LEADING ARTICLE

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Immunotoxicology
Role in the Safety Assessment of Drugs

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Abstract

The immunotoxic effects of drugs are divided into immunosuppression, immunostimulation, hypersensitivity and autoimmunity. The major adverse consequences of immunosuppression are infectious complications and virus-induced malignancies. Flu-like reactions, more frequent autoimmune diseases and hypersensitivity reactions to unrelated allergens, and inhibition of drug-metabolising enzymes are the adverse effects related to immunostimulation. Hypersensitivity reactions are the most frequent immunotoxic effects of drugs. They include immune-mediated ('allergic') and non immune-mediated ('pseudoallergic') reactions. Drug-induced autoimmune reactions, either systemic or organ-specific, are seemingly rare. A review of drug-induced immunotoxic effects demonstrates that immunotoxicity is a significant cause of morbidity and even mortality.

As immunotoxicologists have long focused on immunosuppression, the nonclinical immunotoxicity safety assessment of unexpected immunosuppression is based on a number of relatively well standardised and validated animal models and assays. However, there is no general consensus regarding the minimal requirement for this assessment. Many different assays can be used to extend the assessment case by case. Few animal models and assays have been validated for use in the nonclinical safety assessment of unexpected immunostimulation. The situation is worse regarding the prediction of hypersensitivity and autoimmune reactions. Our limited understanding of the molecular and cellular mechanisms of immunotoxicity accounts, at least partly, for this situation.

Recent guidelines for the immunotoxicity safety assessment of drugs, even though conflicting on several points, will serve as an impetus not only to refine current animal models and assays, but also to search for better alternatives. The new data generated will have to be interpreted and extended to animal species other than just rodents. Likewise, animal results will have to be compared with findings in humans. The search for immunological endpoints that can be used in several animal species and in humans will therefore become essential. Specific endpoints and clinical criteria that can be included in clinical trials to further investigate the potential for immunotoxicity of new drugs will have to be defined.

Because immunotoxicity plays a key role in drug-induced adverse effects, the role of immunotoxicology in drug safety assessment is indisputable and the systematic nonclinical as well as clinical immunotoxicity assessment of every new drug is deemed essential.

Over the past decades, immunotoxicity has progressively emerged as a frequent cause of drug-induced adverse effects resulting in quite a few drug withdrawals from the market. Shortly after the introduction of potent immunosuppressive drugs into the clinic, infectious complications and more frequent lymphoproliferative disorders were described in organ transplant patients.[1] These clinical findings served as an impetus for experimental studies focusing on immunosuppression.[2] However, even though immunosuppression has long been synonymous with immunotoxicity, it is nowadays widely accepted that immunotoxic effects encompass four distinct categories, namely immunosuppression, immunostimulation, hypersensitivity and autoimmunity. Indeed, each broad category results in markedly different adverse effects and specific approaches must be used to predict and investigate these effects, both in animal studies and in humans.[3] The delineation of these four categories reflects a growing awareness that very diverse adverse effects induced by xenobiotics, including pharmaceuticals, are immune mediated. This, in turn, has led drug regulatory authorities in Europe[4] and the US[5] to release guidelines for the immunotoxicity safety assessment of new drugs.

This paper focuses on the major immunotoxic effects of drugs and the current modalities of their immunotoxicity safety assessment. As many issues are still unsolved, future avenues of research are also considered.

1. Immunotoxic Effects of Drugs

1.1 Immunosuppression

Immunotoxic effects related to immunosuppression are fairly well known owing to the clinical experience gained with the therapeutic use of immunosuppressive drugs. Two types of adverse effects are prominent, namely infectious complications and virus-induced malignancies.

1.1.1 Infectious Complications

In immunocompromised patients, infections are typically frequent, often severe and relapsing, and sometimes atypical, e.g. opportunistic infections.[6] Although all types of pathogens, including bacteria, viruses, fungi and parasites can be involved, some are more common, such as *Listeria monocytogenes*, mycobacteria, human herpes viruses, the cytomegalovirus and Epstein-Barr virus. Infections of the respiratory and gastrointestinal tracts are more frequent, but the CNS and the skin are also often affected.

Even though the most comprehensive data concern organ transplant patients,[7,8] infectious complications have also been described in patients receiving corticosteroid therapy[9] or in patients with varied autoimmune diseases treated with low-dose immunosuppressive drugs, such as methotrexate,[10] or the anti-tumour necrosis factor (TNF)-α drugs infliximab and etanercept.[11] There is limited information whether drugs that are not potent immunosuppressants, but impair immune functions only moderately, can also induce more frequent infections. Because infections are clinically and microbiologically unremarkable in this context, epidemiological studies are needed to identify an increased risk of infections in exposed compared with unexposed patients as was, for instance, demonstrated in Japanese patients with Yusho disease caused by the ingestion of rice oil contaminated with immunosuppressive polychlorinated biphenyls.[12]

1.1.2 Virus-Associated Malignancies

Malignancies are the second major type of clinical complications seen in patients treated with immunosuppressive drugs. Many retrospective and prospective studies have provided evidence of an increased risk of lymphoproliferative disorders in organ transplant patients.[13] Although no differences were demonstrated depending on the immunosuppressive drug(s) used, malignancies are more frequent when immunosuppression is more profound. Although lymphoproliferative disorders attracted much attention, skin cancers are actually more frequent in organ transplant patients.[14] Malignancies caused by immunosuppressive drugs are often associated with latent viral infections and it is widely accepted that impaired cellular functions trigger the proliferation of dormant Epstein-Barr

virus within B lymphocytes, resulting in B lymphomas.[15] The role of the human herpesvirus 8 in the pathogenesis of Kaposi's sarcoma, the incidence of which is extremely high in organ transplant patients, has been underscored.[16]

Lymphoproliferative disorders have also been reported in patients treated with low-dose immunosuppressive drugs, such as methotrexate,[17] or anti-TNF-α drugs.[18] Regression was usually observed following discontinuation of treatment. These findings support the hypothesis of a contributing role of latent infections and demonstrate that mild to moderate immunosuppression can result in lymphoproliferative disorders.

1.2 Immunostimulation

Although the immune-mediated adverse effects of immunostimulatory drugs have long been recognised,[19] attention to the immunotoxic effects of these drugs was fueled by the introduction of recombinant cytokines and other immunotherapeutic drugs into the clinical setting.[20]

1.2.1 Flu-Like Reactions

Moderate hyperthermic reactions (~38–38.5°C) with chills, arthralgias and malaise have been described following initiation of treatment with a number of immunostimulatory drugs.[19] These reactions can also be due to vaccination, particularly recall injections. Flu-like reactions are usually easily prevented or reversed by the administration of paracetamol. In some cases, the reaction is much more severe with marked hyperthermia (>40°C), cardiovascular and neurological disturbances, including marked hypotension leading to cardiovascular collapse or myocardial ischaemia, confusion, obnubilation and seizures. The terms acute or toxic cytokine syndrome are preferred to refer to these severe reactions.

The mechanism is not fully elucidated. It no longer appears to be related to a direct effect of interferons or recombinant interleukin (IL)-2, but rather to be the consequence of the acute release of fever-promoting factors in the hypothalamus, such as eicosanoids, IL-1 and TNF-α. Although the acute release of fever-promoting factors can be attributed to the immunostimulatory properties of recombinant cytokines,[21] a direct releasing effect is also possible as clinically similar reactions have been reported following the intravenous infusion of monoclonal antibodies that are immunosuppressive.[22]

1.2.2 Autoimmune Diseases

There are many case reports of autoimmunity developing in patients following treatment with the interferons and recombinant IL-2.[23] Whereas nearly all types of autoimmune diseases have been described in patients with chronic hepatitis C treated with interferon-α, autoimmune thyroiditis is by far the predominant immune disease in cancer patients treated with recombinant IL-2, and patients treated with either interferon-β or -γ develop autoantibodies, but no overt autoimmune disease. Various theories have been proposed to account for these findings, but none is firmly established. Anyway, this suggests that immunostimulation can be associated with more frequent autoimmunity, possibly in genetically predisposed patients.

1.2.3 Hypersensitivity Reactions to Unrelated Allergens

There are rare reports in the literature that immunostimulatory drugs can result in more frequent hypersensitivity reactions to unrelated allergens.[19] Interestingly though, several studies showed that IL-2-treated cancer patients are at a greater risk of developing hypersensitivity reactions to radiocontrast media[24] or chemotherapeutic agents.[25]

1.2.4 Inhibition of Drug-Metabolising Enzymes

An overlooked adverse effect of immunostimulatory drugs is their potential to inhibit drug-metabolising enzymes.[26] The loss of cytochrome P450 enzymes has been shown to predominate at the level of gene expression. There is a large body of experimental data to substantiate the possible adverse effects of some immunostimulatory drugs and several human studies showed a negative impact of interferon-α, influenza vaccines and BCG potentially resulting in clinically significant drug interactions.[27]

1.3 Hypersensitivity

Hypersensitivity reactions are, by far, the most frequently reported immunotoxic effects of drugs in human beings. Epidemiological studies are rare so that the incidence of these reactions is not known accurately. Many mechanisms are involved, but few have been fully elucidated. They can, however, be divided into immune-mediated and non immune-mediated mechanisms.

1.3.1 Immune-Mediated Hypersensitivity Reactions

Immune-mediated hypersensitivity ('allergic') reactions are caused by a specific immune response directed against the parent molecule or metabolites. The response involves specific antibodies, such as IgE, IgG and IgM, or T-lymphocytes. These reactions have been classified into four pathogenic mechanisms (type I–IV reactions), but the so-called Gell and Coombs classification is clearly no longer valid to encompass all described immune-mediated reactions induced by drugs.[28] These reactions can indeed affect nearly every organ or tissue, either acutely or chronically, and several mechanisms can concomitantly be involved. Because most drugs are small molecules with low or no chemical reactivity, the view has long been held that they could induce immune-mediated reactions only by acting as haptens after the strong binding of reactive intermediates to carrier proteins. Although there is a large body of evidence to support this view,[29] a direct binding to T cells interfering with the normal functioning of the immunological synapse was also demonstrated.[30] The lack of clear understanding of the mechanism involved in a given drug-induced immune-mediated hypersensitivity reaction explains current difficulties of their clinical diagnosis.[31]

1.3.2 Non Immune-Mediated Hypersensitivity Reactions

Immune-mediated hypersensitivity reactions only develop in patients who had a prior sensitising contact to the causative drug. The finding that patients with no prior contact can nevertheless develop clinically similar reactions led to the hypothesis that some hypersensitivity reactions can involve non immune-mediated mechanisms. The term 'pseudoallergy' was coined to refer to these reactions.[32] Clinical similarities are due to the involvement of the same mediators, especially those released in IgE-mediated reactions.[33] At least, three mechanisms have been clearly identified. Histamine stored in granules of mast cells and basophils can be directly released by a number of drugs including opioid analgesics[34] and vancomycin.[35] Another important mechanism is the direct activation of the complement cascade as seen in pseudoallergic reactions induced by the pharmaceutical solvent Cremophor El[36] and to some extent radiocontrast media.[37] Importantly, a careful examination of patients with reactions involving either direct histamine release or complement activation present with clinical features that are not typically those of IgE-mediated (anaphylactic) reactions. These include flush, redness of the skin, abdominal pain, cough, mild tachycardia and unchanged, if not mildly increased blood pressure. Finally, intolerance to NSAIDs, which is characterised by asthma, rhinitis, urticaria, angioedema and/or shock, is certainly not immune mediated, but cyclo-oxygenase inhibition and the compensatory overproduction of leukotrienes cannot explain all clinical signs and symptoms.[38]

1.4 Autoimmune Reactions

In sharp contrast with the high incidence of autoimmune diseases in the general population, reports of drug-induced autoimmune reactions are rare. Our review of all drug-induced adverse effects recorded by the French Pharmacovigilance network between 1991 and 1994 found only 0.2% of possible autoimmune reactions.[39] However, underreporting is a possible bias that should not be overlooked. Importantly, drug-induced autoimmune reactions must be differentiated from autoimmune diseases that are reportedly more frequent in patients treated with certain drugs, such as therapeutic cytokines (see section 1.2.2). In general, a single drug induces only one type of autoimmune reaction in any patient, whereas immunostimulatory drugs are associated with many different autoimmune diseases. The underlying mechanisms are not precisely known in

either situation.[40] Drug-induced autoimmune reactions, like spontaneous autoimmune diseases, are either systemic or organ-specific.

1.4.1 Systemic Autoimmune Reactions

Although relatively rare, systemic reactions are by far the most frequently reported drug-induced autoimmune reactions. However, the majority of case reports involved quite a small number of drugs. They consist of a variety of ill-defined pathological conditions that have received denominations based on more or less close similarities with their spontaneous counterparts, e.g. the lupus syndrome,[41] scleroderma-like reactions,[42] or serum sickness-like diseases.[43] In fact, a close scrutiny of clinical and biological changes demonstrates that marked differences do exist so that most drug-induced reactions do not meet the criteria required for the diagnosis of the spontaneous diseases.

1.4.2 Organ-Specific Autoimmune Reactions

Drug-induced organ-specific autoimmune reactions are extremely rare. Only very few drugs have been reported to induce such reactions and there is an apparent, but unexplained, selectivity of the reaction induced by a specific drug. For instance, penicillamine is by far the most frequent cause of drug-induced myasthenia,[44] and there are extremely few reports of other autoimmune reactions associated with penicillamine treatment. A major difference between drug-induced systemic and organ-specific autoimmune reactions is that the latter are very closely similar, both clinically and biologically, to the spontaneous disease.

2. Current Modalities for the Nonclinical Immunotoxicity Safety Assessment of Drugs

Based on this overview, the conclusion can be made that drug-induced immunotoxic effects are a significant cause of morbidity and mortality. Thus, a systematic assessment of the immunotoxic potential of every new drug is deemed absolutely essential. Drug regulatory authorities have been very slow in making such a requirement a part of the overall safety assessment of drugs,[4,5] and immunotoxicity was only very recently considered a priority in the ongoing International Conference on Harmonisation (ICH) process. Current modalities of immunotoxicity safety assessment will be described separately for each broad category of immunotoxic effects.

2.1 Nonclinical Safety Assessment of Unexpected Immunosuppression

As already stated, immunosuppression has long been the sole focus of immunotoxicologists. This has resulted in the current unsatisfactory status of the evaluation of the other categories of immunotoxic effects. A number of animal models and assays have been relatively well standardised and validated so that it can reasonably be claimed that drug candidates with unexpected immunosuppressive effects can be detected by nonclinical studies, assuming that adequate efforts and resources are devoted to this assessment. It is beyond the scope of this paper to review all available modalities, but the general principles can be discussed.

2.1.1 Minimal Requirements

There is a general consensus that unexpected immunosuppressive effects of drugs can – or should – be predicted in short-term repeated-dose toxicity studies in rodents. Typically, the tested drug is administered daily by the intended route of human administration for 28 days. The rat is the preferred, if not the recommended[4] species. Other animal species including nonhuman primates and different dosing regimens can be used depending on the expected modalities of therapeutic use. In order to avoid immune changes induced by the general toxicity of the tested drug, it is recommended that doses that cause overt toxicity are not used.

Histological examination of the main lymphoid organs including the thymus, spleen, bone marrow, main lymph nodes and Peyer's patches is routinely performed, but special care must be paid to the technical skill of the pathologist to assure the reliability of results.[45] After a long debate, it is now widely accepted among immunotoxicologists that at least one immune function assay is necessary,[46] even though not all regulatory authorities share this view.[5] There is, however, a general consensus that,

if performed, this function assay should be a T-dependent antigen response assay. The plaque-forming cell (PFC) assay has been the most frequently used assay in mice[47] and rats.[48] Although it has been extensively validated, this is a time-consuming assay and results show marked inter-individual variability. Keyhole limpet haemocyanin (KLH) is increasingly the preferred antigen and the specific antibody response is measured by ELISA.[49] Any drug that induces no significant changes in any of these parameters is considered negative and no further safety assessment is normally required.

2.1.2 Extended Evaluation

When a drug induces significant and consistent changes, additional studies should be performed. In addition, the US FDA[5] requires extended safety assessment for drugs that are intended for use in HIV patients or pregnant women, or accumulate in lymphoid tissue.

The selection of animal models and assays is preferably made case by case depending on the nature and magnitude of the early observed changes, the pharmacological properties of the drug or chemical similarities with known immunotoxicants.[50] It is noteworthy that the concept of tiered protocols including a predefined battery of tests to be performed as a screen (tier I) or for mechanistic purpose (tier II),[51] is no longer advocated.

Delayed-type hypersensitivity or lymphocyte proliferation assays can be used interchangeably to measure cellular immunity as they are equally sensitive. Lymphocyte subset analysis and natural killer (NK) cell activity can also be considered. It should be noted that these two assays have been proposed as an alternative to a T-dependent antigen response assay for first-line immunotoxicity safety assessment by the European drug regulatory authorities,[4] but not by the FDA.[5] Innate immunity, e.g. macrophage function or neutrophil phagocytosis, can also be investigated. In fact, there is no limit to the list of animal models and assays that can be used, except their necessary compliance to the rules of good laboratory practices and the validation of results using reference compounds. Finally, host resistance models, such as experimental infections,[52] can be useful as changes in the histology of the lymphoid organs or in immune functions may not be sufficient to demonstrate that the drug is likely to result in significant immunotoxic effects when administered to patients.

2.2 Nonclinical Safety Assessment of Unexpected Immunostimulation

Because potent immunostimulatory drugs have only recently been introduced into the clinic, the nonclinical safety assessment of unexpected immunostimulation poses many unsolved questions. Changes in the histology of the lymphoid organs, e.g. splenomegaly and lymph node hyperplasia, have been observed in rodents treated with immunostimulatory oligonucleotides.[53] In general, immune function assays that are used to detect unexpected immunostimulation are those used to detect unexpected immunosuppression. There are, however, few published data to support this approach and it is not known whether a strong immune response elicited by an optimal dose of an antigen can still be increased by an immunostimulatory drug, or whether a sub-optimal dose of the antigen should be used instead. Because the expected clinical consequences of immunostimulation are totally different from those related to immunosuppression, other host resistance models should be used. The utilisation of animal models of experimentally induced autoimmunity or autoimmunity-prone animals is a logical step, but the predictability of these models remains to be ascertained.[54] In fact, the only adverse effects of unexpected immunostimulation that can be reasonably investigated during nonclinical safety assessment are the inhibition of drug-metabolising enzymes, which does not require a specific approach, and the cytokine-releasing properties involved in flu-like reactions or acute cytokine syndrome, which can be tested in *in vitro* assays.[55]

2.3 Nonclinical Safety Assessment of the Potential for Inducing Hypersensitivity Reactions

Despite the long use of contact sensitisation assays in guinea pigs and more recently in mice,[56] a

major hurdle to the development of hypersensitivity animal models and assays has been the belief that immune-mediated hypersensitivity reactions could not be reproduced in animals. This widely held belief, however, was not shared by all, including leading toxicologists.[57] Overall, predicting drug-induced immune-mediated hypersensitivity reactions is impossible in most instances.[58] The local lymph node assay (LLNA)[59] is widely accepted by regulatory authorities to predict the contact sensitising potential of chemicals. This method, however, is not applicable to the majority of systemically administered drugs since they are rarely soluble in the volatile solvents that the assay requires. Although contact sensitisation can be considered as reflecting the sensitisation potential of a given chemical, comparison of results from contact sensitisation assays in the guinea pig to the clinical experience related to systemic drug-induced immune-mediated hypersensitivity reactions led to conflicting conclusions.[60,61]

In contrast to immune-mediated hypersensitivity reactions, non immune-mediated reactions are more accessible to nonclinical safety assessment. Thus, the direct histamine-releasing properties of drugs can be detected using either mast cells *in vitro*, or following intravenous injection, particularly in dogs whose mast cells and basophils seem to be exquisitely sensitive to histamine-releasing chemicals.[62] Recent flow cytometry techniques using human basophils can also be used to explore immune-mediated as well as non immune-mediated hypersensitivity.[63] Complement activation can also be demonstrated *ex vivo*, preferably in human systems[64] because of marked differences between conventional animal species and man.

2.4 Nonclinical Safety Assessment of the Potential for Inducing Autoimmune Reactions

The prediction of drug-induced autoimmune disorders is beyond reach of nonclinical immunotoxicity safety assessment.[65] The search for autoantibodies in conventional repeated-dose toxicity studies often proved unsuccessful.[66] The popliteal lymph node assay (PLNA) is the only assay that has been extensively studied.[67] However, this assay cannot yet be recommended for routine nonclinical safety assessment owing to the use of variable experimental procedures among laboratories and the lack of comprehensive validation. In addition, the PLNA was proposed to detect systemic drug-induced autoimmune reactions and is not applicable to organ-specific autoimmune reactions that are beyond prediction at the present time.

3. Perspectives in the Immunotoxicity Safety Assessment of Drugs

From the above discussion, the conclusion can be made that currently used procedures for the nonclinical immunotoxicity safety assessment of drugs are far from perfect. With the exception of unexpected immunosuppression, very few reliable animal models or assays can be used. Hypersensitivity reactions, although the most frequently reported immunotoxic effect of drugs in humans, cannot be predicted in many instances. To meet the goal of predicting immunotoxicity prior to drug approval, much progress is necessary.

3.1 Possible Improvements in Nonclinical Immunotoxicity Safety Assessment

The pharmaceutical industry has long been, and is still, hesitant regarding the routine introduction of nonclinical immunotoxicity studies for the safety assessment of drugs. It is beyond doubt that our poor understanding of most molecular and cellular immunotoxicity mechanisms is currently a major limitation to the development of reliable animal models and assays to predict the potential of new drugs for inducing immunotoxic effects. In addition, the validation of most current animal models and assays is based on results obtained either with a few prototypic reference drugs, such as ciclosporin (cyclosporine) and azathioprine,[68,69] or essentially with industrial and environmental chemicals.[47,70,71] Thus, it is unknown to what extent these animal models and assays are applicable to the immunotoxicity assessment of every new drug candidate.

In the past, however, findings from animal testing in other areas of toxicology, e.g. carcinogenicity,

were unexpected or unexplained by the scientific knowledge of the time. This triggered mechanistic studies that improved our understanding of the toxic effects under scrutiny, but also helped design more reliable experimental study protocols for routine use. Therefore, the claim that more insights in the mechanisms of immunotoxic effects are needed before immunotoxicity can be included in the safety assessment of drugs is disputable. As a matter of fact, immunotoxic effects are often described in treated patients, but insufficient efforts have been paid to the design, standardisation and validation of animal models and assays over the 20 past years. As specific resources and expertise will be needed for the industry to comply with recently released guidelines,[4,5] they are likely to serve as an impetus to generate a wealth of immune changes in routine nonclinical immunotoxicity studies and to search for new animal models and assays.[72] The use of new methods, such as toxicogenomics,[73] transgenic animals,[74] or molecular biology techniques,[75] is indeed still in its infancy. In current nonclinical immunotoxicity studies, a major issue is the species differences in the histology of lymphoid organs and immune responses.[76] The inclusion of immunological endpoints in conventional repeated-dose toxicity studies performed not only in rodents, but also in larger animal species is, therefore, advisable, but still not widely accepted.

3.2 The Clinical Immunotoxicity Safety Assessment of Drugs

The primary goal of nonclinical safety assessment is to identify safety issues that warrant further in-depth investigation in clinical studies. Until now, extremely limited attention has been paid to defining those immunological endpoints that can be included in clinical trials.[77] It will, however, prove unavoidable that some immunological endpoints that are measured in nonclinical immunotoxicity studies are also measured in patients enrolled in clinical trials to bridge the gap between animal and human findings. This will require close interactions between (immuno)toxicologists and clinicians. In addition, specific immunological endpoints have to be defined that can assess changes in immune functions, or diagnose sensitisation or autoimmunity in humans. Clinical immunotoxicologists will undoubtedly be faced with the same difficulties as nonclinical immunotoxicologists since reliable and well-validated endpoints remain to be identified. Additional criteria, such as allergy questionnaire or the careful clinical and microbiological evaluation of infections will also have to be considered. The consequence of this lack of clinical immunotoxicity evaluation is exemplified by the anti-TNF-α drug infliximab which could be clearly considered as an immunosuppressive drug on the basis of its mechanism of action and the results of toxicity studies. Nevertheless, the greater risk of tuberculosis in treated patients was proven and finally included in the benefit-risk evaluation of infliximab only after several years of clinical use.[78]

4. Conclusion

Unequivocally, drug-induced immunotoxic effects are a heavy burden on the health of treated patients. After 25 years of hesitation,[79] there is a growing awareness that preclinical immunotoxicity studies should be an essential component of the safety assessment of drugs. No general consensus has, however, been reached in the pharmaceutical industry and among drug regulatory agencies on the most appropriate modalities. This is partly explained by the still limited availability of standardised and well-validated animal models and assays. Our poor understanding of the underlying mechanisms should be emphasised. Although rapid progress can be expected in this area, new issues will arise that will require the extension of immunotoxicity safety assessment from nonclinical to clinical studies.

As immunotoxicity plays a critical role in drug-induced adverse effects, the role of immunotoxicology in drug safety assessment is indisputable and the systematic nonclinical as well as clinical immunotoxicity assessment of every new drug is deemed absolutely essential.

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