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Introduction
The evaluation of investigational new drugs (INDs) for immunotoxicologic effects is presented in a US Food and Drug Administration pharmacology and toxicology guidance. The guidance reflects current FDA thinking on immunotoxicology testing for INDs, providing information and recommendations. Briefly, the guidance was the result of the FDA Center for Drug Evaluation and Research’s (CDER) Immunotoxicology Working Group efforts to develop internal guidance for CDER review staff (1994 - 1996) followed by development as a guidance for industry (1997 - 2001). In 2001, the draft guidance was released in the Federal Register for public comment and in 2002 the present guidance was finalized.

The guidance provides preclinical parameters for routine assessment and timing for further immunotoxicity studies when the need arises for additional mechanistic information to characterize the significance of drug effects on the immune system. Its intent is to evaluate immunotoxicologic potential of drugs based upon findings from standard repeat-dose toxicity studies. These findings include standard histopathology and depletion or hyperplasia of lymphoid tissues. Drug concentration in immune tissues/cells may also indicate the need for further studies. Functional assays may be recommended if these effects are seen in standard toxicity studies. Determination of affected cell types by immunohistochemistry and/or flow cytometry may also be useful. Specific situations in which further studies may be indicated include the testing of topical or inhaled drugs for sensitization potential as well as functional assays for anti-HIV drugs. Immunotoxicity findings in a drug with likely use in pregnant women may indicate the need for evaluation of the drug in the immune tissues of the F1 offspring.

In the standard toxicity studies, indicators of immunotoxicity include unexplained changes in hematological parameters, serum globulin or total protein/serum globulin ratios, findings in gross pathology (e.g. thymic involution), weight and/or histopathologic changes in lymphoid tissues, and increased incidence of infections or tumors. Immunotoxicologic effects are observed through a continuum of immune responses and possible outcomes. These effects include immunosuppression, immunogenicity/antigenicity, hypersensitivity, autoimmunity and adverse immunostimulation. Immunosuppression includes effects on the immune system resulting in decreased immune function. Immunogenicity/antigenicity consists of specific immune reactions elicited by a drug and/or its metabolites. Hypersensitivity is the immunological sensitization from drug and/or metabolite exposure. Autoimmunity involves immune reactions to self antigens. Adverse immunostimulation is the non-antigen specific activation of the immune system.

Immunosuppression
Indications of immunosuppression from nonclinical studies include evidence of myelosuppression (e.g. blood dyscrasia), gross pathology findings (e.g. involution of the thymus), changes in immune system organ weights and/or histology (e.g. hypocellularity), increased incidence of infections (e.g. urinary tract infections in rodents), increase incidence of tumors and decreased serum globulin levels. The nature of these effects must be distinguished between unintended (adverse) and intended (pharmacodynamic) effects. Known drug class effects (e.g. antimetabolites, drugs for organ transplantation), exposure to suspected immunosuppressives during pregnancy and pharmacokinetic effects (e.g. concentration of drug and/or metabolites in macrophages) should be taken into consideration.
The guidance recommendation following any of the above findings includes the T-cell dependent antibody response plaque forming cell assay, ELISA / ELISPOT (enzyme-linked immunoabsorbent assay/ enzyme-linked immunospot) assay for KLH or tetanus toxoid, determination of specific protein components if a serum globulin decrease, and identification of causative organisms in unexpected infections. Mechanistic assays may be used to characterize the target of the immunosuppression. These include NK cell, in vitro blastogenesis, cytotoxic T-cell, delayed type hypersensitivity and host resistance assays [4,9,22,24].

The T-cell dependent antibody response is often recommended as a follow-up assay for immune function studies. The T-cell dependent assay demonstrates most components of the classical immune response, including B cell release of antigen specific antibody, macrophage antigen presentation, and T helper cell lymphokine production for B-cell proliferation. Using sheep red blood cells (SRBC) as the T-cell dependent antigen, the assay demonstrates an organized immune response dependent on the functional capacity and cooperation of numerous cell types. Due to the cellular component and antibody response, the assay is sensitive and frequently an initial immunotoxicity assay. It should be taken into consideration that the SRBC assay does not describe the mechanism of immunosuppression. Use of T-cell independent antigens such as lipopolysaccharide, which requires only B cells for an antibody response, or dinitrophenyl-ficoll, which requires B cells and macrophages, may be of use in characterizing the cell types subject to immunosuppression. The assay may be modified using ELISA and ELISPOT as the readout to quantify antibody response and antibody-producing cells, respectively [15,23].

The value of the plaque assay for immunotoxicology was evaluated by the National Toxicology Program (NTP) in mice [13,28]. Over 50 test compounds were selected for evaluation in a battery of immunotoxicology assays. The predictive value of the plaque assay to affect host resistance was demonstrated with 78% concordance for known immunotoxicants [18,19]. Host resistance assays provide functional and mechanistic insight for evaluation of the effect of drug exposure on resistance to infection [27]. The assay provides a complete immune response to infection, especially useful if previous assays indicate multiple immune parameters are affected [17]. An appropriate infection model may be selected for the immune mechanism appropriate to humans. In an appropriate animal host resistance model, drug metabolism and metabolite exposure more realistically portrays the immunotoxic effect and its biological significance. The host resistance is dependent upon the degree of immunosuppression and the quantity of the infectious agent. Assays for immune parameters and changes in the burden of infectious organism provide endpoints for the assay. Factors of drug dose schedule, infectious inoculation schedule, route and titer, duration of study, host species/strain, endpoints, positive controls, interpretation and cost must be considered in the design of appropriate host resistance assays [4].

Immune cell phenotyping may be useful subsequent to indications of immunotoxicity. Flow cytometry has been demonstrated to significantly correlate with host resistance to infection in the NTP studies cited above and may be used clinically to monitor adverse effects as well. The combination of immune cell phenotyping with immune function assays can provide a comprehensive view of drug immunotoxicity in animals and insight for follow-up clinical studies as well [18,19].

**Hypersensitivity**

Antigen-specific reactions with adverse effects such as drug allergy are the result of hypersensitivity. The types of hypersensitivity include type I (IgE mediated, immediate type), type II (IgG or IgM mediated, antibody-cytotoxicity), type III (IgG mediated, immune complex) and type IV (delayed, lymphocyte mediated) [6].

Type I hypersensitivity occurs in systemic or respiratory forms [14]. Demonstration of IgE or its equivalent should be taken as an indicator of a hazard for both subtypes. No methods are currently recommended for routine safety evaluation of systemic Type I hypersensitivity. The results of guinea pig testing were extracted from NDA submissions and compared with clinical findings reported to the FDA adverse event reporting system. A relationship between incidence of rash in post marketing reports and clinical trial adverse event reports was seen without any association between human data and guinea pig test results. The result of this comparison suggests standard guinea pig assays are not predictive of potential systemic hypersensitivity to drugs in humans [26]. Detection of type I inhalation hypersensitivity may employ guinea pig models, originally developed for chemical exposure-related [1,2,7,10]. The local lymph node assay (LLNA) may also be useful [12].

Type II hypersensitivities include IgG or IgM mediated antibody dependent cellular cytotoxicity, complement-mediated lysis of somatic cells and hemolytic anemia, agranulocytosis, and thrombocytosis.

Type III hypersensitivities include IgM mediated immune complex formation and deposition, complement activation, vasculitis, glomerulonephritis, pneumonitis, serum sickness and lupus-like reactions. For both Type II and Type III
hypersensitivities, animal models are generally not predictive [5]. Tissue damage may be examined by immunohistochemical to demonstrate antibodies or complement responsible for immunopathologic effects related to the particular tissue. Immunoallergic hemolytic anemia may be indicated by a direct Coombs assay.

Type IV hypersensitivity is a T-cell mediated immunopathy typically occurring as delayed-type hypersensitivity skin reactions (allergic contact dermatitis) or as photoallergy. Assay by guinea pig dermal sensitization (induction) and challenge (elicitation) may determine the sensitization potential of topically administered drugs. The most common methods for this are the Buehler assay and the guinea pig maximization test [3]. These methods have demonstrated high correlation with known human skin sensitizers. Study of the induction phase of delayed-type hypersensitivity may employ the murine local lymph node assay. The LLNA detects in vivo lymphoproliferation. Results from the LLNA correlate well with guinea pig tests and are more quantitative [16]. Other advantages of the LLNA over guinea pig assays include the use of fewer animals, more rapid results and less animal discomfort. Photoallergy is a special case of Type IV hypersensitivity involving photoactivation of a drug with the resulting covalent binding of drug to protein (hapten). The hapten then acts as a sensitizer. Animal models have demonstrated photoallergy but need further study for correlation with human effects [21]. The screening of prospective drugs from classes of drugs known to cause photoallergy (e.g. β-lactams, sulfonamides) for covalent binding may be considered.

Pseudoallergic (anaphylactoid) reactions arise from the direct effect of drug on mast cells and basophils to produce degranulation, histamine release and/or complement activation without an antigen-specific immune response. Drug-induced anaphylaxis in animal studies may indicate the need for follow-up studies. Anaphylactoid reactions may be distinguished from IgE-mediated anaphylaxis by in vitro or in vivo methods which detect histamine release from mast cell lines and detection of serum anaphylactic complement in vivo.

Autoimmunity
Autoimmunity is an immune response to self-antigens. Drug-induced autoimmunity may be caused by a drug-specific hypersensitivity reaction. Type II and III hypersensitivity may have autoimmune aspects as well. Common examples include glomerulonephritis, lupus-like syndrome, hemolytic anemia and vasculitis. Tissue damage may result from immune complex deposition with complement activation as well as direct immune activity. The origins of autoimmune reactions may be T-cell or antibody mediated. Receptor stimulation due to stimulatory antibodies may directly cause autoimmunity. No specific assays in animals predict the ability of a drug to produce autoimmunity in humans. The popliteal lymph node assay (PLNA) have been proposed to test for drug-induced autoimmunity [8,11,25]. Modifications of the PLNA and the LLNA have been examined to detect drug-induced autoimmunity as well [20]. Adverse immunostimulation is an antigen-nonspecific, inappropriate or uncontrolled activation of any component of the immune system. Immunostimulation may be a desired pharmacodynamic effect of a drug, particularly as an adjuvant. In other cases (e.g. vaccine adjuvants, implanted medical devices) chronic inflammation may occur as a result. Immunostimulation by cytokines may result in capillary leakage while treatment with monoclonal antibodies may cause cytokine release. No specific tests are recommended at this time to detect adverse immunostimulation.

General Considerations
Interpretation of quantitative results should consider that changes in a given immune cell quantity, function or phenotype may not result in significant immune effects, allowing for the redundancy inherent in the immune system. Statistically significant results may not be biologically significant. Follow-up studies to initial immunotoxicity findings should be conducted at a dose and duration similar to the exposure where the findings were observed. Selection of an appropriate animal model or assay is critical to the validity of follow-up studies.

Summary
Drug-induced immunotoxicity may occur as immunosuppression, immunogenicity/antigenicity, autoimmunity, hypersensitivity, or adverse immunostimulation. Unintended (adverse) and intended (pharmacodynamic) effects, together with known drug class effects should be taken into consideration. Standard preclinical toxicity studies may be used to detect immunotoxicity. Initial preclinical immunotoxicity findings may be characterized by follow-up studies, providing a mechanistic basis of toxicity and informing risk-benefit determination. Follow-up studies may depend upon the intended use of the drug, tolerability of immunosuppression and consideration of combination use with other drugs which may exacerbate immunotoxicity by similar mechanisms or pharmacokinetic effects. The development and validation of assays described in this text as well as new methods may provide more useful endpoints for drug immunotoxicity safety assessment.

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References


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