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SHORT ANALYTICAL REVIEW

Signaling by Environmental Polycyclic Aromatic Hydrocarbons in Human Lymphocytes[1]

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During the past decade there has been significant progress made in understanding how environmental agents, drugs, certain chemicals present in the diet, and occupational agents affect the immune system of animals and humans. Polycyclic aromatic hydrocarbons (PAHs) are an important class of environmentally prevalent xenobiotics that exert complex effects on the immune system. These agents, typified by benzo(a)pyrene (BaP), have been shown to alter antigen and mitogen receptor signaling pathways, leading to suppression of humoral and cell-mediated immunity, and at high exposure levels to activation of genes involved in apoptosis in lymphoid cells. Interestingly, at low exposure levels, PAHs may actually augment cell signaling pathways, resulting in immune enhancement or an adjuvant effect. While the biochemical targets and mechanisms responsible for immune modulation are still under investigation, several themes are evolving. PAHs, principally through their cytochrome-P450-derived metabolites, activate oxidative and electrophilic signaling pathways in lymphoid and nonlymphoid cells, including myeloid, epithelial, and other cells. Although PAHs affect signaling pathways in nonlymphoid cells leading to complex interactions between antigen-specific and nonspecific immune and inflammatory responses, this brief review focuses on the mechanisms of signaling by environmentally prevalent PAHs in human lymphocytes. Understanding the mechanisms by which xenobiotics alter adaptive and nonadaptive immune responses may shed light on the etiology of environmental and occupational immune diseases.

INTRODUCTION AND SCOPE OF IMMUNOTOXICOLOGY

Immunotoxicology is a specialty area of toxicology that examines the effects of foreign agents (e.g., pharmaceuticals, medical devices, environmental chemicals, UV light, and radiation) on the immune system (1). While the field of immunotoxicology has its roots in examining the effects of foreign agents (i.e., xenobiotics) on immunosuppression and hypersensitivity, recent interest has also focused on autoimmunity and inflammation.

Because of the complexity of the adaptive and nonadaptive immune systems, examination of immune effects has traditionally involved a multitiered approach (2). The tiered approach includes measures of humoral and cell-mediated immunity, as well as changes in nonspecific, inflammatory, cell subset, and pathologic markers (3–5). While these original tier tests were designed to assess xenobiotic-induced immunosuppression, and in some cases to correlate these effects with altered host resistance, numerous investigators have also developed tests to examine cutaneous and respiratory hypersensitivity reactions (6, 7). Investigators have also developed models to study chemically induced autoimmunity (8), although in most instances extrapolation of findings from these models to humans has proved difficult.

As the field of immunotoxicology has developed, investigators have increasingly asked mechanistic questions about the cells and systems affected by xenobiotics. It is generally accepted among toxicologists that knowledge of the mechanisms of action of immunotoxic agents will allow for more accurate assessment of risk and the establishment of appropriate exposure limits to environmental toxicants. During the past few years, evidence has accumulated that xenobiotics modulate intracellular and extracellular signaling pathways in lymphoid cells (9). In this brief review we will focus on a class of agents, polycyclic aromatic hydrocarbons (PAHs), that has been extensively examined for signaling effects on the immune system of rodents and humans.

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BACKGROUND ON PAHs

PAHs are naturally occurring environmental pollutants formed through the combustion of fossil fuels and the burning of various substances. Tens of thousands of tons of PAHs are released into the atmosphere each year in the United States, leading to contamination of air, water, and soil (10). PAHs are also found in tobacco smoke, are present in charcoal-broiled foods, and are on the surface of diesel exhaust and other combustion-related particles. Humans are exposed primarily though the diet, on an average, to μg amounts of PAH each day. As shown in Table 1, virtually all humans are exposed to PAHs by inhalation and ingestion. Some populations are exposed to much higher concentrations of PAHs by inhalation and ingestion due to use of fuels for heating or cooking that are rich in PAH emission (11) or from certain occupations that may be associated with dermal exposures (12).

Environmentally, PAHs are found in complex mixtures with many other substances. Tobacco smoke, for example, contains over 1000 distinct chemical entities, with dozens of different PAHs represented in particulate and nonparticulate forms. Common sources of environmental PAHs in the air result from diesel and automobile exhaust, forest fires, and wood-burning stoves (13, 14). As shown in Table 2, many PAHs are produced during combustion and these pollutants exist in complex mixtures in the atmosphere or as particle fallout in water and soil. While some PAHs are relatively innocuous, others, such as benzo(a)pyrene (BaP), dibenzanthracenes, and certain dibenzopyrenes and nitropyrenes, are potent chemical carcinogens. PAHs are also known to cause diseases in other organ systems, especially during organ development and lymphoid cell development (Table 3). Interestingly, there appears to be a strong correlation between the carcinogenicity of PAHs, their immunotoxicity, and perhaps other diseases (15). The finding that BaP is carcinogenic and immunotoxic and that the structurally related benzo(e)pyrene (BeP) is weakly if at all carcinogenic or immunotoxic has proved useful for examining specific and nonspecific effects of these agents on target cells. The remainder of this section will focus on BaP and the immunotoxicologically similar model compound, 7,12-dimethylbenz(a)anthracene (DMBA), because these have been the best studied PAHs.

TABLE 1

Routes and Sources of Exposure to Environmental Polycyclic Aromatic Hydrocarbons

Route of exposure	Primary sources of PAHs
Inhalation[a]	Combustion of fossil fuels, such as coal and petroleum products; particulate and nonparticulate exposures from occupational–industrial sources to tars and fumes; wood smoke and cigarette smoke[b]
Ingestion	Dietary: charcoal-broiled foods
Dermal	Exposure to tars or soot

[a] Inhalation of gaseous (volatilized) PAHs occurs in addition to exposure to PAH-coated particles; these particles are deposited in the upper, middle, and lower airways as a function of their size; in the upper airways, large particles are cleared by the mucociliary escalator and the particles are swallowed, leading to significant exposure via ingestion.

[b] In addition to PAHs, there are numerous other carcinogens present in cigarette smoke, including various oxidants, nitrosamines, and nitropyrenes.

IMMUNOTOXICITY OF PAHs

The immunotoxicity of PAHs has been extensively evaluated in murine models (reviewed in 15). PAHs, such as BaP and DMBA, given orally or subcutaneously at total cumulative doses of 50–150 mg/kg suppress humoral and cell-mediated immunity (16–18). There is some evidence that accessory cell function is also sensitive to the effects of DMBA (19). Interestingly, the immunosuppression produced by DMBA is persistent, lasting long after the PAH has cleared from the body (20, 21). Since immunosuppression can be observed at doses that do not produce significant cytotoxicty, it has been suggested that PAHs may induce a state of immunologic unresponsiveness (i.e., tolerance or anergy). Immunosuppression produced by DMBA is seen in virtually all lymphoid compartments, including the spleen, lymph nodes, and Peyer's Patches (22, 23), suggesting that its effects occur via the systemic circulation. BaP has been found to be more than 1000-fold more immunotoxic to mice than BeP, demonstrating that there are important structure–activity relationships for PAHs (18). In addition, since there is a good correlation between immunosuppression produced by PAHs and decreased resistance to infectious agents or transplantable tumor cells in animal models (16, 17), the immunological effects of PAHs appear to be biologically important.

In vitro exposures of murine splenocytes to concentrations of 10–20 μM DMBA produce immunotoxicity (24). The selective immunotoxicity of BaP in mice, contrasted to BeP mentioned above, has also been observed in human peripheral blood mononuclear cells using *in vitro* mitogenesis experiments (25, 26). Importantly, human peripheral blood mononuclear cells appear to be more sensitive to the immunotoxicity of BaP than murine spleen cells, suggesting that humans exposed to PAHs are likely to be adversely affected by these agents (25, 26). The ability of human peripheral blood B cells to secrete antibody in response to activation by a superantigen has also been shown to be suppressed by DMBA (27).

TABLE 2

Environmental Polycyclic Aromatic Hydrocarbons (PAHs) Present in Wood Smoke and Automobile and Diesel Exhaust[a]

Wood smoke (ref. 12)	Noncatalyst auto (ref. 13)[b]	Diesel exhaust (ref. 13)
Naphthalenes (gas)	Dimethylfluoranthenes	Dimethylphenanthrenes
Phenanthrenes	Benzoperylene	Methylphenanthrenes
Anthracene	Methylbenzanthracenes	Pyrene
Fluorene (gas)	Methylfluoranthenes	Benzofluoranthenes
Fluoranthene	Benzochrysene	Methylfluoranthenes
Retene	Dimethylphenanthrenes	Phenanthrene
Pyrene	Benzofluoranthenes	Chrysene
Benzo(b,j,k)fluorene	Benz(a)anthracene	Benz(a)anthracene
Benz(a)anthracene	Chrysene and pyrene	Naphthalenes
Benzopyrenes	Benzopyrenes	Methylbenzanthracenes[c]

[a] *Note.* Top ten PAHs in approximate concentration rank order based on data in references 12 and 13; the actual composition and concentration of PAHs varies considerably based on the fuel and burning conditions. Under atmospheric conditions in ultraviolet light and in air, many PAHs oxidize to form quinones.

[b] Catalysts contained in engine emission systems in current model automobiles significantly reduce PAH emissions.

[c] Benzo(a)pyrene is formed in nearly equivalent amounts in diesel exhaust.

High doses of DMBA are associated with thymic atrophy and decreased lymphoid cell recovery from the spleen, lymph nodes, and bone marrow (22, 23). Pre-B cells in murine bone marrow appear to be particularly sensitive to apoptosis produced by PAHs (28, 29). Murine and human B cell lines are also sensitive to PAH-induced apoptosis (30, 31). Interestingly, in these studies α-naphthoflavone (ANF), an agent that inhibits induction of metabolism of PAHs by blocking the Ah receptor as well as by inhibiting P4501A and 1B activity, was found to prevent the immunotoxicity of PAHs (25, 26). ANF was also effective in reversing suppression of humoral immunity produced by DMBA in mice (32) and has been found to prevent BaP-induced apoptosis in Daudi human B cells (31). Collectively, these results suggest that BaP, and indeed other PAHs, exert their effects through binding to a specific aromatic hydrocarbon receptor (AhR), upregulating AhR-controlled metabolic enzymes (primarily P4501A and 1B), and inducing the production of immunotoxic PAH metabolites. Therefore, it becomes important to understand how PAHs are metabolized in lymphoid and surrounding nonlymphoid tissues, as well as the biologic and toxicologic effects of these metabolites.

TABLE 3

Environmental Polycyclic Aromatic Hydrocarbons and Suspected Human Diseases

Cancer (skin, lung, breast, lymphoma, testicular,[a] others)
Immunotoxicity
Inhibition of pre-B, pre-T, and myeloid cell development
B and T cell suppression of humoral and cell-mediated immunity
Apoptosis of lymphoid tissues
Disruption myelopoiesis
Altered cytokine production by macrophages and monocytes
Adjuvancy?
Teratogenicity
Environmental lung diseases? (COPD, asthma, etc.)
Endocrine disruption?

[a] The carcinogenicity of PAHs present in soot was first discovered in London in the late 1800s in chimney sweeps.

METABOLISM OF PAHs IS CRITICAL TO THEIR IMMUNOTOXICITY

In general, the mechanisms of immunotoxicity of PAHs appear to be closely associated to the mechanisms responsible for the carcinogenicity of these agents. A general review of PAH metabolism and genotoxic and non-genotoxic biochemical mechanisms of carcinogenesis has been presented by Pitot and Dragan (33). Over the past 35 years there have been numerous studies that have shown that BaP is metabolized by P450 and other pathways leading to production of reactive electrophiles that bind to DNA, producing mutations and tumor initiation. BaP is an example of a chemical that induces its own metabolism. BaP binds to cytosolic AhR that translocate to the nucleus complexed to other factors, such as ARNT, and bind to promoter regions of P4501A and 1B genes that metabolize BaP to a BaP-epoxide (Fig. 1). In the presence of epoxide hydrolase (EH), BP-7,8-epoxide is converted to BP-7,8-diol, which is again a substrate for P4501A and 1B, leading to the formation of BP-7,8-diol,9,10-epoxide (BPDE). BPDE is the major electrophilic form of BaP that binds to DNA and initiates cancer (33). There are poorly understood electrophilic sensors in cells that trigger protective metabolic responses through electrophilic response elements (EpRE) on DNA (34). Other enzymes that metabolize BaP include peroxidases which form 1,6, 3,6, and 6,12 quinones of BaP (BPQs). These quinones are of interest because they undergo

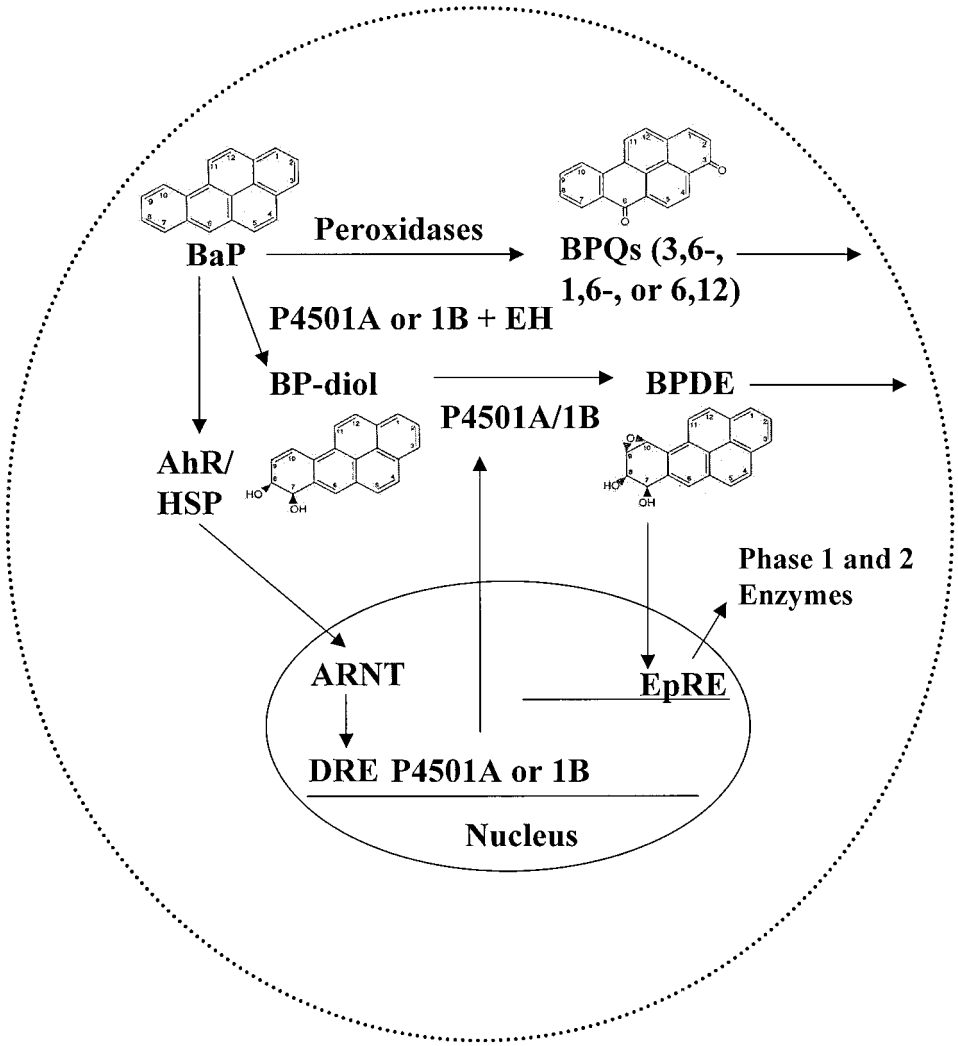

FIG. 1. Simplified schema for metabolism of benzo(a)pyrene (BaP) by P450 and other enzymes. BaP is an example of a polycyclic aromatic hydrocarbon (PAH) that induces its own metabolism. BaP binds to a cytosolic aromatic hydrocarbon receptor (AhR) that is complexed by heat shock proteins (HSPs). Following binding of BaP to the AhR, there is loss of binding of the HSPs, translocation of the complex to the nucleus, and binding of a new partner, known as the AhR nuclear translocator (ARNT, a misnomer because ARNT is already in the nucleus). The BaP–AhR–ARNT complex binds to dioxin-responsive elements (DRE, so called because dioxin, or TCDD, is an AhR ligand and activates this pathway). The activation of DREs leads to the upregulation of P4501A and P4501B enzymes and other genes. P4501A/1B metabolize BaP to various phenolic metabolites and epoxides. The BaP epoxides can then be hydrated by epoxide hydrolase (EH) to form BP-7,8-dihydrodiol (BP-diol). The BP-diol is also a substrate for P4501A/1B, which leads to the formation of BP-7,8-diol-9,10-epoxide (BPDE). BPDE is a strong electrophile that can bind to DNA or activate electrophilic responsive elements (EpRE) to induce further genes. An alternative pathway for BaP metabolism is via peroxidases present in inflammatory and other cells. The peroxidase pathway results in the formation of numerous BaP metabolites, but most notably BP quinones (BPQs, including the 1,6, 3,6, and 6,12 BPQs). These metabolites are important because they can produce oxidative stress in cells due to their propensity to undergo redox cycling in the presence of a NADPH-regenerating system.

redox cycling in the presence of a NADPH reducing system, leading to the formation of reactive oxygen species (ROS, such as H_2O_2, $O_2^{\cdot -}$ and $OH^{\cdot}$) that cause oxidative stress in cells. The specific ROS formed in response to PAHs are expected to be cell type specific, with mononuclear and granulocytic cells likely contributing to the overall oxidative and proinflammatory environments in which lymphocytes are present. We believe that although BPDE and BPQs may act on both lymphoid and nonlymphoid cells via distinct biochemical mechanisms, they both likely play important roles in the immunotoxicity of BaP to human lymphocytes.

PAHs ALTER SIGNALING PATHWAYS IN HUMAN T AND B CELLS

Previous studies in our lab have shown that PAHs produce concentration-dependent signaling effects in

murine and human lymphocytes by both specific and nonspecific mechanisms (reviewed in 35). Early work showed that DMBA inhibited Ca^{2+} mobilization induced by phytohemagglutinin in Jurkat human T cells (36). However, when it was noted that DMBA, but not BeP, increased baseline Ca^{2+} in Jurkat cells, Ca^{2+} mobilization was considered a potential target for immunotoxicity. Thus, premature Ca^{2+} release by PAHs may interfere with normal antigen or mitogen receptor signaling pathways, leading to anergy or apoptosis (Fig. 2). The mechanism of DMBA-induced Ca^{2+} mobilization was later shown to be due to IP_3-dependent src kinase activation in human T cells (37). More recently, the importance of immediate src activation by PAHs has been questioned since it has been found that numerous other non-immunotoxic PAHs at high concentrations (10–20 μM) mobilize Ca^{2+} by apparent nonspecific membrane effects (38). The lack of correlation between immediate Ca^{2+} mobilization and the known immunotoxicity of PAHs argues that this mechanism is not critical for immunotoxicity. Additionally, since the effects are immediate there is inadequate time for PAH metabolism in this process. PAH structure–activity studies performed using inhibition of human T cell mitogenesis as an endpoint showed that the immunotoxicity of BaP and DMBA could be prevented with ANF (26). This observation suggests that metabolism and/or AhR activity is important for immunotoxicity. However, the fact that 2,3,7,8-tetrachlorodibenzo (*p*)dioxin (TCDD), a potent AhR ligand, does not exert similar effects suggests that the AhR agonist activity of BaP is not responsible for the reversal of BaP immunotoxicity by ANF.

PAH-induced Ca^{2+} elevation is not unique to human T cells. We have demonstrated that Ca^{2+} elevation also occurs in human B cells and monocytes (39), and PAHs elevate Ca^{2+} in other cells, including human mammary epithelial cells (40). Studies conducted in the Daudi human B cell line have shown that Ca^{2+} elevation is likely associated with the formation of BPDE (41) and BaP quinones (BPQs, see below). BPDE has been shown to activate Lyn and Syk, two protein tyrosine kinases (PTKs) important in antigen receptor signaling (41). The mechanism whereby BPDE activates these PTKs is still under investigation. However, it is notable that prolonged elevation of intracellular Ca^{2+} in Daudi by BPDE was found to induce apoptosis due to an increase in Bax and a decrease in Bcl-2 concentrations (31). We have recently shown that p53 protein is also rapidly increased in Daudi cells following exposure to BPDE (42). Since BPDE is known to bind to DNA (33, 43) and p53 is a sensor of DNA damage and adduct formation (44), genotoxicity may be responsible for the increase in Bax seen during apoptosis in Daudi B cells (45). However, as a potent electrophile, BPDE may also activate electrophilic response elements (EpRE), also known as anti-oxidant response elements (ARE), that are present in lymphoid cells (34).

ROLE OF OXIDATIVE STRESS IN BaP-INDUCED IMMUNOTOXICITY

It is clear from previous studies that certain BaP metabolites exert oxidative stress in human lymphocytes by depleting reduced glutathione (GSH) (46, 47). It has also been shown that BPQs are formed by peroxidases present in monocytes, macrophages, and neutrophils (48). At this time it is unclear whether B and T cells form BPQs or whether bystander cells, such as macrophages and monocytes, or other cells are the likely primary source of BPQs. We have recently found that BPQs elevate Ca^{2+} in Daudi human B cells (Burchiel *et al.*, submitted for publication). BPQs may directly form ROS through redox cycling or may disrupt mitochondrial electron transport, leading to more ROS formation and depletion of ATP (48). These effects may be exacerbated by Ca^{2+} overload in the cytoplasm. Certain BaP metabolites, BPQs, and/or BPDE may also trigger redox sensors located in the cell that may lead to anti-oxidant responses (49). These agents may also induce oxidative stress in lymphoid cells and may activate PTKs via indirect mechanisms associated with the inhibition of protein tyrosine phosphatases (PTPases). Therefore, at this time the relationship between oxidative stress, altered Ca^{2+} homeostasis, and immunotoxicity produced by PAHs appears to be important; however, the precise mechanisms and pathways have not been elucidated.

IMMUNE ENHANCEMENT AND ADJUVANT EFFECTS OF PAHs

Most early PAH immunotoxicology studies focused on immunosuppression of humoral and cell-mediated immunity. However, during recent years several investigators have demonstrated that PAHs can also enhance immune responses, measured in terms of IgE production, increased cytokine production, activation of macrophages, and increased production of ROS (50–59). Many of these studies have focused on PAHs present in diesel exhaust emissions or indeed on PAH-coated diesel exhaust particles (DEP). DEP and other particulates have been associated with upper airway irritation and perhaps exacerbation of asthma. While DEP are coated with a complex mixture of environmental agents, initial studies suggest that PAHs may play a significant role in lung and airway-associated immune responses. Given the mechanisms of activation of antigen and mitogen signaling pathways discussed in this article, it might not be surprising to predict that at lower levels of exposure than those that produce suppression, en-

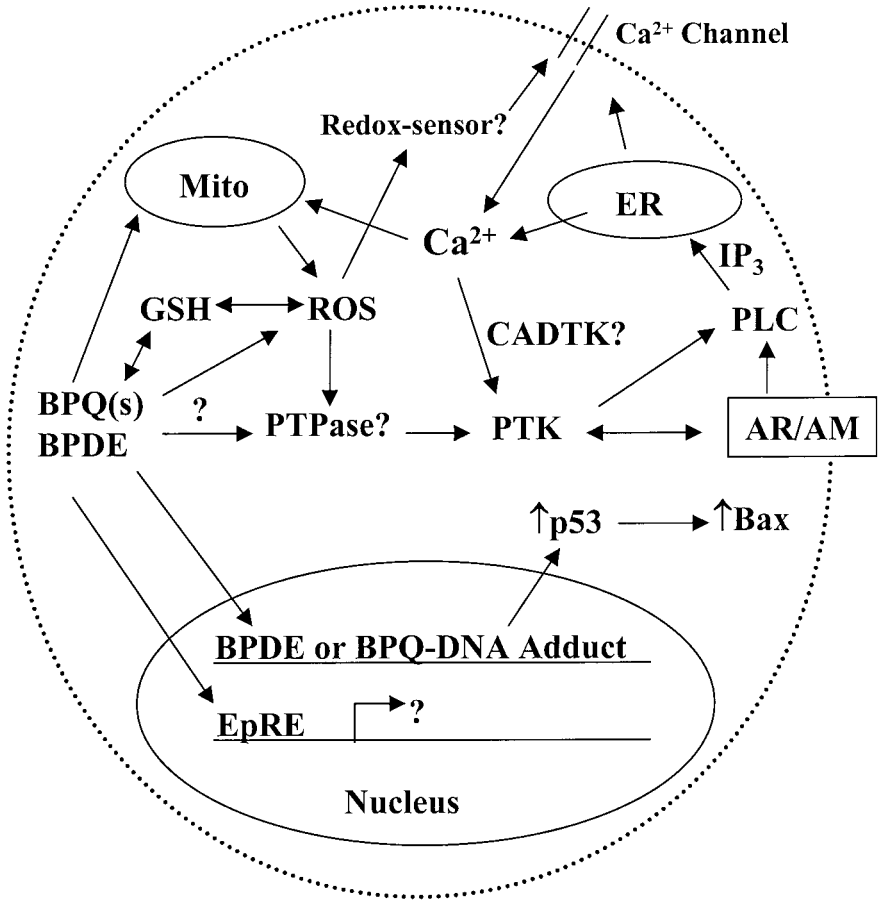

FIG. 2. BaP activates multiple signaling pathways in human B and T cells via its oxidative (BPQs) and electrophilic (BPDE) metabolites. (Note: some BPQs also have electrophilic properties) The activation of BaP to BPQs and BPDE (as shown in Fig. 1) occurs systemically (e.g., by liver and other cells), locally (e.g., by inflammatory cells, stromal cells, certain epithelial cells, and perhaps B cells), and intracellularly (e.g., macrophage/monocytes and perhaps B cells). These metabolites may target immune cells, leading to alterations in Ca^{2+} homeostasis by several mechanisms: (1) activation of protein tyrosine kinases (PTKs) by unknown mechanisms, leading to IP_3-dependent Ca^{2+} release from the endoplasmic reticulum (ER); these same pathways are activated by antigen receptors (AR) and accessory molecules (AM) located on the surface of B and T cells, suggesting that under certain conditions PAHs could also be adjuvants; (2) formation of reactive oxygen species (ROS) that undergo redox cycling in the presence of NADPH, leading to activation of redox-sensitive Ca^{2+} channels. ROS can convert GSH to its oxidized form (GSSG) and BPDE/BPQs can also directly adduct GSH, leading to loss from this anti-oxidant pool; (3) elevation of intracellular Ca^{2+} may lead to a Ca^{2+} "overload" in mitochondria (Mito), resulting in additional ROS formation, further GSH depletion, and oxidative stress that leads to depletion of ATP and the induction of cell death via apoptosis and necrosis. The elevation of intracellular Ca^{2+} in human B and T cells likely produces anergy in the absence of activation of appropriate second and third receptor signaling pathways. Prolonged elevation of Ca^{2+} causes cell death and likely accounts for the thymic atrophy and decreased lymphoid recovery in rodents treated with PAHs. Ca^{2+}-independent pathways of cell death (apoptosis) may also be induced via a p53-dependent pathway. Since BPDE and perhaps certain BPQs can bind to DNA, DNA strand breaks and adducts are sensed by p53, leading to the upregulation of Bax, a pro-apoptotic factor. The role of electrophilic response elements (EpRE) also bound or activated by BPDE/BPQs has yet to be determined in immunotoxicity studies.

hanced signaling might actually be seen. Many immunotoxic xenobiotics exert biphasic effects that are highly dose/concentration dependent. Therefore, further studies are needed to determine the types of immune effects that occur at environmentally relevant exposures of PAHs. In addition, studies are also needed on the role of antigen-specific and nonspecific cellular responses in immunologic and other diseases produced by these environmental pollutants.

SUMMARY

There appear to be complex interactions between PAHs and the immune system involving antigen-specific and nonspecific signaling pathways in lymphocytes and accessory cells. Most of the immunotoxic effects of PAHs are correlated with AhR binding and the formation of reactive metabolites that exert genotoxicity and/or produce oxidative stress. Many of these

pathways are inextricably linked to altered Ca^{2+} homeostasis in T and B cells, leading to disruption of antigen and mitogen signaling as well as initiation of proapoptotic events. Based on a limited number of *in vitro* human studies, it appears that human lymphocytes are quite sensitive to the immunotoxicity of certain PAHs, such as BaP. Since many of the genes that regulate PAH action are differentially expressed in human populations and are altered during environmental exposures, it will be important in future studies to define important gene–environment interactions that may be responsible for susceptibility to these environmental agents.

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