

STP Position Paper: Best Practice Guideline for the Routine Pathology Evaluation of the Immune System

STP IMMUNOTOXICOLOGY WORKING GROUP,

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INTRODUCTION

The European and United States regulatory agencies have recently finalized guidance documents that either include a section referring to immunotoxicity (*CPMP: Note for Guidance on Repeated Dose Toxicity*) or pertain directly to immunotoxicity testing (*FDA: Guidance for Industry, Immunotoxicology Evaluation of Investigational New Drugs*). Both documents state that immunotoxicity testing should be performed on all new investigational drugs or medicinal products. In addition, both documents clearly identify gross and microscopic examinations of lymphoid tissues as necessary and pivotal first steps in the assessment of new drugs for immunotoxic potential. The recommendations contained herein focus primarily on, but are not restricted to, subchronic toxicology studies utilizing rodents. The Society of Toxicologic Pathology (STP), as one of the leading professional organizations devoted to the preclinical safety assessment of new drugs, chemicals, and biologicals, and toxicity studies in general, authorized the formation of the STP Immunotoxicity Screening Working Group (STP IWG) to prepare “best practice” recommendations concerning the collection, interpretation and reporting of organ weights, gross and microscopic observations, and other pathology data relevant to the immune system. The STP IWG consists of anatomic and clinical pathologists from industry, academia, and the National Toxicology Program who specifically identified pathology methods and standardized terminology most appropriate for the detection and reporting of alterations of the immune system. It is the goal of the STP IWG to provide a scientifically sound and well-considered guidance document for routine pathology evaluation of the immune system.

While this document focuses primarily on collection and evaluation of lymphoid tissues from specific pathogen free

rodents, many techniques and approaches presented herein can be considered appropriate for use in studies involving other species, such as dog and monkey. However, whenever non-rodent species are used, it is necessary to be aware of variability of lymphoid organ weight, and gross and microscopic morphology that is associated with age, sex and husbandry of such outbred species. In addition, complete exsanguination of dogs and nonhuman primates is necessary to minimize the variations of spleen weights.

Assessment of Clinical Pathology

This document focuses on anatomic pathology parameters. An in-depth description of clinical pathology evaluation for detection of immunotoxicity is beyond its intended purpose. However, any initial evaluation of immunotoxicity should include routine analysis of hematology and clinical chemistry parameters. Assessment of individual globulin components, i.e., protein electrophoresis, is not recommended as standard procedure, but should be considered if an initial evaluation of other endpoints indicates that a change has occurred. Bone marrow cytology may also be indicated based on observations from bone marrow histopathology and/or hematology results. In particular, bone marrow cytology may be useful if there is a need to differentiate between lymphoid, myeloid and erythroid elements, which can be difficult to distinguish in routine H&E marrow sections. However, cytology for the purpose of determining precise percentages of specific cell types within the bone marrow is often of limited diagnostic value.

Collection and Weighing of Lymphoid Tissue

Pathology practices that include recording and evaluating thymic and splenic weights are supported in the current guidances and should be continued. However, interpretation of these organ weights should only be done in the context of all other clinical, histopathology, and clinical pathology data from the study. Based on general laboratory experience and a review of the literature, the STP IWG contends that alterations of spleen and thymus weights (considered in

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conjunction with histopathology of these tissues) are reasonable indicators of systemic immunotoxicity and are likely to be more reliable indicators than are changes in the weight of peripheral lymph nodes (The ICICIS Group Investigators, 1998; Schulte et al., 2002).

Routine Best Practice for Histopathologic Examination of Lymphoid Tissues as Indicators of Systemic Immunotoxicity

Each animal should receive a thorough macroscopic examination of the lymphoid system and any changes in the spleen, thymus and lymph nodes should be noted. The STP IWG agrees that thymus, spleen, draining lymph nodes, bone marrow in situ, and any gross lesions of a lymphoid organ represent the minimum of tissues for routine evaluation of the lymphoid system, and that microscopic evaluation of thymus, spleen, and bone marrow is a reliable indicator for systemic immunotoxicity. Although bone marrow is considered to be a lymphoid tissue in this document, the STP recognizes that bone marrow is not strictly a lymphoid organ; examination of this tissue is also appropriate to evaluate for potential toxicity to nonlymphoid elements of the immune system and functions not directly associated with immune tissues.

The STP IWG also believes that the most proximal regional lymphoid tissues that drain the drug application site can and should be examined microscopically. Generally, draining lymphoid tissues affected by orally administered drugs are Peyer's patches and mesenteric lymph nodes and should be examined by light microscopy using standard 'best practice' for histopathologic examination in studies involving oral dosing. However, while histopathological examination of the most proximal draining peripheral lymph nodes is appropriate in cases of cutaneous, subcutaneous, or intradermal application of xenobiotic, a thorough understanding of the number and distribution of the targeted nodes must first be established. Additional references concerning lymphatic drainage patterns, and the actual number of nodes involved, in the rat may be necessary and the reader is strongly encouraged to refer to the work of N. Tilney (*J. Anat.* 109:369–383, 1971).

It is the position of the STP IWG that the normal histology of peripheral lymph nodes (i.e., popliteal, auricular, axillary, etc.) that do not drain the site of xenobiotic application can be highly variable, often overlaps with that of altered node morphology, and cannot be unequivocally used as an indicator of systemic immunotoxicity. Moreover, minor differences in collection, embedding, and sectioning combined with high intrinsic variability can seriously limit the value of small lymph nodes for the identification of immunotoxicity. Because of these limitations, collection and examination of peripheral lymph nodes that do not drain the site of xenobiotic application is not recommended for routine assessment of systemic immunotoxicity. In contrast, the normal morphology of the spleen, thymus, and bone marrow is considered more consistent and less subject to differences resulting from collection and processing. Thus, the STP IWG contends that alterations of spleen, thymus, and bone marrow histology are likely to be more reliable indicators of systemic immunotoxicity than are changes in distal peripheral lymph nodes.

Semiquantitative Description of Lymphoid Tissue Changes

Central to many discussions concerning histopathologic examination of lymphoid tissues is the suggestion that semiquantitative descriptive techniques may significantly improve the ability to identify potential immunotoxicants early in xenobiotic discovery or development. In fact, the FDA Guidance specifically comments that: "Methods to enhance detection of immunosuppression in standard toxicology studies have been described, including exact tissues that should be examined and effects that should be noted" (Kuper et al., 1995, 2000). Further: "To better characterize such (histologic) changes, a more quantitative histopathological assessment of lymphoid organs as well as immunohistochemical techniques might be useful" (Ward et al., 1993; Kuper et al., 1995; Mitsumori et al., 1996); additional discussion of such approaches is presented by Kuper et al. (2000, 2002) and the reader is encouraged to review these documents. The recommendations presented herein by the STP IWG have used some of the approaches described by these authors as a starting point.

Based on a review of relevant literature and discussions at international meetings the STP IWG endorses the concept that "best practice" for lymphoid tissue microscopic examination involves "a semiquantitative description of changes in compartments and/or microenvironments of specified lymphoid organs."

Three primary points are emphasized by this approach: (1) each lymphoid organ has separate compartments that support specific immune functions, (2) these compartments can and should be evaluated individually for changes, and (3) descriptive, rather than interpretative terminology, should be used to characterize changes within these compartments.

Such "best practice" does not mandate specialized techniques such as lymphoid tissue immunohistochemistry, blind scoring, morphometry, or flow cytometric evaluation of cell suspensions. These specialized techniques, which may be valuable for clarifying specific lymphoid tissue alterations, should be considered after lymphoid tissue change has been identified and should be directed at answering a specific scientific question; they should not be used as routine screening tools.

Examination of Lymphoid Tissue by Compartments

Current "best practice" for histopathologic examination of any tissue requires that the anatomic pathologist identify and examine all compartments of all tissues, including lymphoid tissue, but only make entries for specific compartments if an abnormality is identified within that compartment. If all tissue compartments demonstrate changes considered to be within normal ranges of variability for that organ, an entry of "No Abnormality Determined (NAD)" or other such term can be entered for the entire organ. This document reiterates that such "best practice" is appropriate for the histologic evaluation of lymphoid tissues. The pathologist should carefully and systematically examine each compartment within each tissue and note any abnormality using appropriate terminology. The use of this approach will add precision to histopathology data tables concerning the relevant lymphoid changes. Nevertheless, the recording of incidental background changes may be performed at the discretion of the investigator(s).

Because such “best practices” are not readily apparent to the nonpathologist, it is recommended that inclusion of a definitive statement regarding the procedures for histopathologic examination of all tissues, including lymphoid/immune system tissues be included within appropriate SOPs. The statement might read as follows: “Histopathologic examination of all tissues will include a detailed examination of all tissue compartments and histopathologic abnormalities will be recorded according to the compartment in which they are identified.”

TERMINOLOGY

In order to achieve an accurate, consistent and useful “semiquantitative description” it is necessary to develop consensus on consistent terminology used in characterization of lymphoid tissue changes. Whenever possible, semiquantitative/descriptive terms (i.e., reduced numbers of lymphocytes) rather than interpretative terms (i.e., lymphoid atrophy) for registering lymphoid tissue changes is recommended. To illustrate this point further, consider immunotoxicological changes of the thymus; a semiquantitative description such as: “thymus, cortex, decreased lymphocytes, marked” would be preferable to “thymic involution.”

Terms such as atrophy, hypoplasia, hypertrophy, and hyperplasia can be applied to either macroscopic or microscopic changes. However, in many cases selection of a more descriptive term, i.e., increased or decreased cells (specify), will provide a more objective semiquantitative description for that change, and is encouraged. In addition, because the term “altered cellularity” can indicate *altered* size of the compartment *or altered* cell density, it is suggested herein that the term “*altered* cellularity” not be used. As it is likely that either increased or decreased cellularity (and the type of cell that is increased or decreased) will be identifiable thereby allowing one to eliminate the ambiguity of the term “*altered*.” A more unequivocal description of the change can be achieved by clearly stating which cell type, i.e., lymphocyte, macrophage, mast cell, etc. is either increased or decreased in number. Nevertheless, there may be cases in which the terms atrophy, hypoplasia, hypertrophy and/or hyperplasia remain the most appropriate microscopic descriptors, for example in carcinogenicity studies. The use of such terms should include appropriate

indicators of severity so as to maximize their descriptive value.

Thus, one may evaluate lymphoid tissues by asking such questions as:

1. Is the lymphoid organ grossly larger or smaller than normal: macroscopically *increased or decreased in size*?
2. Which compartment is specifically involved?
3. Is the change in size of the organ due to a change in components (e.g., cells, stroma, edema fluid) of a particular compartment?
4. Is this change in size due to a change in cell numbers in one or more compartments, i.e., microscopically *increased or decreased number of cells, and if so which cells are involved* (lymphocytes, macrophages, stromal cells, etc.)?
5. Are the changes due to resident cells within the compartment, or due to passenger cells migrating through the compartment?
6. Does the weight, color or texture differ from normal background and/or control group organs (macroscopy)?

This approach emphasizes the use of descriptive terms in the recording of observations as compared to interpretative terms or diagnoses.

Recording of Lymphoid Tissue Changes

In routine evaluation of lymphoid tissues, it is recommended that the pathologist evaluate the individual compartments separately and in the context of the entire organ or tissue. Because the immune system is anatomically and functionally complex and dynamic, it is beyond the scope of this document to provide a comprehensive scheme for each lymphoid organ or tissue. However, a foundation or basis for evaluating major compartments and components in the routine evaluation of these tissues is presented in Tables 1 and 2. The STP IWG has attempted to provide examples of terminology for commonly observed changes for each lymphoid tissue.

The use of descriptive terms should not prevent or interfere with the pathologist’s responsibility to interpret the constellation of changes in a meaningful pathobiologic context. However, interpretation is best reserved for the Discussion section of a pathology report rather than the Results section.

TABLE 1.—Compartments of lymphoid tissues.¹

Thymus	Spleen	Lymph node	Bone marrow
Cortex	White pulp • PALS • Lymphoid follicles • Germinal centers	Cortex • Subcapsular sinus • Follicles • Germinal centers • High endothelial venules	Erythroid component
Medulla		Paracortex	Granulocytic component
Cortex-medulla ratio ²		Medulla • Medullary cords • Medullary sinuses	Fat
	Marginal zone		Lymphoid component
	Red pulp		Stroma
			Megakaryocyte
			Other cells

Note: The table shows only illustrations as to how one might approach the collection of data. It is *not recommended* that these tables be included as part of the data tables or part of the final report.

¹Compartmental descriptors can also be applied to the Peyer’s Patches.

²Not a compartment per se.

TABLE 2.—Possible findings associated with lymphoid tissue alterations.¹

Lymphocytes: increased/decreased
Granulocytes: increased/decreased
Mast cells: increased/decreased
Megakaryocytes: increased/decreased
Tingible-body macrophages
Pigmented macrophages
Vacuolated macrophages
Plasma cells: increased/decreased
Fat necrosis
Inflammation; specify type as appropriate i.e., granulomatous
Sinus erythrocytosis; designate sinus
Sinus histiocytosis; designate sinus
Hemorrhage
Necrotic cells; designate cell type if possible
Infarct
Erythroid component: increased/decreased
Granulocytic component: increased/decreased

Note: The table shows only illustrations as to how one might approach the collection of data. It is *not recommended* that these tables be included as part of the data tables or part of the final report.

¹This is a nonexhaustive and nonprioritized list and represents only some of the more commonly encountered findings.

Moreover, final determination of the most appropriate terminology to be used in a study is the shared prerogative and responsibility of the Study Pathologist and the Peer Review Pathologist.

CONCLUSION

Recently, there has been an increased focus on ensuring consistency in the evaluation of xenobiotics for immunotoxicity. New regulatory guidance documents concerning testing of new agents for potential immunotoxicity recognize the importance that histopathology of lymphoid tissue plays in identification of immunotoxic effects. However, the guidances suggest that current histopathology methods could be enhanced by the application of improved techniques, semi-quantitative terminology, and additional training. Adoption of these procedures will not only ensure that compartments of lymphoid tissues are routinely examined and that abnormalities are recorded with consistent terminology but also will facilitate compliance with these new guidances and help ensure that immunotoxic compounds are identified. Specialized techniques such as lymphoid tissue immunohistochemistry, blind scoring, morphometry, or flow cytometric evaluation of cell suspensions, may be valuable for clarifying specific lymphoid tissue alterations after the initial identification of

such changes and should be directed at answering a specific scientific question; they should not be used as routine screening tools. All changes observed for a given lymphoid organ should be interpreted in the context of the complex interaction of different lymphoid organs and/or other organ systems, as well as hematologic and clinical chemistry data, so as to place the changes in the appropriate toxicologic and pathologic perspective.

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