ILSI® HEALTH AND ENVIRONMENTAL SCIENCES INSTITUTE

International Life Sciences Institute

August 3, 2001

Dockets Management Branch
HFA-305
Food and Drug Administration
5630 Fishers Lane, Rom 1061
Rockville, MD 20852

Re: Docket #01D-0177

Dear Sir or Madam:

The Immunotoxicology Technical Committee (ITC) of the ILSI Health and Environmental Sciences Institute is pleased to provide the enclosed comments on the FDA Center for Drug Evaluation and Research's "Guidance for Industry: Immunotoxicology Evaluation of Investigational New Drugs," in response to your May 11, 2001, request for comments in the *Federal Register* (F.R. Vol 66, #92). The ITC appreciates the opportunity to contribute to the scientific discussion around this issue.

The ILSI Health and Environmental Sciences Institute (HESI) is a global branch of the International Life Sciences Institute, a public, non-profit scientific foundation with branches throughout the world. ILSI HESI provides an international forum to advance the understanding and application of scientific issues related to human health, toxicology, risk assessment and the environment. ILSI HESI is widely recognized among scientists from government, industry and academia as an objective, science-based organization within which important issues of mutual concern can be discussed and resolved in the interest of improving public health. As part of its public benefit mandate, ILSI HESI's activities are carried out in the public domain, generating data and other information for broad scientific use and application. ILSI HESI's programs are supported primarily by its industry membership. ILSI HESI also receives support from a variety of government agencies from the US and internationally.

Please direct any comment or questions concerning this document to Mr. David Sandler, ILSI HESI Senior Project Manager, at 202/659-3306, or dsandler@ilsi.org.

We hope that our comments will be helpful to FDA in finalizing this Guidance document.

Sincerely,

Denise Robinson

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Executive Director

Enclosure
01D-0177

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GUIDANCE FOR INDUSTRY
IMMUNOTOXICOLOGY EVALUATION OF INVESTIGATIONAL NEW DRUGS
US FDA (5/10/01)

Comments prepared by:
ILSI Health and Environmental Sciences Institute
Immunotoxicology Technical Committee
3 August 2001

GENERAL COMMENTS

This document in general provides a good perspective on the state of immunotoxicology testing for pharmaceuticals. We strongly agree with the point made at the beginning of the guidance, and in later sections, that evidence of immunotoxicity can usually be observed in standard nonclinical toxicology studies and that follow-up studies may be necessary to define potential mechanisms.

The guidance also evaluates the concerns and complexities around systemic drug hypersensitivity or autoimmune reactions, and indicates in most instances that there are no standard preclinical tests available for reliably determining the potential for drugs to cause these adverse effects in humans. However, the flow chart presented in attachment two gives the impression that there are defined assays to test for these adverse reactions by listing non-validated assays for hypersensitivity (e.g., MIGET) or autoimmune testing (e.g., PLNA) together with validated assays for determining immunosuppression. We recommend that a clear distinction be made between validated and non-validated assays. Further, for mechanistic studies, non-validated assays could be appropriate if scientifically justified.

The guidance recommends reliance on the SRBC plaque assay to the exclusion of alternative methods that employ immunoassays and other T cell-dependent antigens. We recommend that explicit allowance be made for including the use of T cell-dependent antigens with clinical relevance (e.g., KLH, CRM197, and tetanus toxoid). The utility of these alternative methods is currently being investigated by immunotoxicologists with the idea that they may offer greater flexibility and reproducibility over the plaque assay. To foster continued research in this area, we suggest that the guidance give consideration to the use of these alternatives when they can be justified scientifically.

SPECIFIC COMMENTS

Section III. Evaluating Immunotoxicity Markers

Lines 52-57: We agree with the statement in line 52, that further characterization of immunotoxicity should be based on biologically relevant and not just statistically significant effects to trigger follow up studies, and that a dose response relationship is important. However, with our present state of knowledge, it is often not possible to discern the degree of change that would be biologically relevant. As you are aware, the immune system, like other organ systems, has great reserve and redundancy, and may compensate when only one component is affected.

Thus, a very large change in a single parameter may be required to affect host resistance. We therefore recommend that the uncertainty of what may be considered biologically relevant be emphasized in the guidance.

We would recommend removing reference to specific percent changes (lines 54-56) in lymphocytes and neutrophils, because this suggests a threshold for interpretation, which would likely become dogma. There are species differences in absolute numbers of peripheral WBCs, and several environmental factors can affect the magnitude of change as can normal variability among species. It is also not clear in line 57 if the statement "even if large effects are not observed" refers to biologically significant changes. Perhaps this should read "....observed signs should represent biologically significant effects, however, effects in more than one species would cause concern even if not large." It is not clear if "even if not large" also means not biologically significant.

Lines 70-76: We agree that environmental factors may cause stress-induced immunologic effects, and that in carefully designed studies these effects should be reflected in non-drug-treated control animals. However, it is inherent to the purpose of toxicology studies to induce significant toxicity, which itself can be a source of stress to the animal, and it is often difficult to distinguish a direct immunological effect of the drug at doses that might induce severe stress-related immunologic changes. Thus, we believe the agency should discuss the importance of studying the potential for immunotoxic effects at doses that do not produce significant overt toxicity.

Lines 86-87: The words 'reticuloendothelial tissues' should be replaced with 'lymphoreticular tissues'. We do not believe there is justification for recommending that testing *should* be performed if a drug accumulates in macrophages even when no signs of immunotoxicity are apparent (e.g., accumulation of certain antibiotics). It is not unusual to identify material within the monocyte/macrophage system, and it would be inappropriate to initiate immunotoxicity studies based on this finding alone. Amiodorone is a classic example of a clinically used drug that induces phospholipidosis in pulmonary macrophages without any evident effects on immune function.

Line 107: "Increased incidence of tumors" is cited as an indicator of immunosuppression. It is known from studying immuno-compromised patients that the increased incidence of tumors is essentially virus related (skin/lips cancer and HSV; non-Hodgkin lymphoma and EBV, Kaposi sarcoma and CMV, uterus cervix cancer and HPV). To associate any kind of increased tumor incidence with immunotoxicity, and then recommend (p.22, Attachment 2) the use of tumor host resistance assays when there is evidence of carcinogenicity, might not be appropriate. A direct link between carcinogenicity and immunosuppression should not be made in the absence of other evident signs of immunosuppression (myelosuppression, etc.). A rodent non-myeloid and non-lymphoid tumor response is almost never associated with immunosuppression in nonclinical safety testing.

Lines 111-116: A comment on permanent versus transitory changes due for instance to trafficking of leukocytes could be added. This comment could also be added in lines 152-158.

Section IV. Immunosuppression

Lines 109 and 134-136: Because total serum immunoglobulin is known to be an insensitive indicator of immunosuppression, we recommend that it not be incorporated into a standard testing battery for immunotoxicity. Histopathological changes in lymphoid organs are more sensitive indicators.

Lines 138-150: This paragraph intermingles a description of myelosuppression with autoimmune-mediated effects on erythrocytes. Antibody-mediated, drug-induced hemolysis can occur, but this is not in itself an immunosuppressive effect. The only autoimmune phenomenon potentially relevant to this section might be immune-mediated neutropenia, an exceedingly rare event that is poorly documented. We recommend that this section focus on myelosuppression as being a cause of immunosuppression and move the reference to autoimmune phenomena to the appropriate section of the guidance.

Section IV. B. Immune Cell Phenotyping

Lines 160-186: The guidance recommends the phenotyping of immune cells in follow-up studies if immunosuppression is observed in nonclinical toxicology studies. Phenotyping studies were recommended since immune cell phenotype changes have been demonstrated by the National Toxicology Program (Luster et al., 1992) to be one of the best single correlations with host resistance against pathogens or tumors. This observation, however, was based on only 9 of the 52 chemicals tested where CD4+ and CD8+ subsets were actually measured and the authors cautioned that the predictive value of this data should be interpreted accordingly. We also point out that since the NTP examined the spleen cells of $B_6C_3F_1$ mice for these studies, it is not known if the correlation exists for rats, dogs or monkeys, or with peripheral blood lymphocytes (PBL). With non-rodent species, the analysis of PBL will be much more practical. Moreover, since PBL would be used to monitor adverse effects in clinical trials, it makes more sense that they be used for the preclinical studies. This difference between validation in mouse spleen and practical use of rat, dog, monkey and human PBL needs to be reconciled before stating that immune-cell phenotyping in preclinical studies is a validated approach. This is further supported by recommendations made by a group of experts at a recent workshop on the application of flow cytometry to immunotoxicity testing (ILSI Immunotoxicology Technical Committee, 2001). It was strongly emphasized by the workshop panel that, for regulatory purposes, the application of flow cytometry data is problematic if statistically significant changes are highlighted without evidence for any corresponding biological significance. Further, although changes in human PBL phenotypic markers can be measured, there is no basis at present for establishing how much change is important, or for using this information for making clinical judgements about risk assessment.

Section IV. C. Immune Function Studies

Lines 191-192: Guidance should be given about dose selection for follow-up studies. Should selection be based on multiples of the efficacious dose or of the no observable effect level? Doses that result in overt toxicity should not be evaluated.

Lines 202-204: We disagree that measuring the serum antibody response to a specific antigen challenge by ELISA procedures "is not a true test of immune function," and we recommend that this definition be modified. The plaque assay measures the number of antibody-forming cells in the spleen; the ELISA quantitates the amount of antibody produced from all immune organs, not

just the spleen. We do agree that either assay should be acceptable. In fact, the ELISA method adds a number of advantages since time course can be followed and recovery assessed within the same animals. Also, in section IX, (lines 512-518) there is a strong recommendation made that the SRBC plaque assay be used in conjunction with flow cytometry if follow-up studies are needed. Either approach, measuring antibody responses by ELISA or assessing anti-SRBC plaques, is useful for assessing immunotoxicity to the humoral immune system. We recommend that explicit allowance be made for the use of immunoassays and the assessment of specific antibody responses to T cell-dependent antigens (especially protein antigens with clinical relevance: KLH, CRM197, tetanus toxoid and others). This allows for much greater flexibility in study design (e.g., measuring the time course of antibody response, antibody subclass, and primary versus secondary responses) than does the plaque assay, which is assessed on a single day.

Lines 217-222: It is unclear if the guidance is recommending that all drugs that could be used in pregnant women should be tested for immunotoxicity in reproductive toxicology studies, or only those drugs specifically intended for treating diseases in pregnant women. The area of developmental immunotoxicology is still in its infancy and standard practices have yet to be determined. However, we agree that, at a minimum, evaluation of lymphoid histopathology in the F_1 generation may be appropriate. It is also not clear at what age the F_1 generation should be evaluated. Although this paragraph is under the section of "Immune Function Studies," the parameters the agency recommends to be evaluated are not immune function parameters. We suggest moving this under a different heading to avoid confusion.

Section V. Antigenicity

Lines 257-269: It is stated that an anti-drug assay should be considered as part of the nonclinical safety assessment of drug classes known to be potentially haptenic (e.g., penicillins) (Lines 257-259; 488-490). Many marketed drugs are metabolized to reactive intermediates that bind to various macromolecules, and thereby are potentially haptenic. However, these drugs are associated with a very low incidence of hypersensitivity reactions in humans. Thus, the potential of a drug to be haptenic does not warrant the evaluation of anti-drug responses in standard toxicology assays.

In contrast to lines 257-259 and 488-490, lines 407-412 indicate that anti-drug responses should be conducted if the test compound is known to belong to a class known to produce hypersensitivity reactions through covalent binding. However, studies have demonstrated that these compounds (e.g., sulfonamides, penicillins) do not produce an anti-drug response when administered via a clinically relevant route in rats (without adjuvant or immunizing with drug-protein conjugates) (Kitteringham et al., 1987: Gill et al., 1997). Reasons for the lack of an anti-drug response may be attributed to the amount of reactive intermediates generated and inactivated, and the low immunogenicity of the hapten-protein conjugate in the particular species used for toxicology studies. Thus, evaluating anti-drug responses in routine toxicity studies will not be helpful.

Line 257 states, "Under certain circumstances, attempts should be made to determine the potential antigenicity of large molecular weight drugs." Should that read "small" rather than "large?" As stated in the previous paragraph and in our own experience, large molecular weight

drugs are usually antigenic. Thus, we routinely monitor antigenicity in all our studies with large molecular weight drugs.

Lines 265-267 state, "Assays to identify anti-drug immune responses should be considered part of nonclinical assessment, because peptides, polymer, and protein drugs and classes are known to be potentially haptenic." This sentence is confusing and should be reworded.

Section VI. Hypersensitivity (Drug Allergy)
Lines 272-426: This section is a good, concise review of the area of drug hypersensitivity. It highlights some assays that have been used experimentally to study hypersensitivity, but basically concludes that there are no standard, validated assays that can reliably identify drugs that elicit systemic hypersensitivity responses in humans.

Lines 282-283: Small molecular weight compounds may be antigenic -- not allergenic -- if they bind directly to proteins, either as the parent or via metabolites (lines 282-283). They are allergenic if they produce an exaggerated or pathological reaction. We suggest changing "allergenic" to "antigenic."

Section VI. A. Type I
Lines 289-326: We agree that the guinea pig methods to assess the potential of drugs to produce type I reactions with oral or parenteral routes of administration are not predictive and should not be recommended (Lines 291-309). The mouse IgE test to detect respiratory sensitizers was also discussed. However, since this method has not been validated, a statement regarding its qualified use needs to be clearly stated in the guidance document. The use of the MIGET in connection with the LLNA to assess respiratory sensitizing potential was also proposed. In such assays, cytokine pattern in connection with hapten-specific IgE should indicate whether a drug could act as a respiratory sensitizer. This approach is problematic, since there are numerous reports showing cytokine response patterns following topical exposure to chemicals, which are inconclusive regarding the nature of the allergy, e.g., cellular (Th1) or humoral (Th2) (Ulrich et al., 1998; Infante-Duarte et al., 1999; Zhang et al., 2000; He et al, 2001). In addition, the state of validation of the LLNA with respiratory sensitizers is not sufficient at present. The concept of applying a drug topically to assess its possible allergic effects on the respiratory tract needs to be confirmed and also needs to take into account the physical properties of the chemical.

The guidance document recommends that Karol's (1995) guinea pig method, which "involves dermal or inhalation induction followed by inhalation challenge" be used for inhalation drugs. However, this published method only addresses *inhalation sensitization*, but does not address *dermal sensitization*. In addition, since Karol's (1995) model is difficult to conduct, perhaps other alternatives such as the tiered approach for evaluating respiratory sensitizers of low molecular weight chemical described by Sarlo and Clark (1992) should be considered. In addition, it is not clear when this evaluation would be performed in the clinical development program (e.g., prior to repeat dose studies).

Section VI. B. Type II & III
Lines 342-344: (and also Lines 522-524.) It is suggested that in the case of specific tissue damage such as vasculitis, "immunohistochemical demonstration of antibody or complement in

the affected tissue could suggest immunopathy." Based on the literature, antibody-mediated vasculitis that occurs with drug treatment appears to be very rare. Specific examples of antibody-mediated vasculitis need to be included. Since drug-induced vasculitis may be mediated by several other mechanisms, the deposition of immune complexes and complement may not necessarily demonstrate a direct relationship.

It was also suggested that "specialized biomarker assays can be useful for understanding mechanisms when a drug belongs to a chemical class known to be associated with specific immunopathies." (lines 354-355). Antibodies against trifluoroacetylated proteins were proposed as an example of a potential biomarker for indirectly assessing the sensitizing potential of chemicals related to halothane. Based on this discussion, it is not clear how the biomarker will be used and in which situations it would be helpful. In addition, the types of adducts with liver proteins are highly diverse and lead only in rare cases to immune responses (example halothane: 1 of 10000 patients develops autoimmune hepatitis). Depending on the chemical, the type of immune reaction can differ: halothane may rarely induce antibody responses, whereas autoimmune reactions induced by tienilic acid are cell-mediated. This diversity may also complicate establishment of a testing strategy for such a potential. We suggest that this section be re-written with the inclusion of additional specific examples of how biomarkers will be used in nonclinical studies to identify compounds that may produce Type II or III hypersensitivity reactions.

Section VI. C. Type IV

Lines 367-398: We agree that all dermal drugs should be routinely tested for the potential for dermal sensitization, since validated and predictive assays are available (367-398 and 481-484). We recommend including a reference to the ICCVAM proceedings (NIH publication No. 99-4494), which support using the LLNA as an alternative for contact sensitivity testing.

Line 400-405: This paragraph does not reflect what is described in the Guidance for Industry for Photosafety Testing which states that "Short-term photosensitivity testing in animals, perhaps followed by studies in humans, should be considered for all drug products that absorb UVB, UVA, or visible radiation...". It does not reflect either our recent experience which shows that any topically-applied product showing these characteristics has to be tested for photoallergy. Therefore, we recommend that this guidance be aligned with the CDER Guidance for Industry on Photosafety Testing.

Lines 407-412: It was stated that if a drug belongs to a class known to produce hypersensitivity reactions through covalent binding (e.g., beta-lactams, sulfonamides), demonstration of covalent binding to proteins could be taken as a biomarker of sensitization potential. As stated previously in regards to the Antigenicity section of these comments, studies with beta-lactam and sulfonamide administration to rats have demonstrated that it is very difficult to detect covalent binding in rat tissues even with the administration of high doses. In addition, if covalent binding is observed, it is not known how much should be a concern. Studies that have examined the relationship between the amount of covalent binding and immunogenicity of the hapten have not been reported. Currently, covalent binding as a mechanism for hypersensitivity is a theory, which in the future may lead to the development of *in vitro* systems for predicting the hypersensitivity potential of protein-reactive drugs. But until these assays are available, we

believe it is premature to recommend covalent binding studies for determining the potential immunogenicity/antigenicity of drugs.

Section VII. Autoimmunity

Lines 439-440: It is stated that "Immune stimulation due to specific immune reactions (stimulatory hypersensitivity) may be considered a type of autoimmunity." It is not clear what type of specific immune reaction will result in immune stimulation and why this is considered a type of autoimmunity. To clarify this section, examples of stimulatory hypersensitivity should be provided.

Lines 442-446: We agree that given the lack of "extensive evaluation", the PLNA should not be used to determine if a drug has the potential to produce autoimmune reaction. Markers of T cell-activation and of Th2 cell-induction in Brown Norway rats were also suggested (lines 448-449; Attachment 2). References for these methods should be included as well as the justification for the markers.

Section VIII. Adverse Immunostimulation

Lines 457-460. We are concerned that the definition of adverse immunostimulation as "any antigen-nonspecific, inappropriate, or unintended activation of some component of the immune system" is too broad. Perhaps a better definition would be "uncontrolled immune stimulation".

Lines 469-473. "A relatively common manifestation of immunostimulation is leukocyte infiltration of tissues", could be replaced by "A relatively common manifestation of immunostimulation is leukocyte infiltration of tissues *above the naturally existing level*". We also do not understand the link made between adverse immunostimulation and the diffuse capillary leakage observed with interleukin-2 (lines 472-473).

Section IX. Safety Considerations

It is recommended that for drugs administered by the inhalation route, the sensitizing potential should be screened using an appropriate test such as the guinea pig maximization test (GPMT), Buehler assay (BA), local lymph lode node assay (LLNA) or mouse IgE test (MIGET) (lines 481-484 and Attachment 1). However, justification for using a method for contact sensitivity to determine the sensitizing potential of an inhalation drug needs to be included. In addition, since the MIGET has not been adequately validated, its recommendation does not appear scientifically justified. The recommendations in lines 481-484 are not consistent with the guinea pig assays (Karol 1995) recommended in lines 321-326. The types of assays recommended for assessing the sensitizing potential of inhaled drugs need to be clearly stated.

Line 512-518: Reference is made to two assays that "should" be considered when follow-up immunotoxicity assays are needed. We suggest that consideration should also be given to the assessment of humoral immunity by measuring specific serum antibody to T cell-dependent antigen (e.g., SRBC, KLH or others) by ELISA as mentioned in lines 195-202. In line 198, it is stated that the SRBC plaque assay "could be used" rather then "should be" as referred to in line 515. This is an important issue for companies registering drugs in Europe. The respective CPMP guidance allows the use of other T cell-dependent antigens (SRBC, tetanus) in repeated dose

toxicity studies. The minimal consensus of both guidances would then be to use a clinically-relevant protein antigen for immunization.

Line 521: The statement is made that "when anemia is present, a Coombs test could indicate whether immune-mediated hemolytic anemia is the cause." While this is not an inaccurate statement, we recommend that it be expanded and include "when anemia is present, and other findings are consistent with an immune-mediated hemolytic anemia, a Coombs test...." An additional sentence should then be added such as "Findings consistent with an immune-mediated hemolytic anemia include histopathologic evidence of increased destruction of red blood cells in the spleen and/or bone marrow, hyperbilirubinemia, hemoglobinuria, regenerative response (reticulocytosis or erythroid hyperplasia and/or extramedullary hematopoiesis) without evidence of hemorrhage, and/or spherocytosis." Without further clarification, the original statement could be interpreted to mean that every anemia should be evaluated with a Coombs test.

Line 528: It is stated that the PLNA and specific biomarker assays might provide insight into potential autoimmune mechanisms (lines 528-530; Attachment 2). However, it is stated in lines 444-446 that the PLNA may have promise, but that no extensive evaluation has been reported that would support any recommendation for drug development. In addition, the term "specific biomarker assays" needs to be explained. If this is meant to be markers of T cell-activation and effects of a drug on markers of TH2 cell induction (line 448-449), more information and justification for these markers need to be included. Since the PLNA and biomarkers of T cell-activation are not validated methods to assess for potential autoimmunity induction, these assays should not be recommended.

Lines 532-536: The guidelines recommend that if a compound is found to be tumorigenic in rodent bioassays and is suspected of being immunosuppressive (unintended), follow-up tumor host-resistance should be considered. Specifically, the statement that "tumor host resistance models are appropriate for determining carcinogenic immunosuppressive potential" is inaccurate. These models evaluate immunosuppressive potential, but do not assess carcinogenic potential. In fact, reference is made several times to a causal relationship between carcinogenicity and immunosuppression (lines 107, 132, 532 and 535). There are other mechanisms of carcinogenicity besides immunosuppression. A rodent non-myeloid and non-lymphoid tumor response is almost never associated with immunosuppression in non-clinical safety testing. Further complicating this issue is the fact that neoplasms themselves can cause immunosuppression. Therefore, we recommend that this statement be deleted. We also recommend that if carcinogenic potential is identified in rodent bioassays, then a retrospective analysis of clinical and histomorphologic results should be done to evaluate whether immunosuppression may have occurred.

Finally, we also recommend that the discussion (lines 111-116) regarding the importance of differentiating between unintended and intended impairment of immune function be restated in this section (Section IX, Safety Considerations), along with the statement that indicators of immunosuppression can usually be observed in standard nonclinical toxicology studies.

FLOW CHARTS

In flowchart 1, the validated GPMT, BA, LLNA should be linked to cutaneous hypersensitivity and the inhalation guinea pig assays (without MIGET) should refer to the inhalation route. Therefore, we suggest removing these assays from the first decision box and replacing them with reference to the Sarlo and Clarke (1992) method. The PLNA in flowchart 2 should be deleted or clearly marked as a scientifically meaningful but not validated method.

References:

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ABBREVIATIONS

BA - Buehler assay
CD – cluster of differentiation antigens
CMV- cytomegalovirus
CPMP - Committee for Proprietary Medicinal Products
CRM197 - diptheria toxin carrier protein
EBV - Epstein Barr virus
ELISA - enzyme linked immunosorbent assay
GPMT - guinea pig maximization test
HSV - herpes simplex virus
HPV - human papilloma virus
ICCVAM - The Interagency Coordinating Committee on the Validation of Alternative Methods
KLH - keyhole limpet hemocyanin
LLNA - local lymph node assay
MIGET - mouse immunoglobulin E test
PBL - peripheral blood lymphocytes
PLNA - popliteal lymph node assay
SRBC - sheep red blood cells
Th – T helper 1 or 2 cells
WBC - white blood cells

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