Immunotoxicology

Unit 3 (a and b) – immunotoxicity

The immune system is a very complex and regulated organ system that involves the cooperation and interaction of a number of different cell types, cell products, tissues, and organs. The immune system consists of fixed primary (i.e., thymus and bone marrow) and secondary (i.e., spleen, lymph nodes, and gut-associated) lymphoid tissue, and various circulating immunocompetent cells. This unique organization may contribute to the immune system's vulnerability as a target organ for xenobiotics. For example, cells of the immune system undergo continual proliferation and differentiation for self-renewal to maintain immunocompetence and thus can be affected by xenobiotics that alter this cellular balance. In addition, data indicate that immune responses can be regulated by other organ systems such as the nervous and endocrine systems . Although the immune system is not typically thought of as a primary organ involved in the absorption or metabolism of xenobiotics, parts of the immune system (e.g., gut-associated lymphoid tissue) receive significant initial exposure after ingestion and immune cells have been reported to metabolically activate xenobiotics , and thus along with the liver, may play an important role in mediating a particular metabolites effect on immunocompetence. Since immunocompetence is dependent on a series of complex, time-dependent cellular and cell product interactions, there are numerous potential target sites within the immune system that a xenobiotic may directly or indirectly (i.e., through interactions with other organ systems) affect and thus alter the regulatory function of the immune system, and ultimately immunocompetence (Figure 4.1). The interaction of xenobiotics with the immune system may result in several immunotoxic alterations: immunosuppression leading to alterations in host defense mechanisms against pathogens or neoplasia; uncontrolled proliferation (leukemias and lymphomas); or dysregulation of the immune response (i.e., allergy/hypersensitivity reactions; autoimmune reactions) [6]. The objective of this chapter is to describe the direct and indirect mechanisms that may contribute to the immune system as a target organ for xenobiotics. Importantly, many xenobiotics may affect the immune system by both direct and indirect mechanisms depending on the dose. Due to space limitations, only a select few xenobiotics will be discussed to illustrate prototype agents exhibiting different mechanisms of immunosuppression. A more comprehensive review of the immunotoxicity of various xenobiotics can be obtained from several excellent reviews .

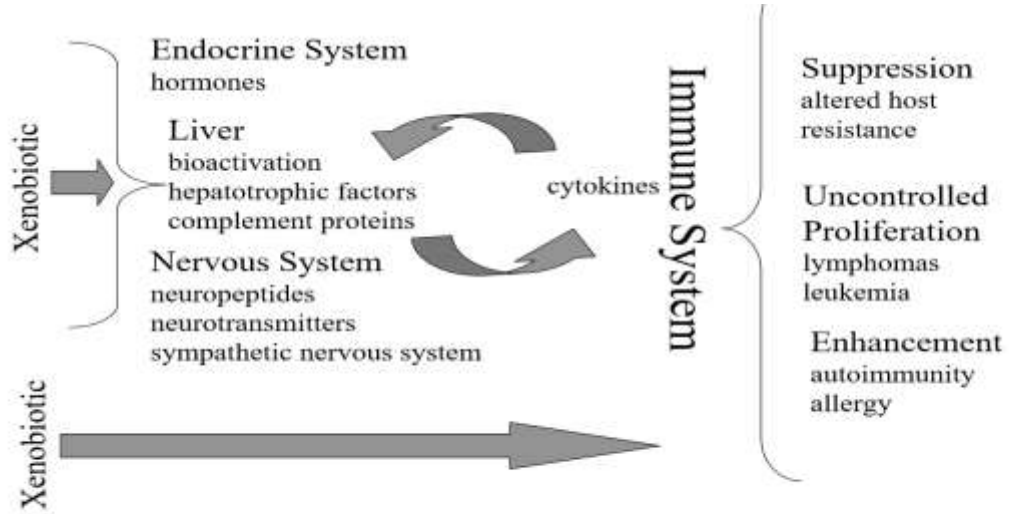

FIGURE 4.1 Scenarios by which chemicals, drugs and other xenobiotics may lead to alterations in immune function.

Overview on the Mechanisms Underlying Chemical-Induced Immunotoxicity

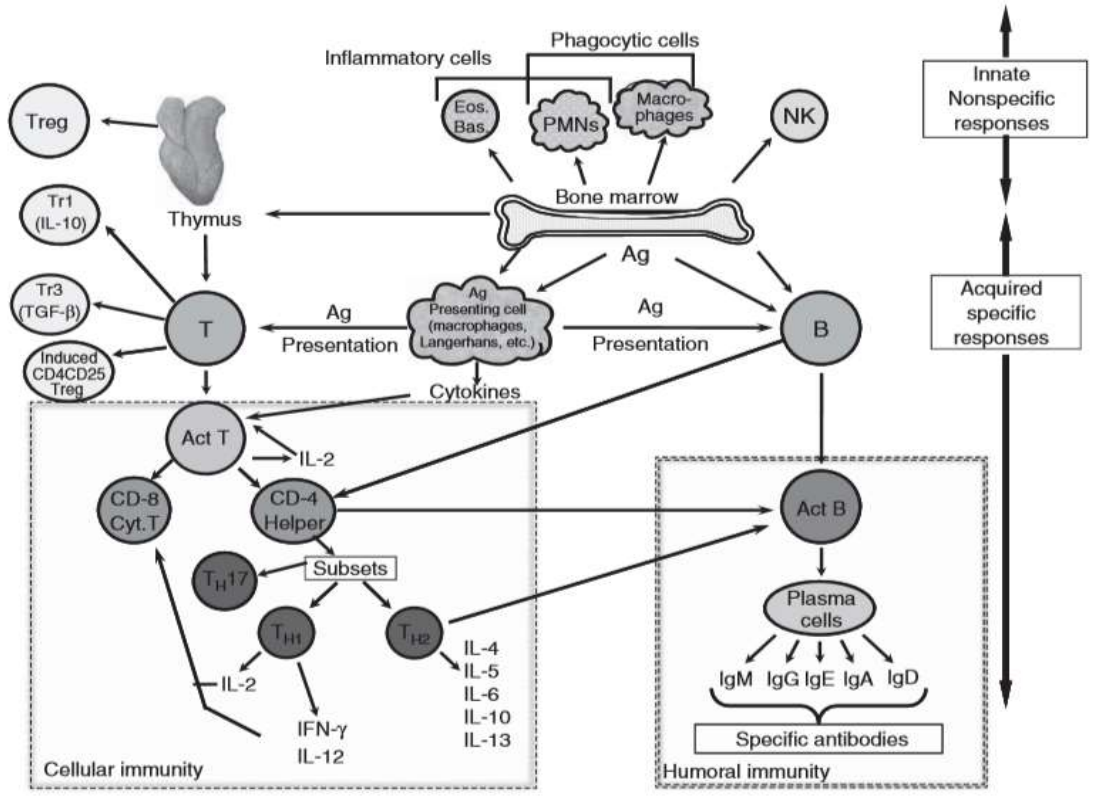

Figure 2.1 Schematic representation of the immune system. All lymphoid organs and immune cells can be targeted by xenobiotics. Act, activated T cells; Ag, antigen; Bas, basophils; Eos, eosinophils; NK, natural killer cells; PMNs, polymorphonucleates.

Mechanisms of Immunotoxicity

In susceptible individuals, drugs and chemicals may initiate, facilitate, or exacerbate pathological immune processes, resulting in autoimmunity, allergy, and cancer. In principle, chemicals can induce mutation or influence the regulationof genes coding for immunoregulatory factors;they can modify immunetolerance and regulation, leading to inappropriate immunostimulation and immunosuppression . There are examples of immunotoxic compounds interfering with all basic signal transduction pathways.

Experimental evidence suggests that reactive oxygen species (ROS) are also important mediators of cellular injury, either as a result of macromolecular damage or by interfering with extracellular and intracellular regulatory processes. through reversible oxidation of critical thiols: ROS has been implicated in a variety of responses from transcriptional activation to cell proliferation and apoptosis.Increased or prolonged free radical action can overwhelm ROS defense mechanisms, contributing to disease and toxicity. ROS influence the expression ofmany early response genes involved ininflammation,immuneactivation,and carcinogenesis by activating two important transcription factors, NF-κB and AP-1.Among immunotoxic compounds acting through ROS generation particulate matters and organotin compounds can be mentioned.Furthermore, ROS also play a key role in chemical-induced allergy.

Different mechanisms can lead to immunotoxicity:

1) Chemicals can kill immune cells, resulting in bone marrow toxicity and immunosuppression. Compounds that can damage or destroy the bone marrow will often have a profound immunotoxic effect, since the effectors of the immune system itself will no longer be available. Antitumoral drugs, benzene ,and ionizing radiations are examples of myelotoxic compounds.

2) Chemicals that can interfere with general or immune-specific signaling pathways,resulting in changes in the expression of surface markers, cytokine production, cell differentiation, and activation. Immunotoxic compounds can act via areceptor-mediatedornon-receptor-mediated effect.Examples of chemicals acting through a receptor-mediated event include glucocorticoids (GCs), polycyclic aromatic hydrocarbons, and cannabinoids, while calcineur ininhibitors, metals,and some pesticides are among immunotoxic compounds acting through an on classical receptor-mediated event.

3) Small molecular weight chemicals (<1000Da) can bind to proteins, forming complete antigens or modifying protein processing exposing cryptic self-proteins, leading to allergy or autoimmune disorders. More than 3000 compounds have been identified as potential skin sensitizers, while ∼30 compounds have been recognized as respiratory allergens. Among chemicals associated with auto immune disorders mercury, silica,L-Dopa, penicillamine, procainamid,solvents,somepesticides,silica can be mentioned.

IMMUNOSUPPRESSION

There are two basic mechanisms by which xenobiotics may induce suppression of the immune system: (1) by direct action of the xenobiotic upon the lymphoid organs or cells involved in the immune response and (2) by indirect action of the xenobiotic on other organ or physiological systems, such as neuroendocrine interactions, metabolic activation of xenobiotics to toxic metabolites, or hepatic modulation, which then impact the immune response. Xenobiotics that suppress the immune system may display lympholytic, antiproliferative, or immunomodulatory effects (e.g., reduced cell number or organ weights; decreased production or release of cytokines; alterations in expression of surface receptors; alterations in immune cell communication), or a combination of these effects. A unique characteristic of the immune system is the ability of immunocompetent cells to be removed from the host, separated into various cell populations using a number of different techniques [9], and functionally evaluated in vitro. This allows for the conduct of a number of in vitro or ex vivo experimental approaches (e.g., cell stimulation and secretion, phagocytosis, separation-reconstitution studies utilizing the in vitro stimulation of specific antibody production) to identify the primary cell type(s) targeted by a xenobiotic and to help distinguish between xenobiotic-induced direct or indirect effects on the immune system. Immunotoxic compounds that act indirectly on immune cells will not have an effect on an in vitro-generated immune response. Lymphocyte subpopulations can also be enumerated via fl ow cytometry using fl uorescently labeled monoclonal antibodies specific for cell surface markers , which permits the identifi cation of the immune cell subtypes that are targeted by a xenobiotic. In addition to the evaluation of cell surface markers, fl ow cytometry can be used to further assess mechanisms as they relate to immunotoxicity .

DIRECT EFFECTS OF XENOBIOTICS

Xenobiotics may directly affect immune function (humoral, cell-mediated, innate, or host resistance); the size, composition (e.g., alterations in the numbers, or differentiation and maturation of B- or T-lymphocytes), or architecture of lymphoid organs; hematological parameters; cytokine production and/or release; the expression of receptors or ligands on the surface of immune cells; and receptor mediated signal transduction [8]. An example of direct acting xenobiotics is the cannabinoids. Cannabinoids have been reported to decrease the cellular and humoral immune responses, NK activity, and host resistance . Cannabinoids are believed to affect the immune system through their interaction with a pertussis toxin-sensitive Gi-coupled cannabinoid receptor on lymphoid cells. Cannabinoid receptor transcripts have been reported in human tonsils, macrophages, spleen, and lymphocytes.. Following binding, cannabinoids inhibit adenylate cyclase and thus prevent an increase in the intracellular cAMP that is associated with lymphocyte stimulation. Immunotoxicity of inorganic metals (e.g., lead, arsenic, chromium) may also occur via direct effects on the immune system or by inhibiting immunoregulation, which may then result in immunosuppression and decreased host resistance, autoimmunity, or hypersensitivity.

Lead immunotoxicity

Recent epidemiological studies have demonstrated that exposure to lead is correlated to several diseases such as blood pressure increased levels, kidney disease, neurodegenerative disease and cognitive disorders , which are all associated to oxidative stress. Oxidative stress is known as an imbalance between the production of reactive oxygen species (ROS) and antioxidants in favour of free radicals (ROS) . Several studies reported that lead can induce oxidative stress in occupationally exposed workers as well as in general population, by two different mechanisms, the first is the pro-oxidative effect of δ-aminolevulinic acid dehydratase (δ-ALAD) and the second is connected with the direct effect of lead on the lipid composition of cellular membranes (55,56). The inhibition of δ-ALAD by lead accounts for the accumulation of its substrate δ-ALA, that can be rapidly oxidized to generate free radicals as superoxide ion (O2 •-), hydroxyl radical (•OH), and hydrogen peroxide (H2O2). Lead has also the capacity of stimulating ferrous ion initiated membrane lipid peroxidation . The decrease of the levels of glutathione (GSH) and protein bound sulfhydryl groups and the changes in the activity of various antioxidant enzymes indicative of lipid peroxidation have been implicated in lead-induced oxidative tissue damage . Lead is known to have toxic effects on membrane structure and functions by altering changes in the fatty acid composition of membrane which are more susceptible to lipid peroxidation. This effect is particular evident on erythrocyte membranes because erythrocytes have a high affinity for lead and are more vulnerable to oxidative damage than many other cells. Besides, lead is able to dysregulate the antioxidant defenses, including the antioxidant enzymes and the non-enzymatic antioxidants, such as uric acid. Another mechanism of lead-induced toxicity implicates the immune system. Epidemiological and experimental studies suggest that lead can influence levels of immunoglobulins, alterations in the numbers of lymphocytes, peripheral blood mononuclear cells (PBMCs) and macrophages, impaired responses to mitogens and depression of neutrophil functions . In vitro studies demonstrated that the treatment of macrophages with lead induces the disregulation of the production of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1α (IL-1α) and IL-6, and promote the synthesis of Th1 cytokines [interferon (IFN)-γ and IL-2] . CD4+ Th cell function is mostly sensitive to the immunotoxic effects of lead. Several studies have demonstrated that lead is able to improve Th2 cell development and affect Th1 cell proliferation . The differential influence of lead on Th1 vs. Th2 activation may be at the level of the antigen-presenting cells (APCs) or the T cell itself. Lead may induce the differential activation of Th subsets by modulating the activity of APC through the modulation of antigen density, a change in the expression of costimulatory molecules on APC, and/or a change in membrane fluidity of the APC. On the contrary, little is known about the possible associations between lead exposure and the function of Th17 and Treg cells. Besides, lead exerts proinflammatory properties, as reported in studies conducted particularly on occupationally exposed populations.

INDIRECT EFFECTS OF XENOBIOTICS

Neuroendocrine Modulation- A highly complex bidirectional interrelationship between the immune and neuroendocrine systems has been reported [2–4] and is discussed in detail in chapter 30 of this book. Nerve fi bers containing neuropeptides and neurotransmitters are observed in various lymphoid tissues where they directly contact immune cells such as lymphocytes. Conversely, products of the immune system (i.e., cytokines) have been reported to affect neuroendocrine functions , while various hormone receptors have been found on immune cells and a number of hormones have been reported to enhance (e.g., growth hormone, thyroid stimulating hormone, and prolactin), attenuate (e.g., gonadal steroids and endogenous opioids), or suppress

(e.g., glucocorticoids and adrenocorticotropin) responses of the immune system. Immune cells have also been reported to produce various peptide and protein hormones such as growth hormone, prolactin, luteinizing hormone, thyrotropin-stimulating hormone, and adrenocorticotropin . Immune cell function is altered following the exogenous addition of neurotransmitters, neuropeptides, or cytokines in vitro. Thus, if a xenobiotic found in the environment alters the production or release of neurotransmitters and neuropeptides, it may also alter the function of the immune system .These interactions are discussed in detail in chapter 30. There is increasing concern that many environmentally persistent xenobiotics such as insecticides, herbicides, fungicides, as well as several industrial chemicals (e.g., polybrominated biphenyl, sytrenes, lead, mercury, 2,3,7,8-tetrachlorodibenzo-p-dioxin [TCDD]), may mimic or antagonize endogenous hormones and adversely affect not only the endocrine and reproductive systems but also the immune system. Laboratory studies with EACs suggest that the reproductive and endocrine systems, and not the immune system, are the primary target organs of toxicity in young adult rats. Further studies, however, are needed to evaluate the effects of low-dose chronic exposures to EACs on humoral, cell-mediated, and innate immunity as well as the developing immune system. Administration of xenobiotics, particularly at high doses that induce signifi cant alterations in body weights, may also lead to a stress-induced, nonspecifi c immunotoxic response due to increased levels of circulating adrenal glucocorticosteroids (e.g., corticosterone), which have potent immunosuppressive activity .

TCDD Toxicity

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD; ‘‘dioxin’’) is the most toxic member of a large group of structurally similar halogenated aromatic hydrocarbons (HAH) that includes other polychlorinated dibenzodioxin congeners (PCDDs) as well as polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs). When these molecules are halogenated in specific lateral positions, they assume a planar conformation and are able to bind to a specific receptor in the cytoplasm of cells known as the aryl hydrocarbon receptor (AhR). The liganded AhR together with its dimerization partner, AhR nuclear translocator (ARNT), act as a transcription factor to regulate gene transcription. The best characterized response to AhR activation is the induction of the Phase I enzyme, P4501A1, which is capable of metabolizing a variety of xenobiotics including the inducer itself [1]. In addition, the transcription of a number of other genes is also altered following AhR activation and this altered transcriptional response is hypothesized to underlie the broad spectrum of toxic effects produced by AhR ligands. AhR-binding core sequences, also known as dioxin response elements (DREs), have been identified.

A common theme emerging from recent studies is that TCDD activates T cells and perhaps other cells (DC, B cells) leading to a premature induction of cell death. However, there has been a long-standing and ongoing controversy over whether or not TCDD can directly induce apoptosis in T lymphocytes. Unfortunately, interpretation of most of the studies has been complicated by an inability to clearly and reproducibly document direct effects of TCDD on purified T cells in vitro as well as difficulty in measuring apoptosis in vivo. In addition, TCDD has been shown to alternately induce, suppress or not affect apoptosis in a variety of other cell types.

TCDD is a highly immunosuppressive chemical that induces potent suppression of immune responses in laboratory animals. However, apart from the requisite role of the AhR and the identification of bonemarrow-derived cells as critical AhR-expressing targets, the specific cells and the underlying biochemical mechanisms by which TCDD disrupts immunological functions remain unclear. Recent data suggest that a new paradigm for the mechanism of immunotoxic action of TCDD may be more accurate, moving from one focused on the suppression

of immune functions to one focused on the inappropriate activation of cells, leading to anergy or death, and the consequent premature termination of the immune response. Enhanced activation of B cells, DC and CD4 + T cells by TCDD has been described as well as the earlier disappearance of the latter two populations from peripheral lymphoid organs. Although much remains to be learned about how inappropriate cellular activation via the AhR induces immune suppression.

Effect of UV radiation on immune system

Ultraviolet (UV) radiation is a ubiquitous component of the environment that has important effects on a wide range of cell functions. Short-wavelength UVB radiation induces sunburn and is a potent immunomodulator, yet longer-wavelength, lower-energy UVA radiation also has effects on mammalian immunity.

The primary source for UVR, a non-ionizing radiation of the electromagnetic spectrum, is the sun, which emits radiation in the UVA (320–400nm), UVB (280–320nm) and UVC wavelength. Under physiological conditions, human skin is only exposed to UVBR and UVA radiation (UVAR), with ambient sunlight on a summer day containing approximately 6% UVBR and 94% UVAR5 . UVR is important for normal physiology, mediating cell growth and differentiation, melanogenesis and vitamin D production. However, at large doses, UVR can cause sunburn and hyperplasia, and chronic exposure can cause skin ageing and increase the risk for certain types of skin carcinogenesis. For example, acute episodes of sunburn, particularly in early childhood, increase the risk of both melanoma and basal cell carcinoma. UVBR is immunosuppressive and this property of UVR is at least partially responsible for skin carcinogenesis in both animal models and humans6,7 . The toxic effects of UVBR UVAR is also immunosuppressive, but differs from UVBR in that it supresses the recall response rather than primary immune responses8,9 . Moreover, UVAR has a bell-shaped dose–response10 curve compared with the linear dose–response curve of UVBR11,12. UVBR is thought to make the greatest contribution to sunlightinduced immunosuppression as it induces immunosuppression more potently than does UVAR, but a larger percentage of UVA rays reach the earth's surface and can penetrate more deeply into the skin than UVB rays, which suggests that UVAR also contributes substantially to immunosuppression.are increasing owing to changes in human behaviour with regard to sun exposure and to longer life expectancy.

UVAR can be further divided into UVA2R (320– 340nm) and UVA1R (340–400 nm). UVA2R has similar properties to UVBR, whereas UVA1R has properties distinct from UVA2R and from adjacent wavelengths in the visible (blue) light range. These wavelengths are absorbed by different chromophores and, therefore, have different biological effects. For example, UVBR and UVA2R are mainly absorbed by nuclear DNA, whereas UVA1R is absorbed by endogenous porphyrins, leading to singlet oxygen generation.

Cis-urocanic acid. Cis-UCA, an imidazole derivative, is produced by the isomerization of trans-UCA following UVR absorption and is found at high levels in the stratum corneum. The idea that trans-UCA is the chromophore for, and cis-UCA is the mediator of, UVBR-induced immunosuppression is supported by two arguments: first, the action spectrum for UVBR-induced immunosuppression of contact allergy in BALB/c mice was shown to be very similar to the in vivo absorption spectrum of trans-UCA; and second, there is ample evidence that topical or parenteral administration of cis-UCA is immunosuppressive and can mimic some aspects of UVBR-induced immunosuppression16. It should be noted, however, that the action spectra for the trans to cis

photoisomerization of UCA in vitro and, importantly, in vivo in mouse skin differ from the action spectrum for UVR-induced systemic suppression of CHS in mice. Therefore, the relationship between cis-UCA formation in skin and UVR-induced immunosuppression may be more complex than originally thought. Cis-UCA induces immunosuppression by binding to the serotonin receptor subtype 5-HT2A (ref. 18). Although mast cells, LCs and keratinocytes all express 5-HT2A, the primary cell types responsible for the immunosuppressive activity of UCA are unclear. In the context of UVRimpaired CHS, cis-UCA stimulates the release of tumour necrosis factor (TNF) from keratinocytes, a mechanism that is thought to trap LCs in the epidermis and prevent antigen presentation to T cells in the draining lymph node19,20. This is in contrast to other studies that suggest dermal TNF can promote LC migration to the draining lymph node21. These contradictory data may be the result of differences in terms of TNF localization within the skin (epidermis versus dermis) and of cell origin. Cis-UCA also stimulates histamine release from mast cells and the subsequent production of prostaglandin E2 (PGE2).

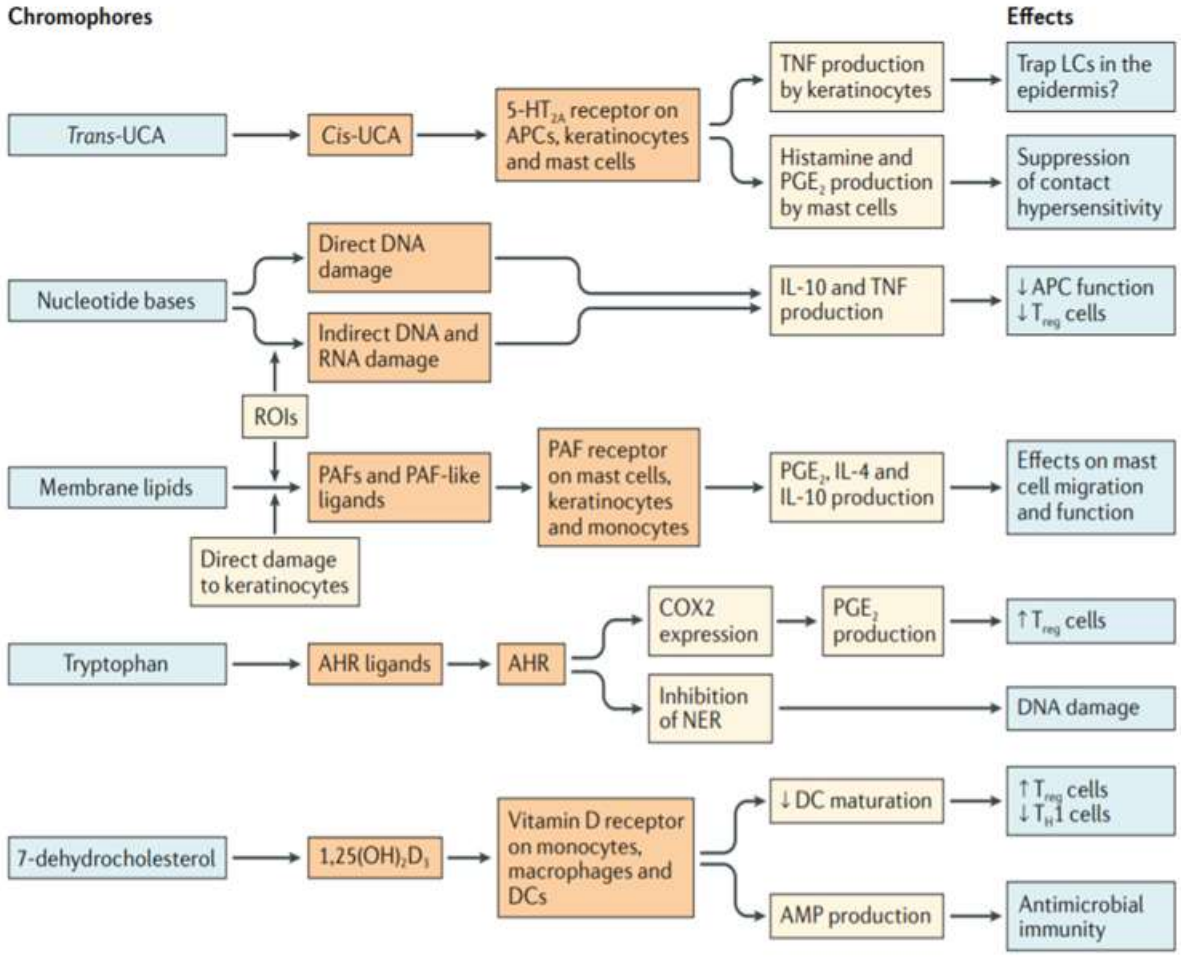

Fig. 1 | **UVR absorption by chromophores and damage recognition.** Cis-urocanic acid (cis-UCA), an imidazole

UVR absorption by chromophores and damage recognition-Cis-urocanic acid (cis-UCA), an imidazole derivative produced by the isomerization of trans-UCA following absorption of ultraviolet radiation (UVR), initiates many of the immunomodulatory effects of UVR by binding to the serotonin receptor (5-HT2A) on antigen-presenting cells (APCs), keratinocytes and mast cells18. Cis-UCA stimulates the release of tumour necrosis factor (TNF) from keratinocytes, which might trap Langerhans cells (LCs) in the epidermis and prevent

the presentation of antigen to T cells in the draining lymph node19,20. Cis-UCA also stimulates histamine and prostaglandin E2 (PGE2) production by mast cells22,23. UVR causes direct damage to DNA, which induces the release of IL-10 and TNF and also impairs the function of APCs, resulting in impaired induction of regulatory T (Treg) cells32. In addition to direct UVR-induced DNA damage, the generation of reactive oxygen intermediates (ROIs) by UVR causes indirect damage to both DNA and RNA. The oxidation of phosphatidylcholine by ROIs results in the production of platelet-activating factor (PAF)-like molecules at the cell membrane. PAFs (produced by direct damage to keratinocytes) and PAF-like ligands induce immunosuppression by binding to the PAF receptor on mast cells, keratinocytes and monocytes and stimulating the release of PGE2, IL-4 and IL-10. The absorption of UVBR by cytosolic tryptophan results in the formation of high-affinity aryl hydrocarbon receptor (AHR) ligands57–59. AHR signalling results in increased expression of cyclooxygenase 2 (COX2), which can contribute to UVR-induced immunosuppression through PGE2 production and the induction of Treg cells. Also, AHR signalling inhibits nucleotide excision repair (NER) by inducing the proteasomal degradation of the p27KIP1 tumour suppressor protein. 7-Dehydrocholesterol absorbs UVR and converts to pre-vitamin D3, which then isomerases to the active metabolite 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) 68. 1,25(OH)2D3 modulates immunity by directly stimulating antimicrobial peptide (AMP) production74,197 and by inhibiting dendritic cell (DC) maturation. 1,25(OH)2D3-modulated DCs have suboptimal APC function, thereby inducing the expansion of Treg cell populations and suppressing T helper 1 (TH1) cell responses69,

Tobacco smoke is a complex mixture of toxicants and the chemical properties change—rapidly in some cases—as smoke ages. Toxicants measured at one point in time may not be what the smoker actually experiences. It is estimated that there are more than 2,000 chemical constituents of tobacco. Almost twice that number results when tobacco is burned incompletely during smoking. Smoking-related changes in the peripheral immune system in humans include elevated white blood cell counts, increased cytotoxic or suppressor and decreased inducer or helper T-cell numbers, slightly suppressed T-lymphocyte activity, significantly decreased natural killer (NK) cell activity, lowered circulating immunoglobulin titers (except for IgE, which is elevated), and increased susceptibility to infection. Similar effects have been observed in animals (Johnson et al., 1990; McAllister-Sistilli et al., 1998; Sopori et al., 1994), suggesting that animal models can be used to test for harm reduction to the immune system from use of new tobacco products or nicotine delivery devices. The effect of tobacco smoke on the immune system of humans and rodents depends on the duration and level of exposure. In general, short-term, low-level exposures do not affect the immune system or may be stimulatory, whereas longer-term exposures (six months or more) or high levels of exposure are immunosuppressive.

Animals exposed to cigarette smoke for extended periods are more susceptible than naïve animals to tumor and infectious agent challenge. Mice exposed to cigarette smoke for six months or longer were more susceptible to intratracheally instilled Lewis lung or TKL5 tumor cells in terms of increased tumor growth, metastases, and early death than unexposed mice (Chalmer et al., 1975; Thomas et al., 1974b). Such changes are not observed in mice exposed to cigarette smoke for short periods of time (days). Chronic exposure of mice to cigarette smoke results in increased susceptibility to infectious agents such as murine sarcoma virus (Thomas et al., 1974a) and influenza virus (Mackenzie et al., 1976; Mackenzie and Flower, 1979). Cellular immunity, as evaluated by phytohemagglutinin (PHA)-induced lymphoproliferative response or development of tumor-specific cytotoxic T cells, was initially increased but, on continued exposure, greatly decreased in mice exposed to cigarette smoke

(Chalmer et al., 1975; Holt et al., 1975; Thomas et al., 1973). Lymphocytes from mice exposed chronically to tobacco smoke have a decreased response to the mitogen PHA and release factors that inhibit the cytotoxic activity of NK cells against tumor cells. T-cell suppression may be due to defective antigen processing or antibody production. The humoral immune response is also suppressed by chronic exposure of mice to tobacco smoke, while acute exposures may stimulate the humoral response. The primary and secondary antibody production by lymphocytes in the lung, lymph nodes, and spleen of mice exposed to tobacco smoke for longer than 26 weeks and challenged by inoculation with sheep erythrocytes was decreased (Thomas et al., 1975). Laboratory test animals can be used to demonstrate the ability of cigarette smoke to slow the mucociliary clearance of particles from the lung and to alter the function of pulmonary macrophages. This effect has been observed in humans (Bohning et al., 1982; Cohen et al., 1979) and animals (Mauderly et al., 1989). In the latter study, rats exposed for eight weeks to cigarette smoke were exposed one time to cerium^{144} dioxide particles. Smoking increased the half-time of the short-term clearance of these particles by 63% and long-term clearance twofold. The slowing of clearance of inhaled particles is an adverse health effect that should be considered in studies of tobacco product toxicity. Alveolar macrophages from rats exposed to tobacco smoke for six months have a decreased ability to phagocytize *S. tuphylocoesus aureus* (Drath et al., 1979; Huber et al., 1980). Alveolar macrophages from rats exposed to tobacco smoke for 36 days or longer had an increased ability to release reactive oxygen species, a property dependent on the particulate faction of the smoke and not the gases. Clearance of *Pseudomonas aeruginosa* from rodents exposed to cigarette smoke for 36 weeks was slower than in controls (Holt and Keast, 1977). The decreased ability to clear particles, including pathogens, and the increased release of reactive oxygen species contribute to enhancement of inflammatory processes in the lung.

Hepatic Modulation The liver is a common target organ for injury resulting from exposure to various xenobiotics and has been reported to modulate immune responses through the release of a number of factors, such as acute-phase reactive proteins, L-arginase, or low density lipoproteins [26]. Following hepatic injury induced by xenobiotics (e.g., carbon tetrachloride, phenobarbitol, ciprofibrate, or α-hexachlorocyclohexane), a regenerative process is initiated by the release of a number of hepatotrophic mediators into the serum [26]. Such factors include complete hepatic mitogens [i.e., transforming growth factor-α, epidermal growth factor, hepatocyte growth factor/hepatopoietin A (HGF), hepatopoietin B, and acidic fi broblast growth factor], a number of co-mitogenic hepatotrophic factors, and transforming growth factor-β1 (TGF- β1) . In addition, other factors such as α-fetoprotein or prostaglandin E2 are released but do not cause hepatocyte proliferation . A number of these factors have been reported to be immunomodulatory. For example, CCl4-induced immunosuppression has been reported to be secondary to hepatotoxicity through the release of serum-borne hepatotrophic factors such as TGFβ1 . CCl4-induced serum factors were also found to increase the functional activity and number of B-cells in the spleen, while Con A-activated spleen cell supernatants from CCL4-treated mice produced larger amounts of IL-2 than untreated spleen cells [26]. α-Fetoprotein also suppresses T-cell-dependent immune responses. Conversely, HGF may indirectly cause an increase in the number and activity of B cells, while other hepatotrophic factors have been reported to induce the proliferation of immune cells [26]. Thus, certain liver-derived factors released following liver damage can have both enhancing and inhibitory infl uences on immunocompetence depending on when the immune response is initiated relative to the hepatic damage.

Metabolic Activation Xenobiotics may exert an indirect action on the immune system by being metabolically activated into toxic metabolites. An example of a xenobiotic requiring bioactivation is cyclophosphamide (CYP), the prototypical member of a class of drugs known as alkylating agents . Upon entering the cell, the inactive, noncytotoxic drug is converted into phosphoramide mustard, a DNA alkylating agent that inhibits cell replication. CYP-induced immunosuppression involves a general reduction in lymphocytes as well as alterations in lymphocyte function . CYP is often used as a positive control in immunotoxicology studies because it has been demonstrated to suppress both humoral and cell-mediated immune responses . Benzene exposure results in bone marrow toxicity, and alters hematopoietic cell profi les and immune function , but data indicate that benzene-induced effects are due to reactive quinone intermediates that result from benzene metabolism . Similarly, afl atoxin B1, a mycotoxin derived from fungi, requires metabolic activation in order to produce immunotoxic effects hydrocarbons (PAHs) [5]. The macrophage was identifi ed as the immune cell subtype capable of metabolizing the PAH benzo(a)pyrene (B(a)P) within the murine spleen . Consistent with this finding are several studies demonstrating that the major cell type affected following exposure to B(a)P is the macrophage. Macrophages have also been reported to metabolize certain drugs by a cytochrome P-450-dependent mechanism. Aryl hydrocarbon hydroxylase (AHH) has also been reported to be present and inducible in monocytes, while human peripheral blood lymphocytes and lymphocytes from a number of species have been shown to possess cytochrome P-450. Although the metabolic capacities of extrahepatic tissue, including cells of the immune system, are substantially lower than the liver, metabolic activation of xenobiotics in potential target tissues, such as the spleen or other immune organs, may play a critical role in determining immunotoxicity. The metabolic activation of xenobiotics in immune tissues may result in the formation of reactive electrophilic metabolites, which may then bind to cellular nucleophilic target sites such as DNA, RNA, and proteins that are important in mediating an immune response or maintaining cellular homeostasis.

DEVELOPMENTAL CHANGES THAT IMPAIR IMMUNE FUNCTION

The immune system has been identifi ed as possibly having heightened susceptibility during maturation. Data suggest that some xenobiotics may cause effects that are more persistent or severe than those observed in adults (qualitative differences), or may induce immunotoxicity at lower doses (quantitative differences). The chronology of immune ontogeny and the development of normal immune capacities are important in understanding the susceptibility of the immune system during development. The development of the immune system during the gestation and neonatal periods is a dynamic process involving proliferation and differentiation of immune cells as compared to that of adults. Although the effects of xenobiotics on developing offspring have been reported, our ability to detect changes before maturation of the immune system is hampered by a lack of validated assays. Therefore, most of these studies have exposed animals (predominantly mice) in utero to xenobiotics and evaluated potential developmental immunotoxicity based on an assessment of the immune status of adult animals. A number of recent publications have reported on the most appropriate methods to assess developmental immunotoxicity , and this topic is discussed in detail in chapter 20 of this volume. These reports suggest the rat as the species of choice for screening for developmental immunotoxicology (DIT) potential and that an exposure design encompassing all critical windows of immune system development, which can then be integrated into existing developmental toxicity protocols, be employed to evaluate the DIT potential of xenobiotics.

IMMUNE-MEDIATED DISEASE

If one considers the immune system as a continuum, dysregulation of immune function can also be expressed in the development of immune mediated diseases via enhanced immune responsiveness. These immune mediated disorders are commonly thought of in terms of allergy and autoimmunity. The mechanisms for these immune responses result from heightened responsiveness or loss of tolerance, and it is generally accepted that pathogenesis requires a combination of genetic and environmental factors. While there is complexity resulting from the interaction of susceptibility factors (e.g., health status, socioeconomics, lifestyle), at least 25 loci have been associated with allergy (or asthma) susceptibility. Likewise, forms of autoimmunity have been associated with major histocompatibility complex (MHC) restrictions; autoimmune hepatitis can be associated with HLA DR3 and DR4 for smooth muscle and antinuclear antibody, respectively, and HLA DR7 for microsomal antibody . Polymorphisms that impact metabolism may be a less obvious consideration but also could play a role in autoimmune reactions as the availability and reactivity of such xenobiotics as procainamide and hydralazine (acetylation), and D-penicillamine and sulfonamides (poor sulfoxidizers), might be altered . While the specifi c mechanism(s) for the development of immune-mediated diseases remain vague, general attributes of most xenobiotics linked to immune dysregulation include chemical reactivity, resulting in the formation of neo-antigens, and the production of (pro)infl ammatory mediators.

Xenobiotic allergy is most frequently attributed to a hypersensitivity reaction towards a chemical-specifi c, haptenized molecule. Hypersensitivity towards xenobiotics generally presents itself either as contact dermatitis, occurring 24–72 hours after dermal exposure, or as immediate, systemic allergy (e.g., urticaria, asthma) occurring soon after exposure. While CD8+, cytotoxic T-cells and Th1 cells are critical to allergic dermatitis and eczema reactions, CD4+ Th2 cells and IgE are primarily associated with Type 1 allergic responses [48]. Haptenation of a low molecular weight (LMW) xenobiotic serves as the initial event in the mechanism of hypersensitivity. Allergenic LMW chemicals are generally reactive and can bind to endogenous proteins (e.g., serum albumin, kerotinocyte proteins) following penetration through dermal and epithelial barriers to form new antigens. In some cases xenobiotics (e.g., sulphamethoxazole) can be considered as “prohaptens” which need to be metabolized before binding endogenous proteins [49]. Likewise, Cheung and colleagues [50] reported the bioactivation of cinnamic alcohol into the protein-reactive chemical cinnamaldehyde, a well accepted skin sensitizer. In the case of photoallergic contact dermatitis, a “photohapten” becomes activated via ultraviolet A irradiation and binds to proteins, including those on the surface of antigen presenting cells, leading to the sensitization of specifi c T-cells . A preference for particular amino acids (e.g., cysteine) has also been reported for reactive haptens . For example, ofloxacin (a halogenated quinolone) demonstrates a propensity for binding to lysine residues found within the sequence of MHC class II [53]. Details on the cellular immune responses occurring following the recognition of xenobiotic haptens as antigens by the immune system are described in chapters 33–35 of this volume. Ultimately, a certain combination of mediators is selectively activated and subsequently helps determine and differentiate the characteristic immune response (e.g., Th1 vs. Th2). For example, dermal sensitizing chemicals (e.g., oxazolone and dinitrochlorobenzene) elicit a higher proportion of Th1 cytokines such as IFNγ and IL-2 ,which helps drive immune reactions primarily mediated via CD8+ T-cells. Conversely, diisocyanates and anhydrides are classes of chemicals associated with Th2 allergic responses that depend on cytokines such as IL-4 and IL-10, and the prototypic mediator of Type 2 allergic reactions, IgE antibody. These responses should not be considered all or none, but rather degrees of Th1/Th2 balance. Much remains unknown regarding the characteristics of these specifi c responses. Using diisocyanates as an example, both human and animal data demonstrate diversity in immune responses where IgE and cytokine profiles are

concerned . In cases of toluene diisocyanate (TDI) induced occupational asthma, the involvement of cells such as neutrophils, eosinophils and CD8+ T-cells support the possibility of non-Th2 mechanisms. Partially due to limitations in the identifi cation of hapten conjugates, the accurate detection of chemical-specific IgE antibody via immunoassay methodology is frequently questioned. Understanding the specifics of haptenation has contributed to diffi culties in clearly elucidating mechanisms for low molecular weight xenobiotic hypersensitivity and thus, predictive models for hazard characterization. There are many more reactions that play a role in the generation of sensitization responses but which extend beyond the scope of this chapter (e.g., tissue remodeling, neuronal intervention and mediator release) . This brief review greatly oversimplifies the current knowledge associated with allergic immune responses towards xenobiotics. Thus, understanding the pathogenesis of autoimmunity is a bit of a misnomer and not a matter of defining a single series of cellular and molecular events. Despite this, the immunological reactions for any given autoimmune response are similar to those identified for allergic responses, and “autoallergy” can be synonymous with autoimmunity, especially in the context of pharmaceutical agents. Drug and chemical associated autoimmune diseases typically disappear once the xenobiotic is discontinued. in the context of allergic responses, inflammation frequently plays a role in autoimmune reactions as the associated cytokines are important in sustaining immune responses, and anti-infl ammatory therapy has been shown to be effective in the development of certain disorders . Oxidative stress has also been implicated in the induction of autoimmunity in cases of xenobiotics, partially due to the effectiveness of some antioxidants in the treatment of specific autoimmune disorders. There are several hypothesized mechanisms by which xenobiotics might induce autoimmune disorders. One is through neo-antigen formation via modification or adduct formation with endogenous cellular molecules. Auto-antigens can be intracellular or extracellular proteins, nucleic acids, or other macromolecules that are slightly modified or bound by xenobiotics. The formation of adducts by xenobiotics is essentially equivalent to haptenation, described above for allergenicity. The primary difference appears to involve the ultimate antigen, as allergenicity results in immune recognition of a hapten conjugate with no specifi city for native protein, while autoimmunity typically results in immune recognition of the endogenous protein or macromolecule. For example, hydralazine, halothane, and tienilic acid are three agents that have cytochrome (CYP450) reactive metabolites and form CYP450 adducts that subsequently elicit anti CYP450 antibodies leading to autoimmune hepatitis. Metabolic activation can also occur within the monocyte/macrophage and has been reported for many of the same xenobiotics (e.g., hydralazine) suggesting multiple mechanisms for xenobiotic autoimmune potential. Auto-antigens can also result from novel immune recognition of unaltered endogenous molecules. During the process of clonal deletion, some auto-reactive T-cells may not be eliminated if respective antigens are not available within the thymus . There may also be xenobiotics (e.g., cyclosporin and procainamide (hydroxylamine), which interfere with T-cell selection. If an auto-reactive T-cell repertoire exists, immune stimulation may serve to expose the atypical repertoire. Some xenobiotics such as procainamide and hydralazine have the potential to inhibit DNA methylation, which has been shown to increase expression for many cytokines and surface molecules with relevant associations to autoimmune responses. In addition, xenobiotics such as procainamide and chlorpromazine have been associated with non-bilayer phospholipid arrangements (NPA) with anionic lipids of cell membranes. NPA are very immunogenic and have been identified as a potential trigger for lupus-like disorders. While the key event(s) that trigger expression of an autoreactive disorder are still unknown, the self-antigens (e.g., liver enzymes and glycoproteins on platelets) for many diseases have long been identified.

Some xenobiotics may have divergent mechanisms of autoimmune responses. For example, hydralazine demonstrates adduct reactivity as well as inhibition of DNA methylation, while procainamide inhibits DNA methylation, forms immunogenic NPA, and disrupts clonal selection in the thymus . It is this complicated pattern of effects that makes assessment of autoimmune potential in the laboratory for new xenobiotics almost impossible. Animal models can sometimes be recreated to resemble human disease, and thus may be useful for therapy considerations, but are difficult to utilize for screening chemicals for hazard potential due to the diverse nature of autoimmunity mechanisms and physiological presentation. While evidence supports many different mechanisms for xenobiotic-induced autoimmune reactions, none have conclusively demonstrated the critical events necessary to lead to the development of autoimmune disease. Therefore, it is difficult to predict or identify xenobiotics that might possess the potential to elicit autoimmune disorders.