Involvement of the Immune System in Idiosyncratic Drug Reactions

Xiachu Zhang, Feng Liu, Xin Chen, Xu Zhu and Jack Uetrecht*

Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Canada

Summary: There is strong evidence that most idiosyncratic drug reactions (IDRs) are immune-mediated and are caused by reactive metabolites of a drug rather than by the drug itself. Several hypotheses have been proposed by which a drug could induce an immune response. The major hypotheses are the hapten hypothesis and the danger hypothesis; however, the characteristics and spectrum of IDRs are different with different drugs, and this likely reflects mechanistic differences; therefore, no one hypothesis is likely to explain all IDRs. Some IDRs appear to involve epigenetic effects, direct activation of antigen-presenting cells, or disturbing the normal balance of the immune system. It has been suggested that many cases of idiosyncratic liver injury are not immune-mediated, and other mechanisms such as mitochondrial injury may be involved. It is essential that any hypothesis be consistent with the clinical characteristics of the IDR. Although the characteristics of most idiosyncratic liver injury do not suggest that mitochondria are the major target, it is quite possible that milder mitochondrial injury could stimulate an immune-mediated reaction. The observation that IDRs can vary widely among different drugs and different patients is most easily explained by an immune mechanism in which the target of the immune response is different.

Keywords: idiosyncratic drug reactions; drug-induced liver injury; autoimmunity; hapten hypothesis; danger hypothesis; mitochondrial injury

Background

Idiosyncratic drug reactions (IDRs) are also referred to as type B (bizarre) adverse drug reactions. These adverse drug reactions (ADRs) only occur in a small proportion of patients who take a drug and are not related to the therapeutic effects of the drug. Overall, ADRs are a major cause of patient morbidity and mortality. Although IDRs make up only about 5% of ADRs, given the large variety of drugs that cause IDRs and the number of people who take drugs, the number of cases is significant. In addition, they can be very severe, e.g., idiosyncratic drug-induced liver injury was responsible for nearly 13% of acute liver failure in the United States from 1997 to 2001. Furthermore, the unpredictability of IDRs makes it very unlikely that they will be discovered in clinical trials. From 1975 to 2000, about 10% of new drugs approved in the US were either withdrawn or received a black box warning due to unexpected IDRs. This uncertainty significantly increases the overall cost of drug development. In addition, much of the preclinical testing is performed to try to prevent IDRs, and although such testing is not very effective at predicting IDR risk, it adds to the time required for drug development, which further adds to cost.

A major characteristic of IDRs is the delayed time to onset. With very few exceptions there is a delay of a week or more in onset of an adverse drug reaction on primary exposure to a drug. This is typical of an immune-mediated reaction because it requires at least a week on first exposure for the few T lymphocytes that recognize a specific immunogen to proliferate and result in a clinically evident immune response. When a patient who has had an IDR is rechallenged with the same drug, there is usually, but not always, a more rapid onset of the IDR. IDRs are often characterized as being dose-independent. This is not true: in fact, the likelihood that a drug will cause a significant risk of IDRs is related to the therapeutic dose of the drug. Drugs given at a dose of less than 10 mg/day rarely cause IDRs, and 77% of 598 cases of idiosyncratic drug-induced liver injuries were found to be from drugs given at a dose of greater than 50 mg/day. What is true is that most patients will not have an IDR at any dose, and there may not be any difference in incidence within the narrow range of doses used therapeutically. In addition, the dose required to cause an IDR may be lower in a patient who has been previously sensitized to the drug, but there will always be a dose below which no one will have an IDR. Some IDRs are more
common in women. For example, women were more susceptible to nevirapine-induced severe skin rash; however, this is not true for all types of IDRs. Genetic factors have been found to be a very strong risk factor for a few IDRs. For example, it appears that hypersensitivity reactions to abacavir is associated with the HLA-B*5701 allele. However, even most patients who carry the HLA-B*5701 allele will not have a hypersensitivity reaction if they take abacavir, so there must be other factors that are required. All of the genetic factors that have been found to be strongly related to an increased risk of IDRs are either MHC I or MHC II alleles. These characteristics suggest that most IDRs are immune-mediated.

**Mechanistic Hypotheses**

Several hypotheses have been proposed for how the immune system may be involved in the mechanism of IDRs. The major hypotheses are the hapten hypothesis, the danger hypothesis, and the pharmacological interaction hypothesis. An immune-mediated reaction can also be induced by direct activation of antigen-presenting cells, by alteration in immune balance, and by epigenetic effects. These hypotheses are summarized in Figure 1. There are also hypotheses for the mechanism of IDRs that do not require the adaptive immune system: mitochondrial damage and the inflammagen hypothesis. These hypotheses for nonimmune mechanisms will be discussed in the section on idiosyncratic liver toxicity, which is the organ toxicity to which they have been applied.

**Hapten hypothesis:** In 1935, Landsteiner reported that some small molecules such as 2,4-dinitrochlorobenzene and p-nitrosodimethylaniline induced skin rashes in guinea pigs, but only if they covalently bound to proteins. Small molecules that bind to proteins leading to an immunogenic protein are referred to as haptens. Later, the ADRs caused by several drugs were found to be related to the formation of reactive metabolites that bind to proteins. A good example of covalent binding leading to a hypersensitivity reaction is penicillin-induced allergic reactions. A characteristic feature of penicillin is a β-lactam ring, which is chemically reactive and does not require metabolic activation in order to irreversibly bind to amino and sulphhydryl groups on proteins. In some patients this leads to IgE antibody formation and an allergic IDR to the penicillin-protein adduct. The hapten hypothesis is clearly true for penicillin-induced allergic reactions because it is the anti-penicillin IgE antibodies that mediate the IDR. For other drugs that are not chemically reactive, reactive species can be formed during metabolism, and these reactive metabolites can act as haptens. A good example is halothane-induced hepatotoxicity. Halothane is oxidized by cytochrome P450 to the reactive trifluoroacetyl chloride, and antibodies have been found against trifluoroacetyl chloride-modified protein in most halothane allergic patients. However, unlike anti-penicillin antibodies, it is not clear that antibodies against trifluoroacetylated proteins mediate halothane-induced liver injury. They do indicate that
halothane has induced an immune response, and even if these antibodies are not pathogenic, it is likely that halothane-induced hepatotoxicity is immune-mediated.

Drug bioactivation and covalent binding risk assessment are a focus in drug development in the pharmaceutical industry. At Merck & Co., Inc., the quantity of covalent binding to proteins has been used to guide drug development. If a drug candidate was found to covalently bind to proteins, especially if the binding is more than 50 pmole/mg protein, the basis for that binding was investigated, and new analogs without the structural feature responsible for the binding would be synthesized and tested until a structure is found that has minimal binding and is likely to be safer.\(^{(17)}\) When corrected for daily dose, covalent binding is related to the risk of liver toxicity; however, it is not a perfect predictor of IDRs.\(^{(18)}\) There are some drugs such as ximelagatran that do not appear to form reactive metabolites and yet are associated with an unacceptable risk of liver toxicity that appears to be immune-mediated.\(^{(19)}\)

**Danger hypothesis:** A classic principle of immunology is that the immune system responds to foreign material, and this is consistent with the hapten hypothesis where the binding of hapten makes the protein immunogenic. It was found that not all autoreactive T cells are deleted during the maturation of the immune system, and there is no difference between how self and foreign proteins are presented to T cells. Therefore, there must be some additional mechanism to control immune responses to prevent widespread autoimmunity. It was discovered that activation of antigen-presenting cells (APCs) leading to expression of costimulatory molecules such as B7 is required for activation of T cells. The interaction between MHC antigen complex on APCs and T cell receptor (TCR) on T cells is referred to as signal 1, while the interaction between B7 on APCs and CD28 on T cells is referred to as signal 2 or costimulation. The immune response is initiated when both signal 1 and signal 2 are present, and tolerance will be induced if only signal 1 is present.\(^{(20)}\) Janeway proposed that signal 2 was mediated by toll-like receptors that recognize evolutionarily conserved molecules on pathogens.\(^{(21)}\) In contrast, Matzinger proposed that it is molecules released by damaged cells that activate APCs and control immune responses, and it has been found that many such molecules also bind to toll-like receptors. This is known as the danger hypothesis.\(^{(22)}\)

An alternative hypothesis for the role of reactive metabolites in the pathogenesis of IDRs is that reactive metabolites or their covalent binding to proteins can interrupt cellular functions and induce stress, which may lead to the release of danger signals from stressed cells to trigger an immune response.\(^{(20,23)}\) However, the hapten and danger hypotheses are not mutually exclusive, and the danger hypothesis could explain why not all drugs that covalently bind to protein are associated with a significant incidence of IDRs. Specifically, unless the reactive metabolite not only modifies protein but also causes cell damage, it will not induce an immune response. This raises an important question: does the danger signal have to be caused by the drug or can other forms of cell damage such as viral infections provide the costimulation required to lead to an immune response, which in the presence of covalently bound drug, can lead to an IDR? There are examples where a viral infection has been found to increase the risk of an IDR.\(^{(24)}\) An obvious increase in ampicillin-induced IDRs was observed in patients with mononucleosis,\(^{(25)}\) and HIV-infected patients have an increased risk of developing an IDR to sulfonamides and other drugs.\(^{(26)}\) Another source of a danger signal could be physical injury, and surgery appears to increase the risk of procainamide-induced agranulocytosis 10 fold.\(^{(27)}\) However, most IDRs occur in patients without viral infections or other obvious sources of a danger signal.

Some documented danger signals include high mobility group protein 1 (HMGB1), IL-1a, cytosolic calcium binding proteins of the S100 family, heat shock proteins (HSPs), and uric acid.\(^{(28)}\) HMGB1 is a non-histone nuclear protein, of which the identified receptors are for advanced glycation end products (RAGE) and toll-like receptors 2, 4, and 9.\(^{(29,30)}\) An interesting characteristic of HMGB1 is that it goes through posttranslational modification in activated monocytes, which leads to its translocation from the nucleus to cytosol and further to the extracellular matrix.\(^{(31)}\) Lipopolysaccharide (LPS) treatment results in hyperacetylated lysines on HMGB1, while TNFa induces phosphorylated forms of HMGB1.\(^{(31)}\) High serum levels of HMGB1 have been found in acetaminophen-induced liver toxicity, and it may act as a proinflammatory factor to initiate an immune response.\(^{(32)}\) S100 proteins, which interact with toll-like receptor 4, also appear to act as danger signals and play an important role in the development of autoimmunity.\(^{(33)}\) S100 A7/A15 (psoriasin) in the epidermis was found to play an important role in the pathogenesis of psoriasis.\(^{(34)}\) Another group of proteins that has been referred to as danger signals is HSPs. However, not every member of this group of proteins can act as a danger signal. While HSP70 was found to be an endogenous danger signal in activating the effector function of natural killer (NK) cells,\(^{(35)}\) HSP27 was found to act as an anti-inflammatory protein.\(^{(36)}\)

**Pharmacological interaction (p-i) hypothesis:** Another hypothesis is the pharmacological interaction (p-i) hypothesis, as proposed by Pichler. In this hypothesis the parent drug acts as a superantigen to bind reversibly to the complex formed by MHC II on APCs and the T cell receptor on T cells to initiate an immune response. This hypothesis was based on the observation that T cell clones from patients with a history of IDRs to sulfamethoxazole were activated, as measured by proliferation, when incubated with sulfamethoxazole in the absence of drug metabolism.\(^{(37)}\) Although the same observation was made with other drugs, sulfamethoxazole is a primary aromatic amine, and virtually all primary aromatic amine drugs given at a dose of 100 mg/day or more are associated with a
significant incidence of IDRs.\(^3\) Presumably, this is because aromatic amines are readily metabolized to reactive metabolites. A key assumption of the p-i hypothesis is that what lymphocytes respond to is what initiated the immune response. In an immune-mediated skin rash induced by nevirapine in rats, we found that lymphocytes from these animals respond to nevirapine better than to the 12-hydroxy metabolite, even though we had shown that oxidation to the 12-hydroxy metabolite is required to induce a rash. Furthermore, T cells from animals in which the rash was induced by treatment with the 12-hydroxy metabolite (and the animals had never been exposed to nevirapine) still responded better to nevirapine.\(^38\) Therefore, the response of T cells is not an accurate indication of what induced an immune response. In a more recent study of human T cells from three patients with hypersensitivity to sulfamethoxazole, lymphocytes proliferation was detected for sulfamethoxazole and both hydroxylamine and nitroso metabolites; however, higher numbers of antigen-specific T-cell clones were generated with the two metabolites than with the parent drug sulfamethoxazole.\(^39\) An IDR that may involve the p-i mechanism is ximelagatran-induced hepatotoxicity. Ximelagatran is structurally similar to a small peptide and does not appear to form reactive metabolites. It may be able to initiate an immune response through a p-i type of interaction, and there is evidence that it binds reversibly to MHC.\(^19\)

**Immune balance:** The immune system is highly regulated, and balance is maintained by various arms of the system. Many new biological drugs, e.g., antibodies and cytokines, have been developed to treat autoimmune diseases such as multiple sclerosis.\(^10\) However, paradoxically, even though most are used for their immunosuppressant effects, they can also induce autoimmunity. For example, anti-tumor necrosis factor (TNF) antibodies can induce various autoimmune syndromes such as lupus and vasculitis; however, there are few such models, and the specific characteristics to the rash that nevirapine causes in humans. It has been very useful for studying the metabolic pathway responsible for the rash\(^54\) and the specificity of the T cells involved.\(^38\) However, there are very few such models, and there appear to be significant differences in the mechanism of different IDRs in humans, as evidenced by the characteristics described below; therefore, several models would be required to determine the range of mechanisms that can lead to an IDR. In the absence of a variety of valid animal models, we are left with using the clinical characteristics of IDRs in humans to try to infer mechanism and also to judge potential animal models. The following is a description of the characteristics of several types of human IDRs and a discussion of how well these characteristics can be explained by the mechanistic hypotheses described above.

**Autoimmunity:** Autoimmunity is the failure of an organism to recognize its own constituent parts as self, which allows an immune response against its own cells and tissues. Many drugs such as hydralazine, procainamide, isoniazid, methyldopa, quinidine, minocycline, and chlorpromazine are able to trigger autoimmunity.\(^55\) Such reactions are characterized by autoantibodies, and some are similar in character to idiopathic systemic lupus erythematosus, the cause of which is unknown. The manifestations of drug-induced autoimmunity can be
classified as either generalized autoimmune reactions that resemble idiopathic lupus, or organ-specific autoimmune reactions such as autoimmune hemolytic anemia, autoimmune hepatitis, and pemphigus vulgaris. Depending on the type of reaction, patients may develop autoantibodies to nuclear antigens, to erythrocytes, or to other protein antigens similar to idiopathic autoimmune diseases. These autoantibodies do not disappear immediately after withdrawal of the offending drug, but the clinical symptoms usually resolve within weeks even though, by definition, the autoantigen is still present.

Many drugs can induce generalized autoimmune syndromes; such as drug-induced vasculitis and drug-induced lupus-like syndrome, a generally milder version of the idiopathic disorder that is usually associated with production of antihistone antibodies. However, the clinical and serological phenotypes of the drug-induced autoimmune reactions overlap with the idiopathic forms so that, other than exposure to drug and resolution when the drug is stopped, it is hard to differentiate them. Common clinical manifestations of drug-induced lupus are myalgias, arthritis, fever, and serositis involving the pleura and/or pericardium. About 10% of lupus is estimated to be drug-induced, with 15,000–30,000 drug-induced cases occurring in the United States. Similarly, about 10% of cases of cutaneous vasculitis is reported to be drug-induced, with purpuric and maculopapular rashes being the most common symptoms. Many drugs are suspected of causing a lupus-like syndrome; it is difficult to determine an accurate number, but to date at least 38 medications have been strongly implicated.

Biological drugs such as anti-TNF-α antibodies and cytokines such as interferon-α can also cause a lupus-like syndrome. One study found that approximately 14% of rheumatoid arthritis patients treated with anti-TNF-α antibodies developed anti-DNA antibodies, although less than 1% developed lupus-like symptoms. It often takes more than 1 year of treatment with the offending drugs before the syndrome becomes clinically evident, although antinuclear antibodies are detectable much earlier. The presence of antinuclear antibodies is virtually a sine qua non for the diagnosis.

There are several hypotheses for the mechanism of drug-induced lupus-like syndrome. One likely mechanism is the inhibition of DNA methylation. Some drugs can result in T-cell DNA hypomethylation, leading to the activation of T cells and lupus-like disease, either through decreased ERK pathway signaling (hydralazine) or through inhibition of DNA methyltransferase (procarainamide). Another proposed mechanism for drug-induced lupus is the oxidation of a drug by macrophages or other APCs, leading to the formation of a reactive metabolite that binds to APCs leading to their activation. A few drugs, such as penicillamine, hydralazine, and isoniazid, react irreversibly with aldehydes on APCs leading to their activation, and they are also associated with a high incidence of drug-induced lupus. A third theory is that biologics such as TNF-α inhibitors may shift the T-helper profile: by blocking this Th1 cytokine, it may shift the immune system to a Th2 profile with the production of autoantibodies and the development of lupus-like features.

In addition to generalized autoimmunity, some drugs can cause organ-specific autoimmunity; the most common are autoimmune hemolagic and hepatic IDRs, as discussed below.

Skin rashes: There are many types of skin rashes induced by drug administration, ranging from the less severe maculopapular rashes and urticaria to Steven-Johnson Syndrome (SJS) and toxic epidermal necrolysis (TEN). Most rashes are immune-mediated.

Drug-induced urticaria represents approximately 5% of all cutaneous drug reactions and is the second most common form of skin eruption after exanthematous reactions. Urticaria lesions, otherwise known as hives, are characterized by raised, itchy, red blotsches or weals that are pale in the center and red around the outside. They are widely scattered on the body, but they can also be accompanied by deeper swelling of submucosal tissues called angioedema. Urticarial lesions often fade within a few hours without a trace, but angioedema takes longer to resolve. The major mechanism for drug-induced urticaria involves the hapten hypothesis. The best studied is penicillin-induced urticaria. β-Lactams are chemically reactive and can covalently bind to proteins, eliciting the production of IgE against the hapten-modified protein. Sufficient IgE production will result in significant allergic reactions such as urticaria and anaphylaxis. This reaction is clearly immune-mediated due to the presence of protein-specific IgE. However, what remains unclear is why different patients have different responses to the β-lactam protein adduct. Some recent studies reported a genetic association between β-lactam allergies and IL-13 and/or IL-4Rα polymorphisms. This also supports an immune mechanism. However, not all drug-induced urticaria is mediated by antibodies. For example, the inhibition of kinin degradation caused by angiotensin-converting enzyme inhibitors, altered arachidonic acid metabolism by aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs), as well as a receptor-mediated release of histamine by opiates all involve a non-immune mechanism, at least not an adaptive immune mechanism.

Exanthematous or maculopapular drug eruptions are probably the most common IDRs and account for almost 95% of all drug rashes. Usually the rashes develop in 1 or 2 weeks following the initiation of the drug, and more rapidly on rechallenge or in previously sensitized patients. Maculopapular rashes are considered to be immune-mediated, specifically, T cell mediated. Pathology studies reveal a cellular perivascular infiltration of TH lymphocytes in the dermis, consisting mostly of CD4+ T cells and fewer CD8+ T cells. The expression of skin-homing receptors such as cutaneous lymphocyte antigen (CLA) and chemokine...
receptors such as CCR6 and CCR10 on circulating T lymphocytes, together with the elevated levels of their ligands (CCL20 and CCL27 in the skin), suggest the migration of T cells from peripheral blood to the inflammatory sites. Both CD4+ and CD8+ T cells have been shown to produce cytotoxic molecules such as perforin and granzyme B. Increased levels of IFN-γ and TNF-α in the serum were also reported. Other than secreting cytotoxic mediators and cytokines, T cells also play an important role in attracting other immune cells. For example, the production of IL-8 may contribute to accumulation of neutrophils, whereas IL-5 and eotaxin are key attractants of eosinophils.

Details of the initial steps in the pathogenesis of drug-induced maculopapular rashes remain unclear, and many studies involve the lymphocyte transformation test. However, the untested assumption of this assay—what T cells respond to is what induced the immune response—has been proven wrong. Therefore, the lymphocyte transformation test cannot be used to investigate the initiation of the disease. Despite this, the presence of drug-specific T cells responding to the parent drug and/or its metabolites by proliferation and cytokine production provide strong evidence for a T cell-mediated mechanism.

SJS and TEN are two forms of life-threatening skin rashes, both characterized by fever and blister formation, and differing only in the degree of severity. In SJS, epidermal detachment is less than 10% of the body surface area; TEN is more severe with involvement of ≥30% of the body surface area and is associated with a >30% mortality. Involvement of between 10% and 30% is termed transitional SJS-TEN. Histologically, both of SJS and TEN are characterized by extensive keratinocyte apoptosis, which results in the separation of the epidermis from the dermis, and this is believed to be induced by T cells. One proposed mechanism is that the interaction between soluble Fas produced by peripheral blood mononuclear cells and the Fas ligand expressed on diseased keratinocytes initiates the extensive apoptosis of keratinocytes. Another possible mechanism is through the release of cytotoxic mediators, such as perforin and granzyme B. It is suggested that the elevated levels of TNF-α and Fas ligand originate from keratinocytes, which may increase the expression of MHC I expression on keratinocytes, making them more sensitive to cytotoxic cells.

Carbamazepine- and allopurinol-induced SJS/TEN are clearly associated with HLA-B*1502 and HLA-B*5801, respectively. These genetic predispositions are drug-specific and vary with ethnicity. The former was only found in some Asian populations (Han Chinese and a Thai population) but not in Europeans, and the latter was found in both Han Chinese and Europeans. The association between HLA and drug-induced SJS/TEN also provides evidence for an immune-mediated mechanism.

**Hematologic idiosyncratic adverse reactions:**
The most common immune-mediated hematologic IDRs are hemolytic anemia, thrombocytopenia, agranulocytosis, and aplastic anemia, and these affect red blood cells, platelets, neutrophils, and all blood cells, respectively.

Drug-induced immune hemolytic anemia is characterized by increased red cell destruction through antibody-mediated complement activation. Three different types of antibodies—hapten-specific antibodies, drug-dependent antibodies, and drug-induced autoantibodies—have been associated with this IDR. A common drug leading to the formation of hapten-specific antibodies is penicillin.Penicillin treatment, especially with high doses for more than 10 days, can induce antibodies that bind to red cells and cause their destruction. In contrast to hapten-specific antibodies, drug-dependent antibodies appear to modify specific red cell membrane glycoproteins. The causative drugs, e.g., quinine, quinidine, and cefotetan, must be present for hemolysis to occur, but the antibodies do not bind to the drug. The third group of antibodies that can induce hemolytic anemia comprises drug-induced autoantibodies, which bind to red cells even when the causative drugs, e.g., α-methyldopa, i-dopa, and procainamide, are not present. Unlike the other forms of hemolytic anemia, which usually occur after a week or two of treatment, the onset of autoimmune hemolytic anemia typically occurs only after 4–6 months of drug treatment. However, only a small fraction of patients with positive antibodies had significant hemolytic anemia. Clearly these are immune-mediated reactions, and the anemia mediated by hapten-specific antibodies support the hapten hypothesis. The mechanism by which drugs induce the other types of antibodies is not clear.

Another common IDR is thrombocytopenia. The normal platelet count is above 150,000 platelets/µL of blood, and when it falls below 10,000 platelets/µL the patient is at very high risk of life-threatening hemorrhage. Drugs are a common cause of thrombocytopenia. Compared with drug-induced immune hemolytic anemia, more drugs are involved in the induction of thrombocytopenia. A typical manifestation of drug-induced thrombocytopenia is spontaneous bruising, and the time to onset is usually after a week or more of treatment with the offending drug. A clear example of immune-mediated thrombocytopenia is caused by heparin which is mediated by antibodies against the heparin-platelet factor 4 complex. Even though it is clearly immune-mediated, it is not associated with immune memory, a common feature of immune-mediated reactions. Specifically, if a patient with a history of heparin-induced thrombocytopenia is rechallenged with heparin, they usually do not develop thrombocytopenia, or if they do, it does not occur more rapidly. As in hemolytic anemia, three groups of antibodies—hapten-specific antibodies, drug-dependent antibodies, and drug-induced autoantibodies—have been associated with the pathogenesis of drug-induced thrombocytopenia. Penicillin is the major cause of hapten-specific antibody, while i-dopa, procainamide, penicillamine, and sulfamethoxazole are implicated in the drug-induced
platelet-specific autoantibodies. More recently, biopharmaceuticals such as rituximab (anti-CD20) and infliximab (anti-TNFα) have been associated with autoimmune thrombocytopenia. Drug-dependent antibodies induced by quinine and many antibiotics bind to glycoprotein (IIb/IIIa complex and GPib/IX complex) on platelet membrane leading to platelet damage.

Agranulocytosis is defined as a neutrophil count of less than 500 neutrophils/µL of blood. This places patients at high risk of serious infections. Most cases of agranulocytosis are induced by drugs including analgesics, antipsychotics, antithyroid medications, and anticonvulsants. Cancer chemotherapy can cause agranulocytosis that is not idiosyncratic, but when it is idiosyncratic, in some cases there is evidence that it is immune-mediated. In one study, rechallenge experiments were performed on two patients with aminopyrine-induced agranulocytosis, and after a single dose of aminopyrine, a precipitous drop in leukocyte count occurred within 2 hours. In another experiment, transfusion of blood from a patient with agranulocytosis into a normal person who had just ingested aminopyrine resulted in a rapid drop in neutrophil count. These experiments indicate that aminopyrine-induced agranulocytosis is mediated by drug-dependent antibodies. In patients with agranulocytosis induced by propylthiouracil, antibodies that reacted with granulocytes, monocytes, and hematopoietic precursor cells were detected, in another report, antineutrophil cytoplasmic autoantibodies against myeloperoxidase were detected in propylthiouracil-induced agranulocytosis patients. In quinine-induced neutropenia patients, quinine-dependent antineutrophil antibodies reacted with several surface neutrophil glycoproteins. However, there are also cases of drug-induced agranulocytosis in which the typical characteristics of an immune-mediated reaction are not present. (Clozapine-induced agranulocytosis is well documented because all patients on the drug have to have a weekly blood count. It is an example in which the agranulocytosis does not recur rapidly on rechallenge, and no drug-dependent antibodies have been reported in clozapine-induced agranulocytosis patients.) However, the lack of typical characteristics of an immune-mediated reaction does not prove that clozapine-induced agranulocytosis is not immune-mediated, as demonstrated by the example of heparin-induced thrombocytopenia discussed above. Clozapine-induced neutropenia is related to its metabolism in neutrophils, where it is oxidized by myeloperoxidase to a reactive nitrenium ion metabolite that binds to neutrophils. The covalent binding between clozapine and neutrophils from patients taking the drug has been detected in our lab, and has potential to initiate an immune response. Specific HLA genotypes that are markers for susceptibility to clozapine-induced agranulocytosis in Ashkenazi Jewish patients were found to be DRB1*0402, DQB1*0302, and DQA1*0301, and in non-Jewish patients, HLA-DR*02, DQB1*0502, and DQA1*0102. However, the number of patients in the study was small (52 patients in total) and the associations were relatively weak. Since the time to onset of clozapine-induced agranulocytosis is usually 6–12 weeks, and rechallenge with clozapine does not shorten the time to onset, autoimmune mechanisms may be involved. It has been shown that clozapine increases the rate of apoptosis in vitro and leads to an increase in neutrophil turnover in vivo in rabbits without leading to neutropenia. It is possible that in a few patients the neutrophil damage implied by these results leads to immune-mediated agranulocytosis, possibly with an autoimmune component.

The diagnosis of aplastic anemia is based on examination of the bone marrow and a finding that most of the hematopoietic cells have been replaced by fat, leading to a deficiency of all of the blood cells described in the previous paragraphs. Although drug-induced aplastic anemia is less common than drug-induced agranulocytosis, this severe adverse drug reaction has limited the use of several drugs, e.g., chloramphenicol and felbamate. It appears that idiosyncratic drug-induced aplastic anemia is immune-mediated; specifically, it is mediated by cytotoxic T lymphocytes that cause bone marrow destruction. Idiopathic aplastic anemia is sometimes associated with viral infections, and it appears to be an autoimmune immune reaction. The observation that both idiopathic and drug-induced aplastic anemia usually respond to immunosuppressive therapy further supports an immune-mediated mechanism. It is still not clear how this adverse reaction is initiated. Because drug-induced aplastic anemia and drug-induced agranulocytosis can be induced by many of the same drugs, most of which can be oxidized to reactive metabolites by the myeloperoxidase system of neutrophils, macrophages, and some of their precursors, such bioactivation may be the common factor in these hematological IDRAs.

Idiosyncratic liver toxicity: The liver is the major site of metabolism for drugs and other xenobiotics. Given that most IDRAs appear to be caused by reactive metabolites, it is not surprising that the liver is involved in many IDRAs. In the United States, it has been reported that idiosyncratic drug-induced liver injury (IDILI) accounts for about 13% of all acute liver failure. In addition, IDILI is also a major issue for drug development. It is the most common reason leading to drugs being withdrawn from the market. The mechanisms of IDILI are not well understood and the involvement of the immune system is more controversial than for other types of IDRAs.

As with other IDRAs, there is generally a delay between starting a drug and the onset of IDILI. The most typical delay is 1–3 months, but in some cases the delay can be significantly longer, especially if the IDILI is clearly autoimmune, in which case it usually requires more than a year of treatment before it becomes symptomatic. There are some cases in which the serum transaminases were normal when the drug was stopped and levels did not become elevated until a month later. In some cases there
is a rapid onset of symptoms when a patient is rechallenged with a drug, but this is not universal. In a few cases, IDILI is associated with fever, rash, and eosinophilia, which are classic symptoms of an immune-mediated allergic reaction, but more often than not these symptoms are absent. In addition, antidrug antibodies or autoantibodies have been detected in some cases of IDILI. Although it is unclear what role they play in the pathogenesis of the injury, such antibodies provide strong evidence that the drug has induced an immune response. The histology of hepatocellular IDILI can mimic almost any other type of liver injury, but most commonly resembles viral hepatitis with mild to moderate inflammation and infiltration of mostly lymphocytes and sometimes eosinophils.

IDILI caused by drugs such as isoniazid and ketoconazole has been classified as metabolic idiosyncrasy. However, there are clear cases of both isoniazid- and ketoconazole-induced IDILI with a very rapid onset on rechallenge. This provides strong evidence of an immune-mediated reaction. Although it can be a risk factor, there are also no examples where polymorphism of a metabolic pathway is sufficient to explain the idiosyncratic nature of IDILI. Another proposed hypothesis is the inflammasome hypothesis, which proposes that the idiosyncratic nature of IDILI is based on the chance superposition of drug treatment and an inflammatory stimulus such as lipopolysaccharide from the intestine. However, this model simply does not have the same characteristics as clinical IDILI.

Another hypothesis for the mechanism of IDILI is that the drugs involved cause mitochondrial damage, which leads to the death of hepatocytes. There are drugs such as valproic acid and perhexiline that cause IDILI that is characterized by microvesicular steatosis and/or lactic acidosis. These are clear indications that the drug has compromised lipid and energy metabolism, which occur in mitochondria. In addition, mitochondria control cell death, which also makes this an attractive hypothesis. However, it does not explain the delay in onset or the inability to readily produce animal models by simply giving large doses of the drug. There is an animal model of IDILI that utilizes mice that are partially deficient in mitochondrial superoxide dismutase. It was found that when these mice were treated with troglitazone and a few other drugs they developed liver toxicity, however, the toxicity was relatively mild and other groups have not been able to reproduce the results. It is possible that the difference in results was due to a difference in the solvent and route of exposure used for administration of the troglitazone. The original study used a solvent that contained polyglycol esters of 12-hydroxystearic acid, which may also have effects on the metabolism of fatty acids, and the troglitazone was given by i.p. injection rather than orally. There are examples in which drugs such as fialuridine and nucleoside reverse transcriptase inhibitors that cause damage to mitochondrial DNA lead to delayed and cumulative liver damage in humans, but this toxicity is not idiosyncratic. The delay and cumulative liver toxicity observed with mitochondrial DNA damage happens because mitochondrial DNA does not have the same repair mechanisms as nuclear DNA. This toxicity is also characterized by microvesicular steatosis and/or lactic acidosis, which is not a common feature of IDILI. It is quite possible that a drug could cause less severe mitochondrial damage that does not result in steatosis or lactic acidosis, but this is unlikely to result in liver failure. If a drug caused damage to mitochondrial proteins instead of DNA, the effects should not be delayed and cumulative because of the relatively rapid turnover of proteins. Milder mitochondrial damage may not directly lead to liver failure, but it could act as a danger signal, and in some patients, this might lead to an immune response that results in liver failure. In fact, even IDILI such as that associated with valproic acid could involve an immune mechanism, and this would explain its idiosyncratic nature. Therefore, mitochondrial damage could be an important component of the mechanism of IDILI, even if it is not sufficient by itself to explain most cases of IDILI.

We are still left with cases of IDILI that do not have characteristics typical of an immune-mediated reaction. In some cases there is no recurrence on rechallenge, such as in the case of isoniazid, or they occur very late as with some cases of troglitazone-induced hepatotoxicity. However, as mentioned above in the case of heparin-induced thrombocytopenia, which is clearly immune-mediated, there is also no immune memory. Furthermore, the delay in onset is actually longest for IDILI that is clearly immune-mediated, i.e., for drug-induced autoimmune hepatitis. For example, minocycline can cause two different types of IDILI: one that is typical of IDILI and occurs after 1–3 months of treatment, and the other that is autoimmune and occurs after more than a year of treatment. Nitrofurantoin and α-methyldopa can also cause typical autoimmune hepatitis, which is characterized by a long delay in onset. A likely explanation for these observations is that most reactive metabolites bind to a variety of proteins, and the pattern is different for different drugs. If the dominant immune response is to a liver protein, it can lead to liver toxicity, and if it is to a skin protein, it can lead to a skin rash. The response can also be to different parts of the drug-modified protein, so that in some cases the major epitope will be the drug, and in other cases the
immune response will be to the native protein. The dominant response will be different in each patient, at least in part, because the T cell receptor repertoire is different for each individual, even in identical twins. If the dominant response has a major autoimmune component, even if it is not classic autoimmune hepatitis, it could lead to a longer delay in onset and possibly lack of immune memory. An autoimmune component could also explain why IDILI could begin a month after the drug had been discontinued and the drug is no longer present or why it sometimes progresses after the drug has been stopped. Even though drug-induced autoimmunity usually resolves rapidly when the drug is discontinued, this is not always the case, and obviously the antigen is still present in an autoimmune reaction.

The liver can be considered to be a lymphoid organ and an important part of the reticuloendothelial system. The liver blood supply comes from both the systemic circulation and the intestine. Each minute, almost one third of the total blood passes through the liver, and about 10^9 peripheral blood lymphocytes can be recruited by the liver in 24 hours. The liver itself contains resident T cells, B cells, natural killer (NK), and natural killer T (NKT) cells. It also contains a large number of macrophages (Kupffer cells), stellate cells, and dendritic cells. These cells are continuously exposed to antigens derived from various sources, such as food, medications, or endogenous toxins. Thus the liver plays an important role in determining how the immune system will respond to different antigens.

A new subtype of helper T cell, the T helper 17 (Th17) cells, which appears to be a major component of the immune response in autoimmune reactions, has been recently identified. A signature cytokine produced by Th17 cells is interleukin (IL)-17, hence the name. If some cases of IDILI have a significant autoimmune component, it would be expected that the patients would have elevated IL-17 levels. In a model of acute halothane hepatotoxicity, anti-IL-17 antibodies attenuated the increase in plasma ALT levels. However, some patients with acetaminophen-induced liver failure, and many did, in fact, have elevated IL-17 levels. However, some patients with acetaminophen-induced liver failure also had elevated IL-17 levels, and it has been found that other innate immune cells such as NK cells and γδ-T cells also produce IL-17. Therefore, we need other methods to study the involvement of Th17 cells and the role of autoimmunity in the mechanisms of IDILI.

Conclusions

The idiosyncratic nature of IDR is most easily explained by an immune mechanism, because even if many people are exposed to an antigen, e.g., pollen, only a proportion will develop an allergic response. By definition, drug-induced autoimmunity is immune-mediated, and those IDRs such as antibody-mediated cytopenias that can be shown to be mediated by specific antibodies are also clearly immune-mediated. Given the characteristics and histology of drug rashes, there is little doubt that most are immune-mediated. The area of greatest controversy is IDILI. Some cases are clearly autoimmune or associated with a generalized hypersensitivity reaction and therefore must be immune-mediated. Some cases of IDILI are either associated with a rapid onset on rechallenge or with anti-drug antibodies or autoantibodies that strongly suggest, but do not prove, that they are immune-mediated. However, there are cases that do not have any of these characteristics. The same drug can cause IDILI that is clearly immune-mediated in one patient, but without the typical characteristics of an immune reaction in another. It is most likely that this represents differences in the type of immune response rather than that some of the reactions are not immune-mediated. Some of the characteristics such as a long delay in onset may be a sign that the IDR has an autoimmune component because cases of clear drug-induced autoimmunity are characterized by a long delay in onset.

The mechanisms by which drugs induce an immune response are not clear. Reactive metabolites appear to be responsible in most, but not all, cases. It is likely that the mechanism is different, at least in detail, with different drugs and in different patients, and many probably involve a combination of mechanisms. In some cases the hapten mechanism is clearly at work. The danger hypothesis is attractive but difficult to definitively prove, and it is unclear whether the danger signal must come from the drug or may come from another source. Although it is unlikely that mitochondrial damage is the sole mechanism for most IDRs involving the liver, mitochondrial damage may produce a danger signal that can help to induce an immune response. With a better mechanistic understanding, it should be possible to develop valid animal models of IDRs, and these models should, in turn, help to increase our mechanistic understanding.

Acknowledgements: JU is the Canada Research Chair of Adverse Drug Reactions. This work was supported by grants from the Canadian Institutes of Health Research.

References

6) Lammert, C., Einarsson, S., Saha, C., Niklasson, A., Björnsson,


81. Abe, R.: Toxic epidermal necrolysis and Stevens-Johnson syndrome: soluble Fas ligand involvement in the pathomechanisms

Copyright © 2011 by the Japanese Society for the Study of Xenobiotics (JSSX)


