

Immune Mechanisms in Drug Allergy

Jean-Claude Roujeau¹

ABSTRACT

Clinicians had suspected for years that drug eruptions were probably mediated by immune mechanisms because their timing suggested sensitization and specific immunologic memory rather than direct toxicity. An immune response to medications was also demonstrated by positive skin tests and by several types of *in vitro* tests that evidenced immediate or delayed hypersensitivity.

In the last decade several teams of researchers obtained *in vitro* drug-specific human T-cell clones, in a variety of clinical types of drug eruptions. These clones were produced from blood or skin mononuclear cells of patients with a history of drug reaction by stimulation *in vitro* with drug. They were either of CD4 or CD8 phenotypes. Drug specific clones were stimulated by the parent drug much more often than by reactive metabolites. That challenged the classical "hapten hypothesis" that the immune response was initiated by reactive metabolites combined to self proteins. The medication usually stimulated specific T-cells after non-covalent binding to major histocompatibility (MHC) molecules on antigen presenting cells. In toxic epidermal necrolysis, T-lymphocytes present at the site of lesions, exhibited a drug specific cytotoxicity against autologous target cells, or allogeneic cells that shared the same HLA than autologous cells. This MHC class I restriction and mediation of death by perforin/granzyme release, is the classical behavior of cytotoxic T lymphocytes, like those operating in the reject of a transplanted organ. MHC restriction could explain the key role of HLA genes as predisposing factors to severe drug reactions. A strong association between HLA and hypersensitivity to abacavir, SJS or TEN to carbamazepine or allopurinol has been recently demonstrated. Resemblance to graft rejection points to the possible therapeutic value of immuno suppressive agents.

Most drug eruptions appear to result from T-cell mediated delayed hypersensitivity. The secondary activation of different cascades of cytokines, may contribute to the heterogeneity of clinical presentations.

KEY WORDS

autologous, cytotoxicity, drug hypersensitivity, epidermal, HLA, immunologic, T-lymphocytes, toxic

INTRODUCTION

Drug reactions are a public health problem because of their frequent occurrence, occasional severity and impact on the use of medications.¹ The skin is among the organs most often affected by adverse drug reactions. The list of conditions that can be triggered by medications includes nearly all dermatological diseases. Many of these adverse reactions result from mechanisms that do not involve an immunological process. For example the accumulation of drug derived compounds in the skin can result in hyperpigmentation (amiodarone, antimalarials, minocycline, quinolones). A pharmacologic interaction with the proliferation and differentiation of epidermis, hair follicles or sebaceous glands can induce alopecia (cytostatics), folliculitis (anti-EGF) or severe dryness (isot-

retinoin). Such side-effects are usually delayed by weeks or months, and sometimes relatively common among long term users of these drugs, *i.e.* very specific and limited populations. In such groups these reactions are expected, and the prescribing physician can easily evaluate the benefit/risk ratio and often also provide advises for preventing or alleviating the adverse effects.

The situation is different for drug eruptions that develop soon after the introduction of a medication. Several large cohorts have shown that such reactions occur in 2 to 3% of unselected hospitalized patients.^{2,3} More and more data suggest that these eruptions have an immunological basis. Fortunately about 90% of these reactions are benign and transient "maculopapular rashes",³ but severe cutaneous adverse reactions (SCAR) to drugs nevertheless affect about 1 per

¹Université Paris XII, Department of Dermatology and Inserm U 659, Hôpital Henri Mondor, Créteil, France.

Correspondence: Jean-Claude Roujeau, M.D., de Dermatologie, Hôpital Henri Mondor, 94010 Créteil cedex, France.

Email: jean-claude.roujeau@hmn.ap-hop-paris.fr

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1000 hospitalized persons.⁴

In this situation the occurrence of the reaction is non predictable, even if some factors are known to increase the risk. The severity is also rather difficult to assess at the early onset of the rash, often leading to the discontinuation of a useful treatment. Further use of medications is compromised because patients consider themselves as potentially "allergic" to every medication. It is therefore of tremendous importance to better understand the basic mechanisms of benign and severe drug reactions in order to improve the management of a variety of situations, from the early pre-clinical phase of the development of a new drug, to the design of tests that could help in clinical practice for assessing drug causality and providing clear recommendations for future use of medications in individual patients.

Taking for granted that most drug reactions are allergic, many questions still need clarification. Is chemical reactivity a key determinant for a medication being antigenic? Which differences in the immune response lead to rare and life-threatening reactions rather than to benign and common eruptions? Can we suspect that some risk factors for drug reactions play a role through interaction with the immune response? How should we use our improved knowledge of mechanisms for prevention and treatment of drug allergy?

MOST DRUG ERUPTIONS ARE PROBABLY "ALLERGIC"

Up to recently, the main argument was the timing of drug eruptions. Most begin one to three weeks after the introduction of the medication, while recurrences after re-challenge begin within 2 days. This timing suggests sensitization and a specific immunologic memory rather than direct toxicity, which should occur when a dose related threshold is reached. It is not yet understood why mild eruptions usually occur 9 ± 5 days after initiation of the medication, when most severe reactions often begin later: 14 ± 7 days for Stevens-Johnson syndrome (SJS) or Toxic Epidermal Necrolysis (TEN), 28 ± 14 days for "drug hypersensitivity syndrome" (DHS) also called "drug reaction with eosinophilia and systemic symptoms (DRESS). Some eruptions occur much sooner: minutes to hours for urticaria and anaphylaxis, 1 to 3 days for "acute generalized exanthematous pustulosis (AGEP) and fixed drug eruption (FDE). It is usually proposed that AGEP and FDE are recall reactions after prior overt or latent sensitization.

There is a large amount of data in the medical literature on *in vivo* and *in vitro* immunologic tests to medications. Penicillin allergy has been extensively studied. The antigenic determinants of immediate IgE-related reactions were determined and the positive or negative predictive value of prick-tests has been evaluated as rather good. For the more frequent

delayed eruptions, to penicillins or to other drugs, the sensitivity and predictive value of skin tests is poorer.⁵ Even if not as helpful as expected in daily clinical practice, positive skin tests to drugs demonstrate a specific sensitization to medications and biopsies of positive tests have been used for isolation of drug-specific T-cells.⁶ The situation is similar for the many *in vitro* tests that had been developed. The overall relationship between positive *in vitro* tests and eruptions strongly supports the existence of an immune response to drugs, but the predictive values are not high enough to be useful for clinical decisions in individual patients.

Many studies of drug eruptions using immunobelling of skin biopsies demonstrated the presence in the lesions of activated T-cells, with usually a predominance of CD4 + lymphocytes in the dermis and a predominance of CD8 + cells in the epidermis.^{7,8}

In guinea-pigs immunized by injection of cephalosporins in complete Freund adjuvant, peritoneal injection of the sensitizing drug resulted in a widespread eruption. The reactivity was transferred to naïve recipients occasionally by the serum and nearly always by lymphocytes from the spleen or lymph nodes from sensitized animals. T lymphocytes were probably responsible for the reaction since it was not abrogated by the depletion of B lymphocytes from the transferred cells.⁹

MEDICATION SPECIFIC T-CELL CLONES

In the last decade the key role of drug specific T-cells in drug allergy was definitely demonstrated by the establishment of human T-cell clones, derived from the blood lymphocytes or from skin lesions of patients with a variety of reactions.¹⁰⁻¹⁵ Since these clones had been obtained after several stimulations *in vitro* with the drug, their relevance to explain the original manifestations of allergy can be questioned. Anyhow T-cell clones provided the demonstration that drugs can be recognized by human T-cells and suggested original pathways of activation. Clones have been obtained with most medications that induce allergic reactions in man including penicillin G, amoxicillin, cephalosporins, sulfamethoxazole, phenobarbital, carbamazepine, lamotrigine. They were often of both CD4 and CD8 phenotype, whatever the original type of eruption had been, with a majority of CD4+ clones. Some clones produced a Th0 profile of cytokines (simultaneous release of IL4 and IFN-gamma). A Th2 orientation was frequent in CD4+ clones while CD8+ clone were usually Th1 and often cytotoxic. Drug presentation to T-cell was MHC restricted, usually as expected by HLA class II for CD4+ cells and by HLA class I for CD8. But there was also less classical situations like HLA class II restricted cytotoxic CD4 clones.^{16,17} With many drugs a very original observation was that the drug could be presented to the TCR and activate specific clone without prior processing

by the antigen presenting cell and through a non covalent binding to the MHC or its embedded peptide.^{12,14,17} Actually some specific TCR could recognize sulfamethoxazole presented either in covalent or noncovalent bound form, but the former was the exception and the later the rule.¹⁸

Since the non covalent binding is reminiscent of the pharmacological interaction between a drug and its receptor, the denomination of pharmoco-immune (p-i) concept has been proposed.¹⁷

Often the recognition by the TCR was not absolutely specific. For example, lidocaine specific clones also reacted to mepivacaine and vice versa¹⁹ and SMX specific clones reacted to a few other antibacterial sulfonamides but neither to Cox-2 antagonist NSAIDS nor to furosemide.¹⁸ Anyhow since the *in vivo* response to medications is not clonal, these *in vitro* data on cross-reactivity should not be extrapolated too quickly to clinical practice.

WHAT IS THE ANTIGEN: PARENT DRUG OR A REACTIVE METABOLITE ?

Twenty years ago it has been proposed that reactive metabolites played a key role in hypersensitivity reactions to drugs, by allowing the covalent binding of metabolites to proteins, when the parent forms of most drugs are not reactive. According to the hapten theory such a binding to proteins was required for initiating an immune response to small non protein molecules like medications. In the serum of patients with hepatitis due to tienilic acid antibodies were found that reacted against a covalent complex of the reactive metabolite with the CYP450 enzyme that produced it.²⁰ The hypothesis of drug reactive metabolites was essentially developed about sulfonamides. It was initially based on a report that among 5 patients with hypersensitivity to sulfamethoxazole (SMX) 4 were slow acetylators and 1 had an "intermediate phenotype".²¹ From this observation the theory was proposed that impaired acetylation of SMX led to increased metabolism through oxidation pathways, with elevated production of reactive metabolites (SMX-NHOH and SMX-NO) that behaved as haptens.²¹ That was rapidly accepted as a dogma after being comforted by a few additional data. Some series found that slow acetylation phenotype or genotype was a risk factor for allergy to sulfonamides,^{22,23} especially in patients with AIDS.²⁴ On the other hand two large prospective cohorts in HIV positive patients treated with sulfonamides did not find any significant impact of slow acetylation genotype or phenotype on the risk of cutaneous reaction.^{25,26}

More recently it was demonstrated that SMX-NO was immunogenic in rats, mice and rabbits while SMX was not.²⁷ But lymphocytes from immunized animals reacted only to metabolites and not to the parent drug. There is therefore no indication that priming of T-cells with reactive metabolites of SMX

may result in reactivity towards the parent drug.

In humans the list is already long of T-lymphocyte clones that react to the parent form of drugs and only occasionally to reactive metabolites. That was shown for SMX¹⁸ phenobarbital¹⁴ and lamotrigine.¹⁵ Reduced glutathione by modifying the relative concentration of SMX and SMX-NO abrogated the response of SMX-NO specific T-cell clones and enhanced the proliferation of SMX specific clone. That indicated that T cells from allergic humans recognize the non covalently bound parent drug rather than SMX-NO fixed on the membrane of antigen presenting cells.²⁸

One important point in this discussion is to estimate the concentration of reactive metabolites that can be expected *in vivo* in human in the intercellular milieu of epidermal cells, or on the membrane of keratinocytes that are the targets of cytotoxic T cells in drug eruptions. There are no vessels in the epidermis. Cells and nutrients have to cross the basement membranes of dermal capillaries and of the dermo-epidermal junction. Precisely because of their very high chemical reactivity it is unlikely that free and still reactive nitroso-SMX can reach the epidermis from the blood. Several teams have demonstrated that keratinocytes can metabolize drugs and produce reactive metabolites locally. But unlike hepatocytes, epidermal cells are not "professional" metabolizers and the amounts of reactive metabolites that they are capable to produce are very low. For example normal human keratinocytes in culture incubated with 1 mMole SMX (the upper range of concentration in the blood of patients taking high daily doses), produced 1 nMole *i.e.* about 250 picogramme/ml SMX-NHOH, precursor of SMX-NO.²⁹ It has not been demonstrated that such *in situ* production of reactive metabolites was capable to initiate an immune reaction. *In vitro* elicitation of a T-cell response to SMX-NHOH and to SMX-NO was observed at concentrations of 25 µg/ml and 1 µg/ml respectively,²⁷ *i.e.* 10⁵ times more than what was produced by keratinocytes in culture.

WHY IS THE IMMUNE RESPONSE TO MEDICATIONS LEADING TO SO DIVERSE REACTIONS ?

We will not discuss here the mechanisms of immediate hypersensitivity, IgE specific or not, but will focus only on delayed reactions, mediated by T-cells. Specific T cells were actually detected in a variety of drug eruptions.

Some answers were provided by studying effectors cells obtained at the site of abnormal reactions. Drug-specific cytotoxic T-cells were found in the bronchoalveolar fluid of patients with minocycline hypersensitivity pneumonitis³⁰ as well as in the skin lesions of fixed drug eruptions³¹ and of toxic epidermal necrolysis.³²

Fixed drug eruption is a fascinating model. Localized blisters recur at the same site a few hours after

drug reintroduction. This is usually not harmful, allowing provocation tests, with sequential biopsies allowing to decipher the mechanisms. In this disease there are CD8+ effector memory cells remaining at the site of the lesions within the epidermis or at the junction between epidermis and dermis. Even when resting these cells express early activation markers (CD69) and a few hours after drug re-administration produce high amounts of IFN gamma, before the onset of apoptosis of epidermal cells.³¹

In TEN, blisters that result from accumulation of interstitial fluid under the apoptotic epidermis³³ contain T-lymphocytes with a phenotype of cytotoxic cells. In 4/6 patient suffering from TEN, these cells killed autologous lymphocytes and keratinocytes in a drug specific, class I restricted and perforin/granzyme mediated pathway.³⁴ The only cytokine that was produced by these effector cells was IFN-gamma.³⁵

On the other hand, drug specific CXCL8 producing clones were obtained from skin tests of patients with acute generalized exanthematous pustulosis, a neutrophil mediated drug reaction.⁶

One may therefore assume that the final phenotype of drug eruptions results from the nature of effectors: cytotoxic T-cells in blistering reactions, T-cells releasing specific chemokines for reactions mediated by neutrophils or eosinophils.

But that is probably too simple since drug specific, MHC restricted and perforin/granzyme mediated cytotoxicity was also observed in T-Cell clones (remarkably CD4+ or CD8+) isolated from patients with maculo-papular benign eruptions.^{13,16}

It is not yet clear whether the huge differences between clinical patterns of drug allergies that are related to delayed hypersensitivity result from an immune response of different intensity, different quality or both.

Comparing fixed drug eruption and TEN leads to some other key questions. Both are drug induced blistering diseases, characterized by apoptosis of epidermal cells, and seem related to the same cytotoxic effectors. The former is localized, with few constitutional symptoms. The later is disseminated with high morbidity. The finding of IL10 producing CD4 T-cells in the lesions of fixed drug eruption suggests the role of regulatory T-cells in limiting the extension of the reaction.³⁶

Recently a transgenic mouse model of TEN suggested that specific anti-OVA cytotoxic T-cells killed OVA expressing keratinocytes only in a context of immune suppression.³⁷ The absence of lesions in animals with a normal immune system depended on both CD4+CD25+ regulatory T-cells and on CD11c+ dendritic cells.³⁸

This concept of negative regulation should be integrated in the theoretical models of drug allergy.

RISK FACTORS OF DRUG ALLERGY

Medications have been elaborated for treating diseases. It is therefore not surprising that among patients with adverse reaction to anticonvulsants there are more epileptics than in a control population. But epilepsy does not appear to increase the risk in comparison with patients using the same drugs for neuralgia or for psychiatric disorders. In other words there is no positive interaction between epilepsy and allergy to anticonvulsants. Epilepsy is not a risk factor by any other way than leading to anticonvulsants intake.

On the other hand there are evidences that some diseases increase the risk of drug allergy.

Several authors pointed to a striking frequency of severe drug eruptions in patients with brain tumors treated with an association of X-Ray therapy, anticonvulsants and corticosteroids.

Systemic lupus erythematosus and more generally collagen vascular diseases were shown to increase the risks of benign drug eruptions and also of SJS/TEN.³⁹

More attention was devoted to viral infections as possible risk factors of allergy to medications. The most striking, but poorly evaluated, model is the nearly constant eruption that occurs when adolescent are given aminopenicillins during infectious mononucleosis. Since the eruption usually does not recur if the same drug is taken again after remission of acute EBV infection, it is often considered to be a non-allergic phenomenon. Anyhow specific immune reaction to amoxicillin was recently evidenced in 3 of 4 patients investigated.⁴⁰ If confirmed in larger numbers, this finding would suggest that the amoxicillin rash occurring in infectious mononucleosis is drug specific and that its expression is enhanced by viral infection. It has been also established that HIV infection increases the risk of drug allergy. That was true for many drugs but mainly evaluated with SMX, a drug already known for a rather high rate of reactions in non HIV infected population and which was largely used by AIDS patients in the first decade of the pandemic. Up to 40% of patients had skin reactions when treated with high doses and about 15% reacted to usual dosage that induced 3 to 5% eruptions in non HIV populations. How AIDS promoted allergy to medication has not received yet a plausible explanation. Most hypotheses focused on abnormal metabolism, but two large prospective studies found no metabolic explanation to this increased risk. The principal factor of risk was a higher number of CD8+ T-cells. Activation of other viruses (CMV, EBV, HHV6) appeared to play no role.⁴¹

It has been also demonstrated that a special type of drug reactions, the so-called "Drug Hypersensitivity Syndrome" or "Drug Reaction with Eosinophilia and Systemic Symptoms" was often associated with reac-

tivation of herpes viruses (HHV6, CMV, EBV).^{42,43} This multi organ reaction to a variety of drugs (anti-convulsants, allopurinol, dapsone, minocycline) is characterized by a later onset than for other allergic reactions (usually 2–4 weeks instead of 1–2 weeks) and an expansion of activated lymphocytes. Virus reactivation may explain some clinical symptoms (rash, hepatitis, encephalitis) as well as lymphocytosis. In some instances virus DNA was not detected in the blood at the onset of the reaction but only several days later. The mechanisms of interactions between drugs and viruses are therefore probably different here from what happens during EBV primary infection and AIDS.

Several hypotheses were proposed to explain the interactions between viral infections and drug allergy.

The first is that viruses impair drug metabolism either directly or through the inflammatory response to infection.

Some medications may induce hypogammaglobulinaemia and promote virus reactivation, the symptoms being those of viral disease and not of drug allergy.⁴³ That has been proposed for DHS/DRESS but does not explain cases with evidence of drug specific immune reactivity (positive skin tests, positive rechallenge).

Viral infection could trigger recognition of drugs as antigens, as well as other “danger signals” that are suspected to enhance the immune response.

Another hypothesis is that recognition of drugs as antigen may be rather common, while the expression of the immune response is repressed under normal circumstances. Viral infection may impair the negative regulation and promote a reaction.

Recent literature showed that genetic factors may be strong predictors of severe drug reactions. Hypersensitivity to abacavir was strongly associated with HLA B*5701.^{44,45} In Taiwan, within an homogeneous Han Chinese population a 100% association was observed between SJS or TEN related to carbamazepine and HLA B*1502⁴⁶ and another 100% association between SJS, TEN or DRESS related to allopurinol and HLA B*5801.⁴⁷ These observations have important theoretical implications. By pointing to HLA genes they strongly support a key role for immune mechanisms. But since the associations found in Taiwan are far from being so strong in other countries,⁴⁸ the practical applications are probably not at hand.

FROM IMPROVED KNOWLEDGE OF MECHANISMS TO PREVENTION AND TREATMENT OF DRUG ALLERGY

We are not yet very close of clinical application of the above progresses. Even though most drug reactions are related to immune response *in vitro* and *in vivo* tests have too low sensitivity and predictive values to be really helpful, and the role of a specific drug remains difficult to demonstrate in individual patients.

There is also no consensus on what of the new findings on genetics could be applied now. In some restricted groups of patients, for treatments that can be delayed, such as antiretroviral therapy in AIDS some physicians already recommend HLA typing before initiating abacavir. But there is no consensus on such attitude, because the predictive values of the test are not yet definitely settled.

Because of growing suspicion that genetics matters it seems already prudent to recommend avoidance of the suspected drug to all blood relatives of a patient with a severe adverse reaction.

The more we learn on cross reactivity, the more it seems restricted to medications with close structural resemblance. It is probably useless to provide patients with broad lists of medications that should be contra-indicated.

We should also reconsider the use of treatments like corticosteroids, cyclosporine or other immunomodulating agents for blocking the cytotoxic reaction in severe life-threatening drug allergy.

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