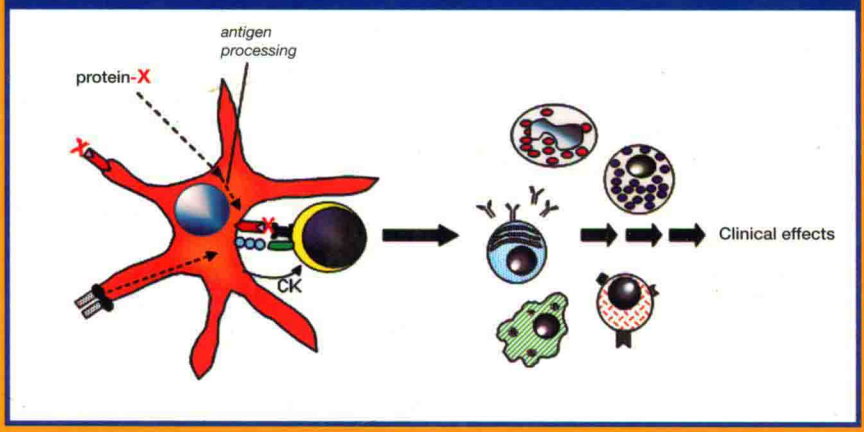
Immunotoxicology Strategies for Pharmaceutical Safety Assessment



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IMMUNOTOXICOLOGY STRATEGIES FOR PHARMACEUTICAL SAFETY ASSESSMENT

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IMMUNOTOXICOLOGY STRATEGIES FOR PHARMACEUTICAL SAFETY ASSESSMENT

PREFACE

This book is the first edition entirely dedicated to immunotoxicology testing during pharmaceutical drug development. Immunotoxicology is a highly specialized discipline that addresses potential adverse effects on the immune system, including immunosuppression, immunogenicity, hypersensitivity and other immune system functional disorders. A broad spectrum of xenobiotics, including agents present in our environment as well as pharmaceutical molecules may adversely affect the immune system. In pharmaceutical drug development, testing of drug candidates involves not only potential immuntoxic hazard identification but also risk assessment in the context of therapeutic use of a given drug. Thus, testing for potential immunotoxicity of drug candidates should be an integral part of overall safety evaluation in both preclinical and clinical phases of drug development. This approach is different from immunotoxicity testing of environmental agents where any immunotoxicity is hazardous and unacceptable in light of potential uncontrolled exposure of healthy population.

In the development of novel therapeutic entities (chemicals, proteins and vaccines), strong immunotoxicity signals can be detected by hematology and/or lymphoid tissue histopathology evaluation as part of standard toxicity studies. However, potential immunotoxicity related to immune dysregulation by drugs, may only manifest at the functional level during an immune response to a challenge with an antigen (e.g., foreign protein, pathogen, toxin). Thus, evaluation of the functional immune system requires studies involving 'activated' immune cells, organs or entire hosts in response to an outside challenge. To detect and characterize such hazards, immunotoxicology assessment involves not only conventional toxicology endpoints (i.e., hematology, clinical chemistry and histology) but also a broad spectrum of specialized testing to evaluate potential immune dysregulation, including specific immune response tests (cellular or humoral), immunophenotyping, cytokine expression, immunoassays to address immunogenicity, and *in vivo* models of immune disorders to characterize potential impairment of host defense to infections, tumors and autoimmune diseases.

This book is focused on discussions of strategies for application of immunotoxicology testing during drug development, in other words it addresses 'what' and 'how' can be performed in such evaluation.

We hope this new book will be found valuable to both the experienced and novice immunotoxicologists who work, teach and study immune-related aspects of safety assessment of drug candidates in pharmaceutical development.

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INTRODUCTION TO IMMUNOTOXICOLOGY

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It is important to briefly describe the organization and importance of the immune system to set the stage for the more focused chapters that follow. It is equally important to explain how the interest in immunotoxicology developed and flourished among toxicologists within regulatory bodies and the pharmaceutical industry. The earliest interest in immunology among toxicologist started with the observation that certain environmental chemicals (e.g., dioxins, PCBs, some pesticides, etc.) appeared to target the immune system and alter its function (Vos, 1977). In parallel, the discipline of immunopharmacology caught the interest of many in the 1970s, including this author, as the search for new chemical entities (NCEs) possessing immunological activity of therapeutic potential flourished. In many pharmaceutical companies, immunological active NCEs and cytokines became a development target (see reviews of Talmadge and Dean, 1994) for the treatment of cancer, viral diseases, and immune deficiencies. During the 1980s, toxicologists in industry were confronted for the first time with the safety assessment of protein therapeutics (e.g., monoclonal antibodies, IFNs, lymphokins, cytokines, etc.) and NCEs with novel immune activity. Consequently, most of the early scientists working in the developing discipline of immunotoxicology got their training in either basic immunology or immunopharmacology.

Recognizing the importance of this topic, the Gordon Research Conference on Drug Safety in the summer of 1978 devoted 2 days to the topic of Immunotoxicology which represented one of the first symposia in this area. Shortly thereafter, I organized the first Workshop on Methods and Approaches for Assessing Immunotoxicology in Williamsburg, VA (Dean, 1979), which was attended by 50 scientists and physicians. The Williamsburg meeting was imme-

diately followed by an NIH Consensus Meeting at Research Triangle Park, NC, with the objective of defining specific research needs for this newly emerging field. The discipline of immunotoxicology soon captured the imagination of the International Program on Chemical Safety (IPCS) of the World Health Organization (WHO) and the first international meeting was held in 1984 during which a formal definition was developed (Berlin et al., 1987). By the mid- to late 1980s, most major pharmaceutical companies began recruiting toxicologists or immunologists trained in immunotoxicology and established small groups to monitor for inadvertent immune side effects of new drug candidates. In 1998 we reported (Dean et al., 1998) that there were 12 pharmaceutical companies with groups involved in the safety assessment of NCEs for immunotoxic potential. During this period there were also efforts at standardizing and validating these methods and the tiered approaches in rodents (Luster et al., 1988), as well as multiple ring studies. The field of immunotoxicology has matured considerably since this early period when most of the focus was on methods development and validation in rodents. This maturation has been successfully natured by the Immunotoxicology Specialty Section, charted by the Society of Toxicology (United States) in 1985, and the Immunotoxicology Technical Committee of the Health and Environmental Scientists Institute (HESI) of ILSI established in 1992.

THE IMMUNE SYSTEM: ORGANIZATION AND FUNCTION

It is now well established that the immune system is a complex multicellular organ system consisting of granulocytes, macrophages, lymphocytes, and dendritic cells with various functions and unique phenotypic characteristics, which can produce various soluble mediators (for detail, see Dean et al., 2007). The cells that constitute the immune system are of hemopoietic origin and in adults, are found in the peripheral blood, lymphatic fluid, and organized lymphoid tissues, including bone marrow, spleen, thymus, lymph nodes, tonsils, and mucosa-associated lymphoid tissue. Since the immune system is in a constant state of self-renewal involving cell proliferation, differentiation, activation, and maturation, it is vulnerable to agents that disrupt any of these cellular processes. The immune system appears to exist principally to defend the host against invasion by infectious and opportunistic microorganisms and spontaneously arising neoplasia. This network of cells and soluble mediators that contribute to host defense are highly regulated and interdependent, and must not only discriminate self from nonself, but also be able to react to nonself with a variety of defensive responses (Paul, 1999). In addition, the immune system can occasionally develop a response to a chemical or drug or their reactive metabolite that might bind to or alter a host protein, resulting in an allergic or autoimmune response. It is now well established that the immune system of experimental animals, although exhibiting some obvious differences

from that of humans, is still sufficiently similar that data obtained from lower species are instructive of a potential human response (Haley, 2003) and these data can be used for risk assessment.

Nonspecific and Acquired Host Defense

The host defense functions of the immune system are provided by two major mechanisms: a *nonspecific* or *innate* mechanism that does not require prior sensitization with the inducing agent to elicit a response, and a *specific or acquired* mechanism directed against the eliciting agent to which the individual has been previously sensitized (immunological memory). Penetration of the skin or mucosal defense barriers by an invading microorganism results in nonspecific reactions by phagocytic cells (granulocytes and macrophages [MØ]). If the microorganism is not controlled by these cells and persists, specific responses involving antibody production and the induction of effector lymphocytes can follow. Effector lymphocytes respond through cytokine mediators to seek out and destroy the invading microorganism. Both antibody-producing lymphocyte responses (B cell mediated) and thymus-dependent lymphocyte responses (T cell mediated) are triggered by the presentation of foreign antigen to appropriate lymphocytes by dendritic cells, macrophages, or other antigen-presenting cells (APCs). Following antigen-induced activation, B cells proliferate and differentiate into plasma cells (PCs), with the support of T-helper 2 (Th2) cells, which produce large quantities of antigen-specific immunoglobulins (antibodies). Antibodies enter the plasma, where they bind the foreign material and neutralize, lyse, or facilitate phagocytosis of the agent. Antibody–antigen interactions are expanded by actions of the complement (C′) system and other inflammatory mediators (e.g., prostaglandins and leukotrienes). With the support of Th1 cells, another population of T cells, referred to as cytotoxic T cells, proliferate and recognize viral infected cells that they can destroy before viral replication is complete.

The immune responses that characterize acquired host defenses represent a series of complex events that occur following the introduction of foreign antigenic material into the body. The two major types of specific immune response are (1) cell-mediated immunity (CMI), which is initiated by specifically sensitized T cells and is generally associated with delayed type hypersensitivity (DTH), rejection of tumors or foreign grafts, and resistance to viral agents; and (2) humoral immunity (HI), which involves the production of antibodies by PCs following sensitization to a specific antigen and is important in resistance to extracellular pathogens.

Origin and Development of the Cellular Constituents

The cellular elements of the immune system arise from pluripotent stem cells, a unique group of unspecialized cells that have self-renewal capacity. These cells are found in the blood islands of the embryonic yolk sac and in the liver of the

fetus during fetal development, and later in the bone marrow. The pluripotent stem cell differentiates along several pathways, giving rise to erythrocytes, myeloid series cells (i.e., MØ and PMNs), megakaryocytes (platelets), or lymphocytes. Maturation generally occurs within the bone marrow, although lymphoid progenitor cells are disseminated through the blood and lymphatic vessels to the primary lymphoid organs where they undergo further differentiation under the influence of the humoral microenvironment of these organs. The primary lymphoid organs include the thymus in all vertebrates and the bursa of Fabricius (in birds) or bursa-equivalent tissue in other vertebrates; the latter are believed to be bone marrow and gut-associated lymphoid tissue in mammals. The primary lymphoid organs are lymphoepithelial in origin and are derived from ectoendodermal junctional tissue in association with gut epithelium. During the beginning of the second half of embryogenesis (days 12 to 13 in the mouse), stem cells migrate into the epithelia of the thymus and bursa-equivalent areas, where they differentiate independently of antigenic stimulation into immunocompetent T and B cells, respectively. The thymus is an organization of lymphoid tissue located in the chest, above the heart. Thymus development occurs during the sixth week of embryological development in humans and day 9 of gestation in the mouse. The thymus reaches its maximum size at birth or shortly thereafter in most mammals and then begins a slow involution that is complete between the ages of 5 and 15 years in humans. Histologically, the thymus consists of multiple lobules, each lobule containing a cortex (outer) and a medulla (inner). Lymphocyte precursors from bone marrow proliferate in the cortex of the lobules and then migrate to the medulla. In the medulla, they further differentiate, under the influence of thymic epithelium and hormonal factors, into mature T lymphocytes before emigrating to secondary lymphoid tissues. The neonatal/postnatal thymus has a significant endocrine function supported by nonlymphoid thymic epithelium cells. These cells produce a family of thymic hormones essential for T lymphocyte maturation and differentiation.

The mammalian bursa-equivalent tissue, where B cells are formed, is believed to be the fetal liver, neonatal spleen, gut-associated lymphoid tissue, and adult bone marrow, depending on age. Mature B lymphocytes migrate from the bursa-equivalent tissue to populate the B-dependent areas of the secondary lymphoid tissues. Neonatal removal or chemical destruction of primary lymphoid organs prior to the maturation of lymphocytes into T or B cells or prior to their population of secondary peripheral lymphoid tissue dramatically depresses the immunological capacity of the host. However, removal of these same organs in adults has little influence on immunological capacity. In addition, neonatal thymectomy in mammals dramatically impairs the development of CMI but does not generally influence the generation of immunoglobulin-producing cells involved in antibody-mediated immunity. In contrast to the removal of primary lymphoid organs, removal of secondary lymphoid organs does not inhibit the development of immune competence, although it may suppress the magnitude or alter the tissue location of the responsive cells.

CELLS AND CYTOKINES: FUNCTION AND RESPONSES

Humoral Immunity

The principal function of B lymphocytes is production of specific antibody in response to antigenic stimulation. B cells recognize antigen *via* a specific receptor, comprised of membrane immunoglobulins associated with accessory proteins either directly or in the presence of an APC. Binding of the receptor with its cognate antigen triggers transmembrane signaling, leading to activation of the B cell. The antigen is subsequently internalized, where it is processed and associated with class II major histocompatibility complex (MHC) molecules. Antigen-derived peptides, along with MHC proteins, are then transferred to the cell surface, where they are free to interact with helper T cells. Within 3 to 5 days following antigen exposure, this T/B cell interaction results in the B lymphocytes differentiating into blast cells, then into immature PC, and finally into antibody-secreting PC. The establishment of humoral immunity is characterized by an early rise in IgM antibody titer in the serum, followed several days later by the appearance of IgG antibodies. During this differentiation process, some of the lymphocytes develop into long-lived or memory cells (sensitized but non-blast cells), so subsequent antigen encounters result in an enhanced (secondary) response. This secondary response is characterized by a shorter latency for antibody appearance, as well as an increased affinity and synthesis of IgG antibodies.

Antibody molecules react with specific antigenic determinants (epitopes) on their target, facilitating its removal (e.g., lysis or enhanced phagocytosis). Based on chemical structure and biological function, the five classes of antibody molecules in mammals are IgM, IgG, IgA, IgD, and IgE. Antibodies operate *via* several mechanisms to protect the host from infectious agents. Some of these mechanisms include virus neutralization, in which antibodies bind and prevent virus particles from infecting target cells; opsonization, the process by which antibody molecules react with infectious agents and thus enhance their phagocytosis; and antibody-dependent cellular cytotoxicity, the process whereby antibody-coated target cells are killed by Fc receptor-bearing lymphocytes. Of increasing interest is the concept that naturally occurring IgM antibodies (that is, antibodies that are secreted in the absence of antigen stimulation) may play an important role in immune surveillance against neoplasia (Vollmers and Brandlein, 2005).

Cell-Mediated Immunity

Cell-mediated immunity (CMI), often referred to as T cell-mediated immunity, refers broadly to any host resistance mechanism in which cellular elements play a direct role and which is part of the acquired arm of immunity. This is in comparison to humoral immunity, in which there are certainly cellular interactions but the final host resistance products are soluble factors such as

antibody. A number of host defenses are mediated directly by cells including MØ-mediated cytolysis, antibody-dependent cellular cytotoxicity, and natural killer (NK) cell cytotoxicity although cytotoxic T lymphocytes (CTLs) usually predominate, particularly in the destruction of virus-infected cells. Functions associated with CMI are commonly considered the province of T lymphocytes, although immune cells (e.g., B cells and MØ), as well as nonimmune cells (e.g., fibroblasts and dendritic cells) contribute to the development of CMI. As the primary effector cell in CMI, the T cell represents one of the most complex and multifunctional immune cells. Antigens that generally elicit CMI include tissue-associated antigens, chemicals and drugs that covalently bind to autologous proteins, and antigenic determinants on persistent intracellular microorganisms. The route of exposure also plays a major role in the type of response generated; for example, sheep erythrocytes elicit antibody production (but not CMI) when injected intravenously in humans, but elicit both when injected intracutaneously. The induction of CMI proceeds when small lymphocytes differentiate into large pyroninophilic cells and ultimately divide, giving rise to cells responsible for effector function, as well as immunological memory. In contrast to humoral immunity, which is more effective against extracellular pathogens, CMI helps protect against intracellular bacteria, viruses and neoplasia, and is responsible for graft rejection. T cells can differentiate into populations responsible for either regulatory or effector function. For example, regulatory and inducer T cell functions are provided by CD3/CD4+ T-helper cells. The T-helper function facilitates antibody responses by B cells and assists in other T cell responses. For most antigens, B cells require assistance from T cells for differentiation into PCs. T-helper cells are integral in the B cell response by participating in two distinct mechanisms: (1) major-histocompatibility locus-restricted B and T cell collaborations, and (2) cytokine-mediated differentiation. Helper function is a result of interactions between surface molecules on T-helper cells and B cells, as well as the production and secretion of immunoregulatory cytokines. Effector functions take the form of cytotoxic activity (CD3/CD8 phenotype), manifested by cytotoxic T lymphocytes (CTLs). These cells are able to specifically lyse target cells *via* the release of various bioactive molecules. Another effector function is the ability of T cells to mediate suppressor activity for both T and B cell responses. Suppressor activity is also mediated by cells bearing the CD3/CD8 phenotype, although recent studies suggest that this activity may be the result, at least in part, of differential cytokine production by this population. This responsibility for both helper and suppressor activities indicates the crucial roles of T cells in normal immune function.

T-Helper 1 and T-Helper 2 Cells

An important conceptual breakthrough in immunology was the finding that two major populations of T-helper cells exist that have different, sometimes opposing functions. Mosmann et al. (1986) first established the concept by