

Immune System as a Target Organ for Toxicity

by Peter H. Bick*

Recently, interest has centered on the immune system as a target organ for toxic effects. This seems a reasonable choice, since it can be argued that alterations induced in this system as a result of a toxic insult could lead to impaired immunity. Such an alteration may be manifest in altered disease susceptibility. Documenting toxic effects upon the immune system is a difficult task due to the multifaceted network of specialized cells that carry out immune functions. Because of this complexity, it is necessary to develop a panel of immunologic assays to adequately cover the diverse functions that may be affected by a toxic substance. In the interest of efficiency and cost, it is preferable to organize the assay systems into a tier structure to generate the most information in the shortest time. An overview of the immune system is presented, and an example of one possible tier system of assays is described.

Introduction

As a target organ for toxicity the immune system is, at first glance, a logical choice to investigate. One could reason that prolonged exposure to a substance of low toxicity or an acute exposure to a highly toxic chemical may alter immunological functions and predispose an individual to increased disease susceptibility. In practice, however, documenting such effects is not the easy task one would hope for. This is due to the nature of the immune system, which comprises a large network of organs and varied cell types that carry out interrelated functions. Many of the cellular subpopulations are highly specialized, and there exist numerous avenues of communication and a complex regulatory network that governs responsiveness (1). Of selective advantage to the host is the fact that the immune system offers several pathways for handling a challenge. This fact alone complicates toxicologic studies by allowing

the possibility of a protective immune response to coincide with a toxic effect. A component of one pathway may be compromised by a toxic insult, yet the host's ability to survive a challenge by an infectious agent may be unchanged. Immunotoxicological studies therefore must be multifaceted to detect such situations.

It is desirable to establish a set of immunologic tests that could be applied to the study of the toxicological properties of a wide range of suspected substances. Such a battery of tests should have the following characteristics: (a) a standardized test panel should be based on sound cellular immunologic techniques; (b) the test panel should be as sensitive and reproducible as possible to facilitate data comparison between laboratories; and (c) the test systems should be as automated as possible to allow sufficient data to be generated from a statistically large sample population. The limitations of each test in relation to what is learned concerning the function of the immune system as a whole should be considered. In order to reach this goal, one must have an overall appreciation of the complexity of the immune system.

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Development, Structure and Function of the Immune System

Cells of the immune system originate from a pluripotent stem cell pool found in the yolk sac and fetal liver during fetal development and in the bone marrow and spleen of the adult (2-4). As shown in Figure 1, stem cells may follow one of several lines of maturation. Influential in the development of one major lymphocyte class is the thymus, which originates from the third and fourth pharyngeal pouches at 9 days of gestation in mice, (9 weeks in man). As stem cells pass through and are influenced by the thymus, they mature and acquire the characteristics of thymus-derived T-lymphocytes on their way into the peripheral lymphoid organs. Mature T-lymphocytes seed the thymic-dependent areas of the spleen and lymph nodes and comprise 55-74% of the peripheral blood lymphocytes (5, 6). These cells can be distinguished from other lymphocytes by genetically defined alloantigenic cell surface markers: Thy 1 (theta) and Lyt 1, 2, and 3 (in mice), among others (7-9). Such cell surface markers provide handles with which to identify functional subpopulations of T-cells in normal and disease states. T-lymphocytes also respond to the mitogens phytohemagglutinin (PHA) and concanavalin A (con A), which can be used as a gross probe for the integrity of T-cell function (10, 11). T-cells carry out functions of immune regulation by participating as amplifying "helper" cells and "suppressor" cells active in antibody responses to thymic-dependent antigens, mixed lymphocyte responses, delayed-type hypersensitivity (DTH) reactions and the activation of cytotoxic effector T-cells. Effector T-cells participate directly as killer cells against virally infected host cells, malignant cells and foreign tissue, function in DTH reactions and elaborate soluble lymphokine mediators capable of modulating inflammation. T-cells are long-lived lymphocytes, and the thymus atrophies in the adult animal. Some estimates of longevity place some T-cells as existing for 15-20 years. Since there is little replacement of T-cells, alterations in this compartment may be very detrimental. Lesions in the T-cell system result in enhanced susceptibility to viral, fungal and parasitic infections and alter the levels of most antibody responses. Depletion of the thymus gland or stem cell pool during fetal development decimates this branch of the immune response as demonstrated by patients with DiGeorge syndrome. Alterations in discrete T-cell subsets may alter immune responses in a selective manner. For

example, patients with systemic lupus erythematosus have below normal suppressor T-cell function, resulting in unregulated antibody production (12-16). Evaluation of T-cell function should include test parameters that detect functional activity of the various T-cell subpopulations.

The second major route of lymphocytes differentiation is well defined in avian species. Here the Bursa of Fabricius processes stem cells to become mature B-lymphocytes. No such organ can be defined in mammals, and several sites for B-lymphocyte maturation have been suggested, including bone marrow, spleen and the gut-associated lymphoid tissue. Lymphocytes that follow this pathway become mature B-lymphocytes that possess specific immunoglobulin receptors on the cell surface that are of the same specificity as the antibody to be produced by the cell following activation. The high density of cell surface immunoglobulin (sIg) is a central characteristic that separates B- from T-lymphocytes. As B-lymphocytes mature from stem cells, a number of cell surface changes occur. B-lymphocytes progress from a "pre-B" cell with no surface immunoglobulin to an immature sIgM-bearing cell. Subsequent differentiative steps see the B-cell sequentially acquiring receptors for the Fc portion of IgG, cell surface IgD, immune-response associated (Ia) antigens coded for by the major histocompatibility complex and receptors for complement components (17-21). B-lymphocytes with differing cell surface phenotypes represent functionally different subpopulations that respond differentially to tolerance induction and antigenic and mitogenic stimulation (22-24). The cell surface markers can be used to delineate and identify alterations in

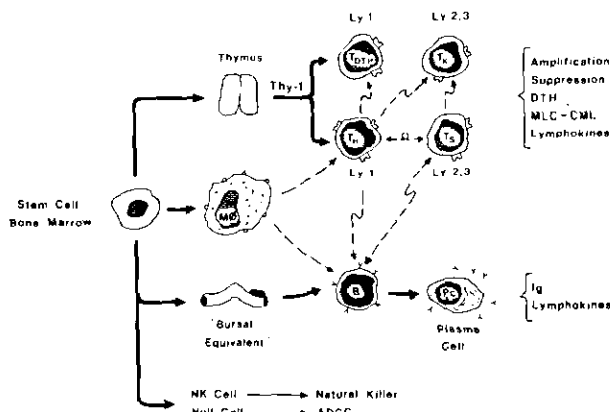


FIGURE 1. Development and organization of the cellular immune system

subpopulations in normal and experimentally modified B-cell populations. Antisera specific for many of the cell surface markers (sig, Ia) are commercially available, some as hybridoma antibodies, allowing widespread application of phenotype determination and cell depletion. Mature B-lymphocytes are found in the thymic-independent areas of spleen and lymph nodes and represent approximately 25-45% of peripheral blood lymphocytes.

Following activation, B-lymphocytes proliferate and/or differentiate into antibody-producing cells and plasma cells. As this process proceeds, many cell surface markers are lost, and the end-stage plasma cell expresses a new antigen (plasma cell antigen, Pca) and produces quantities of specific immunoglobulin. In contrast to the long-lived T-cell, B-cells turn over frequently and are replaced by newly differentiating stem cells. Alterations in the B-cell compartment may be reversible following the differentiation of new adult B-cells. Toxic insults that result in depletion of B-cell function can result in lowered immunoglobulin levels and increased susceptibility to bacterial infection.

Other lymphocytic cells active in immune cytotoxic functions are the natural killer (NK) cell and the "null" cell active in antibody-dependent cell-mediated cytotoxicity (ADCC). Natural killer cells have cytotoxic potential for certain malignant cells in the absence of prior sensitization. These cells probably represent pre-T cells but do not exhibit traditional T-cell phenotypes (25-27). The null cells active in ADCC do not bear traditional B-cell markers but possess receptors for IgG Fc and some T-cell markers and are capable of killing antibody-coated target cells (28). The differentiation pathways of these cells are not as well defined as are those of the T and B lymphocytes. The observation that cells active in ADCC bear some T-cell markers suggests that these cells evolve from the T-cell lineage and that the ADCC and NK effector cell populations may overlap (29-31). Although there exist mouse strains that are deficient in NK activity [Beige mice; C57BL/6J-bg (32, 33)], it is not clear what the long-range effects of defects in ADCC or NK activity may be. These functions may be compensated for by T-lymphocyte functions.

The pluripotent stem cell pool also contains the precursors of monocyte-macrophage and polymorphonuclear leukocyte (PMN) populations. The macrophage has an important role in genetically defined antigen-driven immune activation and tumor-cell killing in addition to its phagocytic role. Defects in stem cell differentiation into PMN's or macrophages will predispose the host to bacterial infection and in the case of a macrophage defect will

interrupt normal B- and T-lymphocyte activation.

As should be clear from the above discussion, toxicological alterations may affect the immune system in a very subtle or gross fashion depending upon the level of interference. If a single T- or B-cell subpopulation is affected, the loss of overall responsiveness and increase in disease susceptibility may be minimal. Such a loss of response may also be transient. However, an alteration in stem cell, thymus or bone marrow differentiative pathway may result in severe nonreversible impairment. It is important, therefore, to study a wide range of parameters in order accurately to assess any effect on the immune system as a whole.

Assessment of Immunologic Potential

Various immune assay systems measure different levels of cell involvement and complexity in response to a stimulus. As such, assays that measure responses that are a result of complex cell interaction mechanisms are more likely to reveal alterations in immune function. For this reason, one can order the immunologic parameters into a tier structure, based on sequential levels of cellular cooperation reflecting this degree of complexity. It is anticipated that studies of drug- or chemical-induced immune alterations would focus on the complex assay systems first. Once a drug-induced lesion is found, the cause can be dissected using assays that involve less complex systems. Such an approach will ultimately lead to the assays that will pinpoint the cellular basis of dysfunction. Table 1 shows one possible tier structure. This tier system is not meant to be exhaustive but to serve as an example of one possible experimental design. The components of Tier I measure reactions that depend upon complex multicellular interactions involving T and B lymphocytes and macrophages. Genetically controlled regulatory networks play an important role in these responses. It would be anticipated that immune alterations at one of several sites in the cell interaction network would result in a modified response. However, since these systems are complex, it may be possible to compensate for an immune lesion by using an alternative response pathway. Furthermore, since these systems only assess a limited area of the total immune system several should be employed to provide good coverage.

The assay systems in Tier II involve cell interaction mechanisms that are less complex than those in Tier I, often inducing only M ϕ -T-cell or B-cell-M ϕ -suppressor T-cell interactions. These assay

Table 1. Hierarchy of some immunologic assay systems.

Tier	Reaction
Tier I	Complex cell interactions <i>In vivo</i> spleen cell IgM and IgG responses to thymic-dependent antigens Popliteal lymph node proliferation following immunization Delayed cutaneous hypersensitivity Quantitation of circulating antibody levels following immunization with thymic-dependent and thymic-independent antigens
Tier II	Immunologic interactions of intermediate complexity Determination of basal immunoglobulin levels Mitogenic stimulation of T-lymphocytes with concanavalin A or phytohemagglutinin Stimulation of B-cell proliferation and antibody synthesis by <i>E. coli</i> lipopolysaccharide One-way mixed lymphocyte reactivity Enumeration of thioglycollate-induced peritoneal exudate cells
Tier III	Direct cellular assays Determination of resident mouse peritoneal exudate cells Determination of phagocytic capacity of peritoneal exudate cells Cytostatic and cytotoxic capability of adherent normal and activated resident peritoneal exudate cells against malignant cells Reticuloendothelial system clearance

systems allow a more defined evaluation of a discrete portion of the immune response. As such they may allow a chemically induced effect detected in a Tier I assay to be localized to a particular cell type. Further definition of a toxic effect may be accomplished using direct cellular assays like those presented in Tier III.

Assays shown in Tier III represent some of the assays available to determine the function of a distinct cell population. Once it is suspected that a discrete cell population is affected by a chemical, assays of this type are useful in supporting the initial findings. By ordering the experimental protocols in a tier structure, a drug-induced alteration in immune responsiveness is likely to be rapidly detected and efficiently defined.

Conclusion

The immune response offers a well-defined system in which to study the toxic effects of suspected drugs or chemicals. Through many years of immunologic research our understanding of the complexity of the cellular interactions involved in any immune response allows us to ask specific toxicological questions. The following considerations are important in any immunotoxicological study. The immune system represents a complex

multicellular, multiorgan network that may be affected at several sites. Alterations in one arm of the response (humoral or cellular) may not affect responses governed by the other limb. The immune system shows a great deal of resiliency and harmful effects may be transient. Some components of the immune system are long-lived (T-cells), whereas others are not (B-cells). Alterations in short-lived, replaceable systems may have little long-term consequence to the host. Immune response capability and the influence of various organs (thymus) changes with ages. Therefore deleterious effects may be a function of age. Since many immune responses are genetically controlled, alterations in responsiveness to one challenge in a given animal model may not hold true in second animal model. Genetic differences may also be seen between sexes.

In view of the above considerations it is important in any toxicological study of immune capacity to consider the immune system as a whole and design experimental protocols that consider the dynamic complexity of the system.

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