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MOLECULAR AND CELLULAR BASIS OF CHEMICALLY INDUCED IMMUNOTOXICITY[1]

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INTRODUCTION

Immunotoxicology is the study of adverse effects on the immune system resulting from occupational, inadvertent, or therapeutic exposure to drugs, environmental chemicals, and, in some instances, biological materials. For the purpose of this chapter, we refer to these substances collectively as "xenobiotics." Immunotoxicity is subdivided into three main research areas: (*a*) studies of altered immunological events associated with exposure of humans and animals to xenobiotics, including altered host resistance to infectious disease; (*b*) studies of allergy and autoimmunity resulting from xenobiotic exposure; and (*c*) implementation of analytical immunological methods into toxicology research. This chapter focuses only on chemical-induced immunomodulation and describes several classes of immunotoxic xenobiotics, emphasizing cellular and molecular targets.

Laboratory studies, conducted primarily in rodents, have provided evidence that the immune system is very sensitive to chemical injury (reviewed in 1–6). This sensitivity is probably due as much to the general properties of a xenobiotic (e.g. reactivity with macromolecules) as to the complex nature of the immune system, which encompasses antigen recognition and processing;

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cellular interactions involving cooperation, regulation, and amplification; cell activation, proliferation, and differentiation; and mediator production. Since these cellular events are also involved in embryogenesis, many immunosuppressive xenobiotics are also teratogenic. The immunological effects associated with exposure to xenobiotics are often accompanied by increased susceptibility to challenge with infectious agents or tumor cells. In several instances, effects similar to those observed in rodents have been reported in humans through therapeutic, inadvertent, or occupational exposure to xenobiotics exemplifying certain characteristics of secondary immunodeficiency disease. These effects include altered immune responses in: Michigan residents and farmers exposed to polybrominated biphenyls (PBB) through the consumption of contaminated livestock and dairy products (7, 8, 8a); Chinese and Japanese exposed to polychlorinated biphenyls (PCBs) and dibenzofurans through contaminated rice oil used in cooking (8a, 9); residents of Missouri chronically exposed to dioxin from a mobile home park (10); Spanish residents exhibiting "toxic oil syndrome" following ingestion of isothiocyanate-derived imidazolidinethione adulterated rapeseed oil (11); factory workers with aplastic anemia and leukemia occupationally exposed to benzene (12); and patients with suppressed natural killer (NK) cell activity (13) and cell-mediated immunity (CMI) (14) receiving conventional diethylstilbestrol therapy for prostatic cancer. Infectious disease and neoplasia have been a recurring consequence of chronic immunosuppression or aberrant lymphoid cell differentiation in several of these cohorts. For example, the frequency of neoplasia among Michigan PBB-cohort members exhibiting immune dysfunction is approximately 15-fold greater than that observed in the control Wisconsin farmer cohort (J. G. Bekesi, unpublished observations). These examples and our current knowledge about the pathogenesis of disease support the possibility that xenobiotic-induced damage to the immune system may be associated with a wide spectrum of diverse pathologic conditions, some of which may only become detectable after a long latency. However, whether exposure to xenobiotics present in the environment influences immunocompetence of the general population under normal circumstances is still not known.

PHARMACOLOGY OF THE IMMUNE SYSTEM

Despite the increasing list of immunosuppressive chemical xenobiotics described, researchers have made little effort to delineate the cellular and molecular events of these chemicals. These efforts have probably been hampered by the fact that much of the technology to evaluate immunosuppression, at least at the molecular level, has only recently been developed. Further, many immunotoxicants have multiple effects on immune function, making

it difficult to construct the actual sequence of events or determine the specificity of these cellular–chemical interactions. Chemical immunosuppressants, like immunotherapeutic agents, may vary from those that demonstrate high specificity (i.e. targeting a subpopulation of lymphocytes by interacting with specific proteins), intermediate specificity (i.e. altering specific biochemical or cellular events that are shared by several cell types), or little specificity (i.e. general antiproliferative or cytolytic activity). Immunosuppressive xenobiotics, due to their random reactivity (e.g., sulfhydryl or nucleophilic binding), might be expected to demonstrate little specificity for immune components, although in many instances xenobiotics have been shown to target specific subpopulations and molecular sites. While it is not our intent to review the organization and function of the immune system, an understanding of the biochemical and physiological processes that occur during the development of immunocompetent cells is necessary to understand how xenobiotics can influence these events. The following is a brief overview of recent developments in our understanding of macrophage and lymphocyte maturation events.

Macrophages

Mononuclear phagocytes (MPs) or macrophages originate from granulocyte–macrophage progenitor cells in the bone marrow, where they mature into promonocytes and monocytes. Monocytes are transported via the blood to organs and tissues, where they develop into macrophages. The macrophages of the body include histiocytes, Kupffer cells, alveolar macrophages, free and fixed macrophages in lymphoid tissue, and macrophages associated with serous membranes. Bloodborne monocytes and local replication can replenish these macrophage compartments with new cells. The recruitment of monocytes from the blood is greatly enhanced and local macrophage multiplication is increased in sites of inflammation (reviewed in 15). MPs provide a major defense against mechanical injuries as well as those produced by chemicals or biological toxins, infectious agents, and neoplastically transformed cells. Dysfunction of MPs can lead to indirect tissue damage through altered host resistance to infectious agents and to neoplastically transformed cells. Additionally, direct tissue injury by the MPs or their cellular products (e.g. autoimmune diseases) can occur. Environmental agents, especially fibers, particulates, and gases (reviewed in 16), and to some extent lipophilic compounds (reviewed in 17–18), can alter MP function.

Studies from several laboratories have demonstrated that murine macrophages develop in stages (19, 20) and that the stages of development can be identified by quantifying the expression of biological and chemical markers. Some of the more commonly used markers for maturation and differentiation include secretion of reactive oxygen intermediates such as H_2O_2 (18) and

quantitation of ectoenzyme levels (21). In brief, these stages are defined by the inductive signals that must be applied to induce full activation (Figure 1, Section 1). Immature MPs, when taken from sites of inflammation (i.e. responsive macrophages), express a variety of functions including increased phagocytic capacity, increased spreading and adherence to glass or plastic surfaces, secretion of neutral proteases, increased production of acid hydrolases, depressed levels of 5'-nucleotidase, and an increased capacity to generate O_2 (15). Responsive macrophages are closely akin to inflammatory macrophages, as they display the markers of increased spreading, increased phagocytosis, and increased secretion of plasminogen activator (22). Inflammatory macrophages can, in turn, be activated to kill tumor cells and facultative intracellular parasites by exposure to lymphokine and/or endotoxin. Primed macrophages also display these markers, and in addition bind neoplastic targets selectively (23). Fully activated or cytotoxic macrophages, which share the properties of the primed macrophages, kill neoplastic or virally infected targets and spontaneously secrete cytolytic protease (22, 23).

Gery et al (24) detected a factor that promotes murine thymocyte proliferation in culture supernatants produced by MPs, which was subsequently termed interleukin 1 (IL-1). Stimulants that induce IL-1 synthesis by MPs act on the plasma membrane and include lipopolysaccharide (LPS), phorbol myristic acetate (PMA), immune complexes, IFN-γ, and activated T cells (reviewed in 25). Conversely, there are a number of immunosuppressive agents including corticosteroids and prostaglandins that inhibit the production and/or release of IL-1 by macrophages. IL-1 has multiple effects on cells involved in inflammation and immune responses. Subcutaneous injection of IL-1 leads to margination of neutrophils and maximal extravascular infiltration of polymorphonuclear leukocytes (PMN). IL-1 is a chemotactic attractant and activator of PMNs, causing increased glucose metabolism, reduction of nitroblue tetrazolium, and release of lysosomal enzymes (reviewed in 25). In lymphocytes, IL-1 is not required for entry from G_0 to G_{1a} of the cell cycle, but once having entered G_{1a}, T cells respond to IL-1 by proceeding through G_{1b} to S phase. These events involve the intermediate formation of IL-2 and DNA synthesis.

B Cells

B cells originate from hematopoietic stem cells. Precursor B cells differ from immunocompetent B cells in lacking receptors for antigen on their membrane. Maturation of immunocompetent B cells into antibody-producing cells or plasma cells is divided into three stages, which include activation, proliferation, and differentiation (Figure 1, Section 2) (reviewed in 26, 27). Substantial effort has focused on early activation events that occur following antigen binding. In B cells, membrane-associated immunoglobulins serve as

receptors for specific antigens. Like many agonists, including hormones, neurotransmitters and other biologically active substances, antigen binding and subsequent immunoglobulin cross-linking mediates transmembrane signaling (28). This event leads to increased phospholipid metabolism, specifically phospholipase C–catalyzed hydrolysis of phosphatidylinositol to inositol triphosphate (IP_3) and 1,2-diacylglycerol (DAG). IP_3 liberates Ca^{2+} from intracellular stores, and DAG activates protein kinase C (28). Formation of these products is associated with protein phosphorylation events and in B cells has been associated with autophosphorylation products, activation of tyrosine-protein-kinase activity, and phosphorylation of guanyl cyclase.

Progression of B cells from G_0 to G_{1a} of the cell cycle is characterized by increased expression of a Class II restricted antigen (Ia) on the surface membrane and cell enlargement. Activated cells rapidly progress into G_{1b} characterized by increased RNA synthesis and responsiveness to specific growth factors. Most of the growth-promoting factors are provided by T helper cells and include B cell–stimulatory factor (BSF or BCGF I), B cell–growth factor II (BCGF II), and, in certain instances, IL-2 (reviewed in 27). The macrophage product, IL-1, also contains growth-promoting activity for B cells. Entry into S phase of the cell cycle is accompanied by responsiveness to several differentiation signals collectively referred to as B cell–differentiation factors (BCDFs). These factors signal the cells to produce and secrete IgM antibody. The differentiation factors are also involved in the isotypic switching of antibody classes (e.g. IgM to IgG antibody-producing cells).

T Cells

Like B cells, T cell precursors originate from hematopoietic tissue (reviewed in 29). Subsequently, T cells enter the thymus and, under the influence of the thymic microenvironment, differentiate into a heterogeneous population with characteristic cell surface antigens and distinct functional properties (Figure 1, Section 3). These surface antigens are referred to as Lyt in mice and OKT in humans. The T subpopulations include T helper, T suppressor, and cytotoxic T cells (CTLs). The former two subpopulations are involved in regulation, whereas the latter is responsible for effector functions. As with transmembrane signaling events in B cells, cross-linking of the antigen receptor on T cells (T3) or mitogenic substances such as phytohemagglutinin or concanavalin A stimulate the breakdown of phosphatidylinositol, giving rise to IP_3 and DAG (30). T helper cells, in addition to antigen, require corecognition of a class II molecule present on the antigen-presenting cell and stimulation by the macrophage product, IL-1, to form lymphoblasts. Following activation, T helper cells produce IL-2, a growth factor that induces DNA synthesis in CTLs that have been activated by antigen to express receptors for

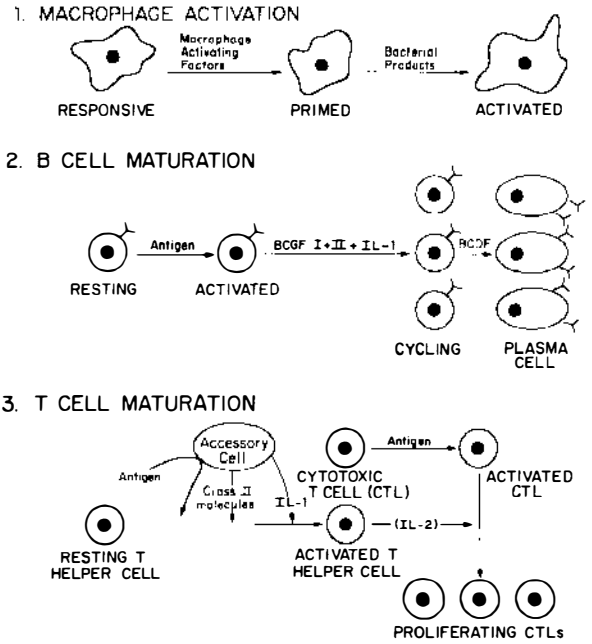

Figure 1 Macrophage and lymphocyte maturation. (*1*) Responsive macrophages are targeted by macrophage-activating factors (e.g. IFNγ), resulting in primed cells. Primed macrophages subsequently respond to bacterial products (e.g. endotoxin, muramyl dipeptide) to become fully activated. (*2*) Resting B cells are activated via antigen cross-linking. Activated B cells proliferate in response to growth-promoting factors and subsequently develop into plasma cells with the aid of differentiation factors. Other than IL-1, these growth-promoting and differentiating factors are derived from T helper cells. (*3*) T helper cells are activated by the combined action of antigens, class II molecules, and IL-1 via accessory cells. Activated T helper cells produce IL-2, which acts as a growth promoter in antigen-activated cytotoxic T cells. These processes can be negatively influenced by T suppressor cell activity. (Adapted from 15, 26, 27, 29.)

IL-2 (reviewed in 31). These cycling cells represent the effector populations responsible for lymphokine production that mediate various aspects of CMI.

IMMUNOTOXIC XENOBIOTICS

Immunotoxicology has only begun to be concerned with the consequences of chemical exposure on specific subcellular events. The following sections describe the effects of several important classes of xenobiotics on immune function. We focus the discussions on alterations in patterns of leukocyte maturation induced by these compounds.

Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PCAs) represent a class of chemicals that are ubiquitous in the environment and consist of carbon and hydrogen atoms arranged in three or more fused benzene rings (reviewed in 32). PCAs arise in the environment from energy production with fossil fuel, motor vehicle exhaust, and refuse burning. Humans are exposed to the various PCAs by breathing polluted air, eating and drinking contaminated food and water, and by tobacco smoke. The level of benzo[a]pyrene (BaP) emitted into the air in the United States is estimated at 894 tons per year.

Many natural and synthetic PCAs are carcinogenic. Interestingly, many carcinogenic PCAs have been shown to be immunosuppressive, whereas their noncarcinogenic analogs have no immunosuppressive effect (33–35). Table 1 lists several studies in which PCAs have been shown to alter humoral immunity, CMI, and/or host resistance (36–39). In addition to suppressing the antibody response to SRBC, BaP and 7,12-dimethylbenzanthracene (DMBA) (carcinogenic PCAs) also suppress antibody responses to T-independent antigens such as trinitrophenyl (TNP)-carrier conjugates including TNP-LPS and TNP-Ficoll (33, 35, 39). The TNP-Ficoll antigen is more dependent on macrophage and T lymphocyte accessory function than is the TNP-LPS antigen. Although not conclusive, data using TNP-LPS and TNP-Ficoll antigens suggest that DMBA and BaP or their metabolites can directly effect B lymphocyte maturation. Recent studies have also implicated that decreased IL-1 production may also be involved in inhibition of antibody synthesis by BaP (40). CTL activity is depressed after in vivo or in vitro DMBA exposure (36, 41). Addition of T helper cells or exogenous IL-2, but not IL-1, to CTL cultures of DMBA-exposed lymphocytes restores CTL function (38). Since T helper cells are the primary source of IL-2, and CTL function was not restored by IL-1, DMBA also appears to alter T helper cell function following in vivo or in vitro exposure.

The mechanism (or mechanisms) by which PCAs produce immunosuppression is not fully understood. To produce their carcinogenic effect, PCAs are believed to require metabolic activation to reactive species capable of forming DNA, RNA, and/or protein adducts (42). Hepatic PCA metabolism is not necessary for immunosuppression, since addition of DMBA or BaP to lymphocyte cultures results in suppression (41, 43). However, PCA metabolism may follow several alternative routes, since lymphocytes and monocytes possess inducible cytochrome P-450 activity capable of generating reactive PCA metabolites (44, 45). Although not demonstrated in lymphocytes, BaP and DMBA can also be oxidized to reactive species by prostaglandin synthetase (46). Another pathway for metabolism of methylated PCAs involves formation of methylene carbonium ions, which are capable of forming DNA

Table 1 Summary of immunologic effects by PCAs in the mouse[a]

PCA	HI	CMI	Host Resistance	Reference
BaP	+	+	–	33, 34, 39
BeP	–	–	ND	33, 34
Anthracene	–	ND	ND	33
BA	+	ND	ND	35, 37
DBA	+	ND	ND	35, 37
3-MC	+	+	+	37, 38, 45
DMBA	+	+	+	33, 39, 41

[a]Abbreviations: (+) = Suppressive; (–) = No Effect; ND = Not Determined; BaP = Benzo(a)pyrene; BeP = Benzo(e)pyrene; BA = Benzanthracene; DBA = Dibenzanthracene; 3-MC = 3-methylcholonthrene; DMBA = 7,12-dimethylbenzanthracene.

adducts (47). Although reactive PCA metabolites are responsible for altering cellular activity, PCA-induced immunotoxicity is also dependent upon the presence of the *Ah* receptor (45, 48).

Polyhalogenated Aromatic Hydrocarbons

Polyhalogenated aromatic hydrocarbons (PHAs) represent a diverse class of compounds that have received considerable attention because of their toxicity in experimental animals, possible risk of carcinogenicity in the human population, and potential widespread environmental exposure (reviewed in 49). PHAs most likely to be of a potential human health risk include selected congeners of polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), polychlorinated dibenzofurans (PCDFs), and polychlorinated dibenzo-p-dioxins (PCDDs). The prototype for this class of compounds is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a contaminant in the synthesis of the herbicide 2,4,5-trichlorophenoxyacetic acid. Numerous studies have shown that administration of toxic PHAs to laboratory animals, and in particular TCDD, causes lymphoid atrophy, immunosuppression, and alterations in host resistance to challenge with infectious agents or transplantable tumor cells (reviewed in 50–52). In fact, thymic atrophy, immunosuppression, and bone-marrow alterations are dominant characteristics of TCDD toxicity that occur in almost all species examined. The relevance of these animal studies has been fostered by clinical observations of immune alterations in individuals inadvertently exposed to various PHAs (7–10).

The specific immunological effects of TCDD and possibly other PHAs in rodents depend, to a great extent, on the age of the animals when the chemical is administered. Perinatal exposure to TCDD (i.e. during thymic organogenesis) is primarily characterized by suppression of CMI, which is not readily apparent following adult exposure (53–55). This immunological profile

shares many features characteristic of neonatal thymectomy (56). Altered differentiation of intrathymic precursor cells, as occurs following neonatal thymectomy, has not been evaluated following perinatal TCDD exposure. However, T cells from treated mice demonstrate altered homing patterns (57), a feature characteristic of undifferentiated T cells. Furthermore, in vitro studies using a thymocyte and thymic epithelial cell coculture system have recently shown that TCDD inhibits the ability of thymic epithelial cells to provide the stimuli needed to induce T cell differentiation (58). Recent studies demonstrating that subchronic TCDD exposure inhibits the generation of CTLs also lend support to the premise that the thymic epithelial cell is a target tissue (59). This suggestion was indirectly evidenced by the use of murine bone-marrow chimeras, which have shown that inhibition of CTLs is due to the *Ah* genotype of the host and not of the grafted stem cell.

In contrast to subchronic or perinatal exposure, acute exposure of adult rodents to PHAs has its major effect on rapidly proliferating cell populations including hematopoietic stem cells (60, 61) and B lymphocytes, the latter manifested as suppressed antibody responses (e.g. 62–65). Unlike CMI, TCDD inhibits hematopoiesis (61) and B cell function (63, 66) by directly inhibiting maturation of the target cell.

Myelotoxicity, thymic atrophy, and immunosuppression by TCDD and PCBs appear to be associated with stereospecific binding to the *Ah* receptor present in lymphoid tissue and lymphoid cells (61, 62, 64–67). This association has been shown in genetic studies using *Ah*-responsive and -nonresponsive mouse strains, including mouse strains congenic at the *Ah* locus, where the immunotoxic effects of TCDD segregate with the *Ah* genotype. This association has also been supported in structure-activity studies where the binding affinity of various PHAs to the *Ah* receptor consistently correlated with its potency to induce immunosuppression. Furthermore, *Ah* receptors have been found in bone-marrow cells (61), lymphocytes (67), and thymic epithelial cells (58), which all are target tissues for PHA immunotoxicity.

Although immunotoxicity of PHAs is associated with binding to the *Ah* receptor, the mechanisms responsible following interaction of the receptor-ligand complex with the *Ah* locus are unknown. In fact, additional loci may be involved, since certain tissue-specific responses, such as epidermal hyperplasia in hairless mice, appear to be regulated by at least two genetic loci, *Ah* and *hr* (reviewed in 68). No consistent findings show that TCDD acts via cellular depletion mechanisms or by inducing qualitative and/or quantitative changes in regulatory products. In epidermal cell lines, TCDD alters normal patterns of proliferation and/or differentiation (reviewed in 68). Likewise, it has been proposed that TCDD induces similar maturational effects in thymic epithelial cells (58) and lymphocytes (66). For example, evidence suggests that

TCDD causes terminal differentiation of thymic epithelial cells, which results in loss of their ability to support thymocyte maturation (58). This altered pattern of differentiation may occur as a result of loss of high affinity epidermal growth factor receptors as occurs in keratinocytes treated with TCDD (69). Studies have shown qualitative and quantitative changes in phosphorylated proteins from purified B cells treated with TCDD (G. C. Clark & M. I. Luster, in preparation). Those proteins with altered phosphorylation patterns are associated with growth promoting activity in B cells. Thus, existing data indicate that TCDD immunotoxicity results from altered patterns of cell proliferation and differentiation in distinct lymphoid targets.

Heavy Metals

Metals may adversely affect the immune system and alter host resistance to infectious agents (reviewed in 4, 6, 70). Although many metals are known to alter immune function, we focus primarily on the effects of the most potent members of this group, which includes mercury (Hg), lead (Pb), and cadmium (Cd). Other heavy metals, such as platinum and gold, are of recent immunological interest. Platinum complexes, used as antineoplastic drugs, have been reported to suppress humoral immunity, lymphocyte proliferation, macrophage chemotaxis, and to induce allergic reactions (71). Gold salts, used pharmacologically as immunomodulators in rheumatoid arthritis (72), can cause nephrotoxicity in some patients that may involve an immunopathologic etiology. Gold injections cause renal disease with the involvement of immunoglobulin complexes and complement in rats, and autologous immune complex nephritis in guinea pigs (73). Gold is also capable of inducing allergic reactions and modulating lymphocyte activation.

Most reviewers have concluded that although heavy metals are immunosuppressive, under certain conditions they may be immunostimulatory (4, 70). Variables implicated in these opposite effects are dose concentration, route and duration of exposure, and strain and species differences. The most consistent effect on immune function in experimental animals following heavy metal exposure is increased susceptibility to infectious agents (74–83; Table 2). For example, increased mortality to *Listeria monocytogenes* was observed in CBA/J mice following subchronic lead exposure (78). Although lead significantly altered host resistance in these studies, it did not inhibit CMI or humoral-mediated immunity (HMI). Altered resistance to bacterial and viral infections produced by heavy metals demonstrates significant strain and species variability; in most murine studies antibody production and T cell activities are not suppressed. However, lead has been reported to inhibit phagocytosis, antigen processing, and other accessory functions in macrophages from several mouse strains (84–86).

Table 2 Summary of studies on heavy-metal effects on host resistance[a]

Host	Metal	Pathogen	Reference
Mouse	Hg	Encephalomyocarditis (EMC) virus	74
Rabbits	Hg	Pseudorabies virus	75
Mouse (SW)	Pb	*Salmonella typhimurium*	76
Rat (CR)	Pb	*Escherichia coli* and *Staphylococcus*	77
Mouse (CBA/J)	Pb	*Listeria monocytogenes*	78
Mouse (CD-1)	Pb	EMC virus	79
Mouse (S)	Pb	*Staphylococcus; Listeria monocytogenes; Candida*	80
Mouse (S)	PB	Langat virus	81, 82
Mouse (B6C3F1)	Cd	Herpes simplex virus	83

[a]Modified from Ref. 70.

The mechanism responsible for the alterations in host resistance by heavy metal exposure is unknown. Lead synergizes with endotoxin causing altered products and activities of reticuloendothelial cells, such as lipid peroxidation, superoxide anion generation, glutathiones, and glutathione-associated enzymes in mice (87), which could account for altered bactericidal and viricidal activity. In addition, although cytotoxic doses were employed (88), in vitro exposure to mercury and lead inhibits macrophage oxidative metabolism.

Mercury, lead, and cadmium reduce antibody production in some animals, but do not consistently produce this effect in all species studied (reviewed in 70). Even in the animals suppressed by heavy metals, it cannot be assumed that the defect is with the B cell. Blakley & Archer (86) have suggested that the ability of lead to inhibit humoral immunity is due to the inhibition of macrophage accessory functions. The effect of metal exposure on CMI is less well characterized. In a comprehensive study in Sprague-Dawley rats, chronic low-level pre- and postnatal exposure to lead suppressed several cell-mediated parameters, including delayed hypersensitivity and lymphoproliferative responses (89). Gaworski & Sharma (90) also noted that splenic lymphocytes from mice exposed to lead had significantly depressed proliferative responses to T and B cell mitogens. In contrast, several laboratories have reported that lead exposure does not suppress T cell proliferation (78, 82, 86). These differences are not easily reconciled, since the lead dosages and exposure periods employed do not easily account for the differences observed.

The mechanism of metal-induced injury to lymphoid cells is complex. Lead, like many metals, is a sulfhydryl alkylating agent with a high affinity for subcellular sulfhydryl groups. The immunomodulatory effects of lead on immune cells may involve an association with cellular thiols, since several studies have indicated that membrane and intracellular thiols are important in

lymphocyte activation, proliferation, and differentiation. Furthermore, the inhibitory effects of lead can be reversed by the addition of exogenous thiol reagents (86).

Although many aspects of metal-induced immunopathology still need clarification, heavy metals have known influences on autoimmunity. The most common observation has been mercury-induced glomerulonephritis in Brown-Norway rats (91) or Wistar rats (92). Mercury injections induce polyclonal activation of B cells and enhance antibody synthesis to several antigens including single-stranded DNA. The precise mechanism of heavy metal–induced autoimmunity is unclear. Metal may directly modulate lymphocyte activation (e.g. enhance B cell or T suppressor cell activity), which could lead to autoimmunity (91). Since mercury and lead biochemically and biophysically alter erythrocytes, they may modify the antigenicity of erythrocytes, which results in an autoimmune hemolytic phenomena.

Organometals (Methylmercury and Organotins)

Methylmercury is readily absorbed through the intestine and passes the blood-brain and placental barriers where it can cause neuropathological changes in humans (Minamata disease). In rodents, methylmercury decreases resistance to infectious agents (reviewed in 4) and chemically induced tumors (93). Subchronic low-level methylmercury exposure in rodents causes atrophy of the thymic cortex and splenic follicles with concomitant suppression of immune functions (94–96). These observations complement Takeuchi's observation (97) of lymphoid and hemopoietic hypoplasia in the spleens of patients with Minamata disease.

Organotin compounds, used primarily as heat stabilizers, catalytic agents, and antimicrobial compounds, are also immunosuppressive in laboratory animals. Both di-n-octyltindichloride (DOTC) and di-*n*-butyltindichloride (DBTC) selectively depress thymic weights as well as T lymphocyte function in rats, without causing nonlymphoid toxicity (98–100). A cellular depletion mechanism is probably responsible for suppression, since decreased T cell–mitogen responsiveness in rats correlates with decreased numbers of lymphocytes with T cell surface markers (reviewed in 101). Suppression of humoral immunity by dialkyltins may be at the level of T cell regulation rather than directly affecting B cells, since T helper cell numbers are also decreased. Immune function is not impaired in mice and guinea pigs fed dialkyltins; this finding correlates with the absence of lymphoid tissue atrophy observed in these species (reviewed in 101). No species specificity is apparent following in vitro treatment; DOTC or DBTC cultured with rat, mice, or human thymocytes causes decreases in cell survival, mitogen responsiveness, and E-rosette formation (98). Organotins may interfere with T cell replication by interacting with plasma membrane sulfhydryl groups essential for amino acid

transport and/or inhibiting glucose metabolism via alteration of pyruvate and α-ketoglutarate dehydrogenase activity (99).

Aromatic Amines

Benzidine (4,4-diaminobiphenyl), employed industrially in the synthesis of dyes as well as analytic reagents in various laboratory tests, is a urinary bladder carcinogen in humans (102). In rodents, benzidine causes hepatomas, mammary tumors, and, to a lesser extent, lymphoreticular neoplasms, primarily lymphomas (103). Because considerable evidence indicates that the immune response to chemically induced tumors may modulate tumor growth and/or progression, it follows that an increased incidence of neoplastic disease may occur by chemical carcinogens that also suppress immune functions. In mice, suppression of CMI occurs at dose levels of benzidine that are tumorigenic (104). In addition, benzidine exposure decreases host resistance to challenge by transplantable tumor cells or *Listeria* (104). Relevant to these observations in mice, an unconfirmed study showed a relationship between immunosuppression and neoplasia in workers engaged in the manufacture of benzidine (105). In this four-year study, only workers who were identified as having suppressed CMI (based on skin tests) demonstrated precancerous conditions and subsequent neoplasms.

The mechanisms responsible for immunosuppression by benzidine may not be the same as those responsible for its carcinogenicity. The addition of benzidine in vitro to mitogen-activated lymphocytes mimics the suppression of lymphocyte responsiveness observed following in vivo exposure. In vitro studies suggest that alterations in metabolites of the arachidonic acid–lipoxygenase pathway by benzidine (benzidine can serve as a co-oxidative substrate for hydroperoxidase) are responsible for inhibiting lymphocyte activation (104).

Estrogenic Xenobiotics

Although sex steroids such as estradiol and testosterone are not xenobiotics, they possess immunomodulatory properties (reviewed in 106). Likewise, a number of xenobiotics demonstrate both estrogenic activity (107), as determined by the rat uterine bioassay, and immunotoxicity. These include DDT, chlordecone, the mycotoxin zearalenone and its derivative zearalenol (a commercial anabolic agent), Δ^9-tetrahydrocannabinol (Δ^9-THC), and diethylstilbestrol (DES); the latter is the most potent estrogen in the series (reviewed in 108). However, other than DES, and to some extent Δ^9-THC, the immunotoxicity of these xenobiotics has not been extensively studied, and any relationship between estrogenic activity and immunotoxicity remains to be determined. The immunological effects of DES, particularly following adult exposure, are similar to those induced by 17-β-estradiol (109, 110) and

following subchronic adult exposure in mice include thymic atrophy, myelotoxicity, stimulation of the reticuloendothelial system (RES), suppression of CMI, and reduction of NK cell activity (reviewed in 108, 111). Suppression of antibody synthesis in mice by DES also occurs, but is probably due either to increased antigen sequestering by macrophages or to depletion of T helper cell function and does not have a direct effect on B cells (112). Mice exposed perinatally to DES also demonstrate marked suppression (113–115). However, although immunosuppression appears to be reversible following adult exposure, the effects following perinatal exposure are persistent and may be under a different mechanism. Unlike mice, rats are resistant to immunosuppression by DES (J. Vos, personal communication).

Clinical observations and animal studies suggest that multiple mechanisms may be responsible for DES immunosuppression. Membrane-reactive estrogen metabolites (e.g. quinone intermediates) can react with the leukocyte cell membrane and modulate cell-surface interactions (116). Estrogens can also influence production of immunoregulatory factors produced by the thymic epithelium (109, 117, 118). This latter effect may be mediated by specific estrogen receptors present in thymic epithelial cells, since suppression can be blocked by steroid hormone receptor antagonists such as tamoxiphen. This putative thymic factor has not, as yet, been characterized, and any relationship with known thymic peptides remains to be determined. However, a recent report indicated that injection of estrogen reduced circulating plasma levels of thymosin $\alpha 1$ (119). Evidence also indicates that DES alters macrophage activation and T cell regulatory functions, which may also contribute to immunotoxicity. A relationship has been established between increased phagocytic activity of the RES and the amount of antigen ultimately available to the spleen, suggesting that depressed antibody responses result from altered antigen distribution or increased sequestering (reviewed in 108). Estrogens have also been reported to increase T suppressor cell activity in spleen cells of pregnant mice (120), decrease the number of T helper cells (111), and inhibit IL-2 synthesis (121). The biochemical mechanisms responsible for these effects have not been examined.

Pesticides

Several classes of insecticides have been examined for immunotoxicity, including organochlorines and organophosphates. Increasing evidence suggests that certain organochlorine pesticides, including DDT (122–124), captain (125, 126), and the chlorinated cyclodiene, chlordane (127, 128), modulate immune function. Immunosuppression by chlordane, however, may require in utero exposure (129). Due to their rapid metabolism by carboxyesterases to nontoxic metabolites, the toxicity of most organophosphate pesticides is relatively low. However, during their manufacture and storage,

contaminants are formed that inhibit carboxyesterase activity. One of these contaminants, O,O,S-trimethylphosphorothioate (OOS-TMP), found in malathion, fenitrothion, and acephate, acts as a cholinergic agent at high dose levels and is immunotoxic at lower dose levels where other toxic manifestations are not observed (130–132). In mice, exposure to OOS-TMP, but not malathion, causes lymphocytopenia, thymic atrophy, suppression of antibody synthesis, and decreased generation of CTLs. However, neither lymphoproliferative responses, nor IL-2 synthesis are affected (130, 131). Methylparathion also suppresses immune function (123) and increases susceptibility to infection with *Salmonella typhimurium* (133). The immunological profile observed appears to be associated with altered macrophage activation. Macrophages treated with OOS-TMP demonstrate characteristics of inflammatory cells (low percentages of Ia antigen, increased IL-1 production, and decreased antigen binding capacity). The subcellular mechanisms for OOS-TMP immunosuppression have not been studied, however suppression does not appear to be related to altered cholinesterase activity, since O,S,S-trimethyl phosphorodithioate, a structural analog of OOS-TMP, inhibits cholinesterase activity without altering immune function (131, 132).

Benzene

Occupational exposure to benzene has frequently produced myelotoxicity expressed as leukopenia, pancytopenia, lymphocytopenia, granulocytopenia, thrombocytopenia, anemia, and bone-marrow hypoplasia (reviewed in 12). In occupational exposure a strong correlation was noted between the most frequently cited symptom, lymphocytopenia, and abnormal immunologic parameters. Benzene exposure in rabbits, rats, and mice has resulted in anemia, bone-marrow hypoplasia, and dose-related lymphocytopenia (12, 134). Benzene-induced myelotoxicity has been correlated with the appearance of benzene metabolites in the bone marrow (135), and the current evidence supports the concept that benzene toxicity is caused by one or more metabolites of benzene (136, 137).

Some investigators have stressed the importance of polyhydroxylated derivatives of benzene and their semiquinones (reviewed in 138). These studies have shown that in vitro hydroquinone and/or para-benzoquinone inhibits microtubule polymerization (139), T and B lymphocyte activation (140), and lectin-stimulated lymphocyte agglutination in rat spleen preparations (141). Since lectin-induced lymphocyte mitogenesis, cell agglutination, and capping are processes dependent upon microtubule integrity, it has been suggested that polyhydroxy metabolites of benzene (e.g. p-benzoquinone) inhibit PHA-induced lymphocyte blastogenesis and agglutination via this mechanism (reviewed in 138). The effects of benzene metabolites on microtubule assembly in vitro suggest reactivity with the two sulfhydryl groups at the GTP-binding

site. However, administration of these compounds to animals does not result in typical effects of benzene toxicity, such as leucopenia, anemia, thrombocytopenia, and, eventually, aplastic anemia. Engelsberg & Snyder (142) have administered the major metabolites of benzene to mice and, using the ^{59}Fe uptake technique, failed to observe decreases in red cell production. Although these compounds are highly reactive and have profound effects when using in vitro systems, their reactivity may limit their ability to reach lymphoid tissue in biological systems. Goldstein et al (143) have suggested that ring-opening products may play a role in benzene toxicity. Thus, the toxic metabolites of benzene responsible for immunotoxicity and bone marrow suppression have yet to be identified.

Studies in rabbits exposed to benzene have described increased susceptibility to tuberculosis and pneumonia and reduced antibody response to bacterial antigens (reviewed in 12). Alterations in host resistance appear to correlate with altered immune function, since subcutaneous administration of benzene to C57BL6 mice inhibited both antibody production and lymphocyte activation (140). Alterations of immune parameters and host resistance that occur in experimental animals following benzene exposure are consistent with severe benzene toxicity in humans, which is often characterized by an acute, overwhelming infection. Evaluation of a large number of workers exposed to benzene revealed depressed levels of serum complement, IgG, and IgA, but not IgM (144, 145). Thus, benzene appears to be an immunotoxicant for humans, although the magnitude of this effect and the exposure threshold for immunotoxicity remain to be established.

Fungal Products

Several groups of mycotoxins, including ochratoxin, trichothecenes, and aflatoxin, are immunotoxic in laboratory animals. Ochratoxins are a series of 3,4-dihydro-3-methyl isocoumarin derivatives produced by several species of fungi belonging to the *Aspergillus* and *Penicillium* genera (146). Human exposure to ochratoxin A (OA) occurs through ingestion of contaminated cereal grains or contaminated animal tissue (reviewed in 147). OA can suppress antibody responses to both T-dependent (148, 149) and T-independent antigens (150) in mice. Administration of the 4-hydroxy metabolite of OA suppressed antibody secretion to the same extent as the parent compound, suggesting that a metabolite formed by liver microsomes in the presence of NADPH is responsible for OA immunosuppression (149). While the mechanism of immunosuppression and toxicity is unknown, Haubeck et al (148) demonstrated that coadministration of phenylalanine prevents OA-induced immunotoxicity. This same protective effect has been shown for OA inhibition of protein synthesis and growth of hepatoma cells in culture (151). With previous experiments demonstrating that phenylalanine inhibits OA toxicity (152), Haubeck et al suggest that increasing the enzyme substrate,

phenylalanine, can reduce the inhibitory effect on the enzyme system and prevent OA-induced immunosuppression (148).

T_2 toxin, one of the most potent trichothecenes, has been linked to cases of alimentary toxic leukopenia in the USSR (153). Immunotoxicological effects of the trichothecenes include decreased antibody responses (154), decreased lymphoproliferation to T and B lymphocyte mitogens (155, 156), increased skin-graft survival time (154), and inhibition of macrophage activation (157). T_2 toxin inhibits protein synthesis in various eukaryotic cells (158), including alveolar macrophages and lymphocytes (157, 159). The inhibitory effect on protein synthesis is believed to be the primary cause for cellular toxicity.

Aflatoxin B_1 (AFB-1) is one of the most acutely toxic aflatoxins known, as it is both a hepatotoxin and a hepatocarcinogen (160, 161). AFB-1 also directly inhibits B and T lymphoproliferative responses in vitro. This effect is markedly enhanced when lymphocytes and AFB-1 are preincubated with hepatocytes (162), suggesting a need for metabolism. Antibody production is only inhibited when AFB-1 is preincubated with hepatocytes (162). Metabolites of AFB-1 have been shown to alkylate DNA. This akylation could account for the need of hepatocyte preincubation (163). The direct inhibitory effect of AFB-1 on lymphoproliferation could be attributed to its ability to act as an antimitotic agent (164).

Dimethylnitrosamine

Dimethylnitrosamine (DMN) has been shown to be carcinogenic and toxic in a variety of animal species (165) and is a suspected carcinogen in humans (166). Metabolic activation of DMN results in a metabolite that alkylates nucleic acids (167). DMN is also immunotoxic in mice, suppressing HMI and CMI (168, 169) and increasing susceptibility to tumor cell challenge (170); however, it also has an additional stimulatory effect on bone-marrow monocyte progenitors (168). DMN suppresses the antibody response to both T-dependent and T-independent antigens in vivo, but has no effect in vitro. Likewise, DMN does not alter mitogen-induced lymphoproliferative responses in vitro, suggesting metabolism may be needed to produce immunosuppression. Inclusion of rat hepatocytes in cell cultures to generate DMN metabolites did not produce suppression, which suggests extrahepatic DMN metabolism or an indirect in vivo effect (162). Examination of antibody production in vitro, using combinations of spleen cells from treated and untreated mice, indicates that suppression is due to altered B lymphocyte function (171).

Pulmonary Irritants

Human exposure to asbestos is associated with pulmonary diseases including fibrosis, asbestosis, and mesothelioma (reviewed in 172). Immunological impairments, such as decreased delayed-type hypersensitivity responses, re-

duced numbers of circulating T cells, and depressed T cell proliferation are associated with the pulmonary abestosis (reviewed in 173). In addition to suppression of CMI, increased levels of serum immunoglobulins and autoantibodies have been observed in asbestosis patients (174). NK cell activity is also decreased in asbestotic individuals (175, 176). Altered alveolar macrophage activity plays a significant role in asbestos-induced immunological dysfunction. Asbestos particles reaching the alveoli are phagocytized by macrophages, resulting in cell lysis and release of lysosomal enzymes, inflammatory products, and the previously ingested asbestos fibers (177). A cytotoxic effect on alveolar and splenic macrophages has also been demonstrated in vitro (178). While macrophages appear to be the most likely target within the lung, data also suggests that asbestos may directly affect T cell function, which may account for immune dysfunction by asbestos (179). The temporal relationship of these immune alterations to the onset of asbestosis and neoplasia remains to be clarified.

Beryllium was utilized in the fluorescent lamp industry and is currently used in the construction of lightweight metal alloys. Exposure to beryllium can result in dermatitis, acute pneumonitis, and chronic pulmonary granulomatosis or berylliosis (reviewed in 180). While most cases of acute pneumonitis are thought to originate from chemical irritation, berylliosis and some acute pneumonitis involve delayed hypersensitivity responses (181, 182). The antigen producing this hypersensitivity reaction is presumed to be a beryllium–protein complex (183). Sensitivity to beryllium may have a genetic basis, since it is transmitted as a non-sex-linked dominant trait in guinea pigs (184). Exposure of lymphocytes from sensitized subjects to beryllium conjugates results in increased lymphocyte transformation (185) and production of macrophage migration inhibition factor (186). Alveolar macrophages phagocytize beryllium and release lysosomal enzymes (187, 188). The pathogenesis of berylliosis and its relationship to immunological changes are not fully defined.

Silica is encountered in many occupational settings including sandblasting and mining. Acute or chronic exposure to silica dust can result in silicosis, a condition characterized by pulmonary fibrosis. Several immunological changes are associated with silicosis, including increased serum immunoglobulin levels, autoantibodies, and an increased incidence of autoimmune disease (189, 190). Increased tumor immunity has also been reported and may be attributed to elevated numbers of macrophages at the pulmonary lesion (191). In contrast to humoral immunity, depression of CMI, as evidenced by prolonged skin graft rejection and decreased resistance to viral mycotic or mycobacterial infections, has been reported (192, 193). Silica can be cytotoxic to macrophages in vitro (194) and can induce their activation (195). A soluble fibrogenic factor, which stimulates hydroxyproline and DNA synthesis in fibroblasts, is produced by macrophages activated by silica (196).

This interaction between activated macrophages and fibroblasts has been suggested to play a role in the pathogenesis of silicosis (reviewed in 197).

CONCLUSION

The immune system is highly complex; in it maturation is subject to orderly control by endogenous (lymphokines, cytokines) and exogenous (bacterial products) mediators. These mediators possess activation, growth-promotion, and/or differentiation properties, and are under the influence of potent, but not well-understood, regulators. A large number of xenobiotics adversely affect the immune system (based on observations in rodents and limited studies in humans inadvertently exposed) through disruption of cell-maturation processes. Considering the widespread distribution and stability of some of these agents in the environment, our concern is that current knowledge on the adverse health effects in humans may represent only the "tip of the iceberg" and that xenobiotic exposure may play a greater role than heretofore suspected in disease causation. Likewise, exposure to immunoalterative xenobiotics might represent additional risk to individuals with already fragile immune systems (e.g. those weakened by malnutrition, infancy, and old age). Due to obvious limitations in human clinical studies, an understanding of these risks depends to a great extent upon the clarification of cellular and molecular events underlying xenobiotic-induced immune alterations in experimental animals.

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