

The danger hypothesis applied to idiosyncratic drug reactions

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Purpose of review

Idiosyncratic drug reactions pose a significant clinical threat and hamper drug development. The idiosyncratic nature of these reactions has made mechanistic studies exceedingly difficult, and yet without a better understanding of the mechanisms involved it is unlikely that much progress can be made in dealing with the problem. Several working hypotheses have been used to study these reactions, but none fits all of the characteristics that are observed. Borrowed from immunology, the danger hypothesis has most recently been used to explain several characteristics of these reactions. The present review describes the danger hypothesis and compares it with previous hypotheses to determine how well each fits with the observed characteristics of the reactions.

Recent findings

Slow progress in the field continues and it is important to use new observations, such as identifying T cells that recognize drugs in the absence of reactive metabolite formation, to test and refine the working hypotheses. However, the development of animal models of idiosyncratic drug reactions as well as progress in basic immunology and genomics are likely to accelerate progress in this area in the near future.

Summary

No one model fits the characteristics of all idiosyncratic drug reactions; however, the danger model provides a new perspective and suggests avenues of research that have the potential to increase our ability to predict and prevent such reactions significantly.

Keywords

danger, hapten, idiosyncratic drug reactions, immunotoxicity, reactive metabolites

Introduction

Idiosyncratic drug reactions (IDRs), also known as hypersensitivity reactions, allergic reactions and type B reactions, represent a major source of morbidity and mortality [1–3]. They also markedly increase the cost and uncertainty of drug development. IDRs are defined here as adverse reactions that do not occur in most patients at any dose and do not involve the known pharmacologic properties of the drug. The characteristics of IDRs, such as the delay that is usually observed between starting the drug and the onset of the adverse reaction, strongly suggest that these reactions are immune mediated [4]. However, with several notable exceptions, very little is known with certainty about the mechanisms that are responsible for such reactions. Bioactivation of drugs to chemically reactive metabolites (for in-depth review [5,6]) is thought to be the initial step that leads to IDRs, but the role of these reactive metabolites in immune responses remains highly controversial. Hence, the driving force for much of the research in the field of IDRs has been centred on current concepts in immunology, and more specifically on how drugs can initiate an immune response. The prevailing theories are that the immune system is designed to respond to putative ‘foreign signals’ [i.e. the self–nonself (SNS) model] or to ‘danger signals’ (i.e. the danger hypothesis [7]). The present review discusses these theories with respect to how they contribute to our understanding of IDRs, and discusses their flaws, which have prevented consensus on a unifying mechanism for IDRs.

The classical view of the immune system

For over 50 years a central tenet of immunology has been that the immune system can differentiate between ‘self’ and ‘nonself’ (foreign), and responds to self with tolerance while mounting an immune response against nonself [8]; this is referred to as the SNS model. An important observation that supported this hypothesis was that tissue transplanted from one organism to another (unless genetically identical) was rapidly rejected unless this transplantation occurred before the determination of ‘self’ was complete (i.e. at around the time of birth) [8,9]. Many aspects of the SNS hypothesis are consistent with experimental data (e.g. T cells with a high affinity for antigens to which they are exposed in the thymus are eliminated). However, this hypothesis is not sufficient to explain the complexity of the immune response or specific observations such as lack of response to antigens expressed for the first time during puberty and pregnancy [10].

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Abbreviations

APC	antigen-presenting cell
IDR	idiosyncratic drug reaction
P-I	pharmacological interaction
SNS	self–nonself
TLR	Toll-like receptor

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A fundamental change to Burnet's SNS model [8] arose when Bretscher and Cohn [11] proposed a two cell–two signal model in which a second signal is required to activate helper T cells that are specific to an antigen. Here, signal 1 is the binding or 'recognition' of an antigen by the T-cell receptor on a T cell that is specific for that antigen, and signal 2 is co-stimulation that must also be provided to the T cell in order for it to become activated. Helper T cells can only recognize antigen (signal 1) if it is processed and presented in the groove of the major histocompatibility complex class II by antigen-presenting cells (APCs). Lafferty and Cunningham [12] further refined this two-signal model by finding that APCs are also responsible for providing signal 2. The signal 2 co-stimulatory signals are now known to be mediated by upregulation of signalling molecules on the APC (e.g. B7), which interact with receptors on the T cell (e.g. CD28). An additional layer of complexity in immune activation involves a third signal, signal 3, which is provided by cytokines that are produced by helper T cells and play a role in the balance between an antibody and cell-mediated response [13]. However, the present review focuses on the mechanism by which helper T cells are activated, because this is believed to be the initial step that determines whether the immune system will actively respond to a stimulus or, more commonly, become tolerant. Therefore, signal 3 and other downstream steps in the immune response are not discussed further.

The addition of signal 2 to the SNS model was very important; however, there is no known mechanism by which an APC can differentiate self from nonself so that it can 'know' when to upregulate co-stimulatory molecules such as B7 (signal 2) in order that a response is specific for nonself. Janeway [14,15] proposed that controlling the level of signal 2 can be achieved by the innate immune system. Cells of the innate immune system, such as macrophages, have receptors that recognize patterns associated with pathogens. These receptors were first described in fruit flies and named Toll receptors, but analogous receptors were later found on mammalian cells and referred to as Toll-like receptors (TLRs). TLRs are specific for molecules such as lipopolysaccharide and unmethylated DNA that are unique to pathogens. Interestingly, TLRs also appear to be involved in detecting endogenous danger signals such as heat shock proteins [16,17], and this point is discussed in more detail below. More recently, a new class of receptors for endogenous proteins has been identified and named nucleotide-binding oligomerization domain receptors, but these receptors are not as well defined as TLRs [18]. The existence of TLRs suggests that the innate immune system plays a major role in the initiation of an adaptive immune response; however, this hypothesis still implies that the immune response is

always directed against exogenous 'nonself' signals and consequently does not explain why autoimmunity exists (idiopathic or drug induced). Moreover, the immune system also rejects transplants and some tumours, and this cannot be explained by receptors for pathogens.

Hapten hypothesis

Early in the 20th century, during studies of the problem of recognition of 'nonself' as applied to small xenobiotics, Landsteiner and Jacobs [19] found that small molecules (molecular mass <1000) were unable to induce an immune response unless they bound to macromolecules such as protein. Their experiments involved chemically reactive agents such as 2,4-dinitrochlorobenzene and p-nitrosodimethylaniline. This work is still the basis for the hapten hypothesis, a major working hypothesis for explaining IDRs, and this hypothesis is best supported by the mechanism of allergy to penicillin.

Penicillin and other β -lactam antibiotics are chemically reactive because of the ring strain involved in the four-membered ring. It is clear that β -lactam-induced anaphylaxis involves the induction of immunoglobulin E antibodies that recognize proteins modified by the β -lactam, and this is the basis for skin testing for immunoglobulin E-mediated allergic reactions to penicillin [20]. Specifically, when small amounts of a synthetic adduct between penicillin and polylysine are injected into the skin of most patients with such an allergy, there is a rapid local weal and flare response. A few penicillin-allergic patients do not exhibit such a response, but in most cases this is due to immunoglobulin E antibodies against reactive degradation products of penicillin – the so-called minor determinants.

A second well-studied example is halothane-induced hepatitis. Halothane is metabolized to a reactive trifluoroacetyl halide metabolite that binds to proteins. Most patients who develop hepatotoxicity have antibodies against trifluoroacetylated proteins. Moreover, such antibodies were not observed in patients who did not develop idiosyncratic toxicity when exposed to halothane [21,22]. In addition, in a guinea pig model, T cells from halothane-treated animals proliferated on exposure to trifluoroacetylated proteins [23].

A third example is tienilic acid, a diuretic that causes idiosyncratic hepatotoxicity in some patients and hence has been withdrawn from the market. It is metabolized to a reactive metabolite by cytochrome P450 isoforms, and because of its very high reactivity the major proteins that this metabolite binds to are P450s [24]. Patients who develop tienilic acid-induced hepatotoxicity have antibodies against the same P450 to which the metabolite covalently binds [25].

Many drugs form several reactive metabolites, but in general there are no clear data that link the formation of a specific reactive metabolite (and hapten formation) to the induction of the IDR associated with the corresponding drug. Therefore, finding a reactive metabolite of a drug does not prove that this reactive metabolite is responsible for the idiosyncratic reactions that are associated with the drug. The SNS model, with a reactive metabolite acting as a hapten to render a self-protein foreign, is depicted in Fig. 1a.

One observation that calls the hapten hypothesis into question is that many drugs form reactive metabolites and yet are not associated with a significant incidence of IDRs. The best studied of these examples is acetaminophen. Much is known about the imidoquinone metabolite it forms, and there are many data to indicate that this reactive species is responsible for the liver toxicity caused by overdoses of this drug [28]; however, acetaminophen is rarely associated with IDRs.

There are three possible reasons why drugs that form reactive metabolites may not cause IDRs. One is simply that the level of covalent binding of these drugs may be too low. In the case of acetaminophen, at therapeutic concentrations of the drug the reactive metabolite is efficiently scavenged by glutathione and the amount of covalent binding is low [29]. Although IDRs are said to be dose independent, this is clearly not true. Most individuals will not have an IDR at any dose of a drug, but among those individuals who are at risk there is a dose-response relationship. This relationship may be shifted to the left of the dose-response curve for the desired pharmacological effect so that a person who is allergic to penicillin can have an allergic reaction at a subtherapeutic dose. However, a dose can always be found that is not sufficient to cause an IDR, and this is the basis for desensitization of patients (i.e. starting with a low dose and gradually increasing it, often leading to tolerance of a therapeutic dose in a previously allergic individual) [30]. In fact, in some cases, such as hydralazine-induced lupus, the dose-response relationship for toxicity is in the same range as that for the desired pharmacological effect [31]. Furthermore, drugs with a low therapeutic dose (i.e. below 10 mg/day) are unlikely to be associated with a high incidence of IDRs [32]. Unfortunately, it is not possible to quantify the amount of covalent binding in the target organ of toxicity in humans *in vivo*. Therefore, any attempt to correlate the amount of covalent binding with the risk that a drug will cause IDRs must involve crude extrapolations from experiments in which covalent binding can be quantified. We simply do not know whether a good relationship exists between the amount of covalent binding and risk for an IDR, but there are indications that at least a crude relationship exists. For example, in the series of

halogenated anaesthetics – halothane, isoflurane and desflurane – the risk for IDRs correlates with the amount of reactive trifluoroacetyl halide metabolite formed [33].

A second likely reason why there may not be a good relationship between the formation of a reactive metabolite and the development of an IDR is that covalent binding to some proteins may be more likely to lead to an IDR than binding to other proteins. It has been suggested that modification of serum proteins is less likely to lead to an idiosyncratic reaction than binding to membrane proteins [34]. Unfortunately, very little is known about the identity of the proteins that are modified by the reactive metabolites of most drugs, and so it is difficult to know what relationships may exist. A related hypothesis proposed by Zinkernagel and co-workers [35,36] is that T cells react against a cell-associated antigen that reaches secondary lymphoid organs in sufficient amounts (but not too much) and in a localized (and not in a generalized) manner. Thus, applied to acetaminophen, only small amounts of haptenated proteins may be formed, and the low concentrations that reach the secondary lymphoid organs may result in immunological ignorance.

Finally, in many cases, the covalent binding of reactive metabolites may not be a crucial step or may be necessary but not sufficient to induce an IDR. These final possibilities are discussed below.

Pharmacological interaction hypothesis

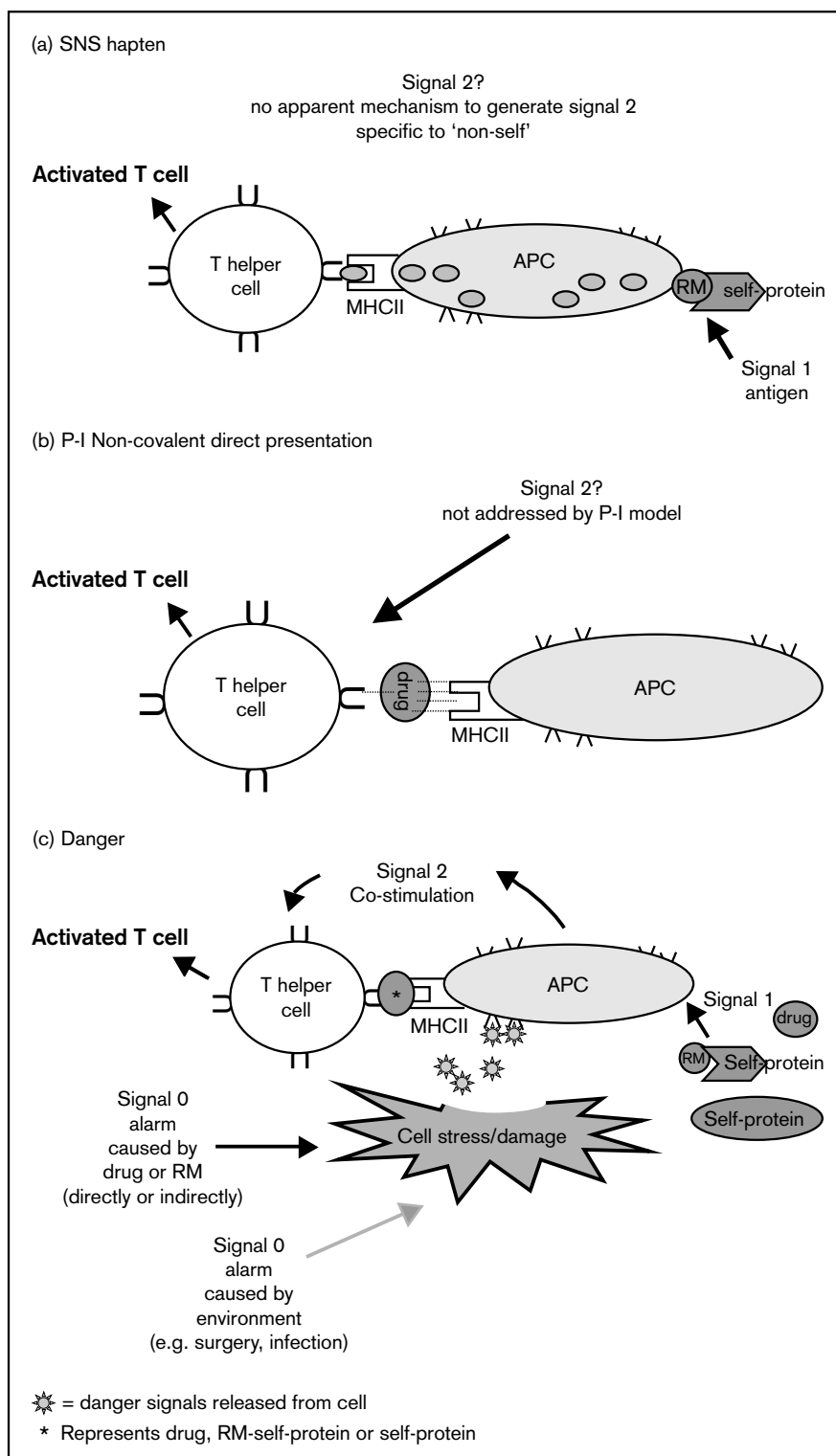
Although the hapten hypothesis provides a good model for penicillin-induced allergic reactions, the requirement for covalent binding of drugs or their metabolites has not been demonstrated for most IDRs. Pichler and co-workers [37,38] isolated T cells from patients that proliferate in response to drugs, such as sulphamethoxazole, in the absence of covalent binding. This suggests that hapten formation is not necessary for the induction of some IDRs and led Pichler [26^{••},47^{••}] to propose the pharmacological interaction (P-I) hypothesis. In brief, this hypothesis proposes that small unreactive drugs can behave like super-antigens and bind reversibly to major histocompatibility complex and the T-cell receptor molecules, leading to an immune response directed against the drug alone. Even though T cells from patients (originally from a polyclonal pool) with an IDR may be able to recognize free drug, there is as yet no evidence that such an interaction can, by itself, induce an IDR [32,39^{••}]. The P-I model is depicted in Fig. 1b. This model does not address the issue of what controls the level of signal 2.

The danger hypothesis

Matzinger [7] proposed an alternative hypothesis in which the immune system need not differentiate

Figure 1. Initial response to a drug

Three models of the initial response to a drug [or its reactive metabolite (RM)] that determine whether the response will be active (activation of helper T cells) or tolerant (lack of activated helper T cells). (a) Self-nonself (SNS) model: the immune system recognizes drug-modified self-proteins as foreign. (b) Pharmacological interaction (P-I) model: drug associates noncovalently with the major histocompatibility complex (MHC)–peptide complex on an antigen-presenting cell (APC) that then stimulates specific T cells. (c) Danger model: an immune response can only be mounted when APCs receive activating signals from stressed or damaged cells. Parts (b) and (c) are adapted from Pichler [26••] and Matzinger [27], respectively.



between 'self' and 'nonself', or even between 'infectious-nonself' and 'noninfectious-self', as suggested by Janeway [14] (i.e. the SNS model), but rather between

harmless and dangerous. This is referred to as the danger hypothesis. Matzinger argued that it would be difficult, unnecessary and potentially damaging to differentiate

between 'self' and 'nonself'. Why would the immune system respond to something that was not causing any damage when an immune response itself could cause damage to the organism? A fundamental difference between the SNS model and the danger hypothesis is that the major stimulus for the initiation of an immune response comes from endogenous rather than exogenous signals and is controlled, not by cells of the immune system, but rather by the damaged tissue itself [27]. Matzinger emphasized the control of the immune response by damaged tissue by referring to the danger signal as signal 0. She proposed that cells that die by necrosis, or are simply stressed, produce danger signals that induce signal 2.

Consistent with this hypothesis, injured or dead cells have been found to act as adjuvants resulting in the upregulation of co-stimulatory factors such as B7 and CD40 on APCs as well as leading to the activation of T cells. The range of factors that can act as danger signals is unknown. It has been suggested that cytokines such as tumour necrosis factor- α and interleukin- 1β are important danger signals [40]; however, stressed or dead cells were observed to stimulate T cells in the absence of protein synthesis [41]. This suggests that some danger signals are constitutively present and need only be released.

The danger model is depicted in Fig. 1c. It does not address the issue of whether covalent binding of drug is necessary for the induction of an immune response and, in fact, suggests how a drug can induce an autoimmune reaction. The danger signal could be caused either by the drug (or its reactive metabolite) or by some environmental factor such as surgery or an infection.

There has been criticism that the revolutionary nature of the danger hypothesis has been overstated [42]. In fact, the danger hypothesis is probably best viewed as representing one aspect of the control of the immune response. Specifically, if the issue is why autoimmunity is not more common, it is best viewed through the 'self/nonself' paradigm, in which T cells with a high affinity for 'self' proteins are normally eliminated, thus limiting signal 1 involving self. Likewise, in trying to understand the response of the immune system to pathogens, it is hard to ignore TLRs specific for exogenous foreign agents such as lipopolysaccharide that upregulate signal 2. However, in trying to understand what controls signal 2 involving a noninfectious agent, such as that believed to be involved in IDRs, the danger hypothesis is a very important addition. In fact, Matzinger [43•] argued that all TLRs were meant to detect endogenous molecules, and as an added bonus they recognize pathogens, while in some cases pathogens may have evolved to use these receptors to their own benefit. This view of TLRs has yet to be demonstrated.

Relevance of the danger hypothesis to idiosyncratic drug reactions

It is currently impossible to predict with any degree of accuracy which drugs will be associated with a high incidence of IDRs or which patients will be affected. If, as appears to be the case, such reactions are immune mediated, an understanding of what factors are involved in the initiation of an immune response is central to achieving such predictive abilities. If danger is a major factor in the initiation of an IDR, then understanding the nature of the danger signal is of obvious importance. There are several possible scenarios for the mechanisms of IDRs, and without more definitive data we are limited to seeing how each fits with the observed characteristics of such reactions.

As indicated above, not all drugs that form reactive metabolites are associated with a significant incidence of IDRs, and one possible reason for this observation is that covalent binding is necessary but not sufficient to induce an IDR. Most foreign proteins do not induce a significant immune response on their own (i.e. signal 2 is required). This is what led Janeway to refer to adjuvants as the 'immunologist's dirty little secret', and therefore it seems that if a reactive metabolite could only act as a hapten then it would be unlikely to induce an immune response or an IDR [44]. Some reactive metabolites can clearly cause cell damage or stress, and this may be an important factor in determining whether a drug will cause an IDR. For example, drugs such as halothane and isoniazid cause a transient rise in transaminases in a high percentage of patients, and this presumably represents a significant danger signal [45,46]. If the P-I hypothesis is true for some IDRs then there is no need to form a hapten, and the induction of a danger signal could be the dominant characteristic of the drug (or reactive metabolite) that determines the likelihood of it causing an IDR. The chemical functional groups on drugs that are most commonly associated with adverse drug reactions (e.g. aromatic amines, nitro groups and quinines) redox cycle and generate reactive oxygen species, which could generate a danger signal. The IDRs studied the most extensively by Pichler [47•] with regard to the P-I hypothesis were caused by sulphamethoxazole, which is an aromatic amine. It seems very likely that the oxidation of this group to a reactive metabolite is related to the association of sulphamethoxazole with a high incidence of IDRs; however, this association may be due to the reactive metabolite causing a danger signal rather than acting as a hapten (for extensive reviews [39•,48•]).

Drug-induced autoimmunity is best explained by the danger hypothesis. For example, α -methyldopa can cause haemolytic anaemia that is mediated by antibodies against red cells (specifically against Rh factor). These

antibodies bind to red cells in the absence of drug and so they are true autoantibodies [49,50]. When the drug is discontinued the haemolysis resolves in about 1 week, although some antibodies can persist for a year or more. Why does the immune response stop when the antigen (red cell) is still present? This is difficult to explain with the SNS model. However, using the danger model, the drug, which is a catechol and can redox cycle, may generate a danger signal. When the drug is discontinued the danger signal also stops and this would be expected to lead to a resolution of the autoimmune reaction.

If the presence of a danger signal is required for the induction of an IDR, a very important question is whether the signal is usually caused by the drug or by some other unrelated environmental factor. In principle, a generalized or systemic danger signal could be damaging to an organism, and therefore it is likely to be uncommon except in life-threatening infections (e.g. HIV) or following major surgery. It has been noted many times that the risk for an IDR is higher in patients who are HIV positive [51]. Likewise, the risk of procainamide-induced agranulocytosis appears to be 10-fold higher in patients whose treatment was started immediately after open heart surgery than in nonsurgical patients [52]. However, most IDRs do not occur in such a context. A more localized danger signal caused by reactive metabolites in cells that are the target of the IDRs seems like a more plausible mechanism for the upregulation of signal 2 leading to an immune response [35]. Such considerations are also relevant to what types of reactive metabolites are most likely to cause IDRs. If IDRs are due to the reactive metabolite acting as a hapten, then it is essential for the reactive metabolite to covalently bind. However, if IDRs are due to the generation of a danger signal then there is no such requirement. In contrast to electrophiles, which usually covalently bind to cellular nucleophiles, free radical reactive metabolites are much more likely to abstract a hydrogen atom from lipids and other molecules, leading to lipid peroxidation, and do not usually covalently bind. Thus, free radicals are more likely to generate a danger signal than to act as haptens. Furthermore, it is possible that some drugs can cause a danger signal in the absence of a chemically reactive species.

As mentioned above, acetaminophen is a bit of a puzzle because it covalently binds to cell proteins and causes cell damage. However, as discussed above, its low risk for causing IDRs may simply be due to very efficient detoxification of the reactive metabolite, except in an overdose, where the antigen (signal 1) may not be sustained long enough to induce an immune response. It is more difficult to understand why anticancer agents are not associated with a higher incidence of IDRs because they both covalently bind (signal 1) and cause cell

damage (signal 2). Most chemotherapeutic agents kill by inducing apoptosis and, according to the danger model, antigens generated from apoptotic cancer cells (but not necrotic ones) may be scavenged by macrophages, thereby releasing very few alarm signals [53,54]. However, recent findings show that apoptotic cells also release danger signals that are capable of stimulating an immune response [41]. Moreover, these agents are quite toxic and must cause cell stress. For example, ionizing radiation, anthracyclines and bleomycin generate reactive oxygen species and could certainly induce signal 2 [55,56]. In support of this view, there are reports of increased incidence of phenytoin-induced Stevens–Johnson syndrome following radiation therapy [57,58]. Furthermore, it has been shown that oxidative stress inhibits the phagocytosis of apoptotic cells, which in turn presumably ‘allows’ the release and ‘detection’ of the danger signals [59]. Finally, at the other end of the spectrum is tienilic acid, which covalently binds almost exclusively to cytochrome P450 [25,60], and it is not clear how such binding would cause a danger signal; yet, it is associated with a significant incidence of idiosyncratic liver toxicity.

Conclusion

From this discussion it is clear that all IDRs do not easily fit any one hypothesis. If there is one thing that characterizes the immune system it is that control of the immune response is exceedingly complex. Although this makes prediction of IDRs difficult, it is probably essential to our survival. If immunoregulatory pathways were simple, then pathogens would have evolved many ways to circumvent them. However, despite the complexities of the immune system, there may be patterns of cell stress that predict a drug candidate’s potential to elicit IDRs. It is almost certain that studies of IDRs will have to be performed *in vivo*, because in-vitro experiments cannot hope to replicate the complexity of an intact organism. We have found preliminary evidence that drugs associated with a high incidence of IDRs do lead to upregulation of protective enzymes [61]. However, until more in-depth studies have been performed it is too early to draw any conclusions. Such studies must also include drugs that form reactive metabolites but are not associated with IDRs, as well as drugs such as tienilic acid that do cause IDRs but would seem less likely to cause a danger signal. Despite the complexities involved in such studies, the possible reward of developing better tests to screen drug candidates for their potential to cause IDRs makes them well worth the effort.

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