October 22, 2003

Lecture 13 Immunotoxicity

I. Overview of Immune System Structure and Function (Physiology)

A. Function of the immune system in organism's survival
   1. Resist infection agents
   2. Destroy neoplastic cells
   3. Reject nonself components

B. Differentiated lymphoid organs do not appear in organisms phylogenetically lower than fish, however, immunocytes (cells with immune functions) of various forms are found in all Phyla from sponges to vertebrates.

C. Immunoglobulins (antibodies) are found in vertebrates only, but functionally analogous proteins called agglutinins are present in invertebrates.
   1. Five immunoglobulin classes are found in mammals
   2. Three in birds;
   3. Two in amphibians and reptiles;
   4. One immunoglobulin class is found in fish.

D. The functional units of the immune system are leukocytes (a.k.a. white blood cells) that develop from pluripotent stem cells.
   1. The stem cells undergo differentiation, maturation, and proliferation into morphologically and functionally distinct cell populations, including (Figure 1):
      a. Granulocytes
         1. neutrophils (a.k.a. polymorphonuclear leukocytes or PMNs)
         2. basophils (granulocytes found in blood)
         3. eosinophils
         4. mast cells (granulocytes found in tissue)
      b. Monocytes (found in blood)
         1. macrophages (designation for monocytes found in tissue)
      c. Lymphocytes
         1. Natural killer cells (NK cells); large granular lymphocytes
         2. T-lymphocytes (a.k.a. T-cells)
         3. B-lymphocytes (a.k.a. B-cells)
      d. Plasma cells (produce antibodies; a.k.a. immunoglobulins)

E. Tissues producing leukocytes
   1. Primary lymphoid tissues include
      a. Bone marrow (Tissue from which immune cells are derived)
      b. Thymus (Tissue in which T-lymphocytes are differentiated)
   2. Secondary lymphoid tissue
      a. Spleen
      b. Lymph nodes (scattered throughout the body)
      c. Tonsils & Adenoids
   3. Localized lymphoid tissue aggregates found associated with bronchus, gut, and skin

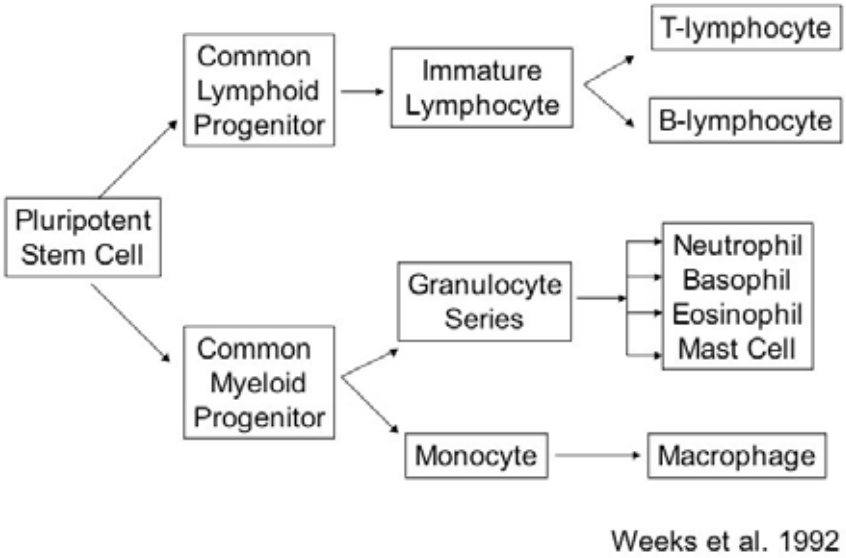

Figure 1. In mammals, pluripotent stem cells differentiate to various forms of leukocytes that form functionally distinct cell populations (from Weeks et al. 1992). The source tissue (or primary lymphoid tissue) of all the immune cells is the bone marrow. The T-lymphocytes mature and differentiate in the thymus gland (which is also a primary lymphoid tissue). Maturation, differentiation, and mobilization of immune cells are controlled by cytokines (e.g., interleukins, interferons, and chemokines), which are soluble mediators produced within immune cells and/or by cells outside the immune system (e.g., epithelial cells and nervous system cells) (Selgrade et al. 2001)

F. Mediation of immunity by leukocytes occurs via two processes
   1. Nonspecific immune responses that are mediated by mononuclear phagocytes (i.e., blood monocytes and tissue macrophages) and granulocytes that recognize foreign material (or cells like bacterial pathogens)
      a. Often called nonspecific immunity
      b. Includes two types of responses:
         1. Phagocytosis
            a. Ingestion and destruction of foreign agents by specialized cells
         2. Inflammation
            a. Infiltration of phagocytic cells into tissue at site of injury or infection
               1. Characterized by activation of clotting mechanisms, increased blood flow, and increased capillary permeability. (Selgrade et al. 2001)
                  (a) These responses facilitate mobilization of immune cells to the site of injury and result in the swelling and reddening associated with inflammation.
      c. Mediated mainly by polymorphonuclear leukocytes (neutrophils) and mononuclear phagocytes (macrophages)

1. Neutrophils: short lived cells specialized for ingesting and destroying microorganisms in the circulation or in tissues following infiltration of an infect site.
    a. Release lysozyme enzyme that is capable of lysing cell components
2. Macrophages: differentiated monocytes that "wander" within an organ or may be infiltrative into tissue.

d. Phagocytic and inflammatory responses can be enhanced by products of lymphocytes, including antibodies and lymphokines
e. Mammals have natural killer cells (NK cells) that are derived from granular lymphocytes
    1. Compose about 5% of the peripheral blood lymphoid cells
    2. Recognize changes in cell surface markers on virus-infected cells and on some tumor cells.
        a. Role in preventing metastases of tumors (movement of tumor cells from the main tumor body through the circulatory system to other tissues)
    3. Can be activated by lymphokines (e.g., interleukin-2 or IL-2).

2. Specific immune responses: two mechanisms
    a. Cell-mediated immunity (CMI) (Figure 2, left-hand side of schematic)
        1. Induced by cells (known as thymus dependent lymphocytes or T-lymphocytes or T-cells) that mature in the thymus.
        2. CMI responses mediate:
            a. Immunoregulation
            b. Delayed-type hypersensitivity
            c. Immunosurveillance
            d. Graft refection
            e. Resistance to infection by pathogens (viruses, bacteria, protozoans, fungi)
    b. Humoral-mediated immunity (HMI) (Figure 2, right hand-side of schematic)
        1. Mediated by lymphocytes (called B-lymphocytes or B-cells) that mature in the bursa of Fabricius in birds or bursal-equivalent tissues and organism in mammals (e.g., gut-associated lymphoid tissue and bone marrow.
            a. B-lymphocytes produce antibodies (immunoglobulin)
        2. In anuran amphibians and reptiles, as in higher vertebrates, a functional bone marrow produces the differentiated B-cells.
        3. In fish and non-anuran amphibians that lack bone marrow, B-cells are generated in other organs.
    c. Both T-cells and B-cells are released into the circulation and taken up by lymphoidal organs (lymph nodes and the spleen) (Figure 2).
        1. Lymph nodes filter the lymphatic fluid; spleen filters the blood.
        2. Exposure to antigens in these lymphoidal organs induces the lymphocytes to assume their genetically determined functional characteristics.
            a. Antigens stimulate only those lymphocytes that have receptors complementary to the antigen configuration.
            b. The antigens can be a piece of cell membrane and associate unique proteins on the surface or it can be a small protein or other biochemical, perhaps a toxin secreted by a pathogen.
    d. CMI Mechanism of Immunity

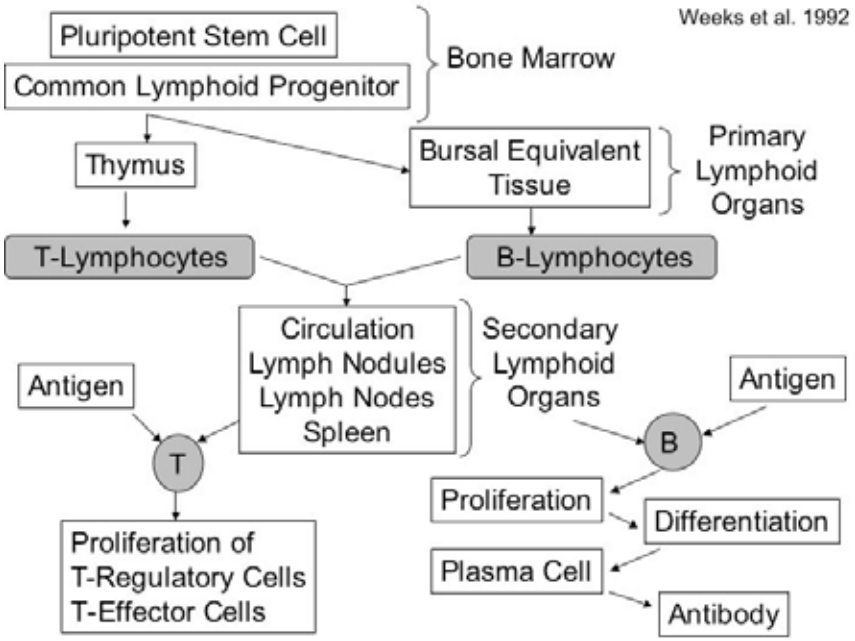

Figure 2. Development of cells of the specific immune system in mammals (redrawn from Weeks et al. 1992)

1. Sensitization of T-cells by antigen followed by differentiation and proliferation of T-cells into effector, regulatory, and memory cells (Figure 3).
   a. Macrophages assist in sensitization by concentrating antigens on their cell surface for presentation to the T-cells, specifically the T-cells known as T-helper cells (T_H cells), T-suppressor cells (Ts cells) and T delayed-type hypersensitivity cells (T_{DTH}).
      1. The macrophages have major histocompatibility complex (MHC) molecules on their surface to which the antigens (which are proteolytically processed short peptide fragments of bigger proteins) can bind. The macrophages are called antigen-presenting cells.
         (a) The antigens are presented to the T-cells that have specific antigen receptors on their surface and therefore recognize and bind them.
      2. T_H and T_S cells are regulatory cells.
         (a) T_H cells also interact with T_S T_C (see below) and T_{DTH} cells to regulate their activity
         (b) T_s cells inhibit or suppress both T-cell and B-cell function
      3. T_H cells also facilitate antibody responses of B-cells.
   b. Effector cells produced in the thymus include cytotoxic T-cells (T_C) that can release lymphotoxins capable of lysing targeted tumor cells or virally infected cells.
   c. Macrophages secrete soluble factors called monokines (e.g., interleukin I), which are types of lymphokines capable of influencing tissue macrophages important in the inflammatory immune response.

1. Movement of inflammatory cells into sites of injury is directed by lymphokines, including migration inhibition factor (MIF) and macrophage activating factor (MAF).

2. Each T-cell type also produces memory cells that are stimulated by antigens.
   a. Memory cells are the basis of secondary (anamnestic) immune responses.

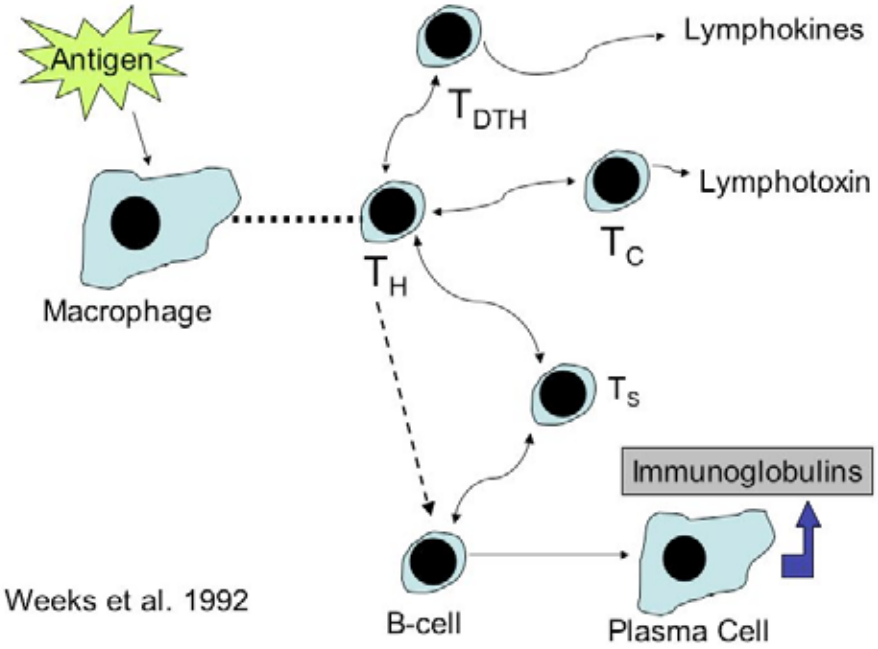

Figure 3. Interactions among lymphocytes in the immune system. Note that the various T-cells are part of the cell-mediated immunity (CMI) process, whereas the B-cells and resulting immunoglobulins are part of the humoral-mediated immunity (HMI) process.

e. Humoral Mechanism of Immunity (Figure 3)
   1. Characterized by the production of antibody molecules (immunoglobulins) that react specifically with antigen.
      a. B-cells are stimulated by antigen and undergo proliferation and differentiation into plasma cells (Figure 2 and Figure 3).
         1. Plasma cells synthesize and secret antibody into the lymphatic and circulatory systems.
         2. Some B-cells differentiate into memory cells that can provide a more rapid response to a second antigen exposure.
      b. Five classes of antibody based on structure and function:
         1. IgM (immunoglobulin M)
         2. IgG
         3. IgA
         4. IgE
         5. IgD
      c. General structure of antibody (Figure 4)

1. Basic unit consists of four polypeptide chains, two identical light chains and two identical heavy chains, that are held together by a number of disulfide bonds.
2. The antibody has a constant structure region common to all antibodies within a particular class. (Figure 4)
   (a) Antibodies have a variable region whose specific amino acid sequence differs from antibody to antibody.
   (b) The antigen binds to the variable region.
      (1) The antigen binding region is called Fab
   (c) The specific molecular structures on the antigen that bind to the receptors on the antibody are called epitopes. (Figure 5)

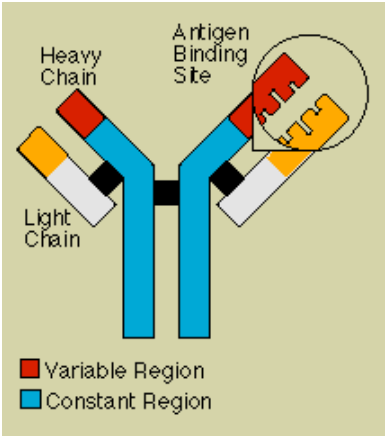

Figure 4. Basic structure of an antibody
(http://www.niaid.nih.gov/final/immun/immun.htm#Defense)

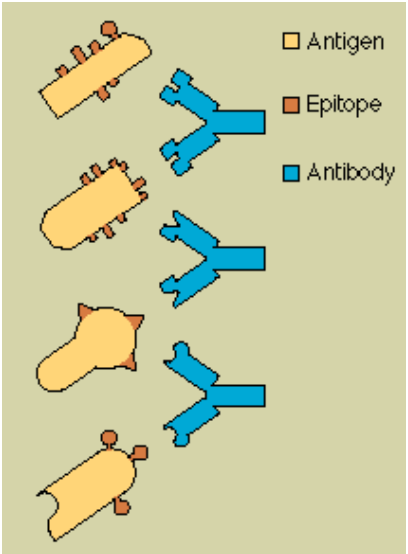

Figure 5. Specific binding of antigen to antibody
(http://www.niaid.nih.gov/final/immun/immun.htm#Defense)

d. Antibodies protect the host from infectious disease via the following functions:
   1. Virus neutralization
      (a) Specific antibody binds to viral attachment sites on target cells, thus blocking attachment by virus.
   2. Opsonization
      (a) Antibody coats (=opsonization) virus or bacteria, rendering them more susceptible to phagocytosis by neutrophils and macrophages that possess antibody receptor sites (called F_C receptors).
   3. Antibody-dependent cell cytotoxicity (ADCC)
      (a) Binding of leukocytes to target cells via antibody bridges, after which the leukocyte can lyse the target cell.
   4. Complement-mediated lysis
      (a) Antibody binds to target cell and activates the complement system (C).
      (b) C consists of >20 serum proteins that interact with one another resulting in the lysis of the target cells (which could be neoplastic cells, transplanted cells, bacteria, or viruses.)

II. Potential Consequences of Interactions Between Immune System and Toxic Chemicals

A. Nature of Interaction
   1. Suppression
      a. Can lead to enhancement of infectious disease (i.e., increase susceptibility to infectious disease)
      b. Can lead to enhancement of tumor formation (i.e., encourage growth of neoplastic cells because they are not suppressed by immune system)
   2. Stimulation
      a. Allergic reactions (overreaction of the immune system to otherwise innocuous proteins or other agents)
      b. Autoimmune disease (failure of immune system cells to recognize "self" tissues or cells)

B. Cases Studies: Frogs
   1. Experiments of Christin et al. 2003
      a. <u>Abstract:</u> In the past 30 years, many amphibian species have suffered population declines throughout the world. Mass mortality have been frequently reported, and in several instances, infectious diseases appear to be the cause of death. The role that contaminants could play in these die-offs through immunotoxic effects has been poorly investigated. In this study, juvenile leopard frogs (Rana pipiens) were exposed for 21 d to a mixture of six pesticides (atrazine, metribuzin, aldicarb, endosulfan, lindane, and dieldrin) and subsequently challenged with a parasitic nematode, Rhabdias ranae. Exposure to the mixture at environmentally realistic concentrations significantly reduced lymphocyte proliferation. Three weeks after the end of the exposure, lymphocyte proliferation had recovered and was stimulated in frogs challenged with parasites with the exception of those previously exposed to the highest concentration. No pesticide effects on phagocytosis and splenocyte numbers were detectable at the end of the exposure period, but these two parameters were diminished 21 d after the infection challenge in frogs

previously exposed to the highest levels of pesticides. In these animals, the prevalence of lung infection by R. ranae also tended to be higher. These results suggest that agricultural pesticides can alter the immune response of frogs and affect their ability to deal with parasitic infection.

1. Note that the innate level of lung infection tended to be high (i.e., the control was high) as evidenced by this statement near the end of the "Results" section:
   a. "The prevalence of lung infection by R. ranae tended to be higher in all treated groups compared to DMSO. Prevalence of infection was 70% in DMSO control (80% in clean water), whereas in frogs exposed to 0.1X, 1.0X, and 10X concentrations of pesticides, prevalence was 80, 100, and 100%. However, among-group differences were not found significant by a Fisher's exact test ($p = 0.2304$)."

C. Case Studies: Marine Mammals
   1. A number of papers describe chlorinated hydrocarbon residues (including PCBs, DDT, and dioxins) in tissues of large marine mammals.
      a. In some cases, disease incidence or unusual behavior, such as beaching, has been correlated with levels of the chlorinated hydrocarbons.
   2. A paper by de Swart et al. (1996), Environmental Health Perspectives 104:823, described the following:
      a. Abstract: Mass mortalities among seals and dolphins inhabiting contaminated marine regions have led to speculation about a possible involvement of immunosuppression associated with environmental pollution. To evaluate whether contaminants at ambient environmental levels can affect immune function of seals, we carried out an immunotoxicological study under semifield conditions. Two groups of 11 harbour seals (*Phoca vitulina*) originating from a relatively uncontaminated area were fed herring from either the highly polluted Baltic Sea or the relatively uncontaminated Atlantic Ocean. Changes in immune function were monitored over a 2 1/2-year period. The seals that were fed contaminated Baltic herring developed significantly higher body burdens of potentially immunotoxic organochlorines and displayed impaired immune responses as demonstrated by suppression of natural killer cell activity and specific T-cell responses. During a 2-week fasting experiment performed at the end of the feeding study, mobilization of organochlorines from the blubber did not lead to a strong increase of contaminant levels in the blood, and no enhancement of the existing immunosuppression was observed. These results demonstrate that chronic exposure to environmental contaminants accumulated through the food chain affects immune function in harbour seals, whereas short-term fasting periods, which are normal for seals, do not seem to pose an additional risk. The seals of this study were not exposed perinatally to high levels of environmental chemicals, and body burdens of organochlorines measured near the end of the study were lower than those generally observed in free-ranging seals inhabiting many contaminated regions. Therefore, it may be expected that environmental contaminants adversely affect immune function of free-ranging seals inhabiting contaminated regions at least as seriously as observed in these studies
   3. Note that the evidence presented in de Swart et al. (1996) is correlative, not causative. It is an example of an ecoepidemiology paper that is basing its conclusions on a known plausible association between organochlorine hydrocarbon contaminants and

immune system effects. However, because exposure was controlled, this paper provides better proof that immune system effects can occur following dietary exposure to organochlorine contaminants. One confounding interactive factor could be handling stress; it is possible there was an interaction with exposure to the contaminants.

D. Case Studies: Natural Toxins Can Affect Immune System
   1. Studies with rats fed mycotoxins (naturally occurring toxins produced by certain fungi of the genera Fusarium and Aspergillus) can alter immune system function as indicated in the abstract of a paper by Hinton et al. (2003).
      a. Abstract: "We investigated the effects of aflatoxin B1 (AFB1) on isolated splenic lymphocytes and the histomorphologic changes in the spleens and liver of Fisher-344 male rats. Weaned animals were fed chow diets that contained 0, 0.01, 0.04, 0.4, or 1.6 ppm AFB1, using an intermittent dosing regimen (4 weeks on and 4 weeks off AFB1), for 40 weeks. An additional group of animals was fed the 1.6 ppm AFB1 diet continuously. The intermittent dosing regimen was designed to evaluate effects of cumulative dose and exposure for risk assessment comparisons. The percentages of T and B cells were affected as shown by flow cytometric analysis after the dosing cycles. The observed changes appeared to reverse or compensate to some extent after the off cycles. Lymphocytes were stimulated in culture for analysis of the production of IL-2, IL-1, and IL-6. Significantly increased production of IL-1 and IL-6 was seen in the second dosing cycle (12 weeks) and the second "off" cycle (16 weeks) at the higher doses. Inflammatory infiltrates were seen in the liver after eight weeks of continuous and intermittent dosing and were increased in size and number at 12 weeks in both 1.6 ppm dose groups correlating with the peak production of Il-1 and IL-6. We concluded that AFB1 effects on the immune system can be either stimulatory or suppressive dependent on a critical exposure window of dose and time. Immune cells in spleen such as T-lymphocytes and macrophages, both important mediators of inflammatory responses to tissue damage, were affected differently in the continuous and intermittent exposures to AFB1.

E. Case Study: Fish ("You win some; you lose some")
   1. PCBs (as the formulation Aroclor 1254) were fed to Chinook salmon that were than challenged with a parasite (Powell et al. 2003). However, no effects on immunocompetence were observed as described in the abstract.
      a. Abstract: Controlled laboratory challenges with pathogenic Listonella (formerly Vibrio) anguillarum bacteria were used to examine potential effects of dietary exposure to polychlorinated biphenyls (PCBs) on the growth and immunocompetence of juvenile Puget Sound (WA, USA) Chinook salmon (Oncorhynchus tschawytscha). Salmon were fed four levels of the PCB congener mixture Aroclor¨ 1254 for 28 d to bracket likely exposure to PCBs in the lower Duwamish waterway near Seattle, Washington, USA. Fish were transferred to five replicate tanks per dose, exposed to L. anguillarum, and monitored for 14 d. Half the PCB-dosed fish were vaccinated against L. anguillarum, and specific immunity was allowed to develop in this group for three weeks prior to challenge. All mortalities following challenge were individually sampled for bacteria to identify the cause of death. The data indicate that dietary PCB exposure, even at

relatively high levels, did not have a significant effect on growth, innate disease resistance, or acquired immunity to L. anguillarum. The controlled laboratory experiments in this study suggest that the immune system of Chinook salmon is not sensitive to orally delivered PCBs at environmentally relevant concentrations.

III. TCDD: A Potent Immune System Toxicant

A. TCDD is tetrachlorodibenzo-*p*-dioxin, the most toxic congener of the polyhalogenated aromatic hydrocarbons called generically dioxin.

B. Chickens and rats fed moderate levels of TCDD results in thymic atrophy and alterations in T-cell mediated immune functions such as delayed hypersensitivity activity (T_{DTH}), T_C activity (cytotoxic T lymphocyte), and T-cell dependent antibody responses.
   1. Both cell and humoral mediated immune responses are affected.
   2. PCBs (polychlorinated biphenyls) can also cause similar adverse effects as TCDD.
   3. In addition to immunotoxicity, TCDD and some of the PCB congeners have the ability to cause hepatotoxicity, embryotoxicity, teratogenicity, dermal toxicity, lethality, carcinogenesis, wasting syndrome and tumor promotion in many different species at low concentrations.

C. Administration of TCDD results in increased susceptibility to challenge with viruses, bacteria, and parasitic diseases, as well as to tumor promoting substances.

D. The exact mechanism of TCDD (and at higher doses, PCB) disruption of immune functioning is not known. However, it is known that all effects of TCDD (and PCBs) occur following binding to a cell cytoplasmic receptor called AhR (aryl hydrocarbon receptor).
   1. The receptor (AhR) is located in the cytoplasm (or cytosol) of the cell (see the figure below, which is taken from Giesy et al. 2002). Heatshock proteins 90 (HSP90) are bound to the receptor. When the appropriate contaminant interacts and binds to the AhR, it is activated and moves into the nucleus where the HSPs disassociate. The contaminant bound AhR forms a ligand with ARNT (Ah receptor nuclear translocator protein). The contaminant-AhR-ARNT complex binds to . This response element is on the chromosome analogous to binding with the estrogen responsive element. However, the response element is called the dioxin responsive element (DRE), and its binding distorts the chromosome allowing access to DNA regions known as promoters and activation of adjacent genes that result in the transcription of DNA to mRNA. The mRNA that codes for a specific isozyme of P450, called P450-1A.
   2. After induction of the P450, a cascade of events occurs to cause cell toxicity and a myriad of physiological responses in many organ systems.
   3. However, the specific turn of biochemical events is still obscure, although bits and pieces of the puzzle of why polyhalogenated aromatics and PAHs cause problems at sufficiently high doses.

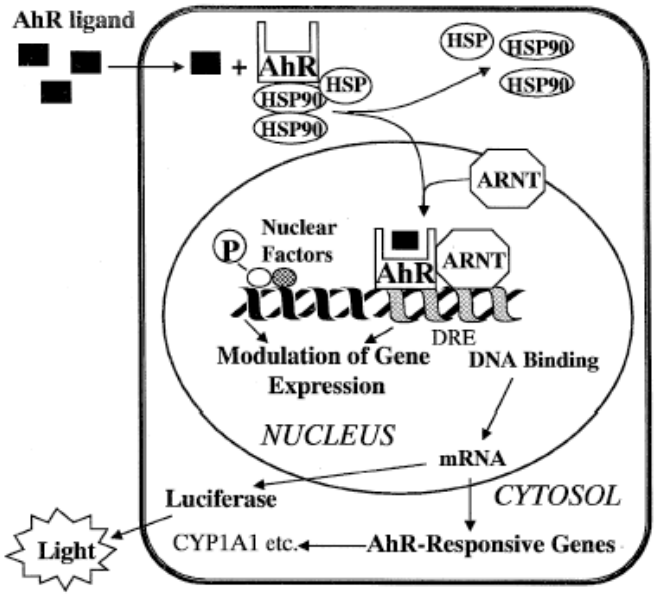

Figure 7. Interaction of TCDD or Ah reactive compound with the Ah receptor and subsequent transport to the nucleus where cytochrome P4501A1 synthesis is induced. This interaction can occur in all cells. Putatively, cells of the lymphoid glands may be especially sensitive targets. Note that the luciferase gene can be cloned into a cell and turned on when the AhR is bound. The light production can be measured for a convenient assay to screen compounds that can bind to AhR (Schematic was copied from Giesy et al. 2002)

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