

Murine models of drug hypersensitivity

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Purpose of review

Drug hypersensitivity reactions are relatively rare but may result in severe morbidity and fatalities. Due to the idiosyncratic nature and multifactorial etiology of these reactions, development of a single animal model to study the immunosensitizing mechanisms of all drugs is impossible. This hampers the development of predictive screening models that are urgently needed to assess the immunostimulating capacity of newly developed drugs. The present review will focus on recent findings on mechanisms of drug hypersensitivity reactions obtained with murine models, and on the use of these models as potential screening tools to assess the immunostimulating capacity of drugs.

Recent findings

Mechanisms of drug-induced sensitization versus tolerance appear dependent on generally accepted immunological paradigms. For instance, co-stimulatory signaling by antigen-presenting cells is decisive in drug-induced immunosensitization and both T cells and antigen-presenting cells are important for the induction of tolerance to orally administered drugs. From recent studies it has been hypothesized that expression of stress-associated transcription factors and the expression of costimulatory molecules or cytokine production within hours or days after the initial exposure may be representative of drug-induced hypersensitivity reactions and may thus be used as predictive parameters to screen for immunosensitizing drugs.

Summary

The development of animal models to study mechanisms of drug hypersensitivity reactions is still in its infancy. Much effort has been made, however, to search for early indicators of immunostimulation in murine animal models that may eventually appear useful in a tiered strategy to assess drug-induced sensitization.

Keywords

animal models, drug hypersensitivity, mechanisms, predictive screening

Abbreviations

ANA	antinuclear autoantibody
APC	antigen-presenting cell
IDHR	immune-mediated drug hypersensitivity reaction
PLNA	popliteal lymph node assay

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Introduction

Some drugs are associated with the development of allergic or autoimmune-like clinical symptoms in susceptible individuals. The clinical manifestation, ranging from mild to severe and life-threatening, can vary considerably for different drugs and for the same drug in different subjects, posing serious problems for patients and physicians. These reactions are idiosyncratic in nature and are here defined as immune-mediated drug hypersensitivity reactions (IDHRs). They comprise drug-allergic (activation of drug-specific T and B cells), pseudo-allergic (interference with immunological effector mechanisms and cells, such as mast cells), and drug-induced autoimmune reactions. Due to the similarities of these symptoms and signs with those observed in spontaneously developing or other (non-drug) induced allergy or autoimmunity, there exists no definitive agreement on the criteria and diagnoses for such adverse drug reactions. For this reason, the exact incidence and prevalence of IDHRs are unknown, but it is estimated that they represent up to one-third of all adverse drug reactions, which can affect 10–15% of hospitalized patients [1]. Idiosyncratic IDHRs also constitute a considerable burden on pharmaceutical industries as these reactions have been reported as the most frequent cause of failure of drugs during clinical development [2].

Despite their significance, the mechanisms of IDHRs are poorly elucidated. Naturally, low incidence, poor diagnostic tools and ethical considerations limit performance of mechanistic studies in humans. In addition, the complex and multifactorial etiology is not easily represented in in-vitro methodologies. Animal models appear vital tools to expand the knowledge on mechanisms underlying the development of IDHRs. Not one model has been found, however, that can be used to study all IDHRs and only few models were shown to mimic clinical symptoms observed in man [3^{*}]. The main reasons are that the low incidence of these idiosyncratic reactions that is observed in patients may also be present in animals, and that the different clinical effects of the

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same drug in different individuals seem impossible to reproduce in animal models.

Even with these limitations, such models have proven useful in providing insight into the mechanisms of IDHRs and have shown that IDHRs seem to be regulated by generally accepted immunological laws of sensitization versus tolerance induction. This review describes the latest findings on the mechanisms of sensitization and induction of tolerance to drugs in murine models. This knowledge is of immediate importance for the development of predictive screening models that may help to prevent the introduction of allergic or autoimmune drugs on the market. No validated tests are currently available to assess systemic IDHRs. However, results obtained in the last year with the reporter antigen approach, in both the popliteal lymph node assay and an oral exposure animal model, hold promise for the predictive value of these assays.

Murine models to study the mechanisms of immune-mediated drug hypersensitivity

Exposure to drugs may lead to IDHR by affecting several aspects of immune responses. According to the hapten hypothesis [4], the drug needs to establish neo-antigens by the formation of haptens that may be recognized as specific targets for immune cells. Drugs may also create immunological targets by cross-reactivity [5], the release of cryptic, non-tolerated epitopes or interference with central tolerance [4]. However, if drugs or their metabolites were limited to (neo-)antigen formation, it would unlikely be sufficient to induce an immune response. The induction of efficient immune responses to a certain antigen also requires the presence of so-called 'danger signals' that are required to activate antigen-presenting cells (APCs), which leads to the release of costimulatory and regulatory cytokines and expression of accessory/costimulatory molecules [6]. These stimulatory properties of drugs may overcome regulatory mechanisms and may result in the prevention or even reversal of tolerance and induction of sensitization.

Recently, Layland *et al.* [7**] studied the ability of CD4⁺CD25⁺ T cells to regulate responses to procainamide and gold sodium thiomalate, which cause lupus and autoimmune syndromes in humans, respectively. Both drugs might be considered prohaptens, because their T cell-sensitizing capacity is dependent on the reactive metabolites N-hydroxylamine-procainamide [8] and gold(III) [9], respectively. These reactive intermediates may generate danger signals in addition to neo-antigen formation and, hence, be capable of priming T cells. In agreement with this hypothesis, transfer of CD4⁺CD25⁺ T cells from drug-treated donors to recipients induced de-novo IgG1 antinuclear autoantibody (ANA) formation. However, transfer of the CD4⁺CD25⁺

regulatory T cell subset, isolated from A/J mice treated with procainamide or gold(I), completely prevented the formation of IgG1 ANA in recipients that had been treated with the same compound. Furthermore, drug-primed CD4⁺CD25⁺ T cells also partially prevented ANA formation in recipients that were cross-treated with the other chemical, indicating non-specific regulation by this cell subset. The authors also concluded that during chemical treatment, T cell reactivity may spread from chemical-induced nucleoprotein-related neo-antigens to peptides of the unaltered nucleoproteins [7**]. This study presented strong experimental evidence that drugs may induce autoreactivity (either stimulating or tolerizing) as a result of epitope spreading.

Mechanisms of tolerance to drugs have also been studied in the brown Norway rat model of D-penicillamine-induced autoimmunity. The clinical manifestations induced by D-penicillamine, which is used for treatment of rheumatoid arthritis or Wilson's disease in man, are resembled by the phenomena observed in brown Norway rats that receive 20 mg/day or more via drinking water [10]. In contrast, pretreatment with a low dose (5 mg/kg) for 2 weeks induces immune tolerance to D-penicillamine-induced autoimmunity, which is associated with a large increase in the production of IL-10 and TGF- β in CD4⁺ T cells. Moreover, the induction of tolerance can be partially prevented by inhibiting T cells with the immunosuppressant tacrolimus during the period of low-dose exposure [11**]. In addition, cotransfer of splenic T cells or splenocytes depleted of T cells from tolerant brown Norway rats into slightly irradiated syngenic recipients can partly prevent clinical symptoms of D-penicillamine-induced autoimmunity [12*]. This indicates that immune tolerance to D-penicillamine-induced autoimmunity may not only require T cells but also APCs. The finding that depletion of macrophages in low-dose-treated rats inhibited the induction of tolerance supports a role for APCs. Moreover, rats failed to develop tolerance when they were treated with the Toll-like receptor (TLR)-4 agonist, lipopolysaccharide, or poly I:C, a synthetic double-stranded polyribonucleotide and agonist of TLR-3 [11**]. Stimulation of pattern recognition receptors with poly I:C appears to suffice as an immunostimulating danger signal to prevent drug-induced tolerance and stimulate drug-induced autoimmunity. As such, APCs seem decisively involved in the regulation of sensitization versus tolerance.

T cells and macrophages were also shown to be involved in the development of D-penicillamine-induced autoimmunity after high dose treatment. T cells appear to be indispensable as tacrolimus totally prevented disease onset, reversed ongoing disease, and prevented disease relapse upon re-challenge with D-penicillamine [13**]. Tacrolimus treatment may therefore be considered

as a potential therapeutic avenue to inhibit severe autoimmune symptoms or prevent the induction of autoimmunity in high-risk patients. Depletion of macrophages with intravenous injections of liposomes in the brown Norway model also moderately decreased the incidence of D-penicillamine-induced autoimmunity. Furthermore, it appears that CD86-expressing macrophages are the major APCs that infiltrate the caecum early in response to drug. In addition, increased proportions of both CD80 and CD86-expressing macrophages were present in spleens of D-penicillamine-exposed mice within 1 week after exposure and these proportions corresponded with the presence of accentuation or prevention of the disease as a result of specific immunomodulatory treatments [13]. These observations in the brown Norway rat model suggest that an early appearance of CD86-expressing APCs may be a significant factor in D-penicillamine-induced immunosensitization and subsequent autoimmunity.

There is a growing interest to elucidate the importance of costimulatory second signals provided by APCs in drug-induced sensitization [14,15]. We previously studied the role of costimulatory interactions in immune responses induced by subcutaneous injections of D-penicillamine and showed increased expressions of CD40 and CD86, but not CD80, on APCs in the draining lymph node early in the response. With the use of monoclonal blocking antibodies, we have additionally shown that interactions of CD40 with CD154 [16] and CD80/CD86 with CD28 (S. Nierkens, unpublished data) are decisive in D-penicillamine-induced immunosensitization to a coinjected bystander antigen. Although expression of CD80 could not be detected, monoclonal antibodies specific for CD80 intensified the inhibiting effects of anti-CD86. Hence, it appears that CD86 is the major costimulatory molecule in this response and that CD80 is functionally linked to and intensifies the effects of CD86. In contrast, the monoclonal antibody anti-CTLA-4 (CLTA-4 being the natural ligand for CD80 and CD86) profoundly increased D-penicillamine-induced production of IL-4 and IgE (S. Nierkens, unpublished data) of which the latter has been associated with adverse drug-induced clinical phenomena [17]. Interestingly, polymorphisms of CTLA-4 have been linked to the intensity of allergic [18,19] and autoimmune manifestations [20]. Hence, people with particular CTLA-4 genotypes may be genetically predisposed to the development of IDHR-associated clinical effects.

Predictive screening models

Increasing knowledge on initiating mechanisms of IDHRs is indispensable for the development of pre-clinical screening tests intended to assess the safety of the use of newly developed and existing drugs. Currently, traditional toxicological studies fail to predict the hazard

of drug-induced immunosensitization so there is a need to identify and validate new markers, in particular markers of cell activation [21]. The popliteal lymph node assay (PLNA), especially with the use of reporter antigens, might be a promising candidate as a first screen because it was successfully used to predict the immune stimulating capacity of a considerable number of drugs [22]. It is a fast, simple and relatively cheap assay that enables measurements of multiple immunologically relevant and mechanism-based parameters. The latter aspect is important as, owing to the probability that multifactorial immunological mechanisms might be involved, changes in an individual endpoint may not be indicative of a potential hazard for human health impairment at therapeutic doses [23]. The PLNA enables determination of influx or proliferation of immune cell subtypes in the draining lymph node after subcutaneous exposure to a drug of interest. In addition, ex-vivo cytokine production after restimulation can be monitored but this does not seem to improve the sensitivity and specificity of the basic PLNA (determination of popliteal lymph node cellularity [24]). However, measurement of the formation of antibody-secreting cells to a coinjected reporter antigens, which may be either T cell-dependent or independent, was shown to allow differentiation between innocent, irritant, and immunosensitizing drugs and might therefore deserve attention as a predictive marker for drug-induced immunosensitization [22]. Using this model, Popovic *et al.* showed that the idiosyncratic drug reactions that are induced in man by the antiepileptic drug felbamate are probably due to the in-vivo formation of its metabolite 3-carbamoyl-2-phenylpropionaldehyde. In this study, felbamate failed to induce popliteal lymph node responses, whereas the metabolite induced the formation of germinal centers, cytokine and antibody production, and the expression of CD54 and CD86 [25[•]]. In addition, we recently showed with the reporter antigen-PLNA that drugs do not sensitize the immune system in the absence of costimulatory signaling (S. Nierkens, unpublished data) [16]. This suggests that the expressions of primarily CD40 or CD86 may be prerequisites for immunosensitization and should therefore be further investigated as first indicators of a drug's immunostimulating potential.

The reporter antigen approach has also been incorporated in a similar model, using intradermal injections at the dorsum of the ear, in which the presence of reporter antigen-specific IgG antibody-secreting cells in the draining auricular lymph node was used as end parameter [26[•]]. This exposure protocol was performed to reduce animal discomfort due to occasionally observed decreased mobility experienced following footpad injection. Both the reporter antigen-PLNA and an auricular lymph node assay (ALNA) positively identified diphenylhydantoin, D-penicillamine, and ofloxacin as immunosensitizing

drugs. Other compounds that are not associated with immune-mediated adverse effects in humans, such as metformin, or ones that require metabolism to become reactive, such as procainamide and sulphamethoxazole, tested negative in the reporter antigen-PLNA. Our results with sulphamethoxazole are in agreement with those of Hopkins *et al.* who showed that subcutaneous injections with sulphamethoxazole in the nape of the neck for three consecutive days failed to induce activation in local lymph node. However, injections with the metabolite, nitroso sulphamethoxazole, stimulated the production of high levels of IL-5 by CD4⁺ T cells [27•].

Lack of metabolism represents the major drawback of these exposure protocols. Therefore, to avoid this problem, we have explored the use of the reporter antigen approach in a murine model with oral drug administrations. BALB/c mice were intragastrically intubated with a drug of interest and received an intraperitoneal injection with the T-dependent reporter antigen trinitrophenyl-ovalbumin. Oral administrations of D-penicillamine and diclofenac increased serum levels of trinitrophenyl-specific IgG1 and IgE. In addition, exposure to these compounds and also to nevirapine-induced delayed type hypersensitivity responses to trinitrophenyl-ovalbumin after a subcutaneous challenge [28••]. These experiments show that orally administered drugs are able to sensitize mice to a systemic bystander antigen, which might well be a (released) auto-antigen. In addition, oral pretreatment with D-penicillamine or diclofenac significantly increased popliteal lymph node responses to the T-independent reporter antigen trinitrophenyl-ficoll, indicating that these drugs initiate the activation of neo-epitope-specific T cells. These results confirm those of Gutting *et al.* [29]; they also suggested that lower doses of diclofenac and also oxazolone-induced oral tolerance in this model [30]. As such, the development of oral exposure models in combination with the reporter antigen approach holds clear promise not only to assess the hazard of immunosensitizing drugs, but also to study the mechanisms of IDHRs.

It must be remembered, however, that the unknown predictability of most observed changes is still a major limitation [31]. Furthermore, the validity of these screening assays remains to be established. For instance, compounds that may directly interfere with immunological effector mechanisms, such as aspirin [32] may be missed and may require separate immunotoxicological testing. In addition, Gerber and Pichler have pointed out that not all drug-induced immune responses depend on primary responses, but may be mediated by preactivated T cells that are cross specific for the drug and other peptides [33]. The activation of these preactivated T cells by certain drugs is not detected in the presented variations of the PLNA. This suggests the use of more dynamic assays that

involve assessment of the response of the immune system to a challenge with a drug or drug-complex, which can be done by oral exposure models.

Moreover, in parallel to T cell sensitization studies, it may also be prudent to validate techniques that detect the immunostimulating properties of a drug, including induction of cell stress. Danger signals, although their exact nature remains largely unknown, seem indispensable for the induction of drug-induced immunosensitization or the reversal of tolerance. Séguin *et al.* have suggested that expression of some stress-associated transcription factors in liver tissue, only 6 h after oral intake of D-penicillamine in brown Norway rats, may discriminate between resistance and susceptibility to the D-penicillamine-induced autoimmune syndrome (B. Séguin, personal communication).

As we uncover the processes that are at play during sensitization or tolerance induction, more potential animal models to study IDHRs may be developed. Careful selection of appropriate strains in relation to effects induced by specific drugs remains an important issue. Although the brown Norway rat model seems very useful to mimic idiosyncratic drug reactions of some drugs (D-penicillamine, nevirapine), attempts with other drugs, such as felbamate were unsuccessful [25•]. The ability to modulate immune regulatory processes with specific agents provides new avenues for model development. For example, the development of drug-induced autoimmunity may be potentiated by an antibody, such as anti-CD25, which deletes the regulatory T cell subset. In addition, genetic manipulations could prove useful in the creation of animal models in species of strains that are otherwise resistant to developing IDHRs [3•].

Conclusion

It has been recognized that more resources need to be devoted to developing urgently needed animal models for detecting potential drug hypersensitivities [34,35]. Improved understanding of the mechanisms of IDHRs will provide insights to prevent or treat these undesired side effects of drugs.

The complexity of IDHRs advocates the use of a rational tiered-approach to assess the immunostimulatory hazard of drugs in the preclinical screening phase. Despite their limitations, both the reporter antigen-PLNA and reporter antigen-ALNA may be used to recognize the immunostimulatory potential and, more specifically, the T cell sensitizing capacity of drugs in a preclinical phase of drug development. In addition, the use of oral exposure models during a second tier provides information about the systemic immunosensitizing properties and may enable detection of drugs that require metabolism. At last, a third tier, including models that show the clinical

phenomena, such as the brown Norway rat model, may be used to explore possible strategies to prevent or treat these drug-induced symptoms.

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