Role of Animal Models in the Study of Drug-induced Hypersensitivity Reactions

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ABSTRACT

Drug-induced hypersensitivity reactions (DHRs) are a major problem, in large part because of their unpredictable nature. If we understood the mechanisms of these reactions better, they might be predictable. Their unpredictable nature also makes mechanistic studies very difficult, especially prospective clinical studies. Animal models are vital to most biomedical research, and they are almost the only way to test basic hypotheses of DHRs, such as the involvement of reactive metabolites. However, useful animal models of DHRs are rare because DHRs are also unpredictable in animals. For example, sulfonamide-induced DHRs in large-breed dogs appear to be valid because they are very similar to the DHRs that occur in humans; however, the incidence is only ~0.25%, and large-breed dogs are difficult to use as an animal model. Two more practical models are penicillamine-induced autoimmunity in the Brown Norway rat and nevirapine-induced skin rash in rats. The toxicity in these models is clearly immune mediated. In other models, such as amodiaquine-induced agranulocytosis/hepatotoxicity and halothane-induced hepatotoxicity, the drug induces an immune response but there is no clinical toxicity. This finding suggests that regulatory mechanisms usually limit toxicity. Many of the basic characteristics of the penicillamine and nevirapine models, such as memory and tolerance, are quite different suggesting that the mechanisms are also significantly different. More animal models are needed to study the range of mechanisms involved in DHRs; without them, progress in understanding such reactions is likely to be slow.

KEYWORDS: animal models, hypersensitivity reactions, idiosyncratic drug reactions, penicillamine, nevirapine, popliteal lymph node assay

INTRODUCTION

To an immunologist, the term hypersensitivity implies an immune-mediated reaction. The term drug-induced hyper-

sensitivity reaction (DHR) is used to describe a syndrome consisting of fever, rash, and involvement of various organs associated with drugs such as phenytoin, carbamazepine, abacavir, sulfonamides, and allopurinol; however, it is also frequently used to describe any idiosyncratic drug reaction. For many of the adverse reactions to be discussed in this review, it has not been conclusively demonstrated that they are immune mediated, and the term hypersensitivity reaction will be used broadly to refer to any idiosyncratic drug reaction that has features that suggest it is immune mediated. Another working hypothesis for this review, which is supported by a large amount of circumstantial evidence,¹ but in virtually no case proven, is that most DHRs are caused by reactive metabolites. Therefore, a major role of animal models is to test whether reactive metabolites are responsible for hypersensitivity reactions and further to determine how reactive metabolites provoke an immune response.

A major characteristic of DHRs is their unpredictable or idiosyncratic nature. This means that at the present time it is impossible to predict which drug candidates will be associated with a relatively high incidence of such reactions, and it is also impossible to predict which patients will have a serious hypersensitivity reaction to a given drug. If we were able to significantly improve our ability to predict such reactions, it would have a major impact on drug development and drug therapy even if the methods were imperfect. It is unlikely that we will make much progress until we have a much better understanding of the mechanisms of hypersensitivity reactions.

Animal models represent a major tool for the study of mechanisms in virtually all of biomedical research. Hypersensitivity reactions, in particular, presumably involve a complex combination of genetic and environmental factors as well as complex interactions between drug or metabolites and the immune system that lead to their unpredictable nature, and a simple in vitro system is very unlikely to be able to mimic such complexity. Although animals do have hypersensitivity reactions to drugs and other xenobiotics, they are just as idiosyncratic in animals as they are in people, so that finding suitable animal models is very difficult and most attempts have failed. For an animal model to be useful it should involve basically the same mechanism as the hypersensitivity reaction in humans. In addition, the

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incidence must be sufficiently high to make it practical. It is also very desirable to have a clinically evident end point because biochemical changes are much more frequent than clinically evident adverse reactions, and a major question is what determines who will have a clinically significant adverse reaction.

If we understood how reactive metabolites provoke an immune response it might be possible to predict which drug candidates are likely to cause idiosyncratic reactions. In addition, animal models should make it possible to determine which factors make a specific individual susceptible to a hypersensitivity reaction to a particular drug. In general, if a patient has a DHR to one drug, the risk that they will have a DHR to another drug does not appear to be significantly increased. Although some patients claim to be “allergic to all drugs,” this does not appear to be the case, and therefore it appears that the susceptibility factors are different for different drugs.^{2,3} This finding also suggests that there are significant differences in the mechanisms of different hypersensitivity reactions, and many animal models will be required to explore the range of mechanisms that can occur. Even if the fundamental mechanism is the same in both the human hypersensitivity reaction and the animal model, there are likely to be some differences; however, as Jim Gillette once said, an animal may be a better model for a specific person than another person. For example, if a large number of people are given nevirapine, most will experience no significant adverse effects, while some will develop a mild rash, a few will develop toxic epidermal necrolysis, and others will develop liver failure. If certain strains of female rats are treated with nevirapine, they develop a rash (as described below) that is similar to the rash that occurs in a few people but they do not develop liver toxicity.

The following is a description of the major animal models that are presently available for the study of DHRs. They will be divided into models used to study mechanism and those used to predict the risk that a specific agent will cause an immune response.

ANIMAL MODELS FOR MECHANISTIC STUDIES

Penicillamine-induced Autoimmunity in the Brown Norway Rat

Penicillamine is used in the treatment of Wilson’s disease, and it has also shown efficacy in the treatment of rheumatoid arthritis but is seldom used for the latter indication because of a high incidence of adverse reactions. It causes a broad range of autoimmune reactions including a lupus-like syndrome, pemphigus, and myasthenia gravis.⁴ It is also associated with rash and agranulocytosis, which are likely immune mediated.^{5,6}

Penicillamine also causes an autoimmune reaction in Brown Norway (BN) rats.^{7,8} It has many of the features of lupus in humans such as antinuclear antibodies and immune complex deposition in the kidneys. The syndrome also includes rash and weight loss, and most animals die if the drug is continued. This syndrome appears to be specific to BN rats and does not occur in Lewis or Sprague-Dawley rats.⁷ The dose-response curve is unusual: the incidence with a 20 mg/d dose is between 50% and 80%, and the incidence is not increased by increasing the dose to 50 mg/d. However, at a dose of 5 to 10 mg/d, the incidence is 0%, and in fact, the lower dose induces tolerance to the 20 mg/d dose. This is clearly immune tolerance as it can be transferred to naïve animals with spleen cells or T cells from a tolerized animal.^{9,10} It was found that CD4 T cells from tolerized rats treated with high-dose penicillamine (20 mg/d) had increased levels of interleukin-10 (IL-10) and transforming growth factor (TGF)- β mRNA, but such elevations were not observed prior to high-dose penicillamine or in naïve animals treated with high-dose penicillamine.⁹ These data suggest that the immune tolerance induced by low-dose treatment is mediated by CD4+, CD25+ regulatory T cells, but the mechanism is likely to involve additional cell types.

Although the incidence of penicillamine-induced autoimmunity is not increased by increasing the dose, the incidence and severity are increased by poly-IC: a polymer of inosine and cytosine that stimulates antigen-presenting cells via toll-like receptor 3.¹¹ Only one dose of poly-IC given on the first day of penicillamine treatment is required even though poly-IC has a short half-life, and on average it takes 3 weeks of penicillamine treatment before the autoimmune syndrome becomes clinically apparent. Poly-IC treatment is also capable of reversing tolerance⁹; however, it does not significantly shift the penicillamine dose-response curve: the combination of penicillamine at 10 mg/d and poly-IC does not lead to autoimmunity. Furthermore, Lewis rats remain resistant: the combination of penicillamine at a dose of 20 mg/d plus poly-IC does not cause autoimmunity in Lewis rats.¹¹ This result indicates that genetic factors are crucial for the susceptibility to penicillamine-induced autoimmunity. Lipopolysaccharide, which stimulates macrophages through toll-like receptor 4, had effects that were similar to those of poly-IC but less pronounced.⁹

In contrast to the effects of poly-IC, misoprostol, a prostaglandin E analog, prevented penicillamine-induced autoimmunity.¹¹ As with poly-IC, only one dose was required even though the half-life of misoprostol is less than 1 hour. Aminoguanidine, an inhibitor of inducible nitric oxide synthase, was also protective. The observation that one dose of agents given at the beginning of treatment has a marked effect on the eventual development of autoimmunity suggests that the “decision” on whether autoimmunity will

develop is made within hours of starting penicillamine treatment. Therefore, we examined the changes in hepatic mRNA expression 6 hours after penicillamine treatment to see if we could predict which animals would develop the syndrome.¹² It was found that 7 of 8 resistant animals had increased expression of cytokine-induced neutrophil chemoattractant (cinc) and serum glucocorticoid-regulated kinase (sgk) but low expression of zinc finger protein 354A or kidney ischemia development protein (kid-1); however, 12 of 25 sensitive animals had the same expression pattern. It was concluded that early changes do occur, but even though thousands of genes were probed with the microarray, it is likely that important genes or other unknown risk factors were still being missed.

Antigen-presenting cells are also likely to be quite important to the mechanism of penicillamine-induced autoimmunity. It was found that partial depletion of macrophages with clodronate-filled liposomes decreased the incidence of penicillamine-induced autoimmunity; however, it appeared that macrophage depletion during low-dose penicillamine treatment may also inhibit tolerance.¹³

Although something has been learned about what cells are involved in the mechanism of penicillamine-induced autoimmunity, as well as factors that influence the incidence and severity of the syndrome, the basic mechanism of how penicillamine initiates a generalized immune response remains unknown. Our current speculation is that the penicillamine reacts with aldehydes on antigen-presenting cells that are normally involved in signaling between T cells and antigen-presenting cells. The normal interaction involves reaction between aldehydes on antigen-presenting cells and amines on T cells to form a reversible imine bond.¹⁴ Penicillamine is known to react with aldehydes to form a thiazolidine ring, which is not readily reversible; this could lead to activation of antigen-presenting cells, and in some cases, an autoimmune syndrome.¹⁵ This hypothesis is being explored.

Most immune-mediated reactions are associated with “memory” T cells that lead to a very rapid response on rechallenge with the same agent. Although penicillamine-induced autoimmunity is clearly immune mediated, the time to onset of autoimmunity in a rat in which autoimmunity has been previously induced with penicillamine is the same as the time course for a naïve animal.¹² It is unclear why this is the case, although since it is an autoimmune syndrome, if autoimmune T cells were not deleted or tolerized when the drug is stopped, the autoimmune syndrome should persist. This does not happen.

Nevirapine-induced Skin Rash in the Rat

Nevirapine is a nonnucleoside reverse transcriptase inhibitor used for the treatment of human immunodeficiency virus (HIV) infections. Its use is associated with a relatively high

incidence of skin rash, sometimes life-threatening, as well as liver toxicity. The incidence of DHRs is higher when the drug is used for prophylaxis, and a normal CD4⁺ T cell count appears to be a risk factor for toxicity.¹⁶ We discovered that nevirapine also causes a rash in rats.¹⁷ The highest incidence (100%) occurred in female BN rats, but this appears to be largely due to kinetic factors. Male BN rats metabolize the drug much more rapidly than females and do not develop a rash at the same dose as females; however, if they are cotreated with aminobenzotriazole to inhibit cytochromes P450, males also develop a rash (J. Chen and J. Uetrecht, unpublished data, October 2003). Likewise, the incidence of rash in female Sprague Dawley rats is only 20%, but at the same dose, the blood level of nevirapine in Sprague Dawley rats is lower than that in female BN rats. Thus it appears that genetic determinants of nevirapine-induced rashes are not nearly as stringent as for penicillamine-induced autoimmunity.

The nevirapine-induced rash in rats is clearly immune mediated, and it is characterized by an infiltrate of inflammatory cells. The time-to-onset of rash is ~2 weeks; however, if the drug was stopped and the rash was allowed to resolve, then the rechallenge resulted in a rapid recurrence of the rash with the ears turning red in less than 24 hours.¹⁷ This result is typical of an immune-mediated reaction and contrasts with penicillamine-induced autoimmunity. This sensitivity can be transferred to naïve animals with spleen cells such that their ears turned red in less than 24 hours on treatment with nevirapine. In some cases we were able to transfer sensitivity with isolated CD4⁺ T cells from sensitized animals. In contrast, transfer of CD8⁺ T cells did not transfer sensitivity. Likewise, partial depletion of CD4⁺ T cells partially protected animals, while depletion of CD8⁺ T cells appeared to make the rash worse.¹⁸ Thus CD4⁺ T cells appear to play an important role in the rash in both humans and rats. In contrast, CD8⁺ T cells may be suppressive.

Like penicillamine-induced lupus in BN rats, treatment of rats with a low dose (50 or 75 mg/kg/d) of nevirapine resulted in tolerance to the dose (150 mg/kg/d), which otherwise led to a 100% incidence of rash. However, in contrast to the immune tolerance induced by low-dose penicillamine treatment, the tolerance induced by low-dose nevirapine treatment was not transferable with spleen cells and appeared to be the result of induction of cytochromes P450 because it could be overcome by inhibition of cytochromes P450 with aminobenzotriazole.¹⁸ The rash induced by nevirapine could also be prevented by the immunosuppressants, tacrolimus and cyclosporin. Treatment with nevirapine plus an immunosuppressant appeared to result in immune tolerance.

Unlike penicillamine-induced autoimmunity, the incidence and severity of nevirapine-induced skin rash was not

affected by poly-IC or misoprostol.¹⁸ The lack of potentiation by the immunostimulant poly-IC was surprising and further serves to emphasize the differences in basic characteristics of different DHRs.

Although the incidence of rash appeared to correlate with blood levels of the parent drug, inhibition of different metabolic pathways by aminobenzotriazole was not equal and aminobenzotriazole shifted metabolism toward 12-hydroxylation. If this benzylic alcohol is sulfated in the skin, loss of sulfate would produce a reactive quinone methide. We are currently trying to determine if this skin rash is due to the parent drug or a reactive metabolite.

Propylthiouracil-induced Autoimmunity in the Cat

Propylthiouracil is used for the treatment of hyperthyroidism but its use is associated with liver toxicity, agranulocytosis, and a lupus-like syndrome. It also causes a lupus-like syndrome in cats.^{19,20} The syndrome in cats is characterized by lethargy, fever, weight loss, Coombs' positive hemolytic anemia, antinuclear antibodies, and antimyeloperoxidase antibodies.²¹ Propylthiouracil is oxidized to a reactive metabolite by myeloperoxidase,²² and this appears to be a characteristic of several drugs that cause a lupus-like syndrome.^{23,24} Like penicillamine-induced autoimmunity in the BN rat, rechallenge did not lead to a shortened time to onset, although a second rechallenge did lead to a more severe reaction. It remains to be determined whether this is a common feature of drug-induced autoimmune reactions.

It is not clear how important genetic determinants are in this syndrome because mongrel cats were used and the incidence was ~40%. When we tried to reproduce the syndrome at a later time we were unsuccessful; the only known change was a significant increase in the level of taurine in cat chow because it was found that taurine deficiency in cats leads to cardiomyopathy and other health problems.²¹ However, we did not perform experiments to determine if taurine deficiency is a risk factor for propylthiouracil-induced autoimmunity.

Sulfonamide-induced Hypersensitivity in Dogs

Sulfonamides are aromatic amines and are associated with a wide range of DHRs including a generalized DHR, skin rashes (including toxic epidermal necrolysis), agranulocytosis, liver toxicity, and a lupus-like syndrome. Sulfonamides also cause a range of DHRs in dogs that are similar to those that occur in humans and include fever, arthropathy, skin eruptions, thrombocytopenia, hemolytic anemia, neutropenia, liver toxicity, and keratoconjunctivitis sicca.²⁵ Although sulfonamide-induced autoimmunity can affect joints in humans it is less common than in dogs. Larger breeds, especially Dobermans, appear to be at higher risk

than small breeds. One likely risk factor for dogs is their inability to acetylate aromatic amines. Cats can also have DHRs to sulfonamides but the incidence is lower. Although the DHRs induced by sulfonamides in dogs appear very similar to the reactions that occur in humans, making it a very attractive mechanistic model, the incidence is only ~0.25%. This small percentage along with the ethical and practical issues involved in experimentation with large-dog breeds, limits the practical usefulness of this model.

Halothane-induced Hepatotoxicity in Rats and Guinea Pigs

Halothane is a model toxin, which forms a reactive trifluoroacetyl chloride reactive metabolite and causes idiosyncratic liver toxicity that has many characteristics suggesting an immune-mediated reaction.²⁶ It almost never occurs on first exposure, although previous exposures are often associated with fever, and most patients who develop toxicity develop antibodies, both autoantibodies and antibodies against trifluoroacetylated protein.²⁷⁻²⁹ This finding has led to several attempts to reproduce this syndrome in animals. Although it is possible to induce mild toxicity in rats by a combination of hypoxia, enzyme induction with phenobarbital, and halothane,³⁰ the toxicity apparently involves the formation of a free radical by a reductive pathway rather than trifluoroacetyl chloride by an oxidative pathway, and it does not have the characteristics of an immune response similar to the liver toxicity observed in humans.

It is possible to induce an immune response to the oxidative metabolite of halothane in Guinea pigs; however, clinical toxicity does not occur and the immune response does not escalate with repeated exposures, suggesting the development of immune tolerance.³¹

Amodiaquine-induced Agranulocytosis/Hepatotoxicity in Rats

Amodiaquine is an antimalarial drug that can cause both agranulocytosis and hepatotoxicity. Consistent with these target organs, it is oxidized to a reactive iminoquinone by human liver microsomes and peroxidases.³² Patients with amodiaquine-induced DHRs were found to have antidrug IgG antibodies.³³ When rats were treated with amodiaquine for 14 days, they also developed anti-amodiaquine antibodies, and at the highest dose, they also had elevated transaminase levels and leukopenia. However, they did not develop agranulocytosis, and there was no histological evidence of liver damage.³⁴ Thus amodiaquine induces an immune response in rats analogous to the DHRs in humans but it is not sufficient to result in clinical toxicity. This model deserves further study to determine if there are factors that could increase the immune response sufficient to cause overt toxicity.

Isoniazid-induced Hepatotoxicity in the Rabbit

Isoniazid is associated with one of the highest incidences of a DHR involving the liver of any commonly used drug. Although the hepatotoxicity is idiosyncratic, it has been proposed that the mechanism involves metabolic idiosyncrasy rather than immune idiosyncrasy.²⁶ It has been suggested that acetylation followed by hydrolysis to acetylhydrazine are the first steps in the metabolic activation.³⁵ However, no idiosyncratic metabolic pathway has been described that would explain idiosyncratic isoniazid hepatotoxicity nor is there definitive evidence against an immune-mediated mechanism.

No animal model has been able to reproduce the characteristics of isoniazid-induced hepatotoxicity in humans. The closest animal model was described by Sarich et al.³⁶ Rabbits were treated with isoniazid at 3-hour intervals for 2 days. This treatment led to increased levels of transaminases that peaked at ~36 hours as well as focal areas of liver necrosis in some of the animals. However, this is much more rapid than the usual onset of toxicity in humans.

Lipopolysaccharide-potentiated Hepatotoxicity

Roth found that ranitidine caused hepatotoxicity in rats pretreated with lipopolysaccharide (LPS).³⁷ Toxicity was accompanied by a marked neutrophil infiltration and inhibited by depletion of neutrophils with antineutrophil antibodies.³⁸ Toxicity was also associated with fibrin deposition and inhibited by heparin. This data suggest that LPS-potentiated toxicity involves a combination of fibrin deposit-induced hypoxia and neutrophil-mediated cell damage.³⁸ It was suggested that this could be a dominant mechanism for hepatic DHRs, and that random exposure to LPS or other inflammatory condition could explain the time course of DHRs.³⁹ Although neutrophils can be part of the cellular infiltrate in hepatic DHRs, the dominant cells are usually mononuclear.²⁶ In addition, unlike this model, there is virtually always a delay of a week or more between starting a drug and the onset of toxicity. Therefore, although this model may represent one aspect of some hepatic DHRs, it is unlikely to be a dominant mechanism.

ANIMAL MODELS FOR SCREENING/PREDICTIVE ASSAYS

There is a great need to be able to screen drug candidates for their potential to cause DHRs.⁴⁰ Present testing is very time-consuming and expensive, and although it eliminates candidates that are directly toxic or mutagenic, it does not predict the risk of hypersensitivity reactions. Many *in vitro* screens have been proposed, such as hepatotoxicity assays using isolated hepatocytes; however, I have never seen convincing evidence that such assays provide even modest predic-

tive value except occasionally in a narrow series of analogs. One exception is covalent binding studies. It is believed that most DHRs involve reactive metabolites and therefore screens that quantify the amount of reactive metabolite covalent binding hold promise for eliminating drug candidates that are likely to cause DHRs⁴¹; however, there are a large number of false positive and false negative results. Most of these screens are performed *in vitro* because of the quantity of radiolabeled drug required for *in vivo* studies; however, *in vitro* systems—even whole cells, lack many processes that can markedly affect the amount of covalent binding.

If we had a better understanding of mechanism, there might be a specific pattern of biochemical changes that would predict that a drug candidate would likely cause idiosyncratic drug reactions. However, as argued above, the limited data that we have suggest that there are many different mechanisms of DHRs; therefore, there will likely be many patterns of biochemical changes that are associated with a drug's potential to cause DHRs.

Popliteal Lymph Node Assay

The major model that has been proposed for predicting the ability of a drug to cause immune-stimulation is the popliteal lymph node assay. In this assay, the drug is injected into the footpad of a mouse or rat and the response of the draining popliteal lymph node is determined 6 to 8 days later and the contralateral node is used as the control.⁴² The response can be assayed by the weight of the node or cell number. Although there is some correlation between response in this assay and the propensity of the drug to cause hypersensitivity reactions, there are many false negatives including sulfamethoxazole, isoniazid, and alpha-methyldopa, all of which are associated with a high incidence of DHRs.^{43,44} It could be argued that isoniazid is not immune mediated; however, that cannot be said for the other 2 drugs. One likely reason for false negatives is that the footpad has limited drug-metabolizing capabilities, and it is assumed that most hypersensitivity reactions are caused by reactive metabolites. However, there does not appear to be a correlation between ease of formation of a reactive metabolite and a positive response of the lymph node. For example, sulfamethoxazole and most other aromatic amines are negative in the assay even though aromatic amines are relatively easy to oxidize, and there is evidence of formation of the reactive hydroxylamine/nitroso metabolites in the skin.⁴⁵ In contrast, phenytoin is more difficult to oxidize and yet it is consistently positive in the assay.^{42,44,46} Any lipophilic poorly soluble drug, such as phenytoin, is likely to precipitate when injected as a dimethyl sulfoxide (DMSO) solution into the footpad, and this may induce an immune response. In fact it has been proposed that hydrophobicity is a common theme

for agents that stimulate the immune system.⁴⁷ It is interesting to note that pristane, which is a relatively inert but very hydrophobic molecule, induces arthritis when injected into some strains of mice.⁴⁸ This concept applies to hydrophobic surfaces (eg, crystals), and so it should not be an issue for drugs such as phenytoin when they are given orally and are unlikely to precipitate; however, it may be an issue for injecting solutions of drug in organic solvents that would precipitate. Thus, dealing with hydrophobic molecules may pose problems in the interpretation of the popliteal lymph node assay.

In principle it should be possible to eliminate false negative reactions that are due to lack of local metabolism by injection of the reactive metabolite. However, there are several problems with this strategy. One is that the reactive metabolite would have to be known and have a sufficient half-life to be injected and most reactive metabolites have a very short half-life, some so short that they do not even escape the enzyme that formed them. In addition, even if a reactive metabolite is sufficiently stable to work with, it will likely cause cell damage and an immune response when injected as a concentrated solution, even if it does not lead to an immune response under conditions when it is formed in vivo. For example, a strong positive response was obtained with the reactive metabolite of acetaminophen even though acetaminophen is not associated with a significant incidence of hypersensitivity reactions (J. Uetrecht, unpublished data, June 1998). Therefore, false-negative results due to the difficulty of generating biologically relevant amounts of reactive metabolite locally is likely a major limitation of the assay.

Reporter Antigen Lymph Node Assay

A variation on the popliteal lymph node assay is the reporter antigen lymph node assay.⁴⁹ In this assay a reporter antigen is injected along with the drug and the antibody response to the reporter antigen stimulated by the drug is determined. The 2 reporter antigens that are used are trinitrophenyl (TNP)-ovalbumin and TNP-Ficoll. Ovalbumin is a protein and, therefore, it can be recognized by T cells that can provide help to the TNP-specific B cells. The type of drug that stimulates the production of anti-TNP antibodies to TNP-ovalbumin is one that acts as an adjuvant to provide signal 2 and increase T-cell help. In contrast, Ficoll is a polysaccharide and cannot be directly recognized by T cells. TNP-Ficoll can stimulate the production of IgM anti-TNP antibodies, but the production of cytokines is required to cause the class switch to IgG antibodies. The type of drug that is believed to increase the response to TNP-Ficoll is one that can produce neo-epitopes and stimulate T cells to produce these cytokines. The reporter assay variation on the lymph node assay is much more sensitive than weighing lymph nodes or determining the number of cells. In addition, it provides further information about the mechanism of

immune system stimulation. A further variant on the assay is to administer the drug orally. This should circumvent the problem of lack of metabolic activation and also avoids the problem of forming crystals of lipophilic drugs. However, in the first report of this method, only 3 drugs were tested and they are not drugs thought to be negative because of lack of bioactivation. Thus, it remains to be seen how generally applicable this assay will be.⁵⁰

CONCLUSIONS

Although there are few reasonable animal models of DHRs, they represent a very important resource for mechanistic studies, and some hypotheses may be virtually impossible to test in any other way. In most cases it is not clear why one drug causes a different spectrum of DHRs than another or why the same drug causes different types of DHRs in different patients. The few animal models that do exist emphasize the differences between different DHRs, and there are probably many different mechanisms of DHRs; therefore, many animal models will be required. Although the popliteal lymph node assay shows some promise, especially if it can be developed as an oral assay, it does not appear that good screening assays currently exist that can predict a drug candidate's potential to cause DHRs, and it will likely require better mechanistic understanding before such assays can be developed. At the present time it appears that the best strategy to decrease the risk of DHRs in drug candidates is to avoid molecules that form significant amounts of reactive metabolite and to make drugs as potent as possible to keep the daily dose to a minimum.

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