**Synopsis**

Understanding the ecotoxicological effects of arsenic in the environment is paramount to mitigating its deleterious effects on ecological and human health, particularly on the immune response. Toxicological and long-term health effects of arsenic exposure have been well studied. Its specific effects on immune function, however, are less well understood. Eukaryotic immune function often includes both general (innate) as well as specific (adaptive) responses to pathogens. Innate immunity is thought to be the primary defense during early embryonic development, subsequently potentiating adaptive immunity in jawed vertebrates, whereas all other eukaryotes must rely solely on the innate immune response throughout their life cycle. Here, we review the known ecotoxicological effects of arsenic on general health, including immune function, and propose the adoption of zebrafish as a vertebrate model for studying such effects on innate immunity.

**Natural sources**

Arsenic is a naturally occurring metalloid element that is found in soil, air and water (Huang and others 2004; Duker and others 2005). Environmental arsenic exists in both organic and inorganic states. Organic arsenicals are generally considered nontoxic (Gochfield 1995), whereas inorganic forms are toxic. The most acutely toxic form is arsine gas (Leonard 1991). Inorganic arsenic exists predominantly in trivalent (As\(^{3+}\)) and pentavalent (As\(^{5+}\)) forms, where trivalent compounds are more toxic than pentavalent ones (Cervantes and others 1994; Smedley and others 1996; Duker and others 2005). Both trivalent and pentavalent arsenicals are soluble over a wide pH range (Bell 1998) and are routinely found in surface and groundwater (Feng and others 2001). Under aerobic conditions, pentavalent arsenic is more stable and predominates, whereas trivalent species predominate under anaerobic conditions (Duker and others 2005).

Arsenic ranks 20th in abundance in relation to other elements in the earth’s crust and high concentrations are found in granite and in many minerals including copper, lead, zinc, silver and gold (NAS 1977). The geochemistry of arsenic in the environment was recently reviewed by Duker and others (2005). Arsenic naturally accumulates as both organic and inorganic forms in soil, surface and groundwater (Attrep and Anirudhan 1977; Lloyd-Smith and Wickens 2000), and seawater (Penrose and others 1977). The primary source of arsenic in soil is the parent rock (Smedley and Kinniburgh 2002). Additionally, volcanoes are a major natural source of arsenic released into the environment (Nriagu and Pacyna 1988; Nriagu 1989) that can generate high arsenic concentrations in natural waters (Smedley and Kinniburgh 2002). The chemistry of arsenic in aqueous environments has been reviewed by Ferguson and Gavis (1972). Arsenic concentrations in lakes are often less than in rivers, due to adsorption by iron oxides, although changes in water levels (Nimick and others 1998; Smedley and Kinniburgh 2002) and geothermal activity can enhance concentrations in some cases (Aggett and Kriegman 1988; Duker and others 2005). Groundwater from alluvial and deltaic watersheds generally has high arsenic concentration due to predominantly reducing conditions (Smedley and Kinniburgh 2002).

**Anthropogenic influences**

Human activities have intensified arsenic accumulation in the environment (Bell 1998) such as fossil fuel combustion and metal smelting, as well as the semiconductor and glass industries. Arsenic is also an ingredient in many commonly used materials including wood preservatives, pigments, insecticides, herbicides, rodenticides and fungicides (Hathaway and others 1991). Although most arsenic in soil is derived from the parent rock, the application of arsenic compounds in agriculture and forestry practices may lead to extreme soil contamination and subsequent groundwater contamination.
contamination, while the burning of coal and smelting of metals may be major sources of airborne arsenic. Mining activities may result in high levels of arsenic contamination in soil, surface water, groundwater and vegetation (Amasa 1975; Smedley and others 1996; Smedley and Kinniburgh 2002). Additionally, human modifications to the natural hydrograph, including the construction of dams (Armah and others 1998), wastewater recycling and irrigation practices (Siegel 2002), can potentiate arsenic accumulation in soil and in water supplies.

**The role of microbes**

Many microorganisms have adapted to arsenic-rich environments, including soils and waters (Nakahara and others 1977; De Vicente and others 1990; Cervantes and Chavez 1992; Ahmann and others 1994; Cervantes and others 1994; Laverman and others 1995; Saltikov and Olson 2002) and may be important factors in arsenic biotransformation (Shariatpanahi and others 1981) and mobilization (Cummings and others 1999) in the environment. Bacterial resistance to the toxic effects of arsenic may be a function of a specific arsenic-resistance operon, *ars* (Carlin and others 1995; Cai and others 1998), and may be facilitated by reduction in arsenic uptake and increased phosphate transport (Willsky and Malamy 1980). Homology across microbial taxa suggests that the *ars* operon is conserved in Gram-negative bacteria and that it has a functional role in arsenic detoxification (Diorio and others 1995). Bacterial populations have been shown to be associated with both oxidation and reduction of arsenic in soils (Macur and others 2004). Degradation of arsenic species has even been shown in bacterial symbionts of marine mussels (Jenkins and others 2003). Under anaerobic conditions, some microbes can reduce the less toxic arsenate to the more toxic arsenite (Andreae 1978; Nies and Silver 1995; Rensing and others 1999) through an energy-generating process (Ilyaletdinov and Abdrashitova 1981). Additionally, other microbes are able to methylate arsenic compounds (Gadd 1993), which may serve as a detoxification process. Seasonal variations in temperature and water levels can have strong effects on arsenic concentration and speciation in soil and water due to changes in microbial uptake (Andreae 1978, 1979). During warm, dry periods arsenic compounds are often oxidized (Maest and others 1992), potentially increasing toxicity (Savage and others 2000), while during wet periods oxidized arsenic is solubilized and distributed throughout the environment (McLaren and Kim 1995; Rodriguez and others 2004).

**Bioaccumulation and metabolism**

Arsenic accumulates across highly diverse environments within the soil, water and air where it is subsequently taken up and processed by microbes, plants and animals. Soluble arsenic taken up by plants rapidly accumulates in the food chain (Green and others 2001). Freshwater plants and peat moss have been shown to contain considerable amounts of arsenic (Reay 1972; Minkkinen and Yliruokanen 1978). Due to the high metal-binding affinity of their soils, wetlands may have elevated concentrations of arsenic (Beining and Ote 1996) when compared with uplands. High arsenic concentrations have been found in the tissues of wild birds (Fairbrother and others 1994) and in many marine organisms, including algae (Lunde 1972, 1973), crustaceans (Edmonds and others 1977), cetaceans, pinnipeds, sea turtles and sea birds (Kubota and others 2003). Ecotoxictants released into the environment, including arsenic, often accumulate most rapidly in aquatic habitats where they enter the biota and are subsequently transferred to higher trophic levels and, in many cases, eventually to humans. Extremely high levels of arsenic have been observed in many fish taxa (Bosnir and others 2003; Juresa and Blanusa 2003) and have been shown to be toxic (Suhandrayatna and others 2002; Tisler and Zagorc-Koncan 2002). Some species possess specific arsenic-binding proteins (Oladimeji 1985) that may increase bioaccumulation. Monitoring arsenic levels and their associated health effects in aquatic organisms, particularly in taxa at high trophic levels such as fish, may provide insight into overall ecosystem health (Zelikoff and others 2000) as well as into potential impacts on human health (Zelikoff 1998; Adams and Greeley 1999).

Exposure from air and soil is usually minimal in humans. The major sources of exposure for humans are food and water (Bernstam and Nriagu 2000). Once ingested, arsenic that is not eliminated from the body may accumulate in the muscles, skin, hair and nails (Ishinishi and others 1986; Kitchin 2001). Food contains both organic and inorganic arsenic, whereas water primarily contains inorganic forms. Seafood may provide higher concentrations of arsenic when compared with terrestrial food products (Sakurai and others 2004), presumably due to increased bioaccumulation through generally longer trophic chains. As elemental arsenic is poorly absorbed, it is predominantly eliminated from the body unchanged (Duker and others 2005). Inorganic arsenic is absorbed through the gastrointestinal tract and is eliminated via renal function (Hindmarsh and McCurdy 1986); however, a small amount is biotransformed...
into "detoxified" forms via methylation and reduction in the liver (Winski and Carter 1995; Bernstam and Nriagu, 2000). Once thought to be a purely detoxification process, it has been shown that methylation of arsenic may, in some cases, actually increase arsenic toxicity in humans and rodents (Petrick and others 2000, 2001; Styblo and others 2000; Del Razo and others 2001). Variation in arsenic metabolism has been shown to occur in humans (Abernathy and others 1999). Interestingly, some mammals, including nonhuman primates, are deficient in arsenite methyltransferases necessary for effective methylation (Aposhian 1997). They may also show different tissue-specific expression (Abernathy and others 1999). The relationships between arsenical exposure, methylation and toxicity are paramount to understanding the risks posed to humans.

**Toxicology of arsenic**

Acute and chronic arsenic toxicities have been shown in a variety of organisms, and the data suggest that most inorganic arsenicals are more toxic than organic forms (Abernathy and others 1999; Duker and others 2005). Toxic effects of inorganic arsenic include denaturing of cellular enzymes through interaction with sulfhydryl groups (Graeme and Pollack 1998; Gebel 2000), causing cellular damage through increased reactive oxygen species (ROS) (Wang and others 1996; Ahmad and others 2000), and altering gene regulation (Rossman 1998; Abernathy and others 1999). Arsenic is known to inhibit more than 200 enzymes (Abernathy and others 1999) and has been implicated in multisystemic health effects via interference with enzymatic function and transcriptional regulation (NRC 1995). A variety of inhibitory effects on cellular metabolism have been shown, affecting mitochondrial respiration (Klaassen 1996; Abernathy and others 1999) and synthesis of adenosine triphosphate (ATP) (Winship 1984). Other effects of arsenic include activation of the estrogen receptor, inhibition of angiogenesis and tubulin polymerization, induction of heat-shock proteins, and oxidation of glutathione (Bernstam and Nriagu 2000). Due to its structural similarity to phosphate, arsenate may replace phosphate (ATP) (Winship 1984). Other effects of arsenic include activation of the estrogen receptor, inhibition of angiogenesis and tubulin polymerization, induction of heat-shock proteins, and oxidation of glutathione (Bernstam and Nriagu 2000). Due to its structural similarity to phosphate, arsenate may replace phosphate (ATP) (Winship 1984).

Among fish taxa, arsenic has been shown to induce apoptosis of fin cells (Wang and others 2004), to cause liver inflammation, hyperplasia and necrosis (Pedlar and others 2002), gall bladder inflammation, fibrosis and edema (Cockell and others 1991; Pedlar and others 2002), kidney fibrosis (Kotsanis and Iliopoulos-Georgudaki 1999), and the induction of various heat-shock proteins (Kothary and Candido 1982). Arsenic has been shown to cause morphological changes, as well as to increase numbers of necrotic bodies, abnormal lysosomes and autophagic vacuoles in fish hepatocytes (Sorensen and others 1985). Additionally, effects on reproduction in fishes include disrupting ovarian cell cycles (Wang and others 2004), inhibiting ovarian follicle development (Shukla and Pandey 1984a), impairing spermatogenesis and changing testicular architecture (Shukla and Pandey 1984b).

There is clear evidence that arsenic can disrupt gene expression, particularly through its effects on signal transduction (Abernathy and others 1999). Arsenic can interact directly with the glucocorticoid receptor (GR), selectively inhibiting GR-mediated transcription (Kaltreider and others 2001). It has been found to inhibit the Janus family of tyrosine kinase-signal transducers and activators of transcription (JAK-STATs) by interacting directly with JAK (Cheng and others 2004), to inhibit IκB-kinase (Roussel and Barchowsky 2000), as well as to inactivate protein tyrosine phosphatases, promote the activation of AP-1 and upregulate levels of MAPK (Cavigelli and others 1996). At low concentrations, arsenic has been shown to affect the DNA-binding capabilities of transcription factors NFκB and AP-1, leading to increased gene expression and stimulation of cell proliferation (Chen and others 2000; Wijeweera and others 2001). However, at high concentrations, arsenic may lower NFκB activation, inhibit cell proliferation and induce apoptosis (Shumilla and others 1998; Wei and others 2005).

It has been suggested that arsenic can disrupt cell division by deranging the spindle apparatus (Abernathy and others 1999). Arsenic induces large deletion mutations (Hei and others 1998), chromosome damage and aneuploidy (Abernathy and others 1999) and causes micronucleus formation, DNA-protein cross-linking, and sister chromatid exchange (Huang and others 2004). It is known to inhibit DNA repair (Lynn and others 1997; Rossman 1998; Brochmoller and others 2000) and even to exacerbate the effects of other mutagenic agents (Abernathy and others 1999), thereby increasing susceptibility to multiple diseases (Duker and others 2005).

**Arsenic and the immune response**

Chronic exposure to arsenic, in addition to its general toxicity and its stimulation of many diseases, may affect lymphocyte, monocyte and macrophage activity in many mammals, resulting in immunosuppression (Blakley and others 1980; Gonsebatt and others 1994; Lantz and others 1994; Yang and Frenkel 2002; Wu and others 2003; Duker and others 2005; Sakurai and
others 2006). Likewise, phagocytic activity of macrophages and other immune responses were found to be significantly reduced by arsenic exposure in birds (Fairbrother and others 1994; Vodela and others 1997). Generally, arsenic can disrupt glucocorticoid regulation of immune function (Kaltreider and others 2001) and arsenic-mediated apoptosis may lead to a diminished immune response in mice (Harrison and McCoy 2001), rats (Bustamante and others 1997) and humans (de la Fuente and others 2002; Gonzales-Rangel and others 2005). Additionally, arsenic exposure in mice has been shown to suppress the primary antibody response (Sikorski and others 1991), reduce macrophage and neutrophil abundance (Patterson and others 2004), increase susceptibility to infection (Aranyi and others 1985), increase mortality due to bacterial infection (Hatch and others 1985), decrease adhesion of macrophages, decrease nitric oxide (NO) production, and reduce chemotactic and phagocytic mechanisms (Sengupta and Bishayi 2002; Bishayi and Sengupta 2003). A field study of the effects of environmental arsenic exposure along a pollution gradient has also been shown to suppress immune function in wood mice (Tersago and others 2004). Arsenic has been shown to affect not only the immune response, but also behavior in rats (Schultz and others 2002). Dose-dependence of the immunotoxicological effects of arsenic is unclear. Dose-dependent immunosuppressive relationships have been observed in mice (Burns and others 1991). However, in some studies the immunosuppressive effects of arsenic were most pronounced at low concentrations of exposure, compared to high concentrations (Blakley and others 1980; Savabieasfahani and others 1998), and it has even been proposed that in some situations arsenic may enhance certain immune responses (Yoshida and others 1997). Generally, arsenic can disrupt glucocorticoid regulation of immune function (Kaltreider and others 2001) and arsenic-mediated apoptosis may lead to a diminished immune response in mice (Harrison and McCoy 2001), rats (Bustamante and others 1997) and humans (de la Fuente and others 2002; Gonzales-Rangel and others 2005). Additionally, arsenic exposure in mice has been shown to suppress the primary antibody response (Sikorski and others 1991), reduce macrophage and neutrophil abundance (Patterson and others 2004), increase susceptibility to infection (Aranyi and others 1985), increase mortality due to bacterial infection (Hatch and others 1985), decrease adhesion of macrophages, decrease nitric oxide (NO) production, and reduce chemotactic and phagocytic mechanisms (Sengupta and Bishayi 2002; Bishayi and Sengupta 2003). A field study of the effects of environmental arsenic exposure along a pollution gradient has also been shown to suppress immune function in wood mice (Tersago and others 2004). Arsenic has been shown to affect not only the immune response, but also behavior in rats (Schultz and others 2002). Dose-dependence of the immunotoxicological effects of arsenic is unclear. Dose-dependent immunosuppressive relationships have been observed in mice (Burns and others 1991). However, in some studies the immunosuppressive effects of arsenic were most pronounced at low concentrations of exposure, compared to high concentrations (Blakley and others 1980; Savabieasfahani and others 1998), and it has even been proposed that in some situations arsenic may enhance certain immune responses (Yoshida and others 1997).

Arsenic and disease

Arsenic compounds have been used directly on humans for treating many diseases including skin conditions, malaria, ulcers, syphilis, sleeping sickness and some forms of leukemia (Luh and others 1973; Nevens and others 1990; Zhang 1999; Miller and others 2002) although it is now rarely used medicinally (Azcue and Nriagu 1994). Organs most susceptible to arsenic toxicity are those involved with absorption, accumulation or excretion, including the skin, circulatory system, gastrointestinal tract, liver and kidney (Duker and others 2005). The primary symptom of arsenic exposure is dermal lesions (Zaloga and others 1985). Skin localizes and stores arsenic, presumably due to high levels of sulfhydryl-rich keratin (Kitchin 2001), potentially explaining this response. Arsenic is associated with multiple health effects, including Blackfoot disease (Abernathy and others 1999), diabetes (Longnecker and Daniels 2001), hypertension (Chen and others 1995), peripheral neuropathy and multiple vascular diseases (see Duker and others 2005). Other effects include anemia (ATSDR 2000), liver damage, portal cirrhosis, hematopoietic depression, anhydremia, sensory disturbance and weight loss (Webb 1966). It has been suggested that multiple factors, including genetics and nutrition, affect susceptibility to arsenic and disease manifestation (Mandal and others 1996; Hseuh and others 1998).
In addition to acute toxicity, long-term exposure to inorganic arsenic is associated with certain forms of cancer of the skin, lung, colon, bladder, liver and breast (Nemery 1990; Abernathy and others 1999; Huang and others 2004; Duker and others 2005) although effects may not appear until more than 20 years after exposure (Jackson and Grainge 1975). It has been suggested that arsenic may act as a carcinogen through DNA hypomethylation and overexpression of protooncogenes (Zhao and others 1997). Cancer may induced by alteration of DNA-repair mechanisms, thus interfering with cell division, differentiation and tumor suppression (Chen and others 1996; Goering and others 1999). Additionally, arsenic may induce certain forms of cancer by enhancing the carcinogenic effects of other substances and by affecting metabolic pathways (Huang and others 2004).

In summary, the effects of arsenic on health include various mechanisms of acute and chronic toxicity, enzymatic and genetic effects, and/or increasing susceptibility to multiple types of disease, both cancerous and noncancerous. Although it is clear that exposure to arsenic alters normal biological functions, resulting in the direct initiation of disease or, at least, predisposition of an organism to it, studying the impact of arsenic on the ability to fight viral and bacterial infections via specific immune responses is particularly important for full understanding of its overall effects on health.

Overall immune response

The immune system functions to protect a host from pathogenic infection. Its complex array of defense mechanisms relies mainly on the ability to distinguish between the host and foreign cells. To effectively manage this, the immune system has 2 separate responses that work synergistically to fight infection. The innate immune response uniquely recognizes molecular patterns, surface structures and cellular products conserved across a diversity of microbes. It is activated as the first line of defense, whereas the secondary adaptive immune response consists of a complex initiation process, followed by clonal selection and expansion of receptors specific to unique epitopes on the pathogen. They are mutually complementary, with the innate system acting as a prerequisite, potentiating factor for the adaptive immune response.

All eukaryotic organisms, from amoebae to mammals, have mechanisms of innate immunity, whereas adaptive immunity, which appeared ~450 million years ago, is present only in jawed vertebrates (Agrawal and others 1998). Some fish, and possibly other species, do not develop functional adaptive immunity until a distinct stage of development, until which time they rely entirely upon the innate immune response (Willett and others 1997). Because of its importance during development, generality of mechanisms of defense against pathogens and taxonomically conserved nature, innate immunity has particularly broad implications for overall health.

Adaptive immune response

The adaptive immune response is mediated primarily by B lymphocytes and T lymphocytes. Antigen-presenting cells transport and present antigenic molecules of pathogens to naïve T lymphocytes, resulting in their differentiation into effector cells. By virtue of rearrangeable immune gene segments, this response can generate a repertoire of lymphocytes with receptors specific to antigenic components of any potential pathogen. The mature T cells either migrate to the site of infection to effect cell-mediated immunity, or circulate in the lymphoid organs to activate the B lymphocytes and participate in humoral immunity. B cells mature to form plasma cells and memory cells, which are responsible for producing antibodies against the pathogen and establishing immune memory, respectively. The adaptive immune response eradicates the infectious agent from the host body and provides an immunological memory against reinfection by the same pathogen.

Innate immune response

Innate immunity comprises a collection of defense mechanisms that protect an organism against infection without depending upon prior exposure and cell memory. As a rapid first response to assault by pathogens, the innate immune system halts infection or keeps it at bay until the adaptive immune response has sufficient time to develop. In addition to circulating cells, the innate immune system comprises epithelial barriers such as skin, scales and mucociliary membranes that separate the organism from the external environment. If this external barrier is compromised, receptors on circulating cells recognize specific molecular signatures and induce an inflammatory response, involving the recruitment and subsequent activation of leukocytes. Inflammation is considered to be the first sign of the wound-healing process and is characterized by localized elevation of temperature, pain, erythema and edema. Infectious agents are eliminated primarily by phagocytic cells, including macrophages, neutrophils and natural killer cells that migrate to the site of infection and respond...
by engulfing and destroying the pathogens. These cells also secrete effector molecules such as chemokines and cytokines that function as chemical messengers of the immune response and facilitate cell-to-cell communication.

Detection of pathogens by the innate immune system is dependent on the recognition of invariant molecules of the microorganism by cell surface-associated receptors, the best characterized of which are the toll-like receptors (TLRs). TLR signaling was recently reviewed by Akira and Takeda (2004). Tolls were first discovered in Drosophila, associated with dorsoventral patterning during development (Hashimoto and others 1988). Further studies revealed the essential role of these transmembrane receptors in innate immunity (Lemaitre and others 1996); they detect conserved molecular patterns shared by large groups of microorganisms, such as lipopolysaccharide and double-stranded ribonucleic acid (RNA) (Akira and Takeda 2004). TLR family members are differentially expressed among immune cells and are characterized by the presence of a leucine-rich, extracellular domain and an intracellular toll/interleukin 1 receptor (TIR) domain. Ligand binding results in conformational changes in the TIR domains that facilitate interactions with downstream signaling molecules. This cascade results in the activation of cytoplasmic transcription factors, NF-kB or interferon regulatory factor 3, which subsequently translocate to the nucleus and begin transcription of proinflammatory cytokines (Medzhitov 2001).

Chemokines are low molecular weight proteins that represent a superfamily of about 30 chemoattractants acting as vital initiators of the inflammatory process. They are secreted by a wide variety of cells including monocytes, neutrophils, epithelial cells, smooth muscle cells and T cells (Rollins 1997). Chemokines function mainly in leukocyte physiology by controlling inflammatory trafficking, but are also important for gene transcription, apoptosis and granule exocytosis (Thelen 2001). Some chemokines are inducible by physiological stress and recruit leukocytes to sites of injury, whereas others are constitutively active and are responsible for basal trafficking of leukocytes. All chemokines are tightly regulated by feedback mechanisms because of their potential for severely damaging host tissues by uncontrolled persistent expression.

Cytokines are small, soluble, pleiotropic proteins that are secreted by virtually all cells in the body and possess both autocrine and paracrine functions. They act on their targets by binding specific membrane receptors and signaling through them to commence the transcription of certain genes, usually those involved in cellular activation or growth and differentiation. The central role of cytokines, however, is to modulate and direct the amplitude of immune responses. There are 2 major groups of cytokines: proinflammatory are secreted by activated macrophages, and anti-inflammatory are involved in the downregulation of inflammatory reactions. Important antiviral cytokines include certain interferons, whereas antibacterial cytokines include interleukins and tumor necrosis factors.

Interferons are a multigene family of inducible cytokines that possess antiviral activity, broadly grouped into 2 categories, type I and type II, based on their biological, biochemical and immunological properties (Samuel 1991). Type I interferons are secreted upon viral infection and almost all virus-infected cells can synthesize them. Type II interferons are upregulated by antigenic stimuli and are secreted mainly by natural killer cells, CD4⁺ Th1 cells and CD8⁺ cytotoxic suppressor cells (Young 1996). All interferons act through membrane-associated receptor complexes. In response to ligand binding, JAK-STAT members become activated via a series of phosphorylation events, ultimately leading to the translocation of STATs to the nucleus, and upregulation of interferon-inducible genes (Stark and others 1998; Horvath 2000).

Among the genes induced by interferons are proteins important in anti-RNA virus activity, including protein kinase (PKR), oligoadenylate synthetase (OAS) and interferon-induced Mx GTPases. PKR and OAS are essential for inhibition of mRNA translation and catalyzing RNA degradation, respectively (Jacobs and Langland 1996; Samuel 1998). Mx GTPases are key components for resisting a wide range of RNA viruses; produced for short periods of time, they function by sensing viral nucleocapsids. Their actions trap essential viral components and make them unavailable for viral replication, thus containing the infection (Haller and Kochs 2002). Mx has strong intrinsic antiviral properties, which makes it competent to induce an effective antiviral state independently (Hefti and others 1999).

Interleukins (ILs) were initially considered to be cytokines essential only for the functioning of leukocytes, but research has shown that they affect nearly all cell types. The IL-1 gene family includes 3 members, interleukin-1α, interleukin-1β and an interleukin-1 receptor antagonist, IL-1Ra. IL-1β is considered to function as a hormone-like mediator, intended to be released by cells, whereas IL-1α is primarily a regulator of intracellular events and of localized inflammation. At low concentrations, IL-1β mediates inflammation by increasing synthesis of cell surface adhesion molecules, thus activating...
neutrophils and macrophages and stimulating their recruitment to the site of injury. At higher concentrations, it exerts systemic effects through the activation of NFκB, AP-1 and activating transcription factor (ATF) (Li and others 2001). Tumor necrosis factor (TNF)α, first discovered for its ability to induce necrosis of tumor cells, belongs to a superfamily of proteins that possess a wide range of proinflammatory functions. TNFα is secreted by activated macrophages and is critical for the normal functioning of T cells, natural killer (NK) cells, macrophages and dendritic cells. TNFα is also responsible for priming the respiratory-burst response in activated macrophages by increasing the production of NADPH-oxidase subunits.

Phagocytosis by neutrophils and macrophages is an essential mechanism for the elimination of invading microorganisms. Upon initiation of phagocytosis, the phagosome fuses with lysosomes and destroys the ingested microbe through the production of reactive oxygen species and superoxides by a process known as the respiratory burst. The enzyme responsible for superoxide production is NADPH oxidase, which has membrane-bound and cytosolic components. The cytosolic subunits translocate to the membrane upon phosphorylation and assist in forming the functional enzyme complex that reduces molecular oxygen to produce superoxide (Smith and others 1996). Hydrogen peroxide, hydroxyl radicals and hypochlorites are reactive oxygen species formed from the superoxide via multiple enzymatic reactions (Hermann and others 2004).

Zebrafish as a model for innate immunity
Zebrafish or Danio rerio is a teleost that has become important as an animal model for studying the embryo development, genetics and immune system. It has several advantages over other animal models currently used. Zebrafish have rapid development, are small in size and easy to manage, and are highly fecund compared with other vertebrate models. Embryos develop externally as transparent larvae, allowing easy observation of cellular and organ development. Genomic libraries, combined with shotgun sequencing methods, have permitted the sequencing of the zebrafish genome, and analyses of genes reveal significant similarities to higher vertebrates (Amemiya and others 1999). Forward genetic screens have identified several mutants with phenotypes comparable to human genetic diseases (Karlovich and others 1998; Leimer and others 1999; Hostetter and others 2003) while reverse genetic approaches have also been designed, allowing knockdown of individual genes (Nasevicius and Ekker 2000). Zebrafish have been used as a model for understanding the genetic basis for both viral and bacterial infectious diseases. Pathogenic viral infections examined in a zebrafish model include the spring viremia carp virus (Sanders and others 2003), pancreatic necrosis virus and infectious hematopoietic necrosis virus (LaPatra and others 2000), and snakehead rhabdovirus (Phelan, Pressley and others 2005). In some viral infection models, zebrafish are able to rapidly clear the pathogen without increased mortality or clinical symptoms, whereas in other models infection results in increased mortality, hemorrhaging and increased expression of genes involved in the antiviral immune response.

Differences in virulence and host immune response have been observed among different bacterial strains used for infection (Menudier and others 1996). In some cases, one strain of bacterium may cause systemic infection in all major organ systems (Neely and others 2002), whereas, in other cases, pathogenesis of a different strain may be restricted to certain tissues (Miller and Neely 2004). Bacterial infection may result in mortality corresponding with a dramatic increase in the amount of bacteria entering the bloodstream (van der Sar and others 2003). It has also been shown that bacterial infection by static immersion results in increased inflammatory cytokine production (Pressley and others 2005). Infections involving both Gram-positive and Gram-negative bacteria in zebrafish have shown similar gene induction to that observed in mammalian systems, suggesting conserved immune mechanisms among fish and mammals (Lin and others 2006). Mycobacterial infection in the zebrafish resulted in granuloma-like lesions similar to those seen in mammals (Prouy and others 2003), while microarray analysis revealed upregulation of genes known to be associated with immune function (Meijer and others 2005).

Functional adaptive immunity is not present in zebrafish until the 4th day of development (Willett and others 1997) such that, until this time, mechanisms of infectious defense rely entirely on the innate immune response. Early zebrafish macrophages develop and have chemotactic functions similar to their mammalian counterparts (Herbomel and others 1999). A bioassay to measure the respiratory-burst response has been developed in the zebrafish (Hermann and others 2004). Multiple chemokines (Long and others 2001; David and others 2002) and cytokines (Altmann and others 2003; Altmann and others 2004; Pressley and others 2005) have been characterized in the zebrafish. Additionally, over
20 putative variants of the toll-like receptors (Jault and others 2004; Meijer and others 2004; Phelan, Mellon and others 2005) as well as 4 adaptor proteins (Jault and others 2004) have been identified in the zebrafish. Thus, the zebrafish provides a unique model for studying vertebrate biology and, in particular, for investigating ecotoxicological effects on innate immunity.

**Arsenic and innate immunity in fish**

As many components of innate immunity are evolutionarily conserved (Hoffmann and others 1999; Ulevitch 2000), and as arsenic often accumulates most rapidly in aquatic habitats, monitoring arsenic levels and their associated health effects in fish may not only provide insight into overall ecosystem health (Zelikoff and others 2000) but may also act as a sentinel for potential impacts on human health (Zelikoff 1998; Adams and Greeley 1999). Because adaptive immunity is developmentally delayed in fish (Alexander and Ingram 1992), the effects of ecotoxictants on innate immunity may be more significant and thus easier to measure in fish than in mammals. The general effects of ecotoxictants (Bols and others 2001) and stress (Fletcher 1986) on innate immunity in fish have been reviewed.

Arsenic has been shown to induce metallothionein (MT), part of the oxidative stress response (Schlenk and others 1997; Hermesz and others 2002; Pedlar and others 2002) in various species of fish. The duration of arsenic exposure may affect the generation of a stress response in some fish, as it has been shown in the snakehead that after initial arsenic exposure and reduction in ROS scavenging enzyme production, an extended exposure upregulated enzymatic activity, resulting in increased arsenic resistance over time (Allen and Rana 2004).

Arsenic has been shown to regulate transcription-factor activation in zebrafish cell cultures (Carvan and others 2000). Effects of arsenic on the innate immune system, including the ability to mount an adequate respiratory-burst response, express essential antiviral genes and produce sufficient levels of TNFα, within the concentration range of arsenic found in contaminated groundwater (Clark and Raven 2004), have been evaluated in the zebrafish model system (Hermann and Kim 2005). The ability to mount an appropriate respiratory burst is an indicator of the general immune health of an organism and reductions in respiratory-burst activity were found in zebrafish on exposure to even low concentrations of arsenic. In an effort to elucidate the possible mechanism for this inhibition, examination of TNFα levels revealed decreased expression upon exposure, supporting the possibility that arsenic hinders respiratory-burst activity by reducing the expression of TNFα, which is essential for priming this response. The antivirus response of the fish was examined upon arsenic exposure before and after infection with snakehead rhabdovirus. Gradual increases in interferon expression were observed over time in arsenic-exposed zebrafish. However, Mx levels failed to mirror this trend. The disruption of Mx induction by interferon indicates arsenic inhibition of interferon signaling. Upon viral exposure, expression levels of both antiviral genes were downregulated when compared with arsenic-unexposed controls. These data suggest that the ability of the zebrafish to mount an effective innate immune response is reduced by arsenic exposure, indicating that the presence of this metalloid in water has potentially adverse effects on the components of innate immunity essential for an antiviral response in fish.

**Concluding remarks**

Mounting evidence of links to human disease underscores the need for in-depth investigations into the health effects of arsenic. Furthermore, from our studies and those of others, it is becoming increasingly evident that fish, and in particular zebrafish, serve as an ideal animal model for studying the effects of arsenic. It is now clear that arsenic disrupts the immune response, potentially playing an important role in the outcome of infection and of host resistance to infectious diseases.

Conflict of interest: None declared.

**References**


Effects of arsenic on innate immunity


