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Immunotoxicology Evaluation of New Drugs

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ABSTRACT *: Drugs can have various adverse effects on the immune system including unintended immunosuppression, induction of both drug-specific immune responses (including drug allergies) and non-specific immunostimulation (including autoimmune reactions), and direct activation of effector mechanisms (such as histamine release). As a practical matter, the Center for Drug Evaluation (CDER) relies on standard nonclinical toxicology studies to detect unintended immunosuppression. Specific assays using guinea pigs and mice are available to identify drugs that can induce immune-mediated dermal hypersensitivity reactions. Respiratory and systemic hypersensitivity and autoimmune reactions are more difficult to model in nonclinical studies. Unintended nonspecific immunstimulation can be detected in animal studies. CDER is currently developing specific guidance for evaluating potential drug immunotoxicity.*

Key Words *: Drug allergy, Immunosuppression, Immunotoxicology*

I. INTRODUCTION

Of the various types of toxicity that can be produced by drugs, immunotoxicity is one of the most complex and difficult to predict. Drugs can produce either down-regulation of the immune system, resulting in unintended immunosuppression, or up-regulation, resulting in one of the various forms of hypersensitivity (including autoimmunity). In addition, drugs can produce dysregulation of the immune system: thus, immune system impairment detectable as im- munosuppression can also result in susceptibility to autoimmunity and hypersensitivity. Examples of immune dysregulation initially observed as immunosuppression include autoimmune reactions associated with cyclosporine and increased susceptibility to drug allergy in patients with human immunodeficiency virus (HIV) infection (Bayard *et al.*, 1992; Carr *et al.*, 1993; Jenkins *et al.*, 1988; Sakaguchi and Sakaguchi, 1989). With the exception of nonclinical tests to detect the ability of drugs to produce immune-mediated dermatitis, immunotoxicology studies have not been commonly conducted as part of routine drug development. However, signs suggestive of adverse immune system effects can be observed in standard nonclinical toxicology studies (Kuper *et al.*, 2000). In the following sections, current practice at CDER in evaluating potential drug immunotoxicity is presented and discussed.

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II. IMMUNOSUPPRESSION

Drug-induced immunosuppression can result in increased susceptibility to infections and/or tumors. In addition, as mentioned above, demonstration of immunosuppression in animals can indicate that the test drug has the potential to produce immune dysregulation with other adverse consequences. Although there are specific assays for detection of immunosuppression, current practice at CDER is to rely on standard repeat-dose nonclinical toxicology studies to detect this effect (Hastings *et al.*, 1997). Signs of immunosuppression include evidence of myelosuppression (decreases in various blood cell types, especially leukocytes), histologic evidence of immune system injury (such as thymic atrophy, bone marrow depletion, and lymph node necrosis), increased incidence and severity of infections in animals on study, evidence of carcinogenicity (especially if the drug is not genotoxic and the tumor types observed are known to be related to immunosuppression, such as lymphomas), and changes in immune system-related clinical pathology parameters (e.g. decreased serum immunoglobulin levels). Although not a direct sign of

immune system impairment, pharmacokinetic/toxicokinetic studies in which the drug appears to be preferentially distributed into cells such as macrophages could also be taken to indicate immunotoxic potential. Other factors that should be considered include the dose-relationship of the observed effects and whether related drugs are known to have immunosuppressive activity. In certain situations, impaired immune function could be related to the pharmacodynamic activity of the drug (e.g. nonsteroidal anti-inflammatory agents) (Goodwin, 1985). Cytotoxic anticancer agents can preferentially target rapidly dividing cells such as are found in the bone marrow and immunosuppression is an anticipated adverse drug effect. Certain drugs (e.g. calcineurin inhibitors) are, of course, developed because of their immunosuppressant activity and animal studies are more likely to be used to assist in risk assessment rather than hazard identification. This discussion assumes that the observed immunosuppressant activity is unintended and unanticipated.

Where signs of unintended immunosuppression are observed, follow-on studies can be conducted to further assess the effect. Currently, CDER would recommend that a study (or studies) be conducted to determine the effect of the drug on two general phenomena: antibody response to challenge with an immunogen and effect of drug on immune cell phenotypes. The most commonly used method to assess antibody response to immunogen challenge is the IgM anti-sheep red blood cell plaque-forming cell assay (generally referred to as the "plaque assay") (Wilson *et al.*, 1999). Although there are a number of variations of the assay, it is commonly conducted in the following manner: mice are dosed with the test drug daily for 14 to 28 days. Four to five days prior to sacrificing the animals, a single injection of sheep red blood cells in suspension is administered via the tail vein. At sacrifice, the spleen is removed and prepared as a single cell suspension in agar, sheep red blood cells and complement are added, and following a five hour incubation, the number of hemolytic plaques counted and compared to findings in untreated (and/or vehicle treated) mice. This assay has been thoroughly evaluated and is considered the most reliable single test for immunosuppression (Luster *et al.*, 1988). There are many variations, including longer dosing periods, use of other immunogens (e.g. tetanus toxoid), and use of enzyme-linked immunosorbent assay (ELISA) to quantitate immunogen-specific IgM. Depending on the conduct of the study, this method can be useful for both hazard identification and risk assessment.

The most common method for determining the effect of drug on immune cell phenotypes is flow cytometry, using either splenocytes, lymph node cells, or circulating leukocytes. In an important and much cited National Toxicology Program study evaluating immunotoxicology methods, flow cytometric detemination of effects on splenocytes was found to be an accurate method for identifying immunosuppressants (Luster *et al.*, 1992; Luster *et al.*, 1993). In addition, the assay can be "built in" to standard repeat-dose nonclinical toxicology studies and can be directly incorporated into clinical trials (using circulating leukocytes). Problems with this method have been cited, however: it is not a measure of immune function and immunosuppressive effects have been observed in the absence of changes in immune cell phenotypes (Phillips *et al.*, 1997). There is considerable uncertainty concerning the value of certain cell surface markers (especially lymphocyte antigens). Under most circumstances, such studies would evaluate drug effects on a minimum set of leukocyte markers, including CD3, CD4, and CD8 T-lymphocyte markers, an NK cell marker, a B cell marker, and a macrophage marker. It is anticipated that additional immune cell markers will be identified resulting in a more robust method for assessment of unintended immunsuppression. Immunohistochemical techniques can also be used and have proven valuable in some circumstances (e.g. *in situ* characterization of lymph node cell population changes).

Where drug-associated unintended immunosuppression has been confirmed in either the plaque assay or immune cell phenotype studies, additional methods are available to determine the probable mechanism(s). These include drug effects on NK cells, cytotoxic T lymphocytes, macrophage function, and *in vitro* and/or *ex vivo* lymphoblastogenesis. Of particular value are the various host resistance assays in which the effects of drug on susceptibility to experimental infections and implantable tumors are determined. These studies can be particularly valuable in

risk assessment where an immunotoxic hazard has been identified.

III. DRUG ANTIGENICITY AND ALLERGENICITY

Drug allergy is one of the most difficult problems in pharmaceutical development. There are essentially two general components in the nonclinical assessment of drug allergy potential: determining if a drug can act as an immunogen (generally referred to as "antigenicity studies") and determining if this immunogenicity can result in immune-based hypersensitivity reactions. It is generally assumed that protein drugs are potential antigens: determing anti-drug immune responses in this case could be potentially helpful in interpreting results of repeat-dose nonclinical toxicology studies (e.g. anti-drug antibodies could alter pharmacokinetic/pharmacodynamic and toxicity profiles). Incorporation of immunoassays into nonclinical toxicology studies with protein drugs also offers the opportunity to develop and validate these methods for potential use in clinical studies. In some circumstances, it could be important to determine if non-protein large molecular weight polymeric drugs are immunogenic. However, under most circumstances, drug antigenicity studies with small molecular weight drugs are not useful in safety evaluation.

The most useful methods for identifying drugs that have the potential to induce immune-based hypersensitivity reactions are the guinea pig models of contact dermatitis. There are numerous methods, but in drug development the Buehler occluded patch test (generally referred to as the Buehler assay; BA) and the Guinea Pig Maximization Test (GPMT) have been used most often (Botham *et al.*, 1991). These assays have been thoroughly evaluated and are useful for identifying contact allergens. Recently, another assay has proven to be useful for the same purpose: the murine local lymph node assay (LLNA) (Kimber *et al.*, 1989). In this test, mice are treated by topical application of the test substance in a liquid vehicle to the ear, followed by injection of a radiolabelled nucleic acid base. The mice are sacrificed and *in situ* blastogenesis determined by scintillation counting of the cells obtained from the lymph nodes that drain the treated ears. Radiolabel incorporation in the test animal lymph node cells is compared to vehicle control: a three-fold or greater incorporation in the treated group is taken to indicate immune system stimulation, a surrogate for skin sensitization. This method has been extensively evaluated and was found to be a validated method by the United States Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM, 1999). CDER currently accepts LLNA results as an alternative to BA or GPMT methods where the latter two would have been appropriate.

For drugs that are to be administered by the inhalation route, it is acceptable to determine respiratory sensitizing potential using the GPMT or certain variations of this method (such as inhalation challenge following a standard sensitization procedure) (DeGeorge *et al.*, 1996). However, it is recognized that this method can result in a high rate of false positive results (many skin sensitizers might not be respiratory sensitizers). Adaptations of the LLNA, such as the mouse IgE test (MIGET) and determining serum cytokine patterns in response to dermal application of the test substance, have been evaluated for identifying respiratory allergens, but these methods should be considered experimental at this time (Dearman and Kimber, 1999; Hilton *et al.*, 1995). Animal models (primarily adaptations of guinea pig methods and the LLNA) have been developed to detect photoallergens, but these methods have not proven to be very sensitive or predictive. Nonclinical methods for identification of respiratory and photo allergens need to be evaluated more extensively before recommendations can be made for usefulness in drug development.

Systemic hypersensitivity is an especially difficult problem in immunotoxicology. The general phenomenon of systemic hypersensitivity includes numerous adverse effects seemingly related only in having some type of immune basis (Park and Kitteringham, 1990). Clinical events classified as systemic hypersensitivity include IgE-mediated urticaria and anaphylaxis, IgG-mediated hemolytic anemias and specific organ systemic toxicities, immune-complex diseases such as "serum sickness", glomerulonephritis, pneumonitis, and vasculitis, and apparently T cell-mediated systemic diseases such as Stevens-Johnson syndrome (Park *et al.*, 1998). Systemic drug-related diseases

have been described that appear to have an immune basis: an example is the anticonvulsant hypersensitivity syndrome (Shear and Spielberg, 1988). Drug-induced autoimmune diseases can present as any of the classic forms of immunopathy (Griem *et al.*, 1998). Methods such as the passive cutaneous anaphylaxis assay (PCA) and the active systemic anaphylaxis assay (ASA) have been used to screen drugs for potential to produce signs thought to be predictive of systemic sensitization. Although these assays appear to model clinical signs associated with systemic hypersensitivity, in fact they are likely only tests for drug immunogenicity. General experience with these assays should be taken to indicate that neither is particularly valuable for detecting systemic sensitization potential. CDER, under most circumstances, does not recommend the PCA or ASA for use in routine drug development.

The popliteal lymph node assay (PLNA), which is normally conducted in mice (although rats have also been used), was originally developed to screen chemicals for autoimmunity-inducing potential (Gleichmann, 1982). In this assay, the test substance is injected into the hind footpad and the draining (popliteal) lymph node is obtained and weighed seven days later. Immunostimulatory compounds produce significant increases in lymph node weight compared to vehicle controls. Many modifications have been made to this simple assay, including use of reporter antigens (co-injected with test compound to determine inherent adjuvant-like activity, thought to be the basis of autoimmunity induction), histology (Brouland *et al.*, 1994; Descotes *et al.*, 1997), and immunhistochemical analysis (Albers *et al.*, 1999). Although originally designed to detect autoimmunogens, the PLNA has been demonstrated to detect other types of immunostimulatory compounds, including drugs known to induce various forms of systemic hypersensitivity (Pieters and Albers, 1999). Use of reporter antigens in particular has been shown to improve the ability of the PLNA to detect chemicals, including drugs, known to cause IgE-mediated immunopathies (Gutting *et al.*, 1999). Fundamentally, the PLNA appears to be mechanistically very similar to the LLNA. In fact, preliminary work has shown that a modification of the LLNA using intradermal or subcutaneous injection of the test compound (rather than skin exposure) allows for the detection of potential systemic sensitizers (Ashby *et al.*, 1995; Meade *et al.*, 1999). The PLNA has been fairly extensively evaluated and is probably a reliable method for detecting immunostimulatory chemicals. The "modified" LLNA could prove to be even more valuable although much work remains to be done to substantiate this possibility.

A serious problem with both assays is the issue of organ-specific metabolism: it is not clear if either method can model this known aspect of some types of systemic hypersensitivity. Susceptibility to drug allergies appears to have a strong genetic component as well: in the future it is possible that genomic techniques might be incorporated into these lymph node assays to improve both hazard identification and risk assessment. Some preliminary work has already been reported in this area (Glatt *et al.*, 2000). Another promising avenue of research is the use of genetic knock-out mice: models deficient in production of various cytokines thought to be important in hypersensitivity reactions have been reported (Karachunski *et al.*, 1999; Rennick *et al.*, 1995; Wynn *et al.*, 1995). It is possible that these models might be found useful in screening drugs for sensitizing potential.

IV. PSEUDO-ALLERGY AND NONSPECIFIC IMMUNOSTIMULATION

These are overlapping concepts that have as their basis activation of immune system functions in the absence of specific antigen recognition. Some drugs produce what appear to be allergic reactions, such as anaphylaxis, but do not act as immunogens. These types of adverse drug reactions are referred to as "pseudo-allergies" and have in common activation of immune effector mechanisms in the absense of specific drug-induced immunity (Descotes, 1986). Some drugs produce pseudo-allergic reactions by interacting with mast cells and producing histamine release in the absence of drug-specific IgE. This type of reaction is referred to as "anaphylactoid" to distinguish it from true IgE-mediated anaphylaxis. An important characteristic of anaphylactoid reactions is that they can be modelled in animals and are generally dose-related and predictable. In addition, some can be demonstrated *in vitro* using mast cell cultures. There are examples of anaphylactoid reactions in which the

mechanism is activation of the alternative complement system resulting in production of complement products known as anaphylotoxins (Benoit *et al.*, 1983).

Nonspecific immunostimulation refers to reactions associated with immunoactive compounds such as therapeutic cytokines. In this situation, many of the adverse effects are in fact not generally associated with the immune system, such as renal toxicity. In fact, the term "cytokine syndrome" has been coined to refer to these types of reactions (Vial and Descotes, 1995). Often it is unclear if these are in fact exaggerated pharmacodynamic effects or true toxicities unrelated to therapeutic activity. As with anaphylactoid reactions, many of these adverse effects are predictable based on findings in nonclinical toxicology studies. Toxicites associated with immunostimulatory proteins oftentimes do not have dose-relationships characteristic of small molecular weight compounds. "Bell-shaped" dose-response curves are frequently observed and should be considered in extrapolating results of nonclinical studies to clinical drug trials.

V. SUMMARY

Immunotoxic effects of drugs includes a broad range of toxicities. Numerous nonclinical methods have been developed which can be useful in both hazard identification and risk assessment. The most difficult area for immunotoxicologists appears to be development of models useful in predicting drug allergies. It is hoped that in the future truly useful methods will become available for this purpose. CDER remains committed to sound science in addressing issues such as unintended immunosuppression and drug hypersensitivity.

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