**ABSTRACT**

Cutaneous toxicity may adopt several forms, among which Allergic Contact Dermatitis (ACD) and Irritant Contact Dermatitis (ICD) are those of greatest prevalence. ACD is the most thoroughly investigated Th1/Tc1 cell-mediated disorder affecting the skin because of the availability of an excellent mouse model and the ability to reproduce the lesions in humans. ICD is used to describe nonimmunologic cutaneous changes caused by contact with substances from the environment. In both entities, keratinocytes, fibroblasts, endothelial cells as well as invading leukocytes interact with each other under the control of a network of cytokines and lipid mediators. Here, we describe the most recent findings regarding the role played by blood and lymphatic vessels in the development of ACD and ICD.

**KEY WORDS:** Lymphatic vessels / Blood vessels / Allergic Contact Dermatitis / Irritant Contact Dermatitis.
INTRODUCTION

Contact dermatitis is defined as any change of the skin or its appendages (hair, nails, mucous membranes) resulting from contact or exposure to an exogenous (chemical or physical) agent. Of work-related cases involving the skin, 90-95% will involve contact dermatitis. In fact, most cases of contact dermatitis treated by physicians are in some way work-related. According to the mechanism of elicitation, the following types of contact reactions may be distinguished: (1) allergic contact dermatitis (ACD); (2) irritant contact dermatitis (ICD); (3) phototoxic and photoallergic contact dermatitis and (4) immediate type contact reactions.

Irritant contact dermatitis (ICD) has been reported to be the most common form (70%). It is identified when the contact-induced inflammatory change is non-immunological, and the clinical picture is the result of cellular damage by the irritating agent. The contact dermatitis is considered ACD only when the contact-induced inflammatory skin changes are compatible with a recognised immunological response to a putative hapten.

The clinical picture of ACD is that of an acute dermatitis (Fig. 1). Lesions usually appear within 24-72 h of contact with the sensitising agent and start with an erythematous reaction, often depicting the area where the hapten 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 sensitising agent can promote the development of a more chronic and less inflammatory form of ACD.

In contrast to ACD, ICD is not a clinical entity, but rather a spectrum of abnormal skin changes. The clinical aspect of ICD is dependent on a dose-effect relationship. A severe, acutely inflammatory reaction caused by strong, primary irritants may include necrosis and ulcerations, whereas chronic lesions present with lichenification, excoriations, scaling, and hyperkeratosis. The hands are most frequently affected by ICD, because they interact most with the environment and have frequent contact with many irritants (Fig. 2).

The histopathological findings are different in acute and chronic ACD and are dependent on the severity of the inflammatory reaction. The most common histological feature is spongiosis, which results from intercellular oedema. There is predominantly perivascular mononuclear infiltration of the superficial dermis, lymphocytes being the predominant cell at early and late stages. The infiltrate ranges from moderate to intense in the positive areas. The infiltrate extends into the epidermis (exocytosis) and accumulates in the spongiotic vesicles.

ICD lesions show a moderate hypertrophy and swelling of endothelial cells with an important infiltration by leukocytes. Infiltrating cells located away from the blood vessels are mostly perivascular polymorphonuclear cells at 24 h. At
48 h mononuclear cells increase in the infiltrate and at this time ICD cannot be distinguished from ACD. However, inflammatory cells found in the epidermis are principally polymorphonuclear cells in most of the individuals with ICD.

Differentiation between ACD and ICD is of major interest in occupational medicine. In spite of their different pathogenesis, their clinical, histological and phenotypical features are remarkably similar. However, spongiosis has been reported more consistently as a feature of ACD, while pyknosis and superficial vesicles containing polymorphonuclear cells have been related to ICD.

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 hapten. The potential hapten are then applied to the skin of the patient in subtoxic concentrations. Besides avoiding contact with the hapten, application of topical steroids is the first choice for treatment. Very resistant and extensive cases require systemic steroids.

Diagnosis of ICD is based on the clinical picture and the dermatological history. Complete patch test may be needed to rule out ACD. Elimination of exposure to the irritant is the most important step in the treatment. If healing does not occur, endogenous factors should be evaluated. Once the offending irritant is removed, the dermatitis often heals without topical treatment except possibly moisturizers.

Topical corticosteroids can be used, depending on the severity of the inflammation.

General characteristics of these entities are displayed in Table I.

### PATHOGENESIS OF ACD

The pathogenesis of ACD can be divided into sensitisation, elicitation and resolution phases, which have been extensively studied in mice but not in humans.

In the sensitisation phase, epidermal Langerhans cells (LC) or dermal dendritic cells (DC) capture the haptens and migrate from the skin to regional lymph nodes where they prime hapten-specific naive T cells. Once activated, these hapten-specific T cells acquire a propensity to migrate to the skin: the expression of skin-homing receptors allows preferential interactions with surface molecules on activated endothelial cells in the skin microvasculature. Further contact of sensitised subjects with the causative hapten induces rapid expansion and recruitment of memory hapten-specific T cells in the skin and eventually gives rise to the elicitation phase promoting the skin damage.

Contact dermatitis has largely been attributed to LC and T lymphocyte actions. However, the role of endothelial cells as major constituents of the skin immune system in contact dermatitis has been neglected thus far. Induction
of ACD does not only require specific mechanisms associated with antigen processing and presentation to T lymphocytes, but it is also associated with non-specific events such as activation of endothelial cells. The latter is a necessary prerequisite for the recruitment of immune cells to sites of prospective inflammation. So far, endothelial cell activation in the initial phase of contact allergy, as it is reflected by induction of adhesion molecules, is supposed to be mediated by the action of cytokines and chemokines released by, for example, leukocytes or keratinocytes.

Interleukin-1 (IL-1) and Tumour Necrosis Factor-α (TNF-α), which have been called primary cytokines, have broad effects that are relevant to inflammation and immunity. The epidermis is a storehouse of IL-1α and can produce large amounts of IL-1β and TNF-α. After binding to their receptors these cytokines activate several cellular signalling pathways, including the nuclear factor-κB (NF-κB) pathway. Among the many genes regulated by NF-κB in skin cells, those that are central to the initiation of cutaneous inflammation include the genes for E-selectin, chemokines and cytokines, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). Type 1 cytokines released by T cells are involved in activating mast cells, which promote neutrophil recruitment through the release of TNF-α and Macrophage inflammatory protein-2 (MIP-2). Mast cells and platelets are indirectly involved in this early T cell recruitment through the release of serotonin, which together with TNF-α activate the endothelium facilitating the cell entry.

During contact dermatitis leucocyte recruitment is under the control of chemokine releases in a sequential and coordinated way from resident and immigrating cells.

The first molecule expressed (2 h after hapten contact) by blood endothelial cells (BEC) during ACD is CS-1 fibronectin, which is the connecting segment-1 motif present in an alternatively spliced variant of fibronectin. Activated lymphocytes express α4β1 integrin, which not only bind to VCAM-1 but also to CS-1 fibronectin. Together with α4β1 integrin, most skin-homing T lymphocytes express cutaneous leucocyte antigen (CLA) which bind to E-selectin expressed on activated BEC of the skin. Adhesion molecules such as ICAM-1, VCAM-1, and E-selectin are upregulated at the transcriptional level in human umbilical vascular endothelial cells (HUVEC) only by NiCl2 and CoCl2, two of the most common haptons leading to allergy, whereas other bivalent ions, which do not cause contact allergy, are not effective.

At 10 h CCL17 is exposed on the surface of activated BEC at the site of skin inflammation. It has been recently reported that skin-seeking CD4+ T lymphocytes express the CCL17 receptor (CCR4) independently of their cytokine-releasing profile. Besides, the axis CCL17/CCR4 promotes the adhesion of skin memory T cells to ICAM-1 in vitro. Therefore, CS-1 fibronectin and CCL17 are probably of great importance in the rapid adhesion step, because they trigger rapid adhesion to VCAM-1 and ICAM-1 of monocytes and T cells rolling on E-selectin. Thus CS-1 fibronectin may work together with CCL17 inducing adhesion of passing cutaneous α4β1+/CCR4+ mononuclear cells under shear.

Kinacitocytes also contribute to early cell recruitment by expressing CCL2 at 6 h after hapten contact, thus clearly preceding infiltration by monocytes and lymphocytes. At 10 h, epidermal expression of CCL2 further increases and also becomes detectable in vascular and perivascular cells. CCL2 was first characterised as a monocyte-chemoattracting protein, but its receptor, CCR2, is also expressed on activated memory T cells, including Th1, Th2 as well as T regulatory lymphocytes (Tr). CCL2 expression is accompanied by epidermal and, to a lesser extent, dermal expression of CCL5 and both are responsible for the recruitment of monocytes and lymphocytes at early and latter stages, respectively.

The inflammatory infiltration in ACD is composed mainly of CD4+ and CD8+ T cells, monocytes and DC with an early and transient presence of neutrophils. The expression of murine contact hypersensitivity (CH) and human ACD correlates with the activity of hapten-specific CD8+ T cells, which exert their effector function through direct cytotoxic activity as well as the release of cytokines. Most of this hapten-specific CD8+ T cells display a type 1 cytokine profile. Expression of ACD to nickel in humans correlates with the frequency of specific CD8+ cells in the peripheral blood, which is high in allergic individuals and low or undetectable in healthy individuals. In contrast, the peripheral blood of both allergic and non-allergic subjects shows comparable nickel-reactive CD4+ responses. These findings indicate that CD4+ T cells are not required for expression of ACD and suggest instead that they may have a role in its regulation. In this regard, most of CD4+ T cells are hapten-specific Th1 cells, with approximately 30% of the cells co-expressing CD25 and Cytotoxic T Lymphocyte Antigen 4 (CTLA-4), a phenotype consistent with either activated effector or T regulatory (Treg) functions.

The preponderance of lymphocytes among the mononuclear cells in ACD may be explained by a broader spectrum of lymphocyte-attractant chemokines (CXCL9, CXCL10, CCL2, CCL5, CCL17, CCL18, CCL22, CCL27) rather than of monocyte-attractant chemokines. Flier et al working with skin biopsies from allergic and sodium lauryl sulphate-induced irritant patch test reactions, showed that expression
of CXCL10, CXCL9 and CXCL11 mRNA could be detected in ACD reactions after 24-72 h, but not in sodium lauryl sulfate-induced ICD reactions. Moreover, up to 50% of the infiltrating cells in ACD expressed the chemokine receptor CXCR3, which specifically binds the three chemokines aforementioned, in contrast to only 20% in ICD reactions. T lymphocytes with polarised cytokine production (Th1 or Th2) show a different distribution of inflammatory chemokine receptors. A large body of evidence indicates that the chemokine receptor CXCR3 and CCR5 are markers for T cells associated with certain inflammatory reactions, particularly Th1 type reactions. This is in concordance with the characterisation of ACD as a Th1/Tc1 immune response.

In a recent report, CCL27-CCR10 interactions were clearly demonstrated to have a pivotal role in T-cell mediated skin inflammation. Most skin-infiltrating lymphocytes of patients suffering from psoriasis, atopic or allergic-contact dermatitis express CCR10 and epidermal basal keratinocytes produce CCL27 protein. This chemokine binds to the extracellular matrix and is displayed on the surface of dermal BEC recruiting CCR10+ lymphocytes from peripheral blood. Moreover in vivo neutralization of CCL27-CCR10 interactions impaired lymphocyte recruitment to the skin leading to the suppression of allergen-induced skin inflammation.

The resolution phase of ACD is likely due to multiple mechanisms. Elimination of hapten from the skin may be due to keratinocyte apoptosis or proliferation. Other mechanisms include induction of T cell anergy by non-professional antigen presenting cells and active suppression mediated by Treg. The inhibition of the inflammatory response might be due to the interaction of several cellular types (keratinocytes, dendritic cells or other antigen-presenting cells and lymphocytes) and be mediated by humoral (anti-inflammatory cytokines) or cytotoxic mechanisms (lysis of effector cells). In this process IL-10-producing T cells may be involved in the production and release of other cytokines such as CXCL8, IL-6 and GM-CSF.

During ICD, ultraviolet and chemical agents can induce epidermal keratinocytes to release inflammatory cytokines (IL-1α, TNFα, chemotactic cytokines (CXCL-8), growth promoting cytokines (IL-6, IL-7, IL-15, GM-CSF, TGFα) and cytokines regulating humoral vs. cellular immunity (IL-10, IL-12, IL-18)). Damage to the keratinocyte releases preformed IL-1α, which essentially is a primary event in skin defence. IL-1α stimulates further release of IL-1α and the production and release of other cytokines such as CXCL8, IL-6 and GM-CSF.

Neutrophil attractant chemokines such as CXCL8 and CXCL1 are only weakly expressed in the epidermis from patients with ACD, but CXCL8 is strongly expressed in skin from ICD patients. This is followed by polymorphonuclear infiltration at early time points in ICD but at 48 h there are only bystander lymphocytes.

In addition to being directly chemotactic for leukocytes, IL-1α induces the expression of intercellular adhesion molecules on the surface of endothelial cells and fibroblasts and stimulates keratinocyte and fibroblast proliferation. Thus, together with other cytokines, it is also involved in wound healing. Another important cytokine in ICD is TNF-α, which is stored in dermal mast cells, and, following...
stimulation it may also be produced by keratinocytes and Langerhans cells\(^{(1)}\).

**THE ROLE OF LYMPHATICS IN CONTACT DERMATITIS**

Contrary to our abundant knowledge about blood vessels in contact dermatitis, little is known about the lymphatics. Interstitial fluid is constantly drained into peripheral lymph nodes (PLN) via afferent lymph vessels. During the elicitation phase of ACD, effector T cells are recruited toward the inflammatory site, but other cells such as monocyte-derived macrophages, DC, LC and likely, lymphocytes also leave the skin due to reasons not very well understood yet. The emigration of cells from skin to PLN is a phenomenon poorly studied in ICD.

In mice three cytokines are known to be required to support the mobilisation and migration of LC through lymphatics in response to skin sensitisation with a chemical allergen: IL-1\(\beta\), IL-18 and TNF-\(\alpha\)\(^{(38,39)}\). All are products of epidermal cells, and there is both direct and indirect evidence that the same, or at least very similar cytokine signals, are associated with the migration of LC from human skin\(^{(40,41)}\).
Cumberbatch et al, using specific neutralising antibodies, have studied the LC migration induced following skin sensitisation and irritation in mice. Oxazolone (hapten) induced LC migration was shown to be dependent on IL-1β and independent of IL-1α. However, following stimulation with the skin irritant sodium lauryl sulphate the loss of LC from the epidermis and the accumulation of DC in PLN required IL-1α and not IL-1β. These data suggest that contact sensitisation and skin irritation employ subtly different cytokine networks in the regulation of LC migration, both involving TNF-α but demonstrating differential requirements for IL-1 cytokines.

The chemokine CCL21 was demonstrated to have a crucial role in the emigration of LC from epidermis toward PLN. There are evidences for CCL21 expression by lymphatic endothelial cells (LEC) in normal peripheral tissues as well as in some human skin diseases. Constitutive CCL21 expression in normal LEC from multiple murine non-lymphoid tissues has been reported by Gunn et al. Later on, Saecki et al. found mature LC expressing CCR7 (the specific CCL21 receptor) and MHC class II within CCL21-staining lymphatic channels in normal mouse dermis. Expression of functional CCR7 by the emigrating LC/DC is particularly important, as DC from mice lacking CCR7 fail to migrate into PLN. We have demonstrated that in patients with ACD and ICD, CCL21 is up regulated by dermal LEC within few hours and shows a very strong expression between 24-48 h. This expression is only observed in areas exposed with the relevant hapten (ACD) or irritant (ICD). Katou et al. have also shown CCL21 expression by LEC from chronically inflamed skin infected with Candida albicans. The importance of CCL21 in LC migration is also inferred from the treatment of wild-type mice with antibodies against CCL21, which inhibits LC migration to PLN, the development of effector T cells and CH responses. Based upon all these results it is very clear that CCL21 and CCR7 participate in the emigration of LC to PLN via lymphatics.

It has been demonstrated that DC exposed to apoptotic cells remain immature in clear contrast to DC, which have been in contact with necrotic cells. Since apoptotic keratinocytes are generated by the inflammatory response in ACD, it could be possible that the epidermal DC after taking up those apoptotic cells migrate to PLN and generate Treg which could later on reach the skin and play a major role in the resolution phase of this type of inflammatory immune responses.

The up-regulation of CCL21 observed in skin LEC during ACD might play two different functions: 1) it could drive tissue inflammatory cells (LC and lymphocytes) towards PLN in order to boost the Th/Te 1 immune response. 2) it could contribute to the emigration of immature LC capable of generating Treg. The circumstances that induce up-regulation of CCL21 and the molecular mechanisms involved are currently being investigated.

Osteopontin is another important molecule secreted by endothelial cells that has been involved in the emigration of LC from epidermis. Weiss et al. made use of a human explant to assess this issue because it reproduced the sensitisation phase of ACD. At 24 h of skin culture osteopontin was strongly expressed in the dermis, in the area of the papillary vascular plexus. LC were found to migrate predominantly towards sites of high osteopontin expression, forming cords in these areas. Due to the lack of available specific markers for LEC they did not determine whether osteopontin was secreted by LEC. Treatment of mice with antibodies against two of the osteopontin receptors clearly inhibited LC emigration. Furthermore, osteopontin-deficient mice have a significantly reduced CH response that correlates with an impaired ability of osteopontin-deficient mice to attract LC/DC to PLN.

Together with the already described functions of lymphatics, these vessels also enable the inflamed peripheral tissues to project their local chemokine profile into high endothelial venules within PLN and thereby exert «remote control» over the composition of leukocyte populations that home to these organs from the blood. This was postulated because skin-derived CCL2 was transported via the lymph to the luminal surface of HEV where it triggered integrin-dependent arrest of rolling monocytes.

In summary, functional integrity of BEC and LEC is crucial for cutaneous immune responses (Fig. 3). These vessels are extremely sophisticated structures, which upon different signals express cytokines, chemokines and other important molecules, in order to control the movement of immune cells.

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