL-4, IL-5 and IL-10, and suppress cellular immune reactions while enhancing antibody-mediated immune responses (Fig. 3). Both subtypes are known to crossregulate one another. In murine ACD, the resulting hapten-specific $CD8^+$ T cells produce IFN-γ and therefore belong to the Tc1 category, while the resulting $CD4^+$ T cells produce IL-4 and IL-10 and are therefore classified as Th2-type T cells. While Tc1 cells are the effector cells that mediate the sensitization process, Th2 cells should counteract the inflammatory process, thus preventing damage to the organism.

Although it is not clear which factors govern the priming of the two different types of T cells by LCs, it is assumed that interleukin 12 (IL-12) might play a role in the development of Tc1 cells. IL-12 is a cytokine that promotes the development of type 1 T cells. In mice, IL-12 is produced by dendritic cells (presumed to be LCs that have migrated from the skin into the lymph node) in the draining lymph node 14 h after the application of allergen to the skin[14]. As a further proof of an important role for

IL-12 in the induction process of ACD and early T-cell development, injection of an antiserum against IL-12 not only prevents sensitization in mice, but also induces tolerance to the respective allergen *in vivo*[15]. All further attempts to sensitize the animal with the same allergen, even in the absence of the tolerance-inducing signal (in this case the blockade of IL-12), were unsuccessful and this tolerance can be transferred from one animal to a syngeneic littermate by the transfer of T cells. It is therefore likely that IL-12 is an essential molecule for the appropriate priming of T cells (probably Tc1 cells) in ACD, at least in mice (Fig. 3).

Conversely, induction of Th2 cells by LCs might result from the influence of IL-10, which is produced by epidermal keratinocytes late after the application of allergen. IL-10 is a counter-regulatory cytokine: it not only inhibits production of proinflammatory cytokines (such as epidermal IL-1 or TNF-α), but also 'distorts' the APC functions of LCs by affecting certain co-stimulatory molecules on the surface of the cell[16]. Co-stimulatory molecules are essential signals for the development of T-cell responses. Different T-cell populations seem to require distinct co-stimulatory signals. Absence of the appropriate co-stimulatory molecule when the TCR has bound its cognate MHC–allergen complex leads to a state of tolerance called anergy[17] (Fig. 4). Even attempts to drive the anergic T cell into proliferation by re-stimulation with a fully competent APC displaying the MHC–allergen complex on its surface, plus appropriate co-stimulation, will not be successful.

This is the case with LCs treated with IL-10; IL-10-treated LCs are no longer capable of inducing Th1 cells (producers of IFN-γ) and they even tolerize the Th1 cells, owing to a lack of co-stimulatory molecules on the LCs (Fig. 4). However, the induction of Th2 cells by IL-10-treated LCs remains unchanged. It is therefore conceivable (although not formally proven) that the LCs that leave the epidermis shortly after allergen contact produce IL-12 and induce differentiation of $CD8^+$ Tc1 effector cells that mediate hapten-specific inflammation. The LCs that leave the epidermis at later stages (when IL-10 is being released by keratinocytes) might not be able to induce IFN-γ-producing T cells and might therefore produce counter-regulatory Th2 cells. This interplay of two different types of T cells might be important for an efficient and balanced immune reaction.

Most of the T cells that have been 'educated' in lymphoid tissue draining the skin express the tissue-specific 'homing receptor' cutaneous leukocyte-associated antigen (CLA)[18]. CLA allows the primed T cells to migrate back to skin via blood and lymph by binding to its counter-receptor, E-selectin, which is preferentially expressed on endothelial cells of the skin. In the elicitation phase of ACD, therefore, no priming or extensive recruitment of T cells is necessary, but the immune response is achieved by T cells already present.

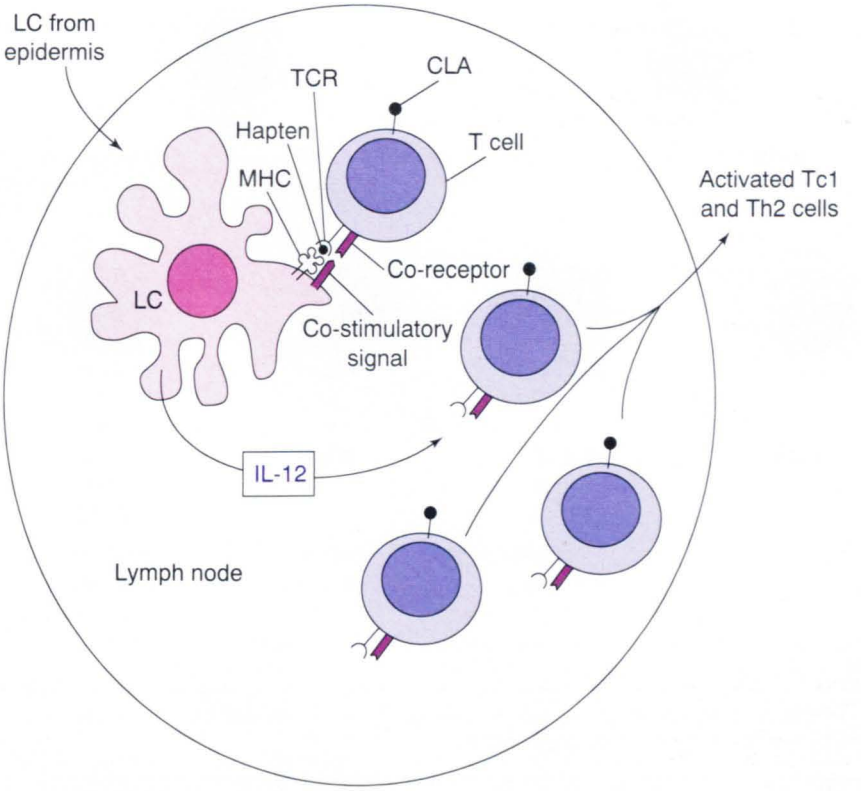

Figure 2. Activation of T cells by Langerhans cells (LC) in draining lymph nodes. LCs that have migrated out of the epidermis into the lymph node bind to appropriate T cells and the corresponding T-cell receptor (TCR) with their major histocompatibility complex (MHC) molecules. Co-stimulatory signals for the activation are delivered by membrane-bound molecules, as well as soluble mediators such as interleukin 12 (IL-12). T cells are activated and start to express the homing receptor CLA (cutaneous leukocyte-associated antigen). The activation process induces Tc1 [$CD8^+$, interferon γ (IFN-γ)-producing] and Th2 [$CD4^+$, interleukin 4 (IL-4)-producing] T cells.

Using cytokines as therapeutic tools for contact sensitivity

The information gained from studying the pathogenesis of ACD can be used in several ways for the development of therapeutic strategies. From the data mentioned above, it has become clear that certain cytokines (such as IL-1β or TNF-α) seem to be essential for the development of sensitization. Furthermore, the inflammatory reaction seems to be carried out largely by IFN-γ-producing $CD8^+$ Tc1 effector cells. Thus, there is a type 1 cytokine pattern that seems to be partially antagonized by Th2-type cells producing IL-4, IL-5 and IL-10.

This suggests three different ways to influence the immune response in ACD artificially. First, abrogating or neutralizing early cytokine signals (such as IL-1β) would prevent the development of an immune response. Second, an ongoing immune response could be modified by strengthening the type 2 reaction pattern observed in ACD reactions. Finally, the immune response in ACD could be reduced by inducing hapten-specific tolerance.

Neutralizing early inflammatory signals by antisense oligonucleotides

Studies using neutralizing antibodies have demonstrated that the cytokines IL-1β and TNF-α that are produced early after application of an allergen in skin both seem to be essential for the development of ACD[7,19]. These results have been confirmed in knockout animals that lack the respective cytokine genes. Blocking these signals might therefore have a therapeutic effect in humans. Unfortunately, antibodies for therapeutic use are usually derived from animals and are often recognized as foreign proteins by the human immune system, thereby inducing side

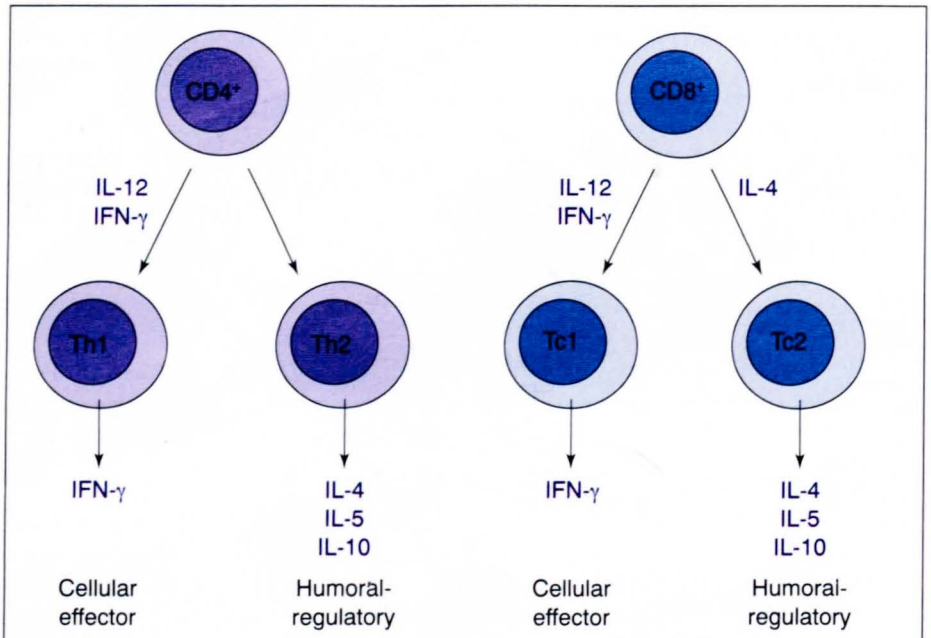

Figure 3. T cells can be divided into various subtypes. $CD4^+$ T cells and $CD8^+$ T cells differentiate further into Th1 [interferon γ (IFN-γ)-producing] and Th2 (which produce the interleukins IL-4, IL-5 and IL-10) and Tc1 (IFN-γ-producing) and Tc2 (IL-4-producing) T cells. The subtypes are induced by the presence of IL-12, IFN-γ or IL-4 during the priming process. The effector functions of these cells vary between cellular effector functions and humoral-regulatory functions.

effects; however, antibodies can be 'humanized' to circumvent this problem.

Antisense oligonucleotides could theoretically be used to block cytokine production; these are small DNA or RNA sequences that precisely complement stretches of mRNA in the gene of interest. Because they are small molecules, they are easily taken up by cells *in vitro*. Once they have entered the cell, they should bind to mRNA molecules that complement their own sequence, thereby preventing translation of the mRNA of interest (for example, that encoding IL-1β). Although there are examples for successful uses of antisense oligonucleotides in animal models and they are starting to be tried in humans, there remain problems with this approach. For example, lack of specificity and difficulties delivering the oligonucleotides to cells in the skin are likely to prove obstacles to their application in the near future.

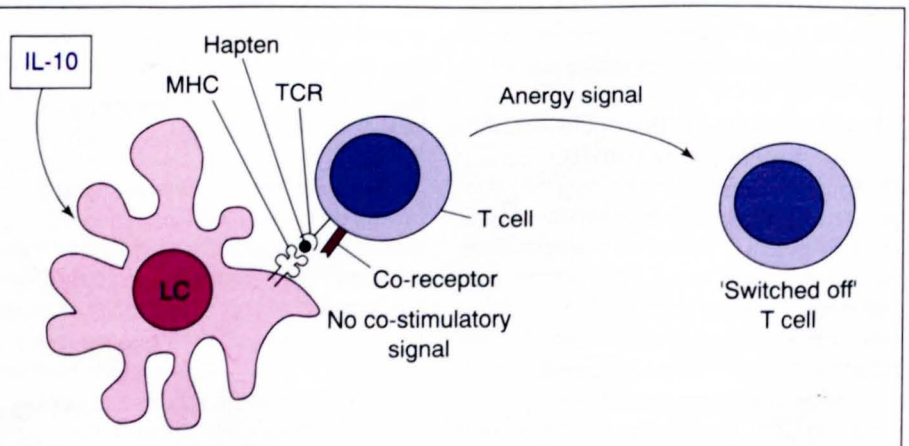

Figure 4. Induction of tolerance in Langerhans cells (LCs) by treatment with interleukin 10 (IL-10). Langerhans cells that are exposed to IL-10 can engage the T-cell receptor (TCR) with their major histocompatibility complex (MHC) molecules and the bound antigen; however, LCs treated with IL-10 do not possess a co-stimulatory signal and therefore induce a state of antigen-specific unresponsiveness (anergy).

Modifying an immune response using cytokines

As described above, ACD is a type 1 immune response characterized by the production of IFN-γ and IL-12 and the presence of Th1 or Tc1 cells. Th2 cells and the cytokines released by these cells (especially IL-4 and IL-10) counteract the effects of type 1 cells in ACD. Therefore, enhancing the type 2 response in ACD might reduce the pathological events. In mice, systemic administration of IL-4 prevented the ear-swelling responses in ACD, proving that IL-4 could indeed counteract the development of IFN-γ-producing T cells[20]. Systemic treatment with IL-4 also had a long-term therapeutic effect, protecting animals against proinflammatory tissue destruction. The number of IL-4-producing T cells was significantly higher in animals treated with IL-4, suggesting that IL-4 production by T cells counteracted the development of an ACD response and tissue destruction.

Similar, but even more pronounced, effects were also achieved with systemic injection of IL-10; this prevented ear-swelling reactions in the elicitation phase of ACD in mice[21]. In mice, local injection of IL-10 before application of an allergen even induced allergen-specific tolerance in these animals[22]. Although these animal experiments indicate that immunomodulation using cytokines is a possible therapeutic strategy, there are certain problems that need to be addressed before these therapies can be used in humans.

First, the systemic application of cytokines in humans will be very problematic in benign diseases such as ACD because of nonspecific effects on various organ systems. Therefore, a targeted application – such as topical application to the skin – would be highly desirable before these agents could be used in humans. Unfortunately, cytokine molecules are too large to penetrate the skin barrier, so intradermal injection would be necessary; this approach would be painful and would rely on the systemic effects of the injected cytokine. The only successful cytokine therapy for ACD in animals by local intradermal injection of cytokines is injection of IL-10 before sensitization. Application of an allergen to the injected site induced tolerance and the animal was protected against further attempts to sensitize with this allergen[22]. This effect was allergen specific, and other immune functions in the mice were not affected. In humans, this approach might be useful as a 'vaccine' against allergens, but it would probably require no prior history of sensitization to the allergen. Applications of this strategy in humans would thererfore be rather limited.

Another strategy to target cytokines is by designing fusion proteins that contain a receptor structure and the cytokine of interest. These

approaches have been used successfully for the treatment of tumors such as malignant melanoma in animal models. In this case, the IL-2 protein was genetically fused to an antibody that recognized ganglioside molecules on certain mouse melanoma cells. Treatment of mice with this chimeric protein resulted in eradication of the tumor cells and long-lived immunity to the tumor[23]. The limiting factor in this approach for use in ACD is to identify a good target structure that is sufficiently specific. For example, to induce expression of IL-4 in T cells, stimulating antibodies against the TCR or other receptors on the T-cell surface, such as CD3, CD4 or CD8, might be fused with the IL-4 molecule. This would allow IL-4 to influence T-cell cytokine secretion directly, either by antagonizing the production of IFN-γ or by enhancing IL-4 release. Such an approach would be most efficient if performed early in the development of ACD, when it is likely to be comparatively easy to modify immune reactions (as has been shown in the IL-4-injection studies in mice).

For targeting expression of cytokines to skin, certain antigens associated with disease and predominantly expressed in skin might be useful to achieve skin-selective expression of cytokines. Two well-characterized examples are the autoantigens of the bullous autoimmune diseases pemphigus vulgaris and bullous pemphigoid[24]. These antigens are predominantly (but not exclusively) expressed in skin and are recognized by autoantibodies in the course of the autoimmune process. Using chimeric proteins containing autoantibodies to these structures combined with IL-10, it might be possible to inhibit cutaneous inflammatory reactions and even tolerize against certain antigens, as has been shown in the injection studies using IL-10 in mice. However, it is important to note that a complete inhibition of the skin immune system would have to be avoided because of its role in immune surveillance against invading pathogens and developing tumors.

Induction of allergen-specific tolerance

Clinicians have long dreamed of the induction of tolerance against allergens in the treatment of ACD. Early studies focused largely on the induction of tolerance by oral application of high doses of allergens[25]. Feeding allergens to animals reduces the sensitivity of these animals to subsequent allergen sensitization. Normal mice cannot be sensitized to the most common allergen in the human system, nickel sulfate, because they are orally tolerized from birth, but if animals are bred for two generations under nickel-free conditions, transcutaneous sensitization of these animals becomes possible. However, toxicological feeding of allergens is problematic in humans because in most cases comparatively high doses of allergen would be needed to achieve tolerance. Therefore, this approach is not being pursued at present.

As an alternative, 'low zone tolerance' to contact allergens could be considered. Steinbrink *et al.*[26] have recently explored the cellular mechanisms responsible for low zone tolerance – a term used for the old observation that low doses of allergen tolerize, rather than sensitize, animals. They showed that low doses of allergen in mice bypass the skin immune system and pass directly to lymphoid organs such as lymph nodes and spleen. Here, they induce a Tc2 suppressor T-cell activity, generating IL-4-producing $CD8^+$ T cells, probably by using resting B cells as APCs. This finding might help to explain why some cases of ACD (such as chromate allergy in bricklayers) take years to develop; perhaps these people constantly 'tolerize' themselves with low doses of allergen until a certain threshold dose of the antigen is reached that is high enough to activate the skin immune system, when sensitization occurs. This model might also provide a useful system for humans. Application of low doses of allergens might serve as a useful preventive strategy for people with jobs that entail a high risk of sensitization to a particular allergen, although the protective effect of low zone tolerance does not seem to be long lasting. Threshold doses for the most common and important allergens in humans would need to be determined, and optimal routes of delivery and timing would need to be established.

Dendritic cell therapies

A further alternative might be the use of dendritic cells (DCs) as tolerizing agents. Although DCs are usually known as potent immunostimulatory cells, recent studies have indicated that they might also be appropriate for tolerance induction. Several laboratories have described the inhibitory effect of IL-10 on the APC function of DCs from various organs or culture systems in mice and humans. LCs, as well as blood-derived DCs from mice and humans, can be converted to tolerance-inducing cells by treatment of the cells with IL-10. IFN-γ-secreting T cells can be 'turned off' in an antigen-specific fashion by IL-10-treated DCs *in vitro*. DCs can now be grown in culture using GM-CSF, IL-4 and other cytokines, and peripheral human blood can be used as a source of precursor cells; applications in autologous human systems are therefore possible[27–29]. It is conceivable that DCs could be cultured from a patient with ACD. After treatment of the cells with IL-10 and pulsing with the respective allergen, these cells could be reinfused into the autologous patient. Because DCs can either 'tolerize' an ongoing type 1 response (IFN-γ-producing T cells) or induce a counter-regulatory type 2 reaction (IL-4-producing T cells), this form of therapy might be able to shut down an immune response such as ACD. This is still 'wishful thinking' because safety studies in animals have to be performed first in order to ensure that IL-10-treated DCs do not completely abrogate immune reactions in mice. Such studies using TCR-transgenic mice are currently under way.

Gene therapy for ACD

As well as the above-mentioned conventional therapies using proteins or peptides to affect immunologic reactivity, novel genetic approaches might also be useful additions to our therapeutic arsenal for ACD.

Skin is especially suitable compared with many target organs for genetic approaches because it is large and readily accessible *in vivo*. Therefore, transfection of skin cells *in vivo* with a gene of interest is technically possible. Theoretically, there are at least three different ways to transfect skin cells *in situ*: naked DNA, bioballistic transfer (gene gun) and liposomes.

Naked DNA and bioballistic transfer have provided encouraging results so far in animal models. In both approaches, cells expressing the transgene were found in the skin and, when naked DNA containing IFN-α was injected into the skin of papillomavirus-infected dogs that had developed warts, there was some therapeutic effect[30].

Almost all the therapeutic strategies outlined above for cytokine therapies using proteins can also theoretically be performed using genetic approaches. It is possible to transfect skin cells with genes coding for antisense oligonucleotides. Even though only transient gene expression can be achieved with these approaches, this might be

The outstanding questions

- What are the early events (before cytokine induction) leading to Langerhans-cell activation *in situ* in allergic contact dermatitis (ACD)?
- What is the essential signal for Langerhans cells to leave the epidermis?
- Why are type 1 and type 2 T cells induced in the same immune response in ACD?
- Which factors decide whether type 1 or type 2 reaction patterns dominate the immune reaction?
- What are the chemical hallmarks that qualify an allergen as 'allergenic' in ACD?

enough to affect immune reactions beneficially. As an example, expression of IL-4 or IL-10 in skin cells followed by local application of an allergen might be sufficient for the induction of a potent type 2 reaction that would then counteract a type 1 immune response such as ACD.

Concluding remarks

ACD is a common and widespread human disease with a significant socioeconomic impact. In certain cases, the causative allergen is difficult to avoid and there is no preventive therapy in sensitized individuals. With the help of animal models, modern molecular techniques have allowed a better understanding of the pathogenesis of ACD in mice and humans. Cytokines play an important role in the development of the disease process; in particular, IL-1β and TNF-α seem to be essential for the induction process of ACD. Other factors, such as IFN-γ and IL-12, are vital for directing the immune reactivity of the effector T cells, and insight into these key molecules has also allowed us to use them as targets for experimental therapeutic intervention strategies in animal models. Although the safety of these modern molecular intervention strategies needs to be established, their potential to cause allergen-specific downregulation of ongoing immune responses certainly favors them over less-specific therapies such as corticosteroids. Inhibiting the early cytokine signal (IL-1, TNF) by conventional protein-based or novel genetic approaches might prevent ACD, and modifiying the immune response by strengthening counter-regulatory cytokines such as IL-4 or IL-10 and the corresponding effector T cells might finally provide a cure for this disease.

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