

Cutaneous symptoms in drug allergy: what have we learnt?

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Purpose of review

A number of studies over recent years have shown the relevance of nonimmediate immunological reactions to drugs and emphasized the need for these reactions to be studied in greater depth, including both in-vivo and in-vitro tests and provocation studies.

Recent findings

Several clinical entities are involved, ranging from mild to severe reactions.

Nonimmediate reactions have a special tropism for the skin, although other organ-specific or systemic reactions may also occur. In the affected organ, the drug acts as a hapten or protohapten, originating an immunological stimulus that is followed by recruitment of T lymphocytes and other types of cells, depending on the reaction. This involves a trafficking phenomenon in which these cells use different receptors that interplay with their corresponding ligands in the tissue. In addition, a number of activation and cytotoxic markers are also expressed, which are related with disease severity.

Summary

Current studies provide overwhelming evidence of the interaction between the organ involved, very often the skin, and immunocompetent cells. This is of relevance for diagnostic and therapeutic approaches.

Keywords

drug allergy, immunopathology, mechanisms, T cells

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Introduction

The skin is the organ most frequently involved in adverse drug reactions mediated by specific immunological mechanisms [1]. Several pathological manifestations are involved, though they are not exclusive to drug reactions and can also be induced by many other agents.

From a mechanistic point of view, drugs interact with the immunological system at one or more specific sites [2]. The reactions can be systemic, organ-specific or both. For example, in anaphylactic reactions to drugs, symptoms appear in different parts of the body but in nonimmediate reactions (NIRs), the inflammatory process occurs in specific tissues. In order to envisage these processes, the following points are relevant: the time interval between the drug intake and the occurrence of the reaction, the clinical manifestations, the concurrence of other factors or agents, the persistence of the reaction and the response to treatment.

The present review focuses on NIRs to drugs, which include a number of clinical entities that usually occur from 24 to 48 h after drug exposure, in a classical sec-

ondary immunological response, although the interval can be shorter and evidence supports the involvement of T cells. We will outline the in-vivo and in-vitro advances that have led to improvement in our understanding of the pathophysiologic mechanisms involved, with special focus on T lymphocytes.

Pattern of responses

NIRs consist of a spectrum of entities that vary from mild reactions, such as maculopapular exanthema (MPE) or urticaria, to severe reactions, such as drug rash with eosinophilia and systemic symptoms (DRESS) or toxic epidermal necrolysis (TEN) [3]. The different clinical manifestations are explained by differences in the pathophysiologic mechanisms, although different T-cell populations are involved in all these entities. These NIRs are included within type IV of the Gell and Coombs classification, with further subclassification [4]. In type IVa reactions (e.g. eczema), Th1 cells producing IFN- γ and activated monocytes are involved. In type IVb (e.g. MPE and bullous exanthema), Th2 lymphocytes producing IL4 and IL5 and eosinophil infiltration are involved. Type IVc (e.g. MPE, eczema, bullous exanthema and

TEN) is a cytotoxic response mediated by CD4 and CD8 T lymphocytes with apoptotic mechanisms mediated by either perforin/granzyme B and/or FAS-FASL. Type IVd involves recruitment of activated neutrophils induced by IL8 produced by T lymphocytes, the only example being acute generalized exanthematous pustulosis (AGEP) [4]. However, this subclassification is more complex and the involvement of different T-cell subsets can be present at different stages of the diseases, with cytotoxic and/or regulatory functions.

MPE, the most common NIR, consists of a cutaneous erythema forming maculopapulae with skin elevation, although vesicles or different degrees of angioedema may appear [5]. Once these reactions are established, they do not usually evolve to more severe diseases, even if drug intake persists [5]. Urticaria is characterized by wheals, sometimes accompanied by angioedema. AGEP consists of edematous erythema followed by small non-follicular sterile pustules that may be accompanied by a nonerosive mucous membrane [3,6,7]. Fixed drug eruptions (FDEs) consist of solitary or just a few pruritic, erythematous macules that evolve into edematous plaques; they recur at the same sites with reexposure and lead to hyperpigmentation [3,8,9]. TEN includes Stevens–Johnson and Lyell's syndromes (<10 and >30% of skin affected, respectively). A massive keratinocyte apoptosis is produced with epidermal detachment and bullae formation, with predominance of lesions at mucosal sites [10]. DRESS is characterized by the production of a severe eruption with edema, lymphadenopathy, fever, hepatitis and hematological abnormalities, of which eosinophilia is the most characteristic feature [11].

Immunopathology

The clinical manifestations described result from the interplay of the immunological system and the cutaneous tissue. In MPE, a mononuclear infiltrate can be found in the perivascular dermis, with T lymphocytes, mainly CD4⁺ T cells [12,13,14[•]], with the presence of neutrophils and occasionally eosinophils [15]. Recent skin patch test studies have shown CD8⁺ T cells in the dermoepidermal junction [16–18] with a cytotoxic capacity [10,19–21]. Whether this occurs at the onset of the acute response needs to be confirmed.

Controversy exists concerning whether nonimmediate urticaria or urticarial-like exanthematic rash is a T-cell reaction, although there is evidence that in some cases drug administration induced an urticaria-like reaction with the presence of angioedema and T-cell involvement [13,22^{••}].

In AGEP, subcorneal pustules with dermal edema, spongiosis, leukocytoclastic vasculitis and focal keratinocyte

necrosis have been found [10]. In this process an intra-dermal infiltration of activated CD4 and CD8 T cells producing granulocyte-macrophage colony-stimulating factors (GM-CSF), with IFN- γ and TNF- α with the release of perforin/granzyme B and FasL, which are responsible for keratinocyte death [23,24]. These T lymphocytes also produce IL8 (CXCL-8), which is responsible for neutrophil chemoattraction and activation [7,23,25–27].

FDEs are characterized by intraepidermal resident CD8⁺ T cells with an effector memory phenotype, responsible for keratinocyte necrosis [8,10,28–30]. CD4⁺ T cells are also found and are thought to act as regulatory cells [10].

TEN is characterized by the keratinocyte production of CCL27, which recruits CD4 and CD8 T lymphocytes expressing CLA⁺ (cutaneous lymphocyte antigen) and CCR10⁺ that produce IFN- γ , TNF- α , perforin/granzyme B/granulysin and FasL, which develop the apoptotic mechanisms followed by a massive necrosis [31^{••}].

In DRESS, in addition to the skin exanthema, there are also hematological abnormalities, with eosinophilia and the presence of atypical lymphocytes. CD4 Th2 cells producing IL5 are involved in the eosinophil activation and trafficking. CD8⁺ T cells also participate, which is the cause of organ damage in this syndrome [32].

Monitoring responses

Follow-up of the immunopathologic response by performing serial determinations in peripheral blood and the affected skin enables the underlying process to be reconstructed. Comparison of NIR versus immediate reactions has shown a Th1 pattern with expression of IFN- γ , IL12 and TNF- α and downregulation of IL4 in the former and a Th2 pattern, with production of IL4 and downregulation of IFN- γ , in the latter [33,34]. These polarizations also extend to the expression of transcription factors, such as T-bet in Th1 and c-maf and GATA3 in Th2 reactions [34]. More detailed studies have shown increases in both peripheral CD4 and CD8 T cells expressing the skin homing receptor CLA, CD69, CD25 and HLA-DR (human leukocyte antigen class-DR) and producing cytotoxic markers such as perforin/granzyme B and FasL; these normalize once the process resolves [13,14[•],35–37]. These changes affect the clinical severity [33]. This process parallels the skin lesion involvement and it is thought that after an antigenic stimulus originated in the skin, the specific immunological process is triggered, with the arrival of lymphocytes via the peripheral circulation with the interplay of different ligands and receptors, including adhesion molecules and chemokine receptors.

Different T-cell subsets are involved in different NIRs and although similarities have been shown, specific differences must exist, contributing to each particular entity. There is a general consensus that CD4 T lymphocytes are mainly involved in MPE, DRESS and AGEP [4,38,39], whereas CD8 T lymphocytes are the effector cells in FDE and TEN [31^{••},40,41], although studies indicate that both CD4 and CD8 T lymphocytes may both be involved in TEN [13] and CD8⁺ T cells are found in the epidermis of nonbullous lesions, including MPE [10].

CLA expression is not enough to explain lymphocyte migration and further receptors are involved, with chemokines playing a fundamental role [42]. In MPE, there is increased expression of Th1 chemokines (CXCL9 and CXCL10) and cutaneous homing chemokines (CCL17, CCL20, CCL27) in the skin [43[•]]. These correlate with a high cutaneous expression of TNF- α and IFN- γ [43[•]] produced by keratinocytes that upregulate CCL27 [44], and CXCL9 and CXCL10, respectively [45]. Recruitment of Th1 cytotoxic T lymphocytes expressing their respective chemokine receptors (CXCR3, CCR4, CCR6, CCR10) to the skin has been observed. In MPE or DRESS, there is an increase in Th2 cytokines, like IL5, and several chemokines, such as RANTES (a chemokine of the CC family that chemoattracts CD4⁺/CD45RO⁺ T cells) and eotaxins, thus explaining the involvement of eosinophils expressing CCR3 [46]. IL8 is involved in the neutrophil migration that occurs in AGEP [25–27,18]. In TEN, T cells expressing CCR10 and its corresponding chemokine CCL27 have been shown to be increased in the skin during the acute phase [47]. In FDE, intraepidermal CD8⁺ T lymphocytes with cytotoxic capacity against keratinocytes have been found [8]. These cells can activate mast cells in the epidermis, leading to the wheal-and-flare-like reaction [8].

The different lymphocyte subsets observed during monitoring of the late response are also responsible for the repair process occurring in NIR to drugs. In FDE, CD4⁺FoxP3⁺ regulatory T (Treg) cells were detected in fully evolved lesions. It is thought that they control the potential harmful immune reactions mediated by intraepidermal CD8⁺ T cells [48].

This monitoring is useful not only to understand the underlying process but also to assess the response to treatment. Because no change occurs in response to corticoids in patients with TEN compared with untreated patients, a sort of refractory response to this drug is thought to occur during this process [35]. On the other hand, administration of γ -globulin has shown a rapid decrease in the cytotoxic markers in TEN [36].

Although these data look promising and it make sense that early monitoring of the response can provide relevant information about the clinical entity and outcomes, including treatment responses, no sufficient number of studies that enable to use this approach for taking decisions have been done. One additional problem is that these determinations should be taken as early as possible – a condition that not very often happens because cases are usually evaluated at a stage when the diagnostic applicability is limited.

T lymphocytes recognition of drug

Although monitoring the immunological response provides clues about the mechanisms involved, definitive proof of the specific immunological mechanism includes demonstrating that the drugs are recognized by T cells and release the mediators involved in the reaction. Studies performed with T-cell lines and clones, from both skin and peripheral blood from different types of allergic reactions, have shown that the majority have a CD4 phenotype with a heterogeneous cytokine pattern [38,49–52]. CD4⁺ and CD8⁺ cells expressing high levels of the activation marker and IFN- γ have been detected in the supernatant of carbamazepine-stimulated T-cell clones [10]. In AGEP, drug-specific T cells have been obtained from patch test sites and blood [25,26,53], producing large amounts of IL8/CXCL8 [7], with a predominant Th1 cytokine profile (GM-CSF and IFN- γ) and TNF- α [47]. Some T-cell clones like CXCL8⁺ display a Th2 pattern (IL4 and IL5) [47]. In blisters, T cells isolated from TEN CD8⁺CD56⁺ T cells have been shown to present natural killer-like features [31^{••},40,41,54], with cytotoxic properties inducing apoptosis to autologous lymphocytes and keratinocytes in a drug-specific, HLA class I-restricted and perforin/granzyme B-mediated Tweak and TNF-related apoptosis induced ligand (TRAIL) pathway [40,41].

Many years after resolution of the clinical symptoms, drug-specific T-cell clones showing the immunological characteristics, effector functions with both cytotoxicity and cytokine production and homing characteristics of the reaction can be detected [32]. This approach is relevant to evaluate these reactions after resolution.

Viruses

There are similarities in the clinical manifestations induced by drugs and different dermatotropic viruses. Exanthematic reactions can be induced by cytomegalovirus, parvovirus, Coxsackie's, toga, paramixovirus and herpes, such as varicella zoster, human herpes virus 6 (HHV-6), and the γ -herpes virus. Exanthema can also be seen in different bacterial diseases, in patients with lymphoma [55], in graft versus host disease [56] and in

diseases with other causes, such as physical agents. With infectious agents, especially viruses, early determination of antibody titer or the in-situ localization of the viral product are useful to identify the culprit agent. Monitoring the reaction shows immunological differences between virus-induced and drug-induced reactions, with a Th0 pattern in the former and a Th1 in the latter [57].

Viruses can interact with the immunological system in many ways, leading to changes in the dendritic cell maturation status, changes in the expression of drug-metabolizing enzymes, and modulating of the cytokine and chemokine production that alter the T-cell pattern and trafficking. These can result in more severe and longer-lasting allergic drug reactions [58].

The first drug reaction to be analyzed was the exanthematic reaction associated with mononucleosis diseases, which occurs in 5–13% of patients, with the occurrence of rashes increasing up to 69% after intake of ampicillin [59,60]. A good studied model is DRESS related with HHV-6 reactivation. Anticonvulsants can activate monocytes and CD4 T lymphocytes during the allergic reaction, leading to reactivation of the HHV-6 from a latent infection and which can cause a delay in recovery [61]. AGEP has also been associated with viral infections [6,62], including Coxsackie B4 [63], cytomegalovirus [64] and parvovirus B19 [65], as well as with *Chlamydia pneumoniae* infection (one case) [66]. In FDE, patients have higher anti-herpes simplex virus (HSV) IgG titers than patients with a history of HSV recurrence [8]. Although few data exist concerning the relation between drug-induced TEN and infectious agents, it has been reported that in AIDS the incidence of TEN is 1000-fold higher than that in the general population. Some studies associated this with Epstein–Bar virus (EBV) reactivation [67].

The diagnostic approach

The need for both in-vivo and in-vitro studies of NIR to drugs has been emphasized [68–70]. Concerning in-vivo testing, a number of studies show that the application of either patch or intradermal testing is a useful approach, especially for betalactams [70,71]. However, recent studies have shown that in exanthematic reactions, the sensitivity is low, suggesting that either previous studies had a selection bias in favor of positive prevalent cases or that the pattern of responses has changed [22^{••},72]. In general, and for all drugs, whether sensitivity is higher in more severe reactions needs to be confirmed with appropriate studies. Evidence also indicates that intradermal testing seems to be more sensitive than patch testing [13], although more data are still required.

The application of the drug to the skin will generally induce a positive response, though for many drugs this is

not always the case. In most drug reactions, the drug reaches the skin via the circulation and often requires metabolic transformation to generate the metabolite involved in the reaction [73]. Skin possesses the capacity for xenobiotic metabolism of highly reactive species, which are able to bind to high molecular weight compounds that are recognized by the immunological system [73]. Drugs such as diclofenac, sulfamethoxazole and anticonvulsants are associated with NIR to drugs and all require previous transformation in the skin before inducing the reaction [74–76].

Because of the limitation of skin testing and the potential danger of eliciting a reaction after cutaneous drug application, different in-vitro assays have been proposed. The lymphocyte transformation test (LTT) has been used since very early on and it has been revitalized in recent years. Studies show good sensitivity and specificity, although often many drugs were used and different clinical entities included [77–81]. In the case of betalactams, the sensitivity in immediate and NIRs was 64.5 and 57.9%, respectively, with a specificity of 92.8% [81,82]. Limitations in the sensitivity of the test can be overcome by the use of dendritic cells as antigen-presenting cells, as has been demonstrated in allergy to betalactams and heparins [83,84], although further studies in large series of patients are required. Since early days, LTT was applied to study drug allergic responses and was proposed as a valuable technique for both delayed and immediate reactions [77–81]. However, and in spite of all the progress and improvements made, there is no standardized commercial kit sufficiently validated. One of the main drawbacks is that although it is clear that LTT can be useful, the validation must be focused on well defined clinical entities group (MPE, DRESS, TEN) and single drug or the same drug family.

Because of the limitation of both in-vivo and in-vitro tests and especially in those cases with mild reactions, the controlled administration of the drug has been considered as an alternative. It is often needed in order to be able to use drugs that are necessary for the patient. This view is supported by the fact that many individuals who claim to be allergic, based solely on a temporal association with the drug intake and mild exanthematic reactions, in fact tolerate the drug after readministration [69]. Controlled administration of the drug must be done only with mild exanthematic reactions and it is contraindicated in all severe reactions induced by drugs that include from TEN to DRESS. In addition, this should be carried out by expertise personnel in appropriated centers.

Conclusion

NIRs include a number of entities with differences in their underlying immunological mechanisms, although

they are all T-cell mediated. These differences are relevant in order to establish the differential diagnosis as well as to assess disease severity and response to treatment. In addition, a certain compartmental division exists and in the event of a reaction, the same entity is elicited after subsequent exposure. Concerning MPE, which is the most frequent NIR, in-vivo tests seem to have a low sensitivity, at least with betalactams. This necessitates the urgent development of in-vitro approaches to establish the diagnosis in these patients.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 485).

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