

# Nonimmediate Allergic Reactions Induced by Drugs: Pathogenesis and Diagnostic Tests

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## ■ Abstract

Nonimmediate allergic reactions (NIRs) to drugs, which are the most common reactions induced by specific immunologic mechanisms, can be induced by all commercially available drugs. NIRs can appear hours, days, or even weeks after drug intake. They elicit a spectrum of manifestations, mostly affecting the skin, ranging from maculopapular exanthema and urticaria to other less common but more severe entities such as acute generalized exanthematic pustulosis, drug rash with eosinophilia and systemic symptoms/drug-induced hypersensitivity syndrome, Stevens-Johnson syndrome, and toxic epidermal necrolysis. The main pathologic event involved in NIRs is a T-cell effector response and the wide heterogeneity of clinical symptoms may reflect differences in the underlying immunologic mechanisms. Despite their clinical heterogeneity, NIRs share certain aspects such as the activation of T cells with increased expression of CD25 and HLA-DR. NIRs are classified as type 1 helper (T<sub>H</sub>1) T-cell responses, characterized by the production of interferon- $\gamma$ , tumor necrosis factor- $\alpha$ , interleukin 2, T-bet, and the cytotoxic markers perforin and granzyme B. Diagnosis is often complicated because of the difficulty of obtaining a reliable clinical history, the important role played by cofactors such as viral diseases, and the low sensitivity of skin tests and in vitro tests. Further studies are thus required in order to improve our understanding of NIRs and refine our diagnostic criteria.

**Key words:** Nonimmediate. Allergy. Drugs. Pathogenesis. T cells. Diagnosis.

## ■ Resumen

Las reacciones alérgicas no inmediatas (RNI) frente a fármacos, que son las reacciones más comunes inducidas por mecanismos inmunológicos específicos, pueden desencadenarse por todos los fármacos disponibles en el mercado. Las RNI pueden aparecer horas, días o incluso semanas después de la toma del fármaco. Provocan un espectro de manifestaciones, la mayoría de ellas afectando a la piel, que abarcan desde el exantema maculopapular y la urticaria a otras entidades menos frecuentes pero más graves como la pustulosis aguda exantemática generalizada, exantema por fármacos con eosinofilia y síntomas sistémicos /síndrome de hipersensibilidad inducida por fármacos, Síndrome de Stevens-Johnson, y la necrolisis epidérmica tóxica. El principal evento patológico implicado en las RNIs es la respuesta T efectora y la amplia heterogénea de síntomas clínicos podría reflejar las diferencias en los mecanismos inmunológicos subyacentes. A pesar de la heterogénea clínica, las RNIs comparten ciertos aspectos como la activación de las células T con aumento de la expresión de CD25 y HLA-DR. Las RNIs se clasifican en respuestas de célula T de tipo 1 colaborador (TC1), caracterizadas por la producción de interferón- $\gamma$ , el factor de necrosis tumoral- $\alpha$ , interleucina 2, el T-bet, y los marcadores citotóxicos perforina y granzima B. El diagnóstico con frecuencia es complicado por la dificultad en la obtención de una historia clínica fiable, el importante papel jugado por cofactores como las enfermedades virales, y la baja sensibilidad de las pruebas cutáneas y pruebas in vitro. Son necesarios estudios adicionales para mejorar nuestra comprensión de las RNIs y refinar nuestros criterios diagnósticos.

**Palabras clave:** Reacción no inmediata. Alergia. Fármacos. Patogénesis. Células T. Diagnóstico.

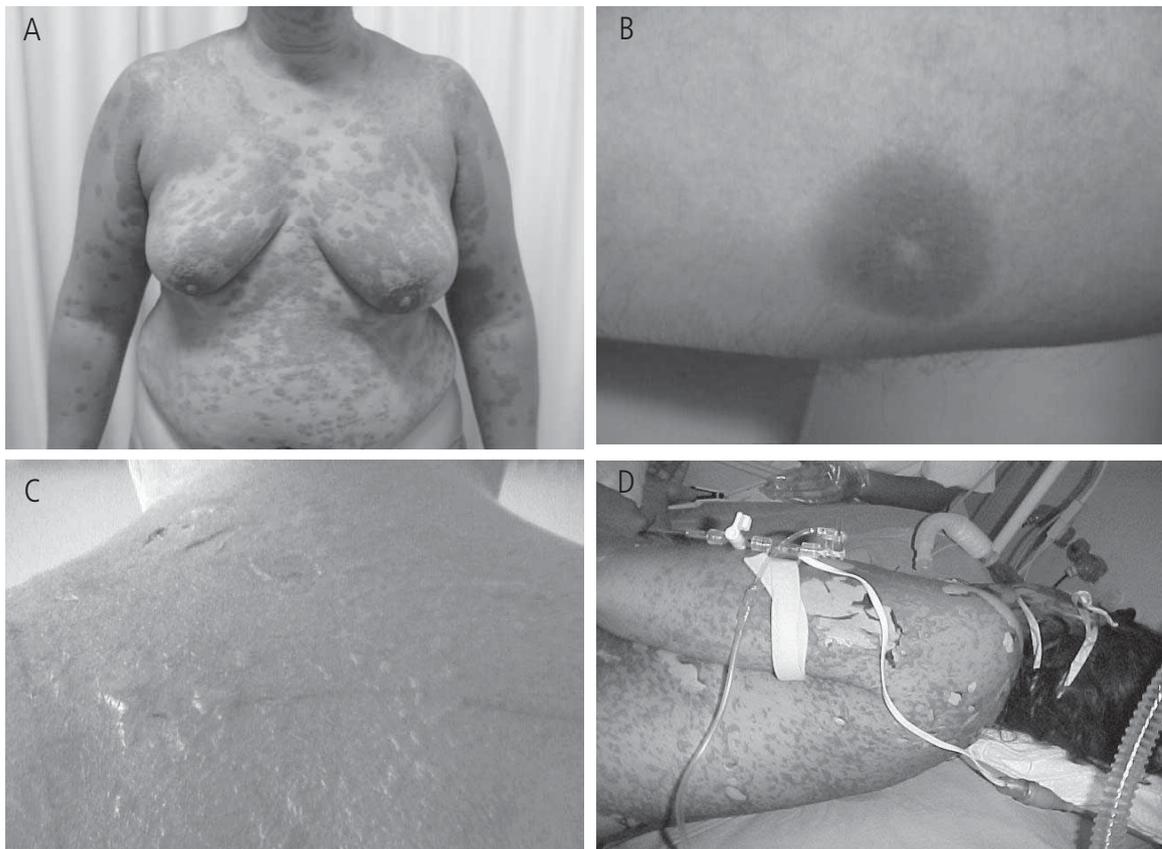
## Introduction

An adverse drug reaction is defined by the World Health Organization as “a response to a medicine which is noxious and unintended, and which occurs at doses normally used in man for the prophylaxis, diagnosis or therapy of disease, or for the modification of physiological function” [1]. Adverse drug reactions are usually classified as type A if they are predictable and related to the pharmacologic actions of a drug, and type B if they are unpredictable and not usually related to the pharmacologic actions of a drug [2]. Type A reactions are most common and account for approximately 80% of all adverse reactions. Immune-mediated adverse drug reactions, also known as allergic drug reactions or drug hypersensitivity reactions, account for approximately one seventh of all adverse drug reactions and belong to the type-B category [3].

Allergic reactions can be produced by any of the 4 immunologic mechanisms proposed by Gell and Coombs [4]. Type I reactions, also called immediate-type reactions, occur within less than an hour of drug administration and are mediated by drug-specific immunoglobulin (Ig) E antibodies. Typical clinical manifestations are urticaria and anaphylaxis. Type II (cytotoxic) and type III (immune complex) reactions

are mediated by drug-specific IgG or IgM antibodies and are less common. Finally, type IV reactions are mediated primarily by T cells; they are also known as delayed hypersensitivity reactions as they typically occur between an hour and several days after drug intake. Delayed hypersensitivity reactions to drugs have proven to be more complex than Gell and Coombs first believed as they are now also categorized according to cytokine patterns and the preferential activation of different immunocytes.

Clinically, allergic reactions to drugs can be classified as immediate, accelerated, or delayed, depending on the time between drug intake and occurrence. Although this classification was developed by Levine [6] on the basis of his experience with penicillin allergy, it can be applied to all types of drug allergies. A working classification for clinical use divides reactions into immediate and nonimmediate reactions (NIRs) (ie, accelerated or delayed reactions). Immediate reactions appear within an hour, and often within just a few minutes, of drug intake and their main clinical manifestations are urticaria/angioedema and/or anaphylaxis [7]. NIRs can occur within hours, days, or even weeks of drug intake and are characterized by a wide range of clinical manifestations. In this review, we examine the latest information published on NIRs.



**Figure 1.** A, maculopapular exanthema induced by metamizole. B, fixed drug eruption induced by a quinolone. C, bullous reaction in Stevens-Johnson Syndrome induced by tetrazepam. D, toxic epidermal necrolysis induced by an anticonvulsant.

## Clinical Manifestations

The skin is the most frequently involved target organ in NIRs, which produce clinical manifestations ranging from maculopapular exanthema (MPE), the most frequent type of drug eruption, to urticaria and other less common but more severe entities such as acute generalized exanthematic pustulosis (AGEP), drug rash with eosinophilia and systemic symptoms (DRESS)/drug-induced hypersensitivity syndrome (DIHS), Stevens-Johnson syndrome (SJS), and toxic epidermal necrolysis (TEN) [8]. Erythema multiforme and fixed drug eruption are less common manifestations [8]. Drug-induced contact dermatitis is an occupational hazard, often affecting pharmaceutical and healthcare workers [9], and serum sickness-like reactions have been reported, mostly with betalactams [10]. Other clinical entities have also been described, including the baboon syndrome, an erythematous reaction that affects the buttocks, inner thighs, and axillae [11]. It can sometimes be difficult to identify NIRs because of the wide spectrum of clinical manifestations and the fact that these can be quite similar to those caused by infectious or autoimmune diseases. Figure 1 shows some typical clinical manifestations of NIRs.

The most common entities associated with NIRs are benign diseases such as exanthematic reactions and MPE, followed to a lesser extent by urticaria. When retrospective-only information is available, it can sometimes be difficult to distinguish between different reactions, even in the presence of angioedema because certain severe exanthematic reactions involve swelling. MPE can sometimes be intense, appear in conjunction with subcutaneous edema, and persist for several weeks despite discontinuation of treatment.

DRESS/DIHS and bullous reactions with mucosal involvement are considered severe diseases. Erythema multiforme, which is less severe, is usually induced by a virus and characterized by the presence of typical target lesions (Figure 2). SJS and TEN are the most severe type of hypersensitivity reactions affecting the skin and are characterized by extensive epidermal detachment and mucous membrane erosion. In general, the more severe the reaction, the greater the likelihood that the reaction has been induced by a drug. There is growing evidence that SJS and TEN are a single disease with common causes and mechanisms; the main difference appears to lie in the extent of detachment, which is limited (<10%) in the case of SJS and more widespread (>30%) in that of TEN (Figure 2) [12]. While rare (2 cases/million population/year), SJS and TEN have high mortality (20%-25%) [13].

## Drug Involvement

The true prevalence of NIRs is unknown, especially in less severe reactions, for different reasons including confusion with viral and autoimmune diseases. Moreover, linking symptoms to a particular drug can also be difficult because of

Classification		EM	SJS	SJS-TEN	TEN
Skin detachment		<10%	<10%	10%-30%	>30%
Typical lesions		Yes	No	No	No
Classification		High	Flat	Flat	Flat



Figure 2. Classification of bullous exanthemas. The less severe the reaction, the more likely it is to be induced by a virus. EM indicates erythema multiforme; SJS, Stevens-Johnson Syndrome; TEN, toxic epidermal necrolysis.

the long interval between drug intake and the onset of clinical symptoms, particularly in patients taking many drugs at the same time.

Several studies have reported the relevance of aminopenicillins in the development of MPE and urticaria in patients with mild or moderate reactions [7]. Although less frequent, these types of reactions have also been described for other drugs such as anticonvulsants [14], systemic corticosteroids [15], and iodinated contrast media [16].

Investigations of the drugs responsible for severe reactions such as SJS and TEN have shown high relative risks for anti-infective sulfonamides (especially cotrimoxazole), carbamazepine, phenytoin, phenobarbital, nonsteroidal antiinflammatory drugs of the oximam type, allopurinol, chlormezanone, aminopenicillins, cephalosporins, quinolones, and tetracycline antibiotics [17]. There have also been reports of strong associations in more recently launched drugs such as nevirapine and lamotrigine [18].

## Immunopathologic Mechanisms

The wide heterogeneity of clinical manifestations found in NIRs cannot be fully explained by the mechanisms described for type IV reactions by Gell and Coombs [4]. One attempt to explain these mechanisms involved subdividing T-cell responses into 4 different types [5]: *a*) type IVa responses, for which T cells produce interferon (IFN)- $\gamma$ -activated macrophages, whose typical clinical manifestation is eczema; *b*) type IVb responses, mediated by T cells producing type 2 helper (T<sub>H</sub>2) cytokines (interleukin (IL) 4 and IL 5, which in turn induce B cells to produce antibodies and mast cell and eosinophil responses, mainly in DRESS, MPE, and bullous exanthema; *c*) type IVc responses, induced by CD4<sup>+</sup> and CD8<sup>+</sup> T cells, which produce cytotoxic mediators that result in keratinocyte apoptosis in MPE and massive apoptosis in TEN; and *d*) type IVd responses, characterized by neutrophil activation and recruitment induced by T cells via the production of a chemokine, CXCL8, whose typical clinical manifestation

is AGEP. The basis of this classification system is that immune cells other than T cells (neutrophils, eosinophils, macrophages, and keratinocytes) are involved in type IV reactions. However, this differentiation of responses into fixed compartments does not clarify the situation since the same clinical manifestations may occur in 2 different subtypes.

It has been proposed that drug-protein conjugates might be processed and presented by antigen-presenting cells to naive T cells after drug intake, inducing tolerance or effector responses such as hypersensitivity reactions [19]. In the case of hypersensitivity, the immune system develops either immediate  $T_H2$ -type responses, mediated by specific IgE antibodies, or non-immediate  $T_H1$ -type responses, mediated by specific T cells. Most information available on NIRs concerns the specific effector immune response mediated by T cells, but little is known about the initial steps mediated by the innate immune system, served mainly by dendritic cells.

## The Role of T cells in NIRs

The involvement of T cells in NIRs has been demonstrated; not only do they prime the immunologic response by interacting with dendritic cells, but they also act as effector cells inducing tissue damage and inflammation [20].

One way of improving understanding of the pathologic mechanisms underlying NIRs is to monitor acute response by obtaining sequential samples (from blood, blister fluid, and skin) during reactions. Such an approach has shown that despite the clinical heterogeneity of NIRs, these reactions share common aspects such as the activation of T cells with increased expression of CD25 and HLA-DR [14]. As mentioned earlier, the skin is the target organ of most NIRs; in such cases, the T cells can express homing receptors such as the cutaneous lymphocyte antigen (CLA) and chemokine receptors such as CCR6 and CCR10 [21-24], which is found in higher levels in more severe reactions such as SJS and TEN [23]. The above markers have been detected simultaneously in both peripheral blood and skin, in contrast to their chemokine ligands, CCL20 and CCL27, which have only been found in increased levels in the skin [24], demonstrating that cell trafficking of the T-cell subpopulation to cutaneous sites of inflammation takes place [14,21-24].

NIRs have been commonly defined as  $T_H1$  reactions, involving the production of IFN- $\gamma$ , tumor necrosis factor (TNF)- $\alpha$ , IL 2, T-bet (a  $T_H1$  transcription factor) [25,26], and the cytotoxic markers, perforin and granzyme B [26,27]. Several studies have found that the levels of these markers vary according to clinical symptoms [14,25-27]. In 1 study, a detailed analysis of the skin-homing CLA showed that this antigen was increased in MPE compared to SJS/TEN, probably because CLA cells are mainly located in the dermis in MPE reactions whereas in SJS/TEN reactions, they migrate to the epidermis and blisters, where there is severe destruction leading to the loss of these cells [14]. Other studies have reported a higher production of  $T_H1$  cytokines (IFN- $\gamma$  and TNF- $\alpha$ ), T-bet, and certain cytotoxic markers in more severe reactions such as those seen in SJS/TEN [26,27], evidencing a correlation with clinical severity.

Another important difference between the different clinical entities associated with NIRs is the subpopulation of cells that participate in the reaction as effector cells. While several authors have shown that  $CD4^+$  and  $CD8^+$  T cells are involved in MPE and bullous exanthema, respectively [28-30], others have reported that both types of cells may be involved in SJS/TEN ( $CD4$  cells in the dermis and  $CD8$  cells in the epidermis) [21,30-34]. Our group has found increased  $CD4$  cell levels in both the skin and peripheral blood in SJS/TEN and to a lesser extent in MPE [14,16,21,22]. There may be several reasons for these differences but the compartment evaluated is likely to have had a considerable influence. The majority of studies in SJS/TEN have been undertaken in skin and blister fluid, where it is more likely to find cytotoxic cells expressing  $CD8^+$  T cells. Peripheral blood mononuclear cells and the dermis, in contrast, are predominantly composed of  $CD4^+$  T cells [35,36]. Recent studies have also demonstrated the involvement of these cells in increased cell counts in peripheral blood and skin and in the increased production of cytokines, chemokines, and cytotoxic markers [24,26].

As already described, T cells act as direct effector cells by producing the cytotoxic mediators, perforin and granzyme B, which induce death in target cells. They also play another important role in NIRs by acting as chemoattractants and activators of other immune cells such as neutrophils, eosinophils, and keratinocytes. This is the case of AGEP, a NIR characterized by the presence of sterile pustules on an erythematous base and increased neutrophils. Several studies have shown that T cells are specifically activated by culprit drugs, as demonstrated by their specific proliferative response. Moreover, they produce the neutrophil-attracting chemokine IL 8, which may contribute to the accumulation of polymorphonuclear neutrophils at the site of the lesion [37,38]. In MPE and DRESS/DHIS, skin-infiltrating T cells are able to produce IL 5 and eotaxin (CCL-11). This is of particular importance since these markers are both known to be key factors in the regulation of the growth, differentiation, and activation of eosinophils, which may be increased in such reactions and contribute to the generation of tissue damage through the release of various toxic granule proteins [39]. T cells can also activate epidermal keratinocytes, inducing the massive apoptosis of these cells in TEN through 2 main mechanisms: the perforin/granzyme B mechanism and the Fas/Fas ligand (FasL) mechanism. Recent studies have shown that apart from acting as target cells in TEN, keratinocytes may also be cytolytically active [36,40]. If this were the case, the recruited memory  $CD4^+$  and  $CD8^+$  T cells expressing CLA and CCR10 in the skin would produce IFN- $\gamma$ , inducing keratinocyte activation to produce TNF- $\alpha$  and FasL and trigger apoptosis. Moreover, the production of TNF- $\alpha$  would increase the expression of MHC class I antigens in keratinocytes, making them more sensitive to cytotoxic cells producing perforin and granzyme B [14,21,23,24].

These data suggest that the final phenotype of drug eruptions results from the nature of cytotoxic effector T cells in MPE and bullous eruptions, and T cells releasing specific chemokines for reactions mediated by neutrophils or eosinophils (Figure 3).

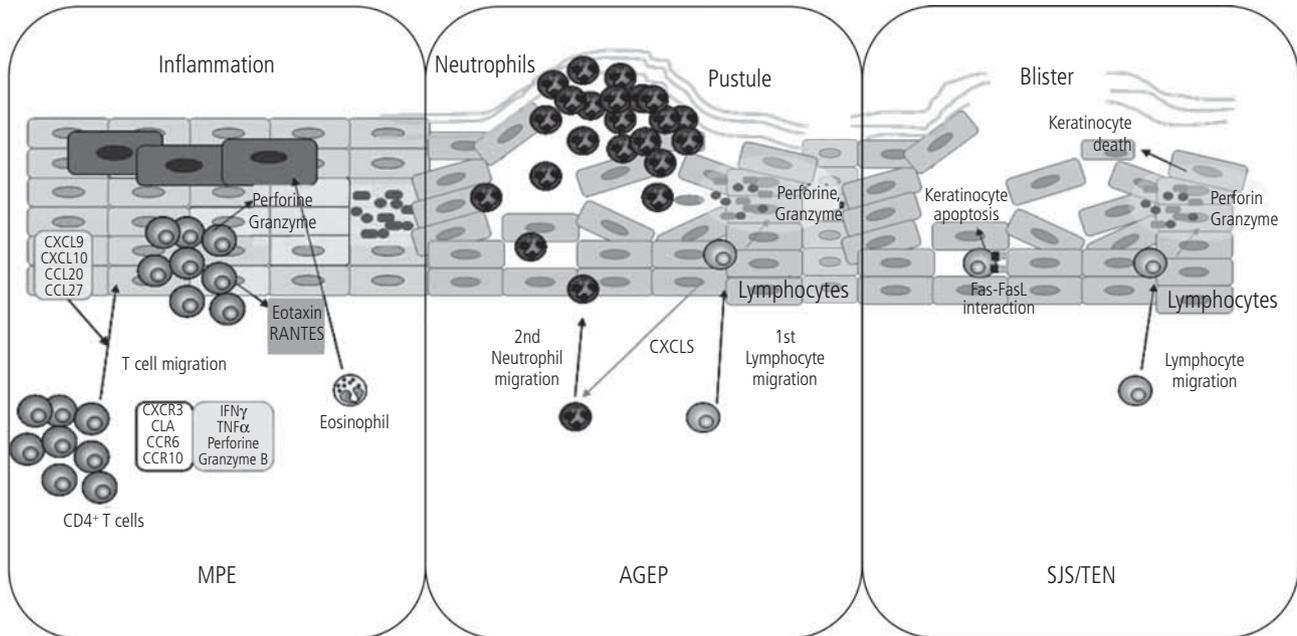


Figure 3. Immunopathologic mechanisms involved in maculopapular exanthema (MPE), acute generalized exanthematous pustulosis (AGEP), and Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN). CLA indicates cutaneous lymphocyte-associated antigen; TNF, tumor necrosis factor.

## The Role of Dendritic cells in NIRs

Most information about T cell-mediated drug reactions concerns specific effector immune responses and the role of drugs in generating interactions with specific T cells, either directly or via drug metabolism and haptentation [41,42]. Little information, however, is available about the initial steps mediated, mainly through dendritic cells, by the innate immune system. Dendritic cells are professional antigen-presenting cells that play a crucial role in the initiation of T-cell responses [43]. Their abilities are regulated in a process known as maturation, during which they modulate the tolerant or effector immune responses mediated by different subtypes of T cells [44]. While immature and semimature dendritic cells—which are highly endocytic but poorly effective as antigen-presenting cells—have been associated with tolerant immunologic responses, mature dendritic cells, which have no endocytic capacity, migrate to the lymph nodes and efficiently present previously captured and processed antigens to the T cells, which induce effector responses. This concept, which is a key factor in the discrimination of self-antigens or innocuous molecules from pathogenic molecules, seems to be more complex, and other factors are probably involved [19].

It is believed that a danger signal is required to trigger a pathologic response in which the hapten-carrier is recognized by the immune system and antibodies or T cells are generated. Different types of danger signals exist, including exogenous signals—related to infectious pathogens, also called pathogen-associated-molecule patterns (PAMPS), bacterial and viral genomes, flagellins, and endotoxins such as lipopolysaccharide [44]—and endogenous signals such as TNF- $\alpha$  and IL 1 [45,46]. These molecules interact

with dendritic cells via a Toll-like receptor [47], inducing the activation of a signaling cascade with the participation of mitogen-activated protein kinases [48]. This dendritic cell maturation process produces a series of cytokines and chemokines that modulate a T<sub>H</sub>1 or T<sub>H</sub>2 response appropriate to the danger signal induced.

A number of findings support the role of dendritic cells in the response to haptens [48-50]. Contact dermatitis, a delayed-type hypersensitivity reaction to low-molecular-weight compounds such as nickel is the best-studied model [51]. The hapten by itself is able to produce a maturative state in dendritic cells that induces a specific T-cell response [48,51]. This is the case of sulfamethoxazole and its reactive metabolite nitroso sulfamethoxazole, which have been shown to be associated with the generation of the costimulatory signals required to initiate a primary immune response [52].

Moreover, our group has studied the role of dendritic cells in the generation and modulation of pathologic T cell-mediated NIRs to drugs and demonstrated that haptens such as amoxicillin and heparins can induce changes in the maturational status of dendritic cells, generating a specific proliferative response in T cells in allergic patients but not in tolerant controls [53,54]. These results suggest that certain drugs may act not only as a target antigen for the immune response in particular but also as a stimulus for dendritic cell maturation. Another possible explanation for the generation of effector responses to drugs is that the interaction with dendritic cells occurs in maturational conditions. This maturation could be caused by concomitant factors such as the presence of exogenous signals such as PAMPS or of endogenous signals, with cytokines such as TNF- $\alpha$  induced by an inflammatory process.

## Role of Viral Infection in NIRs

NIRs can also occur as the result of interaction between a particular drug and different factors. A key factor is the underlying disease, especially in the case of viral infections. Several viruses have been related to allergic reactions to drugs, including human herpes virus 6 (HHV-6), cytomegalovirus, Epstein-Barr virus (EBV) and, more recently, paramyxovirus.

Viruses can interact with the immune system at several points: during drug metabolism, during the presentation of a drug to lymphocytes by dendritic cells, and during the production of cytokine and chemokine in the effector response (Figure 4) [55,56]. Conserved microbial products which interact with pattern-recognition receptors such as Toll-like receptors can induce the maturation of dendritic cells, and this may be of relevance to the increased risk of hypersensitivity reactions associated with viral infections such as EBV, HHV, and HIV [57-59].

The drug-induced clinical entity most often associated with viral infections is ampicillin-induced exanthema in

patients with infectious mononucleosis, an acute disease induced by EBV [60]. Exanthema occurs in 5% to 13% of patients with infectious mononucleosis, but this rate increases considerably in those receiving ampicillin (29%-69% of adults and up to 100% of children) [61]. Although some authors have reported that this is not a true sensitization [56,62], others have shown true immunologic sensitization by skin testing and the lymphocyte transformation test (LTT). Moreover, the response is not transient [63,64]. Several reports have shown an association between a high frequency of SJS and TEN in patients with HIV and early reactivation of EBV and cross-reactivity with drugs [60]. The best studied drug-allergy reaction associated with viral infection, however, is DRESS/DIHS, which has been linked to the reactivation of HHV-6 [65-67]. The clinical symptoms of this disease often resemble those induced by cutaneous viral infections. Some authors have shown that the slow resolution of DRESS/DIHS is due to HHV-6 reactivation [68], favored by hypogammaglobulinemia, which can occur during treatment with certain drugs such as anticonvulsants [69]. This suggests that anticonvulsants may not only activate cytotoxic T cells involved in the allergic

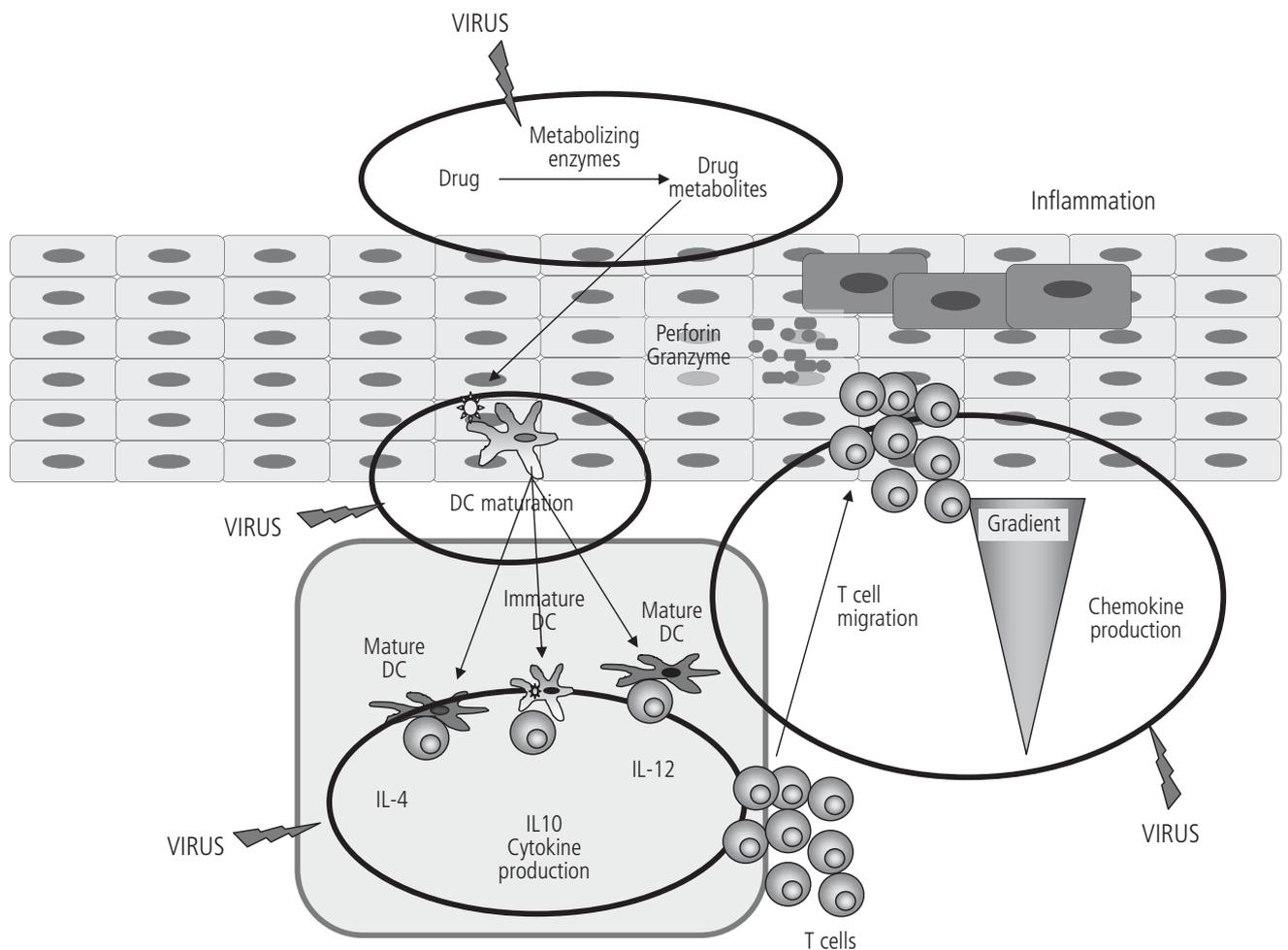


Figure 4. Virus-immune system interaction points in allergic reactions to drugs. DC indicates dendritic cell; IL, interleukin.



Figure 5. Positive delayed-reading intradermal test results for radiocontrast media (A) and aminopenicillins (C). Positive patch test results for anticonvulsants (B) and tetrazepam (D).

reaction to these drugs but also induce immunosuppression that might also promote virus reactivation, thus delaying resolution.

The cooperative role between viruses and drugs in the induction of NIRs is unclear. Our group has demonstrated that amoxicillin induces changes in the maturational status of dendritic cells towards semimature dendritic cells, producing specific T-cell proliferation [53]. The fact that patients may have a confirmed NIR but a negative LTT to a particular drug might be due to the absence of concomitant viral stimulation, which has been shown to induce fully mature dendritic cells. Preliminary studies with drugs and a Toll-like receptor agonist have shown an increase in dendritic cell maturation and a positive LTT response only in patients with drug-induced MPE and an infectious disease (data not published). This indicates that both elements might be necessary in order to mimic the immunologic mechanisms involved in the *in vivo* reaction.

Furthermore, viral infections can induce skin symptoms similar to those produced in drug allergy reactions, an important factor when establishing a differential diagnosis,

especially in children. On comparing viral-induced and drug-induced exanthematic reactions in children, our group found a higher expression of the homing receptor CLA and activation marker CD69, as well as a  $T_H1$  cytokine pattern in children with drug-induced reactions [70]. Children with viral reactions, in contrast, had a nondefined cytokine pattern.

## Diagnosis

A common feature of NIRs is that symptoms appear 24 to 48 hours after drug intake, although they may occasionally appear after a few hours and in some cases even after several days. Because of the wide variety of possible clinical entities, the clinical history does not always provide a well-defined description of the past episode. The time of onset of symptoms and their severity are factors to be taken into account, not only in the evaluation of NIRs but also in the determination of diagnostic tests required.

Difficulties in diagnosis reside in the lack of sensitivity of

currently available tests, the absence of cofactors that may have been present at the time of the reaction, and the possibility that symptoms were not immunologically mediated. The different diagnostic methods currently available are described below.

### *Skin Tests*

The diagnostic value of skin testing has not been fully evaluated and experiences in different centres are rarely shared. Reliable skin test procedures for the diagnosis of NIRs are thus generally lacking and test concentrations are unknown or poorly validated for most drugs. Delayed-reading intradermal and/or patch tests have been used for many years. The main advantage of drug patch tests is that they can be performed with any commercially available drug form. While intradermal tests are more sensitive, they need to be performed with an injectable form or a pure, sterile preparation of the drug (Figure 5).

Both intradermal and patch tests have been widely used in the diagnosis of NIRs to betalactams, with reported sensitivity ranging from 2.6% (positive responses to patch tests in 8 of 298 patients tested with phenoxymethyl penicillin) [71] to 37.8% (positive responses to patch tests and/or delayed-reading intradermal tests with penicillins in 98 of 259 patients) [72]. New evidence seems to indicate that skin test sensitivity is lower than previously believed. A recent study that prospectively evaluated 22 patients with confirmed MPE or urticarial exanthema due to betalactam intake found that only 2 (9%) had a positive delayed-reading intradermal/patch test despite confirmation of the reproducibility of the reaction by a drug provocation test [73]. Similar results have been found in children [74]. This indicates that the sensitivity of skin testing is lower than previously thought, at least in patients with exanthematic reactions to betalactams, although it may be higher in more severe reactions such as desquamative exanthemas.

Similar results have been detected for other drugs. In a group of 21 patients with NIRs to systemic corticosteroids confirmed by drug provocation testing, for example, just 2 patients had a positive patch and intradermal test [15]. Patch testing was recently seen to be useful for the diagnosis of exanthema induced by cardiovascular or antiepileptic drugs (positive patch test results in 10 [4%] of 247 patients for amoxicillin and in 6 [3.9%] of 152 patients for benzylpenicillin) [71].

### *Drug Provocation Tests*

Because intradermal or patch testing often has insufficient sensitivity in patients with NIRs, a large proportion of patients need to be given the drug to establish a diagnosis or, perhaps more often, to confirm tolerance. Drug provocation testing is the best tool with which to confirm a causal relationship between drug administration and a NIR. It involves the careful administration of a suspect agent in a specialized centre and close monitoring for symptoms, in particular skin manifestations [75]. Drug provocation testing, however, is not generally recommended and is actually contraindicated in some cases such as generalized bullous fixed drug eruptions, AGEP, SJS, TEN, DRESS/DIHS, systemic vasculitis, specific-organ manifestations (blood-cytopenia, hepatitis, nephritis, pneumonitis) and drug-induced autoimmune diseases [76].

Of all the drugs suspected to cause NIRs, betalactams have been studied most extensively. Most patients that develop an exanthematic reaction after betalactam administration and have negative skin tests tolerate drug provocation tests well [71-74,76,77]. In a study by Romano et al [72], only 3 (2.5%) of 117 subjects reacted to drug provocation testing with suspect penicillins. Nonetheless, some patients with clear NIRs are diagnosed by a positive drug provocation test. In a study by Padial et al [73], only 2 (9%) of 22 adult patients with exanthematic reactions to betalactams diagnosed by drug provocation testing had a positive delayed-reading intradermal skin test. Similar results were recently found in 20 children with exanthematic reactions and positive drug provocation tests (only 1 positive skin test) [74]. This indicates that drug provocation testing is the most important diagnostic tool in the particular case of exanthematic reactions to betalactams.

### *In-Vitro Diagnostic Tests: The Lymphocyte Transformation Test*

The LTT, which is based on the principle that T cells can proliferate in the presence of a specific antigen, has been the most widely used test over the last 30 years to detect T-cell sensitization to a drug in vitro. The usefulness of this test in the diagnosis of NIRs, however, has been debated. Reports published to date have been characterized by small series, a wide range of drugs and clinical entities, sensitivity rates of 60% to 70% and rather low specificity (85%) [78-80]. Furthermore, this test is not available everywhere and is still considered a research tool.

In a study by our group, 57% of patients with a NIR to betalactams had a positive LTT to at least one of the penicillins tested [80]. We detected diverse response patterns and certain patients with selective in vivo responses to amoxicillin responded in vitro to benzylpenicillin and/or amoxicillin, even though they tolerated benzylpenicillin.

A recent study analyzing the role of dendritic cells in the enhancement of amoxicillin-specific lymphocyte proliferation in patients with NIRs to amoxicillin showed that compared to traditional antigen-presenting cells such as B cells or monocytes, dendritic cells improved LTT sensitivity [53]. Similar results have been obtained for NIRs to heparins [54].

### *Immunohistochemistry*

NIRs usually affect the skin, which is an easily accessible organ for biopsy. Samples can be taken from the acute reaction site or from tissue that has yielded a positive drug provocation or skin test result. While immunohistochemistry findings help in the investigation of underlying immunologic mechanisms, they do not provide information about the drug involved or discriminate between different types of reactions. The most common finding is a mononuclear cell infiltrate composed mainly of activated T cells expressing DR antigens, CD69 activation markers, and the skin-homing receptor CLA in both CD4<sup>+</sup> and CD8<sup>+</sup> T cells (with a general predominance of CD4<sup>+</sup> cells) [14].

There are, however, a number of differences between mild and severe reactions. In MPE, for example, a predominant perivascular mononuclear cell infiltrate with increased numbers

of eosinophils in the papillary dermis has been found, with up to 20% of the cells expressing perforin and granzyme B and enhanced IL 12 expression [81]. SJS/TEN, in contrast, is characterized by the presence of many dead keratinocytes with minimum T-cell infiltration, probably due to the loss of superficial skin layers [81]. In AGEP, keratinocytes express high levels of IL 8, a chemokine that recruits neutrophils to the epidermis.

## Conclusions

NIRs to drugs are the most common hypersensitivity reactions and can be induced by all commercially available drugs. The reactions cause a wide heterogeneity of clinical symptoms, reflecting differences in the immunologic mechanisms involved. A T-cell effector response is the main pathologic event. Diagnosis is often complex because of the difficulty in obtaining a reliable clinical history, the importance of concomitant factors such as viral diseases, and the low sensitivity of skin tests and in vitro tests. More studies are needed in order to improve our understanding of NIRs and refine our diagnostic criteria.

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