Idiosyncratic Drug Reactions: Past, Present, and Future

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Although the major working hypothesis for the mechanism of idiosyncratic drug reactions (IDRs), the hapten hypothesis, has not changed since 1987, several hypotheses have been added, for example, the danger hypothesis and the pharmaceutical interaction hypothesis. Genetic studies have found that several IDRs are linked to specific HLA genes, providing additional evidence that they are immune-mediated. Evidence that most IDRs are caused by reactive metabolites has led pharmaceutical companies to avoid drug candidates that form significant amounts of reactive metabolites; however, at least one IDR, ximelagatran-induced liver toxicity, does not appear to be caused by a reactive metabolite. It is possible that there are biomarkers such as those related to cell stress that would predict that a drug candidate would cause a significant incidence of IDRs; however, there has been no systematic study of the changes in gene expression induced by drugs known to cause IDRs. A major impediment to the study of the mechanisms of IDRs is the paucity of valid animal models, and if we had a better mechanistic understanding, it should be easier to develop such models. There is growing evidence that these adverse reactions are more varied and complex than previously recognized, and it is unlikely that a quick fix will be achieved. However, IDRs are an important cause of patient morbidity and mortality and markedly increase the uncertainty of drug development; therefore, continued basic research in this area is essential.

Contents

1. Past

The term idiosyncratic drug reaction (IDR) means different things to different people, but in this perspective, it will be used to indicate an adverse drug reaction that does not occur in most patients at any readily achieved dose of a drug and does not involve the known pharmacologic effects of the drug [for a more extensive discussion of the definition, see the recent review (*1*)]. The major working hypotheses for the mechanism of IDRs that we use today were developed prior to the first issue of Chemical Research in Toxicology in 1988; the principal hypotheses are the hapten hypothesis, reactive metabolite hypothesis, and metabolic idiosyncrasy hypothesis. Most IDRs appear to be immune-mediated (*1*), and fundamental to any adaptive immune response is the presentation of processed immunogen by antigen-presenting cells (APCs) in the groove of the major histocompatibility complex (MHC) to T helper cells (Figure 1). The innate immune system has received more attention recently, and it undoubtedly plays a role in influencing the adaptive immune response (*2*); however, at present, there are no examples in which there is compelling evidence that an IDR is mediated exclusively by the innate immune system.

1.1. The Hapten Hypothesis. In 1935, Landsteiner reported that he was unable to induce an immune response to small molecules unless the molecules were chemically reactive and bound to protein (*3*). This led to the hapten hypothesis: Small molecules are not immunogenic, but if they bind irreversibly to protein, the modified protein can induce an immune response (Figure 1). The small molecule that binds to protein leading to an immune response is referred to as a hapten.

The first IDR whose mechanism was studied in detail was that of penicillin-induced allergic reactions (*4*). (The definition of an IDR commonly used by allergists/clinical immunologists excludes immune-mediated reactions but that is not how the term is used by most other physicians or how it will be used in this perspective.) It was found that the β-lactam ring of penicillin reacts irreversibly with free amino and sulfhydryl groups on proteins. In some patients, this leads to an immune response against the penicillin–protein adduct, and if the antibody response generates sufficient IgE antibodies, a severe allergic reaction such as anaphylaxis can result. On the basis of this understanding, it was possible to develop a test for penicillin allergy in which penicillin is bound to a polymer of lysine, which has free amino groups, and when small amounts of this material, referred to as the major determinant, are injected into the skin, it causes degranulation of mast cells leading to a local wheal and flare response (*5*). There are also breakdown products of penicillin that can covalently bind to proteins and lead to an immune response, and these adducts are referred to as minor

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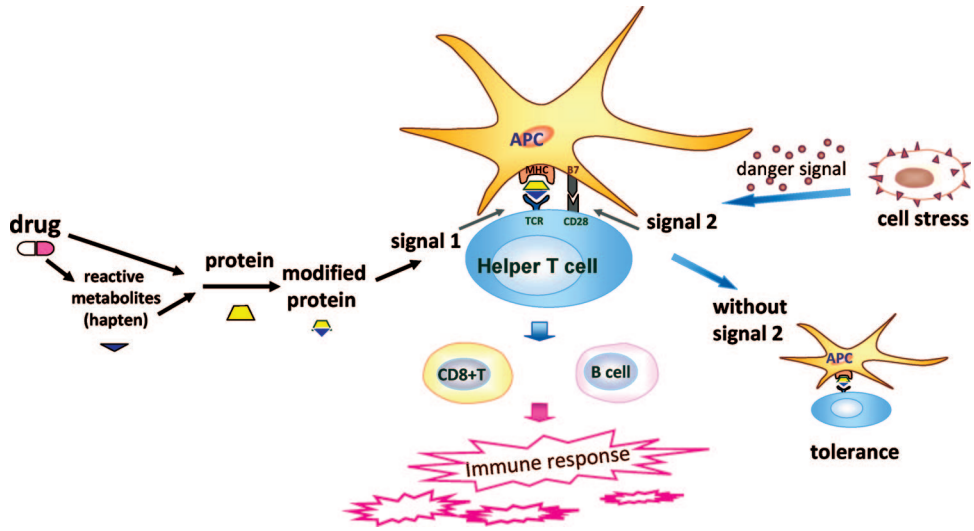

Figure 1. Hapten and danger hypotheses. The hapten hypothesis involves a chemically reactive drug or reactive metabolite acting as a hapten by binding to protein, which is then taken up by an APC and processed. The processed antigen is presented in the context of MHC to a helper T cell; this represents signal 1. The danger hypothesis involves cell damage or stress (possibly caused by the drug or reactive metabolite) causing the release of danger signals that lead to upregulation of costimulatory factors; this is signal 2. Without signal 2, the result is immune tolerance.

determinants. The mechanism of penicillin-induced allergic reactions fits the hapten hypothesis where the chemical reactivity of the penicillin allows it to act as a hapten. Although it is not known why some people develop a predominantly IgE response to penicillin and others do not, the basic understanding of penicillin allergy and the hapten hypothesis provided a framework for the examination of other IDRs.

Unlike penicillin, most drugs are not chemically reactive but many do form reactive metabolites as discussed in the next section. An example is aminopyrine, which causes an IDR that appears mechanistically similar to penicillin-induced allergic reactions. In 1952, an investigator reported an experiment in which he took aminopyrine and then infused serum from a patient with aminopyrine-induced agranulocytosis into himself, leading to very rapid and profound neutropenia (*6*). This demonstrated that aminopyrine-induced agranulocytosis involves the destruction of mature neutrophils and is mediated by aminopyrine-dependent antineutrophil antibodies. We now believe that the metabolite of aminopyrine that acts as a hapten to induce these antibodies is a very reactive dication formed by neutrophil-generated HOCl (*7*). Several other IDRs are associated with antibodies against drug- (or reactive metabolite-) modified proteins; therefore, there is ample evidence to support the hapten hypothesis at least in some IDRs (*8–11*). However, it is important to point out that the presence of antidrug antibodies does not prove that the IDRs associated with that drug are mediated by these antibodies or even that the IDR is immune-mediated. There are also examples of IDRs in which there are antibodies against the cytochrome P450 that is responsible for forming a reactive metabolite of the drug. Examples include tienilic acid-induced hepatotoxicity where the reactive metabolite is formed by Cyp2C9 (*8*) and dihydralazine-induced hepatotoxicity where the reactive metabolite is formed by Cyp1A2 (*12*). Presumably, this is also due to reactive metabolites acting as haptens as discussed in the next section, and in this case, the result is antibodies directed primarily against the protein portion of the hapten–protein complex to produce autoantibodies.

1.2. Reactive Metabolites. A significant influence on the field of IDRs was the finding by the Millers that a common mechanism by which chemicals cause cancer involves the formation of chemically reactive metabolites that bind to DNA and lead to mutations (*13*). At the National Institutes of Health, this idea was applied to the problem of acetaminophen-induced hepatotoxicity and it was found that acetaminophen-induced hepatotoxicity is caused by a reactive imidoquinone metabolite (*14*). Most of this reactive metabolite is detoxified by reaction with glutathione, and significant toxicity does not occur until liver glutathione is substantially depleted. The concept that most adverse reactions that do not involve reversible binding to receptors are due to reactive metabolites has become a pervasive theme (*15*), and it fits with the hapten hypothesis described above in which reactive metabolites can act as haptens.

A classic example that appears to link reactive metabolite formation and the hapten hypothesis is halothane hepatotoxicity. Halothane is oxidized by cytochrome P450 to the reactive trifluoroacetyl chloride (*16*). Although the incidence of liver failure is low, most patients who develop halothane-induced hepatotoxicity have antibodies against trifluoroacetyl-modified proteins (*10*) and this implies that modification of protein by the reactive metabolite has led to an immune response. In addition, these patients also have antibodies against native proteins such as protein disulfide isomerase (*17*). Protein disulfide isomerase is also a target for the reactive metabolite of halothane; thus, protein modification also appears to lead to the production of autoantibodies analogous to tienilic acid and dihydralazine described above. Halothane is administered for a brief period of time, which is insufficient in a single exposure to allow for the development of a full adaptive immune response, and the observation that hepatotoxicity almost always occurs after multiple exposures (*18*) also suggests a process of immune sensitization. However, unlike penicillin allergic reactions and aminopyrine-induced agranulocytosis, there is no evidence that the antibodies observed in halothane-induced hepatotoxicity actually mediate liver damage, and it is possible that they are a marker for an immune response, but it is actually cytotoxic T cells that are responsible for most of the liver damage. However, the combination of drug-related antibodies, the apparent need for prior sensitization, and the fact that halothane-induced hepatitis is often associated with fever and eosinophilia have led most investigators to believe that it is immune-mediated (*18*).

1.3. Metabolic Idiosyncrasy. Although several IDRs fit the pattern of a reactive drug or metabolite acting as a hapten leading

to the induction of an immune response (*1*), many IDRs, especially those involving idiosyncratic liver toxicity, do not have characteristics typical of an immune-mediated reaction. This led Hyman Zimmerman to classify idiosyncratic liver toxicity into immune idiosyncrasy and metabolic idiosyncrasy (*19*). In this classification, if the hepatotoxicity is associated with fever and rash, eosinophilia, antidrug antibodies, and occurs rapidly on rechallenge, it is assumed to involve an immune-mediated reaction, whereas if it is not associated with these characteristics, it is likely to involve metabolic idiosyncrasy. In addition, most of the idiosyncratic reactions that did not have characteristics of immune-mediated reactions, such as those associated with troglitazone, isoniazid, and ketoconazole, also had a long lag time between the start of the drug and the onset of toxicity (*20–22*). This classification has been widely accepted, especially by hepatologists. There are examples, such as with isoniazid, in which polymorphisms in metabolic enzymes (in this case N-acetyltransferase and cytochrome P450 2E1) appear to be a risk factor for idiosyncratic liver toxicity; however, in the case of isoniazid, the odds ratios are low (3.66 for N-acetyltransferase (*23*)), and although it was 5.9 for Cyp 2E1, it was not statistically significant (*24*). Although I accept that such polymorphisms are a risk factor, they are not sufficient to explain an incidence of isoniazid-induced liver failure of approximately 1/1000. Furthermore, if that were the basis for toxicity, it should be possible to develop an animal model with characteristics similar to the IDR reaction in humans simply by giving a larger dose or in other ways manipulating the metabolic pathways. At present, there is no example where a known polymorphism in a metabolic pathway, either involving the drug or any other metabolic pathway, is sufficient to explain the idiosyncratic nature of liver toxicity. (Excluded from this discussion are toxic agents such as mercaptopurine, where a polymorphism in thiopurine methyltransferase can lead to an exaggerated response that would occur in everyone if the dose were increased. Although this is an idiosyncratic response, it is not an example of what I would call an IDR as indicated earlier.)

2. Progress Since 1987

The prevailing hypothesis in immunology in 1987 was the self–nonself hypothesis (*25*). In this framework, the immune system is tolerant of self and responds to anything that is nonself. One mechanism by which tolerance to self is established is that T cells having a high affinity for self peptides are deleted in the thymus and most of this tolerance of "self" is established soon after birth. Using the hapten hypothesis, modification of a self-protein by a hapten would make it foreign and could lead to an immune response. As mentioned earlier, the fundamental step leading to an adaptive immune response is recognition by T cells of processed antigen presented in the groove of the MHC by APCs; this is often referred to as signal 1 as depicted in Figure 1. In addition, costimulation of T cells by other interactions between the APCs and the T cells such as interaction between B7 on APCs and CD28 on T cells is also required (referred to as signal 2; Figure 1). In the absence of costimulation, the response to signal 1 is tolerance. Activation of APCs leads to upregulation of costimulatory molecules, and this is an important addition to the picture of an immune response. Therefore, it is essential to determine what stimuli lead to activation of macrophages.

2.1. Danger Hypothesis. Polly Matzinger proposed that the primary determinant of an immune response is not nonself but rather "danger" (*26*). Janeway described adjuvants as the immunologist's "dirty little secret" because foreign proteins do not, in general, induce much of an immune response in the absence of an adjuvant (*27*), and the primary purpose of adjuvants is to activate APCs. Thus, it appears that to a large degree it is activation of APCs that leads to an immune response. Matzinger reasoned that there is no need for an organism to respond to everything that is foreign unless it is a threat to the organism. We all tolerate gut bacteria and other foreign antigens without problems. In addition, there are several antigens that are not present until puberty and therefore would not have induced tolerance in the perinatal period; yet, they do not evoke an immune response at puberty.

Using the danger hypothesis framework, it is the injured tissue that determines if an immune response will occur and, if so, what kind of response will occur (*28*). The injured tissue produces danger signals that activate APCs leading to upregulation of costimulatory molecules and providing the second signal mentioned above. Several things have been proposed to act as danger signals including hydrophobic biological molecules (she refers to such molecules as Hypos) (*29*) and stress proteins such as heat shock proteins and HMGB1 (*30*). The rationale for involvement of Hypos is that the hydrophobic portion of biological molecules normally aggregates in an aqueous biological environment and if hydrophobic surfaces are exposed it is a sign of cell damage. It appears that endogenous molecules that act as danger signals may bind to the same receptors that recognize molecules on foreign pathogens, that is, toll-like receptors (*28*). I find this an attractive hypothesis; however, more evidence is required to demonstrate the extent to which such a mechanism represents the major control of immune response.

If the danger hypothesis is correct, the reactive metabolites that are associated with drugs that cause IDRs could cause cell damage and generate a danger signal (Figure 1). Thus, the ability of a reactive metabolite to cause cell damage could be a determinant of whether a drug that forms reactive metabolites will be associated with a significant incidence of IDRs (*31*). It is also possible that other factors leading to cell damage such as infection, surgery, etc. can increase the risk of an IDR (*2*). There are specific examples where this appears to be true; however, there does not appear to be a clear and dominant pattern of factors such as infection being associated with an increased risk of an IDR. It may be that in most cases the immune system is very "smart" and unless the danger is directly linked to the drug it will not lead to an immune response against the drug or reactive metabolite.

The hapten and danger hypotheses are not mutually exclusive, and it is likely that both can play a role in IDRs. In some cases, such as penicillin-induced anaphylaxis, the evidence for the hapten hypothesis is compelling. In contrast, it is less clear exactly what role danger plays in the mechanisms of IDRs.

2.2. Pharmacological Interaction (PI) Hypothesis. Pichler found that clones of lymphocytes from patients with a history of an IDR to sulfamethoxazole proliferated in response to sulfamethoxazole in the absence of metabolism. This led him to propose that some drugs are able to initiate an immune response through a reversible interaction with the MHC-T cell receptor complex. He referred to this as the PI hypothesis because the drug was acting more as a pharmacological agent (*32*). This result is surprising because sulfamethoxazole is an aromatic amine, and virtually every drug that has an aromatic amine functional group given at a dose of 100 mg/day is associated with a relatively high incidence of IDRs no matter what the therapeutic class or what the rest of the structure looks like (*33*). Presumably, this is because aromatic amines are metabolized to reactive metabolites. However, the PI and danger

hypotheses are not mutually exclusive, and the reactive metabolite could provide the danger signal.

The implicit assumption upon which the PI hypothesis is based is that what lymphocytes respond to is what initiated the immune response. In an immune-mediated skin rash induced by nevirapine in rats, we have found that the lymphocytes respond to nevirapine even though we have strong evidence that it is a reactive metabolite that is responsible for causing the rash (unpublished observations). Furthermore, when the rash was induced by treating rats with the 12-hydroxy metabolite (an intermediate metabolite in the metabolic sequence leading to the putative reactive metabolite), the lymphocytes from these animals responded far more strongly to nevirapine than to the 12-hydroxy metabolite even though these animals had never "seen" nevirapine (unpublished observations). Presumably, once an immune response is initiated, lymphocytes are recruited that respond to related structures even if these molecules cannot form haptens, and in the case of nevirapine, the response to nevirapine is stronger than to the 12-hydroxy metabolite, possibly because nevirapine is more hydrophobic, allowing stronger interactions.

Even though I am skeptical that the response of lymphocytes proves what initiated an immune response or that the PI hypothesis explains the immune-mediated reactions to sulfamethoxazole, that does not mean that the PI hypothesis is wrong. There are other drugs such as ximelagatran and possibly lamotrigine (*34*) that do not appear to form reactive metabolites. Ximelagatran, in particular, looks somewhat like a small peptide, and it may be able to initiate an immune response through a PI type of interaction; in fact, there is now evidence that it binds directly but reversibly to MHC (*35*).

2.3. Nonimmune Mechanisms. There is strong evidence that many IDRs are immune-mediated. This includes most skin rashes, some types of hematological IDRs that are associated with pathogenic antibodies, generalized hypersensitivity syndromes (e.g., the syndromes caused by anticonvulsants such as phenytoin and carbamazepine), and certainly autoimmune type IDRs such as lupuslike syndromes (*36*). It is less clear that most hepatic IDRs are immune-mediated. As discussed above, Zimmerman classified hepatic IDRs that were not associated with fever and rash as representing metabolic idiosyncrasies even though there are no examples where polymorphisms in drug metabolism or any other metabolic pathway have been demonstrated to be responsible for the idiosyncratic nature of such reactions. There are many immune-mediated reactions that are not associated with rash and fever, so these seem like weak criteria; however, many of the reactions classed as metabolic idiosyncrasies do not have a decreased time to onset on rechallenge. The lack of a rapid onset on rechallenge implies that there are no memory T cells, and memory T cells are a characteristic of immune-mediated reactions. However, I am impressed that antibody-mediated thrombocytopenia, a clearly immune-mediated reaction, often does not recur on rechallenge, and if it does, the time course is not shortened (*37*). We also noticed that penicillamine-induced autoimmunity in the Brown Norway rat, obviously immune-mediated, did not occur more rapidly on rechallenge. Therefore, it does not appear that lack of rapid onset on rechallenge can be used to establish whether an IDR is immune-mediated or not. The question is why are there no memory T cells in these immune-mediated reactions? One possible explanation is that this is a characteristic of autoimmune reactions. When drugs cause an autoimmune reaction, by definition, the antigen is still present when the drug is stopped; yet, the autoimmune IDR usually resolves rapidly when the drug is stopped (although occasionally IDRs such as drug-induced lupus do seem to persist after the drug is stopped). Thus, it appears that in most cases even an autoimmune IDR requires the continued presence of drug, possibly to provide a danger signal, and in the absence of drug, the autoimmune T cells must be either deleted or made anergic. Thus, if an IDR is autoimmune in nature, there may be no memory T cells. Penicillamine-induced autoimmunity is obviously an autoimmune reaction. It is not clear to what degree heparin-induced thrombocytopenia is autoimmune in nature. Some of the antibodies are against platelet factor 4, which is an autoantigen, whereas other antibodies are against the heparin–platelet factor 4 complex (*11*), but even heparin is an endogenous molecule. More recently, it was found that ximelagatran-induced hepatotoxicity often did not recur on rechallenge; yet, it is associated with a specific HLA genotype (*35*), which suggests that it is immune-mediated. In this case, there was no evidence of autoimmunity, although it could have been missed, and the lack of response on rechallenge could be due to the induction of immune tolerance. There may be many examples of immune-mediated IDRs that have been misclassified because of this characteristic. One example is troglitazone-induced hepatotoxicity, which is classed as representing metabolic idiosyncrasy, but recently, it has been noted to be associated with autoantibodies (*38*). Another possibility is that troglitazone-induced hepatotoxicity involves mitochondrial damage. Recently, Boelsterli described a model in which mice heterozygous for the mitochondrial superoxide dismutase (SOD2) treated with troglitazone developed delayed onset hepatic necrosis when treated with troglitazone (*39*), a drug that was withdrawn from the market because of idiosyncratic liver toxicity. It is possible that genetic polymorphisms in mitochondrial DNA could be risk factors for IDRs. Some drugs such as valproic acid are associated with liver toxicity with characteristics such as hyperammonemia and microvesicular steatosis, which suggest that the mechanism involves mitochondrial damage (*40*). Mitochondrial damage and mediation by the immune system are not mutually exclusive hypotheses for the mechanism of IDRs, and it is quite possible that mitochondrial damage can act as a danger signal and initiate an immune response.

One example that has bothered me for some time is clozapine-induced agranulocytosis. The idiosyncratic nature and delay in onset between starting the drug and the onset of agranulocytosis suggested that it was immune-mediated; however, in cases of rechallenge that I observed, the time to onset was the same as on initial exposure (*41*). This forced me to test other possible reasons for the idiosyncratic nature of this IDR, that is, ascorbate and selenium deficiency, but the results of these studies were negative (unpublished observations). It is possible that clozapine-induced agranulocytosis is immune-mediated and possibly an example of an autoimmune reaction. This brings me back to the question of whether a major portion of hepatic IDRs represents immune or metabolic idiosyncrasy. I would put my money on immune-mediated, but we need better evidence to make a definitive judgment, and it is likely that there are at least a few IDRs that are not immune-mediated. It also depends on the definition of an IDR because as mentioned earlier, if the toxicity of mercaptopurine in patients with a deficiency in thiopurine methyltransferase is considered an IDR, then certainly it would be an example of a nonimmune IDR.

2.4. Inflammagen Hypothesis. Another hypothesis for the mechanism of IDRs, especially hepatotoxicity, is the inflammagen hypothesis. Roth found that cotreatment of animals with lipopolysaccaride (LPS) and drugs such as ranitidine causes immediate hepatic damage (*42*). He has proposed that we are

commonly exposed to inflammagens such as LPS, and it is the combination of drug and inflammagen that leads to hepatic damage. Although this mechanism may be responsible for some hepatic IDRs, it is unlikely to be responsible for most IDRs. Most IDRs have a relatively characteristic time to onset rather than the random pattern predicted by this model (*1*). Furthermore, in this model, the LPS was administered iv at doses unlikely to represent clinical exposure and the damage was very similar to that caused by higher doses of LPS alone. Furthermore, ranitidine is available over the counter and is a very rare cause of hepatic IDRs. Therefore, if this model were used to screen drug candidates, it would inappropriately prevent the development of relatively safe drugs such as ranitidine. In addition, we have not been able to develop animal models of hepatic IDRs or other types of IDRs simply by coadministration of LPS or other inflammagens such as poly IC with drugs that are commonly associated with liver toxicity such as isoniazid. Therefore, if this model were used as a screening test, there would be a large number of false positives and negatives. On the other hand, it is likely that environmental factors such as this do play a role in the idiosyncratic nature of some IDRs.

2.5. Genetic Determinants of IDRs. It is likely that genetic factors are a major determinant of the idiosyncratic nature of IDRs. Although links with genetic polymorphisms in drug metabolism have been sought, in most cases, no link was found (*43*), and as mentioned earlier, any observed associations to date are too weak to explain the idiosyncratic nature of IDRs. Several strong associations with specific HLA genotypes have been found, and this strongly supports an immune mechanism for these IDRs (HLA is the acronym for human lymphocyte antigen and is the human version of MHC; thus, it is the molecule involved in antigen presentation.) One example is abacavir-induced hypersensitivity reactions that are strongly associated (odds ratio 960) with the HLA-B*5701 genotype, and to a lesser degree, a haplotypic Hsp70-Hom variant (*44*). In Han Chinese, there is a strong association (odds ratio 895) between HLA-B*1502 and carbamazepine-induced toxic epidermal necrolysis (*45*) as well as HLA-B*5801 and allopurinol-induced toxic epidermal necrolysis (*46*); however, the same association, at least for carbamazepine, was not observed in a European population (*47*). In most cases, strong associations have not been found, and it is likely that, analogous to other immune-mediated diseases such as type I diabetes, the determinants are polygenic (*48*).

2.6. Other Advances. Other advances in our understanding of the immune system too numerous to mention have occurred in the last 20 years. One in particular is the resurrection of the suppressor T cell, which is now referred to as the regulatory T cell (Treg) (*49*). I suspect that many patients treated with a drug that can cause IDRs do have an immune response but the response is the induction of tolerance rather than an IDR. If this is the case, our understanding of why this system fails in some patients could lead to the prevention of IDRs. The innate immune system has also received renewed attention, and it is likely that it plays an important role in the mechanism of IDRs (*50*).

There have also been significant advances in our understanding of how cells respond to electrophiles/oxidative stress. An important mechanism involves the binding of Nrf-2 binding to the antioxidant response element leading to the induction of protective enzymes. In an unstressed cell, the Nrf-2 is bound to Keap1, a protein with many (27) cysteine residues, which in some cases are a target for reactions with electrophiles, and binding of Nrf-2 to Keap1 leads to the degradation of Nrf-2, thus controlling the amount available for binding to ARE (*51*). Depletion of Keap1 leads to protection against acetaminophen hepatotoxicity (*52*).

There have also been advances in our knowledge of the targets of covalent binding. Some 30 proteins modified by the reactive metabolite of acetaminophen have been identified (*53*); however, it is very difficult to determine which binding is responsible for toxicity. The covalent binding of the meta isomer of acetaminophen, 3-hydroxyacetanilide, is comparable to that of acetaminophen; yet, it is not significantly hepatotoxic (it is possible that the meta isomer would cause IDRs, but its potential in this regard is unknown because it has not been used in humans). The pattern of covalent binding of the meta isomer is different than that of acetaminophen (*54*), and other biological effects such as induction of heat shock proteins (*55*), etc. are also quite different. Therefore, not all covalent binding is associated with the same toxic potential. The toxicity of acetaminophen is not considered idiosyncratic, and unfortunately, the binding data for drugs that cause idiosyncratic reactions are sparse; in fact, it is virtually impossible to quantify the amount of covalent binding in humans in the target organs of toxicity. Although the observation that drugs given at low dose are less likely to cause IDRs suggests that some minimal degree of covalent binding may be necessary to induce an IDR, it is unlikely that there is a good correlation between the amount of covalent binding and the risk that a drug will cause a relatively high incidence of IDRs because there are many drugs that form reactive metabolites but rarely cause IDRs. One can only speculate whether this is because binding to specific proteins is important or because the reactive metabolite (or parent drug) must also cause cell damage (danger signal) or because the location of binding is important (intracellular vs extracellular; mitochondrial vs endoplasmic reticulum), and it may even be different for different IDRs.

It is interesting that the chemical reactivity of reactive metabolites toward small molecules does not predict their in vivo pattern of covalent binding; specifically, binding can be selective for specific thiol groups while at the same time binding can also occur to nucleophilic groups in proteins such as imidazole that would not be expected based on the relative reactivity of the isolated amino acids, that is, cysteine vs histidine (*56*). Another example is the comparison of the pattern of covalent binding of procainamide, vesnarinone, and clozapine, all of which are associated with a relatively high incidence of agranulocytosis (*57*). In particular, the reactive metabolites of vesnarinone and clozapine are similar in that they are formed by the same enzyme (myeloperoxidase) and have approximately the same half-life, and both react preferentially with thiol nucleophiles. Yet, the pattern of binding for the reactive metabolites of these two drugs is quite different, thus making it difficult to determine which binding if any is responsible for agranulocytosis. Presumably, the in vivo differences in protein binding of electrophiles that have similar binding to simple amino acids are based on the physical properties of the drug and noncovalent interactions to biological molecules.

In general, intracellular antigens induce predominantly cell-mediated immune responses while extracellular antigens produce predominantly antibody responses (*58*). It appears that this pattern persists in IDRs. Park's group found that reactive metabolites that bind mostly to intracellular antigens produce a cytokine profile associated with cell-mediated immune responses while reactive metabolites that bind mostly to extracellular antigens (in this case serum protein) produce a cytokine profile associated with antibody-mediated responses (*59*). Very reactive

metabolites are more likely to bind to intracellular proteins and generate intracellular antigens.

3. The Way Forward

There are several different strategies that have the potential to decrease the risk of IDRs; some are being utilized at the present time, but others have a long lead time.

3.1. Decreasing Reactive Metabolites. If, as appears likely, most IDRs are caused by reactive metabolites, screening drugs for their ability to form reactive metabolites and designing this feature out of the molecule should lead to safer drugs. This approach has been adopted by some pharmaceutical companies (*60*). Unfortunately, there is no evidence yet that this has led to safer drugs. In addition, there may be some drugs such as ximelagatran that cause IDRs without forming reactive metabolites. Thus, if we eliminate one problem, others may arise. In addition, many new drugs are large molecules such as antibodies or cytokines that cause IDRs by entirely different mechanisms such as the cytokine storm caused by TGN1412 (*61*). Despite these caveats, small molecule drugs will continue to play an important therapeutic role, and if decreasing the risk of IDRs by decreasing covalent binding decreases the risk of inducing an IDR, it is worth doing even if it is not 100% effective. IDRs are uncommon in drugs given at low doses, possibly because this limits the amount of reactive metabolite that can be formed (*2*). Therefore, simply making drugs more potent is likely to significantly decrease the risk of IDRs, although there are classes of drugs where this is unlikely to be possible.

Although there are a few examples where the identity of proteins to which reactive metabolites bind is known, in most cases, this information is lacking. A web site exists that catalogues the known protein targets of reactive metabolites (tpdb.medchem.ku.edu:8080/protein_database/), and as this data expands, a correlation between adducts with specific proteins and specific types of IDRs may be found. If such associations can be found, it would greatly facilitate our ability to predict the risk of IDRs.

3.2. Developing Biomarkers that Predict IDR Risk. If the danger hypothesis is correct, biomarkers that reflect cell damage might predict the risk that a drug would cause a significant incidence of IDRs. In the limited number of studies that we have performed, some drugs meet this expectation but others do not. Even if the danger hypothesis is wrong, there may still be patterns of drug-induced gene expression that predict that a drug will be associated with a significant incidence of IDRs. The degree to which biomarkers will readily predict IDRs depends to a large degree on how many different mechanisms for IDRs exist and how complex they are. The patterns of gene changes appear to be different for different drugs, so it is unlikely that a small number of biomarkers will be predictive. What is needed is a comprehensive evaluation of in vivo changes in gene expression caused by a variety of drugs known to cause IDRs in humans along with appropriate controls using drugs not associated with a significant incidence of IDRs. The best study of this type that I have seen compared the in vivo and in vitro covalent binding as well as changes in gene expression induced by a series of anticonvulsants (*62*); however, it was limited in scope. Several other studies that I have seen were limited to in vitro data and often misclassified drugs as to whether they are associated with IDRs. The animals used to study the effects of drugs on gene expression may respond differently from humans, and where possible, changes seen in animals should be tested in humans to determine the degree to which the response differs. For example, in the study sited above, the metabolic activation of felbamate is much less in rodents than in humans (*63*); therefore, it is likely that the data underestimate the changes that occur in humans. It surprises me that some pharmaceutical companies perform microarray studies on new drug candidates without the background studies on old drugs that would provide information about how such changes correlate with IDR risk. I also believe that for the foreseeable future, high-throughput in vitro screens are unlikely to be of significant value. Most IDRs appear to involve complex pathways involving drug metabolism and cell signaling that cannot be mimicked in vitro. The one possible exception would be if biomarkers of cell stress in hepatocytes, which are able to form reactive metabolites, predicted that the drug being tested would cause hepatotoxicity. However, I still believe that such tests will have poor predictive value. A better mechanistic understanding may prove me wrong; however, I suspect that in vitro screens, as opposed to in vivo screens, will never be able to accurately predict the risk that a drug will cause IDRs.

3.3. Personalized Drug Therapy. Most patients can safely take a drug that is associated with a relatively high incidence of IDRs. Therefore, if we were able to predict the patients who are likely to have an IDR to a specific drug, "dangerous" drugs could be used safely. If genetic factors are the major determinant of risk, genotyping patients might make it possible to determine who is at risk of an IDR. There is one example where this already appears to be true, specifically abacavir-induced hypersensitivity (*64*). Even though the risk factors for most IRRs appear to be polygenic, as genotyping becomes less expensive and the data more complete, it may become possible to predict the individual risk for IDRs involving multiple genes. I believe that this will occur; however, it seems unlikely that it will be of practical value for most drugs for some time to come. I still remember some 25 years ago being told that very soon everyone would be phenotyped for their drug-metabolizing activity with respect to several different enzymes and patient therapy would be based on this information—It did not happen. A very important byproduct of genetic studies is that they have the potential to provide very important mechanistic clues.

3.4. Developing a Better Understanding of the Mechanisms of IDRs. Our current mechanistic understanding of IDRs is superficial (I have been told this is an understatement). Although I believe the data are adequate to indicate that most IDRs are caused by reactive metabolites and are immune-mediated, there are likely exceptions and it is not at all clear how common such exceptions are. As mentioned above, genetic studies are likely to provide important mechanistic clues, and to date, essentially all of the genetic associations have been with genes, such as HLA genes, associated with immune response. Although it may be possible to decrease the risk of IDRs without a better mechanistic understanding, it is likely that real progress will require a much better understanding of the mechanisms involved in IDRs. However, mechanistic studies are difficult. The low incidence makes prospective human studies virtually impossible, and animal models are very difficult to develop because such reactions are also idiosyncratic in animals. Therefore, some of the basic tools used for mechanistic studies in other areas of biomedical research are lacking in this field.

3.5. Animal Models. A fundamental question is why is it difficult to develop animal models? If the key is activation of APCs, it should be possible to develop animal models by appropriate stimulation of APCs. Poly IC (which mimics viral double-stranded RNA and activates APCs through toll-like receptor 3) increased the incidence and severity of penicillamine-induced autoimmunity in the Brown Norway rat (*65*); however,

it did not affect the severity of the immune-mediated skin rash in rats treated with nevirapine (*66*). An important point is that the Poly IC was administered by intraperitoneal injection and the skin is the target of the nevirapine IDR. It is likely that the activation of APCs must be closely associated with the drug and the target organ. For a generalized autoimmune syndrome, intraperitoneal injection may be appropriate, but the same is not true for a skin rash. In addition, it is known from vaccine development research that, as with a pathogen that activates APCs through multiple pathways, more than one pathway of stimulation may be required to achieve adequate APC activation. Another point is that although Poly IC treatment potentiated penicillamine-induced autoimmunity in the Brown Norway rat, treatment of Lewis rats with penicillamine and Poly IC did not lead to autoimmunity; therefore, other genetic factors must play a role in this model.

It could be that a specific MHC is required to present the appropriate processed antigen. This seems less likely in a generalized autoimmune syndrome such as that induced by penicillamine, but it would be consistent with the human data for IDRs such as abacavir-induced hypersensitivity. If this is a general feature of IDRs, we would have to use transgenic animals expressing the appropriate MHC to develop the appropriate animal model. However, nevirapine-induced skin rash occurs in several strains of rat (*67*); therefore, it must not require a specific MHC. Although a specific MHC may be required for some IDRs, it should be remembered that most people can mount an adequate immune response to most pathogens or sensitizing agents such as urushiol, which is the sensitizing agent in poison ivy. This is presumably because most haptens bind to multiple proteins, and in most people, there will be at least one combination of hapten–peptide/MHC that is capable of initiating an immune response under the right conditions. Thus, the degree to which there is an association with a specific HLA gene may depend on the total amount of covalent binding and the range of proteins modified.

Genetic factors controlling other aspects of immune response such as cytokine networks and tolerance are also likely to be important (*68, 69*). Genetic factors controlling reactive metabolite formation/detoxication must be important in some cases, but there are no good examples where such factors are the major factor leading to the idiosyncratic nature of these reactions. Therefore, if we are successful in developing additional animal models, it will provide very important insights into the risk factors involved in human IDRs and the range of mechanisms involved. In addition, animal models such as nevirapine-induced skin rash should make it possible to study the very first events that ultimately lead to an immune response, which, in turn, could lead to biomarkers that predict IDR risk.

4. Conclusions

In looking back at the history of IDRs, progress in understanding the mechanisms of these adverse reactions has been slow. The hapten hypothesis remains a dominant mechanistic hypothesis; however, several additional hypotheses have been added. Thus, returning to Figure 1, although it is still valid for some IDRs, the picture is getting more complex for those IDRs that it does represent and it does not represent the mechanism of all IDRs. It fact, the mechanisms for the same IDR caused by different drugs can be different, and the mechanisms for the IDRs associated with a given drug can be different in different patients. For example, the HLA association that predicts the risk of carbamazepine-induced Stevens–Johnson syndrome does not predict the risk of a carbamazepine-induced generalized hypersensitivity reaction or maculopapular rash, and this implies that the basic mechanisms are quite different (*70*). I suspect that the mechanisms of IDRs are at least as complex as those involved in diabetes or cancer; yet, far fewer investigators have been involved in mechanistic studies of IDRs than for diabetes or cancer. Therefore, it is not surprising that progress has been slow and it is naïve to expect a quick fix. On the other hand, significant progress has been made in the last 20 years, and I hope that in the future the importance of this field will be recognized and more people will become involved in this challenging field of research so that advances can occur at a faster rate.

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