Allergic and irritant contact dermatitis

Pathophysiology and immunological diagnosis

Irritant and allergic contact dermatitis are common inflammatory skin diseases induced by repeated skin contact with low molecular weight chemicals, called xenobiotics or haptens. Although both diseases may have similar clinical presentations, they can be differentiated on pathophysiological grounds. Irritant contact dermatitis (ICD) is a non-specific inflammatory dermatitis brought about by activation of the innate immune system by the pro-inflammatory properties of chemicals. Allergic contact dermatitis (ACD) corresponds to a delayed-type hypersensitivity response with a skin inflammation mediated by hapten-specific T cells. Recent progress in the pathophysiology of chemical-induced skin inflammation has shown that ICD and ACD are closely associated and that the best way to prevent ACD is to develop strategies to avoid ICD. The immunological diagnosis of ICD or ACD requires investigation of the presence (ACD) or absence (ICD) of antigen-specific T cells. The detection of T cells can be performed in the skin (collected from ACD lesions or positive patch tests) and/or in the blood, particularly by using the enzyme-linked immunospot assay (ELISPOT). This method, recently developed in ACD to metals, offers a new biological tool enabling the immunobiological diagnosis of ACD.

Key words: allergic contact dermatitis, irritant contact dermatitis, pathophysiology, T cells, ELISPOT

Clinical aspects of contact dermatitis – similarities and differences between skin irritation and allergy

Dermatitis includes the acute and chronic forms of ICD and ACD. Table 1 summarizes the characteristics of each type of dermatitis in its typical clinical presentation.

Irritant dermatitis

Approximately 70 to 80% of contact dermatitis cases are ICD. Irritant dermatitis is damage to the cutaneous integrity with epidermal lesions of different degrees of severity and an inflammatory reaction in the underlying dermis [4, 5]. The heterogeneous clinical expression ranges from simple dryness of the skin (xerosis) to caustic lesions (burns) and depends on numerous factors including i) the chemical nature of the product (irritant, corrosive or caustic) and its concentration, ii) the length and frequency of the contact (repetition), iii) the environment (tempera-
Inflammation induced by the toxicity of the chemical (nature, dose, skin type)

Inflammation induced by the activated chemical-specific T cells

Chemical toxicity and cellular activation of the skin innate immunity

Activation of cytotoxic CD8+ T cells against keratinocytes (FasL, perforine)

Necrosis, apoptosis, cellular activation

Cytokines

Chemokines

Inflammatory infiltrate

Figure 1. Immune mechanisms in ICD and ACD. ICD and ACD are induced by skin contact with chemicals. The early stages are different as the chemical is pro-inflammatory by its direct "toxicity" on the skin cells in ICD while the active chemical triggers an inflammatory reaction mediated by specific T cells in ACD. The later stages giving rise to an eczema lesion may, on the other hand, be very similar and involve cytokines, chemokines, phenomena of apoptosis and cellular necrosis and the recruitment of a polymorphic inflammatory infiltrate. This explains why ACD and ICD lesions can be confused clinically and histologically.

Table 1. Differential diagnosis between ICD and ACD

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<th>ACD</th>
<th>ICD</th>
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<tr>
<td>Skin lesions</td>
<td>Not limited to the contact site</td>
<td>Limited to the contact site</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Itch</td>
<td>Burning</td>
</tr>
<tr>
<td>Epidemiology</td>
<td>Affects some subjects handling the product</td>
<td>Affects the majority of subjects handling the product</td>
</tr>
<tr>
<td>Histology</td>
<td>Spongiosis, exocytosis</td>
<td>Epidermal necrosis</td>
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<td>Patch tests</td>
<td>Positive (eczema)</td>
<td>Negative</td>
</tr>
<tr>
<td>Skin immunology</td>
<td>Presence of activated T cells</td>
<td>No activated T cells</td>
</tr>
<tr>
<td>Blood immunology</td>
<td>Presence of specific T cells</td>
<td>No specific T cells</td>
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On the hands, the palms are mainly concerned and the affected areas may reflect the professional activity. However, no clinical presentation is absolutely specific for ICDs, which can mimic allergic contact dermatitis (ACD) when the irritant is a strong hapten endowed with potent pro-inflammatory properties (e.g., epoxy resin). The irritant can equally trigger or prolong dyshidrosis or atopic dermatitis. A new contact with the irritant can result in a relapse, which will take place even more rapidly due to the cutaneous alterations which have already occurred.

Allergic contact dermatitis

ACD only occurs in sensitized patients, i.e. individuals who have developed chemical-specific T cells [2]. These cells have pro-inflammatory properties and are referred to as effector T cells. The concentration of hapten necessary to induce an ACD in a sensitized patient is lower than that necessary to induce an ICD in a non allergic individual. In sensitized patients, ACD occurs 24 to 96 hours after contact with the hapten. The initial localization is the site of contact. The edges of the lesion may be well defined, but, in contrary to ICD, they can spread locally or even at a distance. In the acute phase, ACD consists of erythema, oedema followed by the appearance of papules, numerous vesicules, and oozing followed by crusting. In the chronic phase, the skin becomes lichenified, fissured and pigmented, but new episodes of blistering, oozing and crusting can occur with further exposition to the hapten. ACD is generally associated with intense pruritus. Systemic contact dermatitis (SCD) is induced by oral or parenteral exposure to certain types of allergens in sensitized individuals. The best example is an outbreak of eczema occurring on the site of previous eczema after an oral provocation test with the hapten. The molecules most implicated in systemic CD are metals (nickel) and drugs [6, 7].

The pathophysiology of irritant and allergic skin inflammation

ICD has long been considered as a non-immunological inflammation whereas ACD was considered an immunological inflammation. In fact, both types of eczema implicate the immune cells but ICD follows the activation of innate immunity while ACD is the result of acquired immunity and the induction of specific pro-inflammatory T cell effectors [2-4]. It should be noted that the development of ACD initially requires the activation of innate immune cells which permit maturation of the cutaneous dendritic cells. The dendritic cells are then required for the presentation of allergens to T cells in the lymph nodes, and thus to the induction of an acquired immune response [8, 9]. The main characteristics of innate and acquired immunity are summarized in table 2.

Irritant and/or allergenic chemicals

All chemicals, whether they are responsible for ICD or ACD, can be considered as irritants, with very important differences in the concentrations necessary to induce irritation [10, 11]. For example, DNFB is an irritant at 0.05% while geraniol is an irritant at 50%. On the other hand, only those chemicals which behave as haptens are allergens. Indeed, they interact in a covalent manner or otherwise with amino acids, and thus are able to modify the proteins giving rise to neo-antigens [10]. Contact allergens are thus only a minority of chemicals.

Skin contact with an irritant will only induce an ICD. However, contact with a hapten can induce ICD or ACD, the latter occurring only if the individual has been immunized during previous skin exposures to the same chemical.

Skin irritation: activation of innate immunity

Innate immunity

Innate immunity refers to all the cells and molecules capable of distinguishing ‘danger signals’ of an infectious, physical or chemical nature, and of inducing an inflammatory reaction. The inflammation enables the individual to eliminate the infection and repair the damage caused by the physical and/or chemical agents (wound healing). Innate immunity is therefore synonymous with inflammation. In the blood, the innate immune cells are the hematopoietic cells, with the exception of T and B lymphocytes, which form the acquired immune response. In the skin, the totality of the epidermal and dermal cells constitutes innate immunity. The recognition of infectious danger signals

<table>
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<th>Table 2. Distinctive features of innate versus adaptive immunity</th>
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<td><strong>Innate immunity</strong></td>
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<tr>
<td>Synonymous: non specific immunity, natural immunity</td>
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<tr>
<td>Multicellular organism</td>
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<tr>
<td>Immediate response (3-12 hours)</td>
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<tr>
<td>Constitutive effector fonctions encoded in the germline (inflammation, phagocytosis)</td>
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<tr>
<td>Granulocytes, Natural Killer cells, monocytes, macrophages, dendritic cells</td>
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<tr>
<td>Receptors are PRRs (Pattern Recognition Receptors): hundreds of specific receptors bind to conserved molecular structures shared by large groups of pathogens</td>
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<tr>
<td>No memory, no affinity maturation</td>
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<td>Recognition of danger signals</td>
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implicates a set of membranous and intercellular receptors called Toll-like (TLR) and Nod-like receptors (NLR), which induce the activation of the inflammasome and the NF-kB pathways, resulting in the production of inflammatory cytokines and chemokines, among which are IL-1, IL-3, IL-6, IL-8, TNF-α. Molecules of innate immunity also include the complement, the plasmatic enzyme systems of coagulation and fibrinolysis, interferons...

**Skin irritation. Mechanisms of action**

The penetration of a chemical through the different layers of the skin, notably the epidermis and the dermis, is responsible for the release of a large number of cytokines and chemokines by different cell types whose respective roles in the induction of inflammation are not yet well understood [5, 12]. Keratinocytes represent 95% of epidermal cells and are the principal and first cells to secrete cytokines after an epicutaneous stimulus, thus giving them an essential role in the initiation and development of ICD. Other cell types are activated by the chemicals and contribute to the induction of inflammation. Studies currently undertaken with transgenic mice, deficient in certain types of cell, should bring a better understanding of the respective contributions of mast cells, macrophages/dendritic cells (DC), endothelial cells and NK cells in the development of ICD lesions [13, 14].

The profile of cytokine expression during ICD varies over time and also depends on the nature, environment and dose of the chemical [12]. The most frequently found mediators of ICD are IL-1α (Interleukine-1α), IL-1β, IL-6, IL-8, TNF-α (Tumor Necrosis Factor-α), GM-CSF (Granulocyte/Macrophage-Colony Stimulating Factor) and IL-10, which is an anti-inflammatory cytokine. However, initiation of the inflammation seems to be mainly linked to IL-1α, TNF-α, and derivatives of arachidonic acid. Indeed, IL-1α and TNF-α are two primary cytokines capable of inducing secondary mediators (including numerous cytokines, chemokines, adhesion molecules, growth factors) which are essential for the recruitment of leucocytes to the altered skin site. Thus a multistep cascade in the production of inflammatory mediators takes place, finally inducing histological modifications followed by the clinical expression of eczema.

**Direct responsibility of the chemical in ICD**

In ICD, the chemical is directly responsible for the cutaneous inflammation by its “toxic” physico-chemical properties, which are pro-inflammatory. Analysis of the inflammation of an ICD finds all the characteristics of a non specific inflammatory reaction, i.e. a hyperproduction of cytokines and chemokines, the presence of a polymorphic inflammatory infiltrate and lesions of apoptosis/necrosis of the epidermal cells with a compensatory proliferation of keratinocytes. There is no argument for an involvement of T cells.

**Skin allergy: the role of specific immunity**

**Specific immunity**

Specific immunity involves B cells (humoral immunity) and T cells (cellular immunity). Specific immunity is responsible for the immune memory which protects us from re-infection but which is also responsible for the chronicity of eczema in allergic patients.

**Skin allergy. Mechanisms of action**

ACD lesions are secondary to activation, at the site of contact with the hapten, of specific T cells which have been induced during previous contacts [2, 15-17] (figure 1). The specific T cells are recruited in the skin and activated by skin cells which present the hapten to them on MHC class I and II molecules. The activated T cells produce type 1 cytokines (IFN-γ, IL-2, IL-17) and are cytotoxic. They activate and destroy skin cells, including keratinocytes. The cellular apoptosis induces inflammation which allows the recruitment of new cells in the skin, resulting in eczema lesions. Knowledge of the mechanisms of ACD comes mainly from pre-clinical mouse models which illustrate the cytokotoxic pro-inflammatory effector role of CD8+ T cells while CD4+ T cells comprise anti-inflammatory regulatory populations known as Treg cells [15, 16].

**Indirect responsibility of chemicals in skin irritation**

In the case of ACD, the chemical is indirectly responsible for the skin inflammation. It is the T cells which induce specific inflammation to a hapten applied to the skin. T cells multiply the effect of the hapten and make it “toxic” to the skin. The hapten itself is not sufficiently toxic to create an inflammatory reaction, either because its concentration is not high enough, or because, at the concentration used, the patient is not sensitive to the irritant potential of the chemical.

**ICD conditions the development and magnitude of ACD**

**Induction of a specific immune response requires the activation of innate immunity**

The distinction between irritation and allergy is very abstract, as well as that between innate and specific immunity. In practice, the two types of immunity are almost always associated and closely linked. Thus, the induction of an efficient specific immunity requires the activation of innate immunity, which allows the maturation of dendritic cells and antigen-presenting cells. A classic example is the vaccination against an infectious protein (tetanus anatoxin), through which Abs and specific T cells are developed (acquired immunity), associated to an adjuvant (aluminium) which generates inflammation at the injection site (innate immunity). The injection of aluminium alone or the anatoxin alone does not result in the production of antibodies, while the injection of the two molecules at the same time induces a strong specific immune response to tetanus. This holds true for hapten which bear both the pro-inflammatory (adjuvant) properties and the antigenic properties through binding to self-proteins. Strong hapten are those endowed with the most adjuvant properties and are therefore able to sensitize the majority of individuals. Strong hapten are mainly used in experimental settings and are not used in daily life. In contrast, weak hapten have only very limited adjuvant effects and can sensitize a minority of people in frequent contact with the chemical. Weak hapten comprise chemi-
cals which are present in our environment, including perfumes, preservatives, dyes and drugs.

**Skin irritation is the basis of skin allergy**

In the case of eczema, it is known that ICD creates the conditions for ACD, on the basis of observations that patients who have ICD are more easily sensitized to the products they handle than patients who do not present any cutaneous irritation [17, 18]. This hypothesis has been recently confirmed by experimental results showing that the intensity of an ACD response to a hapten is proportional to the cutaneous irritation induced by contact with this hapten during sensitization [18]. In this example, the chemical tested was DNFB, which has both irritant and allergic properties. At low doses of DNFB during sensitization, there is no skin irritation on D1 and no eczema on D5. At higher doses, the intensity of the allergic reaction on D5 is directly correlated to the intensity of the irritation on D1 and is proportional to the concentration of DNFB.

**Pathophysiology of skin inflammation.**

The connection between innate and acquired immunity

*Figure 2* sums up the above discussion and shows the stages involved in the generation of an ACD reaction [reviewed in 19]. The reaction starts with inflammation, clinically visible (ICD) or totally unseen, induced by application of the chemical to the skin. This innate inflammatory reaction has several important consequences for the later development of ACD: i) activation of skin dendritic cells (DC), ii) recruitment to the skin of DC precursors, which are blood monocytes, iii) maturation and migration of skin DC to the lymph nodes draining the site of exposure to the chemical. In the lymph nodes, the immunogenic DCs activate specific T cell effectors which proliferate and migrate to the site of the contact with the chemical. In fact, in the absence of activation of innate
immunity, the maturation of skin DC is incomplete and pro-inflammatory T cell effectors are not able to be activated. On the other hand, immature DCs are capable of activating anti-inflammatory regulatory T cells [19].

How to differentiate between skin irritation and skin allergy?

Considering the strong similarities between irritation and allergy, clinically, histologically, and at a cellular and molecular level, the only way to differentiate the two types of inflammation is by their pathophysiological differences. As skin irritation has no defining characteristics for a certain diagnosis, it is the characterization of hapten-specific T cells in the blood and/or the skin of patients with eczema which allows us to make the diagnosis of allergy (and therefore ACD), eliminating at the same time skin irritation (ICD).

The diagnosis of ACD (allergy) relies on two different methods:

– skin tests, where a positive result, expressing as a dermatitis at the site of contact with the chemical, is considered to be synonymous with contact allergy [2]; we will show that this is far from being true;

– immunological tests showing the existence of allergen-specific T cells in the skin or blood of patients; although these specific T cells still have to be shown to be the effector cells of the ACD!

Skin tests

Skin tests enable the diagnosis of ACD to be made. This is not really true, at least for the most frequently used tests, i.e. patch tests [20].

Patch tests (epicutaneous)

These consist of the application of the chemicals being tested on the back skin, under occlusion, for 24 to 48 hours [21]. These tests maximise, but do not reproduce, the normal use of the products. Although the concentrations of chemical products in patch tests are standardized and theoretically non-irritant, irritation reactions are frequent in patients whose skin is particularly sensitive or irritable, but also when the tests are left longer (72 hours) than the recommended length of time (48 hours) or at times of the year when irritative reactions are more frequent (summer). Concomitant patch testing of non-allergic but irritant chemicals (like sodium lauryl sulphate-SLS) can detect patients with particularly irritable skin and therefore indicates a positive control for irritation. When the control is positive, the results obtained with the other molecules tested should be interpreted with caution. In these cases, we re-test for a shorter time period (24 hours for example), which is insufficient for the development of a clinical response to irritation. The reading of patch tests and their interpretation are also at the origin of confusion between irritation and allergy. Although the tests are very standardized, reading only rarely takes place at two time intervals (48 and 72 (or 96) hours) and the inflammatory responses seen during a single early reading (48 hours) are sometimes difficult to class as irritation or allergy. This is particularly true for weak positive (+/-) and doubtful reactions. The clinical relevance of a positive patch test is directly proportional to its intensity. Patch test techniques are simple but must be learnt and mastered properly [20, 21].

Open-tests or repeated open tests - ROAT

These tests consist of repeated applications, e.g. twice daily for 15 days, of a commercial product (cosmetic, drug (e.g. collyres)) or a solution in water or petroleum of allergens, on the flexor aspect of the forearm, near the cubital fossa [20]. The allergic patient will develop eczema at the site of repeated applications after a few days (1-15 days). Use tests are the only completely relevant tests. Irritation reactions are very limited compared to patch tests but for some patients open tests are less sensitive than classical patch-tests.

Differentiation between irritation and allergy can therefore be established clinically by:

– The systematic use of a positive control for irritation during the tests;

– When a reaction is difficult to interpret or there are positive irritation tests: 1) re-test with patch tests for only 24 hours (or 12 hours if the first reaction is strong); 2) carry out a ROAT test.

Immunological tests

Immunological tests aim to investigate the presence of allergen-specific T cells in the skin and/or the blood of patients, allowing the diagnosis of ACD in a patient with eczema who handles that product.

Presence of allergen-specific T cells in the skin found in a punch biopsy of ACD lesions or in skin tests

Demonstration of T cells by immunohistochemistry in an eczema biopsy is not definitive for ACD. Indeed, all inflammatory reactions are accompanied by the recruitment of a polymorphic infiltrate in which there will be a greater or smaller percentage of T cells. It is necessary to show that the T cells are specific for the hapten manipulated by the patient. Several possibilities exist: i) show that the antigen-specific T cells are infiltrating the eczema lesion; ii) show that the lesion is infiltrated by activated T cells.

– Demonstration of an oligoclonal response of the T cells infiltrating the lesion by a molecular analysis of the T cells. This technique is used for experimental studies in several skin diseases, such as psoriasis [22] but has not yet been developed in ACD. In cases of ACD, there will be recruitment, activation and preferential proliferation of specific T cells (oligoclonal expansion), which form a high percentage of the T cells present in the lesion. In ICD there is no reason why certain sub-populations of T cells would be preferentially activated and a polyclonal infiltration of T cells is found in the skin. This technique is still at an experimental stage.

– Functional analysis (antigen-specific) of T cells infiltrating the lesion by cell culture and expansion of the leucocytes, from a biopsy. In ACD, the T cell lines obtained from the cutaneous sample contain specific T cells which proliferate in a secondary response to the hapten. In ICD, there is no proliferative response. This technique is still at an experimental stage.

– Presence of activated T cells within the cutaneous lesion by analysis of those cytokines, production of which is restricted to T cells. In fact inflammatory cytokines are
often found everywhere and are produced by different cell types, thus they are often found in ACD and ICD inflammation. IL-1, IL-3, IL-6, IL-8, TNF-α are synthesised by keratinocytes, monocytes/macrophages, dendritic cells, mast cells and T cells. An increase in the synthesis of one of these molecules can therefore not be considered as an indication of T cell activation. The main cytokines produced by CD4 and CD8 T cells are IFN-γ and IL-2 (defining the type1 profile) and IL-17 (Type 17 profile). Type 2 cytokines (IL-4, IL-13, IL-5), along with regulatory cytokines (IL-10, TGFβ) with an anti-inflammatory activity, are produced by many cells and their expression in a tissue does not indicate the originating cell. It should be noted that the presence of antigen-specific T cells activated within inflammed skin does not mean that these T cells are effectors of the disease. It is perfectly possible to imagine the presence in an eczema lesion of antigen-specific regulatory or by-stander T cells which do not participate in the generation of inflammation but which are rather involved in its control and resolution. This rapid and simple technique is currently still experimental in both ACD and in drug allergy [23]. But it could easily be transferred to hospital laboratories in the future.

Presence of allergen-specific T cells in patients' blood
Progress in immunology has allowed the development of methods to detect antigen-specific T cells in the blood [24, 25]. Among the possible methods, such as radioactivity (lymphocyte transformation test), flow cytometry (multimers) and molecular techniques (T cell receptor repertoire), the ELISPOT assay (enzyme linked immunospot) is the method most easily transferred from research laboratories to routine biology laboratories (e.g. the TBspot enables a diagnosis of tuberculosis to be made with evidence of antigen-specific T cells to mycobacterium tuberculosis antigens). The technique is based on the detection of cytokine-producing T cells following activation of blood leucocytes by the antigen. The IFN-γ ELISPOT assay is particularly used because IFN-γ is a T cell specific cytokine produced in large amounts by activated T cells.

In the field of allergology, the ELISPOT technique enables a diagnosis of drug allergy to be made in patients who have developed benign or severe drug allergic reactions and who have drug-specific circulating T cells [26, 27]. As the contact allergens are haptons, like the drugs, it seems to be quite possible that ACD immunobiological tests using ELISPOT could be developed. In ACD to metals, recent work by Bordignon et al. has shown that these tests offer an immunobiological diagnosis of allergy [28]. The development of such tests needs to know the exact phenotype of the effector T cells of the ACD and the cytokines produced. As discussed above, it is the demonstration of the presence of specific effector T cells in the patient’s blood which enables a diagnosis of ACD to be made and not only the presence of specific T cells, since certain specific T cells can be non-inflammatory (anergic) or can comprise anti-inflammatory regulatory cells. Fundamental research must therefore be continued to define, for each group of haptons (strong, moderate, weak), the type of effector T cells and the cytokines which are associated to their activation process [29].

Conclusion
In conclusion, progress in the knowledge of the mechanisms at the origin of skin inflammation has brought better understanding of the pathophysiology of eczema with three practical consequences: i) new immunobiological diagnostic methods in eczema; ii) novel therapeutic strategies aiming at re-inducing immune tolerance to chemicals in patients with ACD [30]; iii) justification for preventive measures in ACD. In this respect, recent studies have shown that ICD and ACD are closely associated and that the prevention of ACD implicates the prevention of ICD. This can be achieved by protecting consumers from the most irritating chemicals, using gloves to reduce the risk of hand dermatitis or simply by using chemicals at low, non-irritating doses [31]. The prevention of eczema also requires the maintenance of a good quality barrier function of the skin, which limits the penetration of chemicals and thus the appearance of ICD.

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References


