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Diagnostic evaluation of delayed hypersensitivity to systemically administered drugs

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Introduction

Adverse drug reactions with an immunologic basis (ADRI) can be mediated by antibodies or T cells, but most clinical studies have focused on IgE-mediated reactions, which usually occur less than 1 h after drug administration (immediate reactions) (1). The penicillin model has contributed greatly to the understanding of immediate reactions, which are well-defined clinical entities such as urticaria, angioedema, and anaphylactic shock (2-4).

However, in recent years, increasing attention has been paid to nonimmediate (i.e., occurring more than 1 h after drug administration) hypersensitivity reactions to systemically administered drugs, with clinical and laboratory studies indicating that a cell-mediated pathogenic mechanism may be involved (5, 6). It has been reported that peripheral blood lymphocytes from patients with a drug-induced reaction may be stimulated *in vitro* with the culprit drug, and specific T-cell clones may be generated from the peripheral blood of patients with such reactions to betalactams or sulfamethoxazole (7-10). Immunohistochemical studies have demonstrated that T cells are the main cellular component of the skin inflammatory infiltrate in drug-induced nonimmediate cutaneous hypersensitivity reactions (7, 11). Cells isolated from biopsies of such reactions can proliferate *in vitro* after stimulation with the responsible drug (7, 8, 12, 13).

In view of these findings, it seems advisable not to focus solely on IgE-mediated allergy to drugs, but also to consider those reactions where T cells seem to play a role in the response, as occurs in nonimmediate reactions.

Diagnostic approach

Several factors make the diagnosis of nonimmediate reactions to drugs a complex clinical problem. The clinical

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manifestations are very heterogeneous and may have characteristics which resemble the symptoms of infectious or autoimmune diseases. Moreover, such reactions may even be the result of interaction between infectious agents, such as the human immunodeficiency (HIV) or Epstein-Barr virus, or other agents and drugs (14). In any case, as in all adverse drug reactions, the patient's history is relevant to establishing the diagnosis.

Consideration of a number of findings is essential for a correct evaluation of adverse drug reactions: the illness for which the drug was prescribed, the drug or drugs associated with reactions, presence or absence of prior exposure to the drug, duration of treatment before onset of a reaction, time elapsed between last drug administration and onset of symptoms, careful description of the preceding, recurrence of symptoms upon rechallenge, and the effect of stopping treatment. These data may help in understanding the causal relationship between the offending drug and the reaction induced.

There are not many reliable tests generally available to diagnose drug allergy, particularly the delayed type. Nevertheless, the following ones have proven to be useful in some cases.

Skin tests

Although evidence in some studies indicates that *in vitro* tests can also be reliable, skin testing (prick and intradermal) is the best method for diagnosing type-I IgE-mediated hypersensitivity (15). Some data suggest that the delayed reading of intradermal skin tests can also be an effective way of diagnosing nonimmediate allergic reactions to drugs (16–18). However, standardization requires further studies.

Patch tests

Patch, or epicutaneous, testing is useful in diagnosing cases of eczematous contact forms of allergy such as those observed in pharmaceutical workers. Patch-test positivity can also occur in nonimmediate cutaneous reactions to systemically administered drugs such as penicillins (19–24), anticonvulsants (16, 20, 25), and pyrazolones (20).

Our experience with patients who have developed delayed hypersensitivity reactions to drugs such as betalactams (21–24, 26), diltiazem (27), diclofenac (28, 29), and strepronin (30, 31) indicates that these conditions are associated with late response to both intradermal (more than 6 h after injection) and patch testing. Furthermore, in a previous study, all 18

patients with histories of maculopapular eruptions during aminopenicillin therapy and displaying patch-test and delayed intradermal-test positivity, and who agreed to undergo oral challenges, reacted with diffuse maculopapular rashes; 10 also developed angioedema of the face, hands, and feet. The dose of the drug used in the challenge was variable, but, in most cases, the provocative dose was 1% of the therapeutic one (21). Although the pattern of both patch-test and delayed intradermal positivity seems a clear indication of delayed hypersensitivity, confirmation requires further studies.

In vitro tests

The *in vitro* lymphocyte response to drugs, known as the lymphocyte transformation test (LTT), is used for evaluating T-cell response to drugs. There is not full agreement on the diagnostic value of this procedure. Some researchers who assessed a large number of patients with adverse reactions to drugs such as penicillin, sulfamethoxazole, and carbamazepine with LTT consider this method sensitive and specific (32). Others doubt the diagnostic value of LTT studies (33). These differences of opinion may be due to the fact that a number of mechanisms can induce lymphocyte proliferation, including cross-linking of membrane structures or other types of activation. Another reason may be that in some circumstances the lymphocyte proliferative response occurs when degradation of the parental drug produces a metabolite which is active on lymphocytes, inducing proliferation. In the study of Merk et al., compounds such as pyrazolones and sulfamethoxazole, which cause adverse cutaneous reactions, were incubated with murine liver microsomes and lymphocytes of peripheral blood as well as cloned T cells from the skin lesion. The authors observed marked lymphocyte proliferation after the addition of the microsomes, evidence of the involvement of a cytochrome-P450-dependent metabolism as a prerequisite for an optimal lymphocyte response to culprit drugs (34). According to these authors, the failure of T cells to be stimulated by the culprit drug in the LTT may also result from the low frequency of drug-specific precursor cells. This is supported by the observation of Roujeau et al. that the drug-induced proliferation of peripheral blood mononuclear cells (PBMC) from patients with toxic epidermal necrolysis (TEN) seen *in vitro* at the onset of clinical symptoms was virtually absent months later (35). Another situation that may explain negative LTT results is the absence in peripheral blood of antigen-presenting cells able to interact with T cells to induce the

immunologic response. In addition to these limitations, it has been shown that lymphocyte proliferation in response to drugs represents their immunologic ability to recognize the latter; therefore, PBMC from healthy controls with different types of exposure to the investigated drug, or who have been treated with it without adverse reactions, may occasionally react (36, 37).

The macrophage migration inhibition test (MIF) is another approach that has been used to study immunologic drug reactions (38). In this test, normal macrophages are cultured in the presence of test lymphocytes from patients and the suspect drug. Sensitized lymphocytes produce a lymphokine which inhibits the normal migration of macrophages. Some authors indicate that it can be a useful diagnostic tool in some instances, but their results have not been widely supported, and the test is time-consuming and costly.

Another alternative that has been used in some cases is monitoring of the acute response at the moment of the reaction. The detection in peripheral blood of different cell-membrane markers by flow cytometry may allow the identification of some cell-mediated adverse drug reactions. Some authors have reported an increase in the expression of cutaneous lymphocyte-associated antigen (CLA) on circulating T cells in subjects with delayed allergic reactions to drugs. These cells were activated, and their CLA tended to become normal in correlation with the disappearance of skin symptoms, demonstrating that CD3+ CLA+ cells were involved (39–41).

In conclusion, these data indicate that the available technology can be useful in the evaluation of nonimmediate adverse drug reactions, although more precise validation is required.

Provocation tests

Although it is sometimes contested because of ethical considerations, provocation is often the reference standard for confirming a causal relationship between drug administration and nonimmediate adverse reactions. It consists of the careful administration (i.e., at increasing doses, usually starting with one-thousandth) of the suspect agent. It can be performed only in specialized centres with facilities for early intervention, including intensive care. In the case of severe reactions, such as the Stevens-Johnson syndrome or TEN, this test should be avoided.

Specific drugs

Betalactams

Penicillins may induce nonimmediate reactions such as maculopapular or morbilliform rashes. Several studies have shown that in adults these rashes may represent a cell-mediated hypersensitivity phenomenon, which can be associated with patch-test and/or delayed (i.e., more than 6 h after administration) intradermal-test positivity (11, 17–20, 42, 43). In some studies, challenges with the suspect drug have induced delayed-onset skin rashes, confirming the diagnosis (17, 18, 42).

In our studies, more than 50% of 171 adults and about 10% of 42 children who had experienced maculopapular rashes during aminopenicillin treatment presented delayed hypersensitivity diagnosed on the basis of patch-test and/or delayed intradermal-test positivity (21, 22, 24). In the assessment of nonimmediate reactions to aminopenicillins, intradermal tests appear to be more sensitive than patch tests. In effect, we have observed subjects who presented patch-test negativity and delayed intradermal-test positivity confirmed by challenge. However, we have also found a few cases of false positivity; thus, this pattern of skin reactivity needs further investigation (24). Our experience also indicates that, unlike type-I allergy, delayed hypersensitivity to aminopenicillins is a persistent condition (21, 23, 24).

Another important aspect is the necessity of using the suspected penicillin in both patch tests and intradermal injections because it has been shown that in delayed reactions to aminopenicillins, the aminobenzyl group of the side-chain plays a predominant role (11, 18, 19, 21–24). Thus, skin tests with penicillin determinants (penicilloyl-polylysine, minor determinant mixture, and benzylpenicillin) generally fail to detect side-chain-specific reactions to particular semisynthetic penicillins. Our group has also reported that delayed hypersensitivity to aminopenicillins is related to major histocompatibility complex genes (44).

Sulfonamides

The sulfonamides are frequently associated with non-immediate manifestations such as fixed eruptions and maculopapular rashes. Some reactions may be T-cell mediated, as positive topical provocations by patch tests have been reported in patients with cotrimoxazole fixed eruptions (45, 46). Sulfamethoxazole-reactive lymphocytes have been demonstrated in patients with exanthematic eruptions during sulfamethoxazole therapy (10, 47). In some

subjects, proliferative lymphocyte response in the LTT can be observed only after addition of murine liver microsomes (34).

Anticonvulsants

Adverse reactions to anticonvulsants are characterized by delayed-onset skin eruptions, mainly maculopapular or erythrodermic, often accompanied by fever and hematologic complications. Osawa et al. have reported patch-test positivity to culprit anticonvulsants ranging from 40% (phenobarbital) to 80% (sodium valproate) among subjects who had suffered such eruptions (16). Positive patch-test reactions and/or LTT in carbamazepine skin reactions have been shown in several studies (20, 25, 48-53).

A recent study describes a patient who had suffered severe exanthematic eruption, fever, and leukocytosis with eosinophilia during phenobarbital therapy. There was an increase in CLA expression in CD3+ cells in peripheral blood, as well as an increase in the expression of HLA-DR in the CD3+ CLA+ population. The LTT showed a stimulation index of 3.3 for phenobarbital. These data indicate that

an immunopathogenic mechanism was involved in this reaction (40).

Nonsteroidal anti-inflammatory drugs

Although many cutaneous reactions to nonsteroidal anti-inflammatory drugs appear to be induced by a pseudoallergic phenomenon, a cell-mediated hypersensitivity mechanism may be involved in some nonimmediate ones, and patch testing can be useful in assessing such reactions (28, 29, 54-56).

Concluding remarks

In recent years, considerable progress has been made in understanding the mechanisms involved in allergic drug reactions, but most of these advances concern IgE-mediated reactions. Much research needs to be done on nonimmediate allergic reactions, both to identify the pathogenetic mechanisms involved and to improve diagnostic methods for evaluating such manifestations.

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