Chapter 17

ADDITIONAL TOXINS OF CLINICAL CONCERN

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INTRODUCTION

Several toxins produced naturally by microorganisms and plants are potent, stable, and capable of causing a wide range of effects leading to incapacitation or death. These agents can be ingested, administered percutaneously, or potentially delivered as aerosols at the tactical level. Although these toxins may be lethal, the amount of toxin available from a single organism is typically small. Toxins listed on the Centers for Disease Control and Prevention's bioterrorism threat list are proteins of microbial or plant origin, and include *Clostridium botulinum* neurotoxin, *C perfringens* epsilon toxin, *Staphylococcus aureus* enterotoxin B, and ricin from *Ricinus communis*. Additional, nonproteinaceous toxins that may pose a threat are the trichothecene mycotoxins (eg, T-2 toxin) and marine toxins (eg, saxitoxin [STX], brevetoxins, and domoic acid).

Although any of these toxins have the potential to cause significant effects in humans or animals, their potential as biological warfare/biological terrorism agents varies depending on several factors. These toxins are also clinically relevant because intoxications occur naturally in humans and animals. The toxins in this chapter have been selected for discussion because of their potential for intentional use.

TRICHOTHECENE MYCOTOXINS

History

Mycotoxins are metabolites of fungi produced through secondary biochemical pathways. Various mycotoxins are implicated as the causative agents of adverse health effects in humans and animals that consumed fungus-infected agricultural products.[1,2] Consequently, fungi that produce mycotoxins, as well as the mycotoxins themselves, are potential problems from a public health and economic perspective. The fungi are a vast group of eukaryotic organisms, but mycotoxin production is most commonly associated with the terrestrial filamentous fungi referred to as molds.[3] Various species of toxigenic fungi are capable of producing different classes of mycotoxins, such as the aflatoxins, rubratoxins, ochratoxin, fumonisins, and trichothecenes.[1,2]

Use in Biological Warfare

From 1974 to 1981 the Soviet Union and its client states may have used trichothecene toxins[4] in Cold War sites such as Afghanistan, Laos, and Cambodia. These agents may have been delivered as an aerosol or droplet cloud by aircraft spray tanks, aircraft-launched rockets, bombs (exploding cylinders), canisters, a Soviet handheld weapon (DH-10), and booby traps. Alleged attacks in Laos (1975–1981) were directed against Hmong villagers and resistance forces who opposed the Lao People's Liberation Army as well as the North Vietnamese. In Afghanistan these weapons were allegedly delivered by Soviet or Afghan pilots against mujahideen guerrillas between 1979 and 1981. The attacks caused at least 6,310 deaths in Laos (226 attacks); 981 deaths in Cambodia (124 attacks); and 3,042 deaths in Afghanistan (47 attacks).[5]

The "Yellow Rain" Controversy

Some of the air attacks in Laos, described as "yellow rain," consisted of a shower of sticky yellow liquid that fell from the sky and sounded like rain. Other accounts described a yellow cloud of dust or powder, a mist, smoke, or an insect-spray–like material. More than 80% of the attacks were delivered by air-to-surface rockets and the remainder from aircraft-delivered sprays, tanks, or bombs.[5] The use of other agents, such as phosgene, sarin, soman, mustards, CS gas, phosgene oxime, or BZ, has been suggested by intelligence information and symptoms described by the victims. These chemical agents may have been used in mixtures or alone, with or without the trichothecenes.[5] Evidence for, and against, the use of trichothecenes in Southeast Asia has been fully discussed in previous texts.[6,7,8]

Weaponization

Mycotoxins (especially T-2 toxin) have excellent potential for weaponization because of their antipersonnel properties, ease of large-scale production, and proven delivery by various aerial dispersal systems.[5,7-11] In nanogram amounts, the trichothecene mycotoxins (in particular T-2 toxin) cause severe skin irritation (erythema, edema, and necrosis).[8,11-15] It is estimated that T-2 toxin is about 400 times more potent in producing skin injury than mustard (50 ng for T-2 vs 20 μg for mustard).[9] Lower microgram quantities of trichothecene mycotoxins cause severe eye irritation, corneal damage, and impaired vision.[4,5,9,16] Emesis and diarrhea have been observed at 0.1 to 0.2 lethal doses (LD) of trichothecene mycotoxins.[9-19]

By aerosol exposure, the lethality of T-2 toxin is 10 to 50 times greater than when it is injected parenterally,[20]

depending upon the species and exposure procedure.[21-22] With a larger dose in humans, aerosolized trichothecenes may produce death within minutes to hours.[5-7] The inhaled toxicity of T-2 toxin is in the range of 200 to 5,800 mg/min/m^3 [20-22] and is similar to that observed for mustards or lewisite (range of 1,500–1,800 mg/min/m^3).[23] Percutaneous lethality of T-2 toxin (median LD [LD_{50}] in the range of 2–12 mg/kg)[9,14] is higher than that for lewisite (LD_{50} of approximately 37 mg/kg) or mustards (LD_{50} of approximately 4,500 mg/kg).[23]

T-2 toxin can be produced by solid substrate fermentation at approximately 9 g/kg of substrate, with a yield of 2 to 3 g of crystalline product.[24] Several of the trichothecene mycotoxins have been produced in liquid culture at medium yields and large volumes of culture for extraction.[25] A trichothecene mycotoxin used in phase I and II cancer trials, 4,15-diacetoxyscirpenol (DAS), was produced large scale by a procedure considered proprietary by industry.[10] Thus, using existing state-of-the-art fermentation processes developed for brewing and antibiotics, ton production of several trichothecene mycotoxins would be fairly simple.

The delivery methods allegedly used in Southeast Asia would result in a low-efficiency respiratory aerosol (1–5-μm particles),[26] but a highly effective droplet aerosol could result in severe skin and eye irritation. A National Research Council/National Academy of Sciences expert committee estimated that the offensive use of trichothecene mycotoxins could produce concentrations of approximately 1 g/m^3 in the exposure cloud and 1 g/m^2 on the ground.[10] Much lower aerosol concentrations could be expected to cause significant incapacitating responses (ie, skin and eye irritation at nano/microgram quantities) that would adversely affect military operations.

Description of the Toxin

Natural Occurrence

Potentially hazardous concentrations of the trichothecene mycotoxins can occur naturally in moldy grains, cereals, and agricultural products.[10,16] Toxigenic species of *Fusarium* occur worldwide in habitats as diverse as deserts, tidal salt flats, and alpine mountain regions.[10] A food-related mycotoxic disease has been recorded in Russia from time to time, probably since the 19th century.[27-29] In the spring of 1932, this disease appeared in endemic form throughout several districts of western Siberia (with a mortality rate of up to 60%). From 1942 to 1947, more than 10% of the population in Orenburg, near Siberia, was fatally affected by overwintered millet, wheat, and barley.[16,29,30] The syndrome was officially named alimentary toxic aleukia (alternative names in the Russian literature include septic angina, alimentary mycotoxicosis, alimentary hemorrhagic aleukia, aplastic anemia, hemorrhagic aleukia, agranulocytosis, and Taumelgetreide [staggering grains]).[27,29] Symptoms of this disease include vomiting, diarrhea, fever, skin inflammation, leukopenia, multiple hemorrhage, necrotic angina, sepsis, vertigo, visual disturbances, and exhaustion of bone marrow.[27-29,31] Extensive investigations in Russia indicated that a toxin from *Fusarium* species was the causative agent of alimentary toxic aleukia.[29,32,33] Subsequently, it was demonstrated that T-2 toxin, a potent trichothecene mycotoxin, was the likely agent of the disease.[33,34]

Human cases of stachybotryotoxicosis (another toxic trichothecene mycotoxin) have been reported among farm workers in Russia, Yugoslavia, and Hungary.[35-38] This disease is caused by a mold, *Stachybotrys atra,* on the hay fed to domestic animals. Symptoms of this toxicosis include conjunctivitis, cough, rhinitis, burning in the nose and nasal passages, cutaneous irritation at the point of contact, nasal bleeding, fever, and leukopenia in rare cases.[35,36] A macrocyclic trichothecene (satratoxin) is produced by *Stachybotrys* species, which may be partly responsible for this toxicosis.[37-41] The only apparent human cases of stachybotryotoxicosis in the United States cited in the literature occurred in people living in a water-damaged house heavily infested with *S atra.*[42] Russian scientists have reported a case of "cotton lung disease" that occurred after inhalation of cotton dust contaminated with *Dendrodochium toxicum*, which is a fungus synonymous with *Myrothecium verrucaria* (a natural producer of the verrucarin class of macrocytic trichothecenes).[30,43]

The "red mold disease" of wheat and barley in Japan is prevalent in the region facing the Pacific Ocean.[16,44] In humans, symptoms of this disease included vomiting, diarrhea, and drowsiness. Toxic trichothecenes, including nivalenol, deoxynivalenol, and monoacetylnivalenol (fusarenon-X), from *F nivale* were isolated from moldy grains.[16,44] Similar symptoms were described in an outbreak of a foodborne disease in the suburbs of Tokyo, which was caused by the consumption of *Fusarium*-contaminated rice.[10]

In addition to human intoxication, ingestion of moldy grains contaminated with trichothecenes has also been associated with mycotoxicosis in domestic farm animals.[30,31,44-51] Symptoms include refusal of feed, emesis, diarrhea, skin inflammation, hemorrhage, abortion, cyclic movement, stomatitis, shock, and convulsions. Overall, the symptoms evident in domestic farm animals that eat food contaminated with trichothecene mycotoxins are similar to those observed in humans.

Chemical and Physical Properties

The trichothecenes make up a family of closely related chemical compounds called sesquiterpenoids.[16] All the naturally occurring toxins contain an olefinic bond at C-9,10, and an epoxy group at C-12,13; the latter characterizes them as 12,13-epoxy trichothecenes. The structures of approximately 150 derivatives of trichothecenes are described in the scientific literature.[10,52,53] These mycotoxins are classified into four groups according to their chemical characteristics. The first two groups include the "simple" trichothecenes, and the other two include the "macrocyclic" trichothecenes.[16,30] Because of its relatively high toxicity and availability, T-2 toxin has been the most extensively studied trichothecene mycotoxin.

The trichothecene mycotoxins are nonvolatile, low-molecular–weight (250–550) compounds.[53] This group of mycotoxins is relatively insoluble in water; the solubility of T-2 toxin is 0.8 and 1.3 mg/mL at 25°C and 37°C, respectively.[54] In contrast, these toxins are highly soluble in acetone, ethylacetate, chloroform, dimethyl sulfoxide, ethanol, methanol, and propylene glycol.[53] Purified trichothecenes generally have a low vapor pressure, but they do vaporize when heated in organic solvents. Extracting trichothecene mycotoxins from fungal cultures with organic solvents results in a yellow-brown liquid, which, if allowed to evaporate, yields a greasy, yellow crystalline product believed to be the yellow contaminant of yellow rain. In contrast, highly purified trichothecenes form white crystalline products that have characteristic melting points.[10]

Trichothecene mycotoxins are stable compounds in air and light when maintained as crystalline powders or liquid solutions.[10,54-57] When stored in sterile phosphate-buffered saline at pH 5 to 8 and 25°C, T-2 toxin was stable for a year, with an estimated half-life of 4 years.[54] In contrast, T-2 toxin degrades rapidly over several days in culture medium containing fetal bovine serum[58] or bacteria from soil or freshwater.[59] This suggests that enzymes present in serum or produced by bacteria can stimulate biotransformation of trichothecene mycotoxins. A 3% to 5% solution of sodium hypochlorite is an effective agent for inactivating trichothecene mycotoxins.[56,57] The efficacy of this agent is increased by adding small amounts of alkali, but higher concentrations of alkali or acid alone do not destroy trichothecene activity. Thus, high pH environments are ineffective for inactivating trichothecene mycotoxins. The US Army decontaminating agents DS-2 and supertropical bleach inactivate T-2 toxin within 30 to 60 minutes. These mycotoxins *are not inactivated by autoclaving* (at 250°F for 15 minutes at 15 lb/in^2); however, heating at 900°F for 10 minutes or 500°F for 30 minutes inactivates them.[56,57] This emphasizes the marked stability of trichothecene mycotoxins under varying environmental conditions.

Mechanism of Action

The trichothecene mycotoxins are toxic to humans, other mammals, birds, fish, various invertebrates, plants, and many types of eukaryotic cells in general.[1,2,8,10,30,60-62] The acute toxicity of the trichothecene mycotoxins varies somewhat with the particular toxin and animal species.[8,10,43,60-63] Variations in species susceptibility to trichothecene mycotoxins are small compared to the divergence obtained by the diverse routes of toxin administration. Once the trichothecene mycotoxins enter the systemic circulation, regardless of the route of exposure, they affect rapidly proliferating tissues.[8,10,16] Oral, parenteral, cutaneous, and respiratory exposures produce (*a*) gastric and intestinal lesions; (*b*) hematopoietic and immunosuppressive effects described as radiomimetic in nature; (*c*) central nervous system toxicity resulting in anorexia, lassitude, and nausea; and (*d*) suppression of reproductive organ function as well as acute vascular effects leading to hypotension and shock.[2,10,20-22,30,60,63-68]

These mycotoxins are cytotoxic to most eukaryotic cells.[30,69,70] A number of cytotoxicity assays have been developed that include (*a*) survival and cloning assays,[70,71] (*b*) inhibition of protein[69,72] and DNA[73,74] synthesis by radiolabeling procedures, and (*c*) a neutral red cell viability assay.[75] It takes a minimum of 24 to 48 hours to measure the effects of trichothecene mycotoxins on cell viability.

Uneo et al[76] first demonstrated that the trichothecene mycotoxins inhibit protein synthesis in rabbit reticulocytes and ascites cells. The inhibition of protein synthesis by these mycotoxins occurs in a variety of eukaryotic cells.[59,71,72,77,78] Similar sensitivity to T-2 toxin was observed in established cell lines and primary cell cultures.[59,72] Protein synthesis inhibition is observed rapidly within 5 minutes after exposure of Vero cells to T-2 toxin, with a maximal response noted within 60 minutes.[59] A number of studies have concluded that the trichothecene mycotoxins interfere with peptidyl transferase activity and inhibit either the initiation or elongation process of translation.[77,79-81] Alterations in trichothecene side groups can markedly affect protein synthesis inhibition in in-vitro systems.[59,70,72,75,77]

Substantial inhibition (86%) of RNA synthesis by trichothecene mycotoxins was observed in human (HeLa) cells,[77] but T-2 toxin had minor effects (15% inhibition) on RNA synthesis in Vero cells.[59] In eukaryotic cells, blocking protein synthesis can severely inhibit rRNA synthesis.[77] Because rRNA accounts for

80% of the total cellular RNA, the trichothecene-mycotoxin–related inhibition of RNA synthesis is probably a secondary effect linked to inhibited protein synthesis.

Scheduled DNA synthesis is strongly inhibited in various cell types exposed to trichothecene mycotoxins.[59,71,82,83] In mice or rats given a trichothecene mycotoxin, DNA synthesis in all tissues studied was suppressed, although to a lesser degree than protein synthesis.[83-87] Cells require newly synthesized protein to exit G_1 and enter the S phase of the cell cycle,[88] during which DNA is synthesized. Inhibitors of protein synthesis prevent cells from entering S phase, thereby blocking most DNA synthesis.[88] Thus, the pattern of DNA synthesis inhibited by the trichothecene mycotoxins is consistent with the primary effect of these toxins on protein synthesis. For the most part, trichothecene mycotoxins do not possess mutagenic activity or the capacity to damage DNA in appropriate cell models.[51]

Because the trichothecene mycotoxins are amphiphilic molecules, a number of investigations have focused on various interactions with cellular membranes.[89,90] Yeast mutants with reduced plasma membrane were more resistant than the parent strain to T-2 toxicity.[91,92] Changes in cell shape and lytic response to T-2 toxin were observed in studies with erythrocytes, which lack nuclei and protein synthesis.[93-96] Susceptibility to lysis is species dependent and correlates with phosphatidylcholine.[95] In L-6 myoblasts, uptake of calcium, glucose, leucine, and tyrosine was reduced within 10 minutes after exposure to a low dose of T-2 toxin.[89] These authors concluded that T-2 exerted multiple effects on the cell membrane.

Once trichothecene mycotoxins cross the plasma membrane barrier, they can interact with a number of targets including ribosomes[77] and mitochondria.[92,97-101] These toxins also inhibit electron transport activity, as implied by decreased succinic dehydrogenase activity[97,100,101] and mitochondrial protein synthesis.[98] Toxin-stimulated alteration in mitochondrial membranes contributes to the effects on cellular energetics and cytotoxicity. Although initial investigations on the mechanism of action for trichothecene mycotoxins suggested that protein synthesis is the principal target, current observations indicate that the effects of these toxins are much more diverse.

In cell-free or single-cell systems, these mycotoxins rapidly inhibit protein synthesis and polysomal disaggregation.[10,51,67,102] Thus, it is postulated that the trichothecene mycotoxins can directly react with cellular components. Despite this direct effect, several investigations have been published on the toxicokinetics of the trichothecene mycotoxins.[53]

Very little of the parent trichothecene mycotoxin is excreted intact; rather, elimination by detoxification is the result of extensive and rapid biotransformation. The biotransformation of T-2 toxin occurs by four competing pathways: (1) ester hydrolysis at the C-4, C-8, and C-15 positions; (2) conjugation with glucuronic acid; (3) aliphatic hydroxylation of the C-3N and C-4N positions on the isovaleryl side chain; and (4) reduction of the 12,13 epoxide.

Clinical Signs and Symptoms of Intoxication

The pathological effects and clinical signs can vary with the route and type of exposure (acute single dose vs chronic subacute doses). Local route-specific effects include the following: (*a*) dermal exposure leads to local cutaneous necrosis and inflammation[12,14,103-105]; (*b*) oral exposure results in upper gastrointestinal tract lesions[106-109]; and (*c*) ocular exposure causes corneal injury.[28] For the trichothecene mycotoxins, however, many systemic toxic responses are similar regardless of the exposure route. In contrast, the symptoms and clinical signs of trichothecene intoxication can vary depending on whether the exposure is acute or chronic. For biological warfare use, an acute exposure would be the major concern.

Dermal Exposure

Cutaneous irritations have been observed in individuals exposed to hay or hay dust contaminated with trichothecene-producing molds.[35-38] While working up large batches of fungal cultures from trichothecene-producing organisms, workers suffered facial inflammation followed by desquamation of the skin and considerable local irritation.[110] Applying trichothecene mycotoxins of relatively low toxicity (crotocin and trichothcein) to the volar surface of a human forearm or to the head resulted in erythema and irritation within a few hours of exposure, followed by inflammation that healed in 1 or 2 weeks.[111] The hands of two laboratory workers were exposed to crude ethyl acetate extracts containing T-2 toxin (approximately 200 μg/mL) when the extract accidentally got inside their plastic gloves.[111] Even though the workers thoroughly washed their hands in a mild detergent within 2 minutes of contact, they experienced a burning sensation in their fingers about 4 hours postexposure, which increased in intensity until 8 hours after contact with the toxin. Within 24 hours, the burning sensation had disappeared and was replaced by numbness in the fingers. After about 3 days, sensitivity was lost in all exposed fingers, and by day 4 or 5, the affected skin became hardened and started to turn white. During

the second week, the skin peeled off in large pieces 1 to 2 mm in thickness. By day 18 after contact, normal sensitivity had been regained in the new skin. These observations provide evidence that when human skin is exposed to small amounts of trichothecene mycotoxins, severe cutaneous irritations develop and may last for 1 to 2 weeks after acute exposure. These local skin exposures were too small to cause any detectable systemic reactions.

Several animal models have helped assess the local and systemic toxicity, as well as lethality, from skin exposure to trichothecenes.[14] In a dermal study using a mouse model, T-2 toxin in dimethylsulfoxide was applied to the skin, without the use of a barrier to prevent oral ingestion or removal of the toxin during the grooming process.[112] Characteristic radiomimetic effects in the thymus, spleen, and duodenum were easily recognized by 6 hours after topical application of 5 or 40 mg/kg of T-2 toxin.[112] Severity of the damage was dependent on the organ evaluated and time after topical exposure. Necrotic skin was present within 6 hours after dermal application of T-2 toxin. With the exception of skin damage, lesions were quantitatively and qualitatively similar to those seen after intragastric application of T-2 toxin. Cumulative mortality and early systemic effects in mice were essentially similar for topically applied T-2 toxin, HT-2 toxin, DAS, verrucarin A, and roridin A.[113]

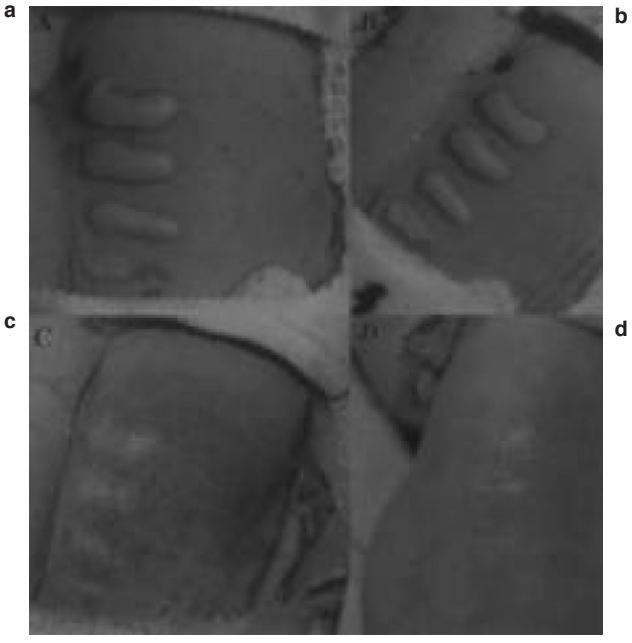

Fig. 17-1. Skin lesions on the back of a hairless guinea pig at (**a**) 1, (**b**) 2, (**c**) 7, and (**d**) 14 days after application of (bottom to top) 25, 50, 100, and 200 ng of T-2 toxin in 2 μL of methanol.

Regardless of the route of administration, systemic histological lesions associated with T-2 toxin are similar—the most prominent being necrosis of rapidly dividing cells such as those found in the gastrointestinal tract and lymphoid tissues.[14] The severity of necrosis, both local and systemic, is dose dependent. Twenty-four hours after rats were exposed to a dermal dose of 2 mg/kg of T-2 toxin in dimethylsulfoxide, cardiac function was altered, as evidenced by decreased arterial blood pressure, peak intraventricular pressure, and resting systolic and diastolic blood pressure.[114] The toxin-treated rats had lower epinephrine-stimulated intraventricular pressure values, indicating reduced contractility. They also exhibited prolonged QT intervals on their electrocardiograms.

Clinical observations and experimental animal studies show that the trichothecene mycotoxins are severe skin irritants (Figure 17-1). If these toxins are applied with absorption enhancers, they cause systemic toxicity at doses comparable to oral or parenteral exposure. Local skin sensitivity and rate of absorption are influenced by a number of factors, including the species, skin thickness and structure, age, nutritional status, and underlying infections.

Ocular Exposure

Ocular exposure may result in tearing, eye pain, conjunctivitis, and blurred vision. A laboratory worker developed burning of the eyes and blurred vision for several days after a powder containing roridin A was accidentally blown into his eyes.[43]

Cultured filtrates containing roridin A and verrucarin A produced ocular lesions in rabbits.[105] When the filtrates were instilled into the conjunctival sac, erythema and edema of the conjunctival membranes were observed within 1 or 2 days. Later, the cornea became opaque and developed scarring, which persisted as long as 5 months.[115] Instillation of trichothecene into the conjunctival sac of a rabbit caused slight inflammation of the conjunctiva, the nictitating membrane, and the eyelids.[116] When T-2 toxin (1 μg) was instilled into the eyes of rats, irregularity of the cornea developed in 12 to 24 hours, which was readily visible with a hand-held ophthalmoscope.[9,117] Occasionally, corneal staining with fluorescein was positive and diffuse. This lesion would be expected to result in photophobia and decreased acuity. Peak injury was at 24 to 48 hours with recovery in 3 to 7 days. Histologically, this dose of T-2 toxin can cause extreme thinning of the corneal epithelium, which may be irreversible. With exposure

to higher doses of T-2 toxin, scleral and conjunctival vasodilatation and inflammation may occur, with corneal irregularities that may persist for 6 months or more.

Because trichothecene mycotoxins can cause severe eye injury that markedly impairs vision, they represent a severe incapacitating problem for unprotected military personnel. No systemic toxicity has been documented from the instillation of trichothecene mycotoxins into the eyes of experimental animals.

Respiratory Exposure

Agricultural workers exposed to hay or hay dust contaminated with trichothecene mycotoxins developed signs and symptoms of upper respiratory injury, including cough, rhinitis, burning in the nose and nasal passages, and nose bleeds.[35,36] The occupants of a water-damaged house with a heavy infestation of *S atra*, who were exposed to trichothecene-mycotoxin–contaminated dust from the air ducts, complained of a variety of recurring illnesses including cold and flu symptoms, sore throats, diarrhea, headaches, fatigue, dermatitis, intermittent focal alopecia, and general malaise.[42]

In animal studies, mice, rats, and guinea pigs were exposed to deeply deposited aerosolized T-2 toxin with an average aerodynamic median diameter of 0.6 to 1 μm.[20-22] At high (lethal) aerosol concentrations of T-2 toxin (2.4 mg/L), mice were lethargic and exhibited no grooming behavior; most were prostrate, and all were dead in 18 hours.[20] When exposed to an LD_{50} aerosol concentration of T-2 toxin (0.24 mg/L), the mice became lethargic and prostrate near death, which occurred in 30 to 48 hours. No significant lesions were observed in the upper respiratory tract or lungs of the exposed mice, rats, or guinea pigs.[20-22] The microscopic lesions were mainly observed in the lymphoid system and intestinal tract. In a [^{3}H]-labeled T-2 toxin distribution study, approximately 11% and 30% of the total radioactivity was associated with nasal turbinates immediately after a 10-minute exposure of mice with a respective LD_{50} or LD of aerosolized toxin.[20] At the end of this exposure time, only 1% to 2% of the retained radioactivity was found in the respiratory tract; the remainder was distributed throughout the carcass. Thus, approximately 70% to 90% of a retained dose from a 0.6- to 1-μm particle aerosol of T-2 toxin was cleared by the alveoli of the lungs, with a half-life of less than 1 minute. The T-2 toxin associated with the nasal turbinates was probably ingested and may have been responsible for intestinal crypt epithelial necrosis in mice receiving the high-dose aerosol.[20]

Ingestion

Although aerosol forms of trichothecene mycotoxins are of the most concern as biological warfare weapons, acute ingestion of foods contaminated with large amounts of these mycotoxins could be devastating to soldiers. Chronic subacute ingestion of trichothecene mycotoxins is responsible for atoxic alimentary aleukia, which consists of gastric and intestinal mucosa inflammation that may be followed by leucopenia with progressive lymphocytosis and bleeding diathesis if large amounts are ingested.

Within 4 hours after gastric intubation of a single dose of T-2 toxin, chickens developed asthenia, inappetence, diarrhea, and panting.[118] Coma was observed in birds given high doses of T-2 toxin. Death of the birds occurred within 48 hours after T-2 mycotoxin administration. The abdominal cavities of birds given lethal doses contained a white chalk-like material, which covered much of the viscera. Necrosis of the mucosal surface lining the gizzard, as well as thickening, sloughing, and epithelium necrosis in the crop were noted in chickens given a high dose of T-2 toxin. Subacute doses of T-2 toxin resulted in decreased weight gain and feed consumption.

Gastric intubation of an acute dose of T-2 toxin in guinea pigs resulted in lethargy and death within 48 hours.[119] Gross lesions included gastric and cecal hyperemia with watery-fluid distension of the cecum and edematous intestinal lymphoid tissue. Histological alterations included necrosis and ulceration of the gastrointestinal tract and necrosis of rapidly dividing cells of bone marrow, lymph nodes, and testes.

Within 20 minutes of a subacute dose of T-2 toxin given by esophageal intubation, a calf developed hindquarter ataxia, knuckling of the rear feet, listlessness, severe depression, loud teeth grinding, and repeated head submersion in water.[120] Three days after the initial intubation, the feces became noticeably loose. At necropsy, acute ulceration and necrosis were observed in the gastrointestinal tract.

Parenteral Exposure

The LD_{50} of T-2 toxin by the intramuscular route in cynomolgus monkeys is 0.75 mg/kg with a 95% confidence limit of 0.4 to 4.2 mg/kg.[14] Similar toxicities were seen for intravenous administration of T-2 toxin in the monkey when administered by a bolus or 4-hour infusion. Mean time to death was 18.4 hours and independent of dose (between 0.65 and 6 mg/kg). Monkeys dosed intramuscularly developed emesis within 30 minutes to 4 hours with doses as low as 0.25

mg/kg.[14] Emesis occurred 15 to 30 minutes after an intravenous dose of T-2 toxin as low as 0.014 mg/kg. The duration and severity of emesis appeared dose-dependent. At 2 to 4 hours postexposure, the monkeys developed a mild to severe diarrhea, especially in the higher dose groups. Listlessness, sluggish response to stimuli, and ataxia occurred 4 to 6 hours postexposure. A progressive hypothermia was evident in dying monkeys. Food intake was reduced in surviving monkeys, even at a dose of 0.014 mg/kg. Severity and duration of food refusal was a function of the toxin dose.

Gross and histological examinations were done on all cynomolgus monkeys that died after exposure to T-2 toxin in various doses. Eight of 16 monkeys showed a mild degree of petechial hemorrhage in the colon and cecum. Three had slight petechial hemorrhages in the small intestine and stomach.[14] Lymphoid necrosis was present in all intoxicated animals. Splenic necrosis was consistently most severe in the white pulp, and lymph node necrosis occurred in the germinal centers, which also affected mature lymphocytes. Gut-associated lymphoid tissue necrosis was a consistent feature ranging from mild to moderate in severity. Thymic necrosis was seen in one of the monkeys, and bone marrow necrosis was observed at higher doses of toxin.[14] Necrosis of glandular elements within the gastrointestinal tract was present in all monkeys, but varied in both severity and distribution, from multifocal to diffuse. The most severe lesions were in the colon. Stomach lesions were inconsistently present in six monkeys. One monkey showed minimal multifocal necrosis of hepatocytes. Seven of the monkeys were diagnosed as having mild nephrosis, consisting of degeneration and necrosis of tubular epithelial cells with no inflammatory response. Heart sections revealed vacuolar change and multifocal degeneration ranging from a mild to moderate degree in eight of the monkeys. One monkey in the high-dose group had a leukoencephalopathy, and three others had minimal focal inflammatory lesions. Multifocal areas of minimal hemorrhage were observed in the spinal cord of four monkeys. Testes from 14 monkeys showed mild multifocal degenerative changes. Minimal to mild hemorrhagic lesions were observed, most commonly in the cecum and heart, in all the monkeys. At doses of T-2 greater than 1 mg/kg, there was minimal hemorrhage in the brain and/or spinal cord. In conclusion, necrosis of lymphoid tissue and glandular epithelium of the gastrointestinal tract were consistent lesions linked to T-2 toxicosis in the monkey. These alterations are also consistent with observations in other species. Among the significant findings was an apparent dose relationship to bone marrow necrosis and leukoencephalopathy, both of which occurred only in the high-dose groups. Mild lesions in the heart, liver, and kidney are consistent with those observed in other species.[14,121-125]

Diagnosis

Presumptive Diagnosis

Diagnosis of an attack with trichothecene mycotoxins would largely depend on the clinical observations of casualties and toxin identification in biological or environmental samples, which would involve a combined effort among medical and chemical units in the field. The early signs and symptoms of an aerosol exposure to trichothecene mycotoxins would depend on particle size and toxin concentration. For a large-particle aerosol (particles > 10 μm, found in mist, fog, and dust similar to that allegedly used in Southeast Asia), the signs and symptoms would include rhinorrhea, sore throat, blurred vision, vomiting, diarrhea, skin irritation (burning and itching), and dyspnea. Early signs and symptoms from a deep-respiratory aerosol exposure (from aerosol particles in the 1- to 4-μm range) have not been fully evaluated but could include vomiting, diarrhea, skin irritation, and blurred vision. Later signs and symptoms would probably be similar (except for the degree of skin rash and blisters) for both large-particle and deep-respiratory aerosol exposure to trichothecene mycotoxins. They could include continued nausea and vomiting, diarrhea, burning erythema, skin rash and blisters, confusion, ataxia, chills, fever, hypotension, and bleeding.

Initial diagnostic tests should include standard clinical laboratories and serum, urine, or tissue samples for toxin detection. Nonspecific changes in serum chemistry and hematology occurred in monkeys exposed to an acute dose of T-2 toxin. Alterations in serum chemistries may include elevated serum creatinine, serum enzymes (especially creatine kinase), potassium, phosphorous, and serum amino acid levels. Prothrombin and partial thromboplastin times should also be evaluated by the laboratory because a decrease in coagulation factors may lead to an increased risk of bleeding. An initial rise in the absolute number of neutrophils and lymphocytes may occur within hours, followed by a decrease in lymphocyte counts by 48 hours. Survival beyond several days may be associated with a fall in all blood cellular elements.[14] Although it is likely that these acute changes will be seen in humans, clinical observations among human victims of acute trichothecene mycotoxicosis have not been reported to date. In patients with chronic toxicity resulting from repeated ingestion of contaminated bread, pancytopenia is an important part of the

clinical picture.[29] Patients that are exposed to mold and mycotoxins in water-damaged buildings may develop mold-specific immunoglobulin (IgG) and IgE detectable with enzyme-linked immunosorbent assays and radio allegro sorbent test protocols using fungal extracts; however, the elevation of these antibodies has not been statistically associated with morbidity. Secretory IgA against molds and mycotoxins in bronchoalveolar lavage fluid and saliva may be produced in the absence of serum antibodies and may assist in making the proper diagnosis; however, these specific antibodies could be elevated from naturally occurring environmental exposure.

After the yellow rain attacks in Southeast Asia, diagnosis of the causative agent was difficult and involved ruling out conventional chemical warfare agents. An attack with mycotoxins alone would not contaminate the environment and clothing with nerve and blistering agents, and these agents were not detectable in such samples from Southeast Asia. The following events should suggest that a biological warfare attack with trichothecene mycotoxins has occurred: (*a*) clinical findings that match the symptoms listed above; (*b*) high attack and fatality rates; (*c*) dead animals of various types in the attack area; and (*d*) onset of symptoms after a yellow rain or red, green, or white smoke or vapor attack.

Several commercial immunoassay kits are marketed for detecting trichothecene mycotoxins (T-2 toxin, deoxynivalenol, and their metabolites) in grain extracts or culture filtrates of *Fusarium* species.[126,127] The US Department of Agriculture has published a manuscript by the Grain Inspection, Packers and Stockyards Administration Technical Services Division that lists approved tests for this use; however, these kits have not been evaluated against biomedical samples that contain typical concentrations of the mycotoxins. Screening tests for presumptive identification of trichothecene mycotoxins in the biomedical samples would involve bioassays, thin-layer chromatography (TLC), or immunological assays, in any combination. At a national laboratory, confirmatory methods involve the use of various techniques that include gas chromatography, high-performance liquid chromatography (HPLC), mass spectrometry (MS), and nuclear magnetic resonance spectrometry.

In areas that have experienced a yellow rain attack, environmental assays have been in the range of 1 to 150 parts per million (ppm) and blood samples in the range of 1 to 296 parts per billion (ppb).[1,128] Ten and 50 minutes after an intramuscular injection of 0.4 mg/kg of T-2 toxin in dogs, plasma concentrations of T-2 toxin were respectively 150 and 25 ppb, and 50 and 75 ppb for HT-2 toxin.[129] Thus, any screening procedure for trichothecene mycotoxins in biomedical samples must have detection limits of 1 to 100 ppb. Most of the analytical procedures require extraction and cleanup treatments to remove interfering substances.

Screening tests for the trichothecene mycotoxins are generally simple and rapid but, with the exception of the immunochemical methods, are nonspecific. Several bioassay systems have been used to identify trichothecene mycotoxins. Although most of these systems are very simple, they are not specific, sensitivity is relatively low compared to other methods, and they require that the laboratory maintain vertebrates, invertebrates, plants, or cell cultures. TLC is one of the simplest and earliest analytical methods developed for mycotoxin analysis. Detection limits for trichothecene mycotoxins by TLC is 0.2 to 5 ppm (0.2 to 5 μg/mL). Therefore, extracts from biomedical samples would have to be concentrated 10-fold to 1,000-fold to screen for trichothecene mycotoxins.

To overcome the difficulties encountered with the bioassays and TLC methods, immunoassays using specific polyclonal and monoclonal antibodies have been developed for most of the major trichothecene mycotoxins and their metabolites. These antibodies have been used to produce simple, sensitive, and specific radioimmunoassays and enzyme-linked immunosorbent assays for the mycotoxins. The lower detection limit for identification of trichothecene mycotoxins by radioimmunoassay is about 2 to 5 ppb,[130] and by enzyme-linked immunosorbent assay, 1 ppb.[131]

Confirmatory Procedures

Gas-liquid chromatography (GLC) and HPLC are two of the most commonly used methods for identifying trichothecene mycotoxins in both agricultural products and biomedical samples; however, extensive treatment to clean up the sample is required before derivatization and subsequent analysis. By the most sensitive procedures, detection limits for trichothecene mycotoxins is 10 ppb. If the analysis is on a sample that contains an unknown toxic material, such as that from a yellow rain attack, then the GLC method can provide only presumptive evidence of a trichothecene mycotoxin exposure. Confirmation requires identification with more definitive physicochemical procedures.

MS is the physicochemical method of choice for characterizing, identifying, and confirming the presence of trichothecene mycotoxins. Picogram quantities of trichothecene mycotoxins are readily detectable by MS methods. In some cases, extensive cleanup steps are unnecessary. The combination of GLC and MS techniques (GLC–MS) has proven to be a more specific method for identifying mycotoxins than GLC

alone,[132,133] and it has become the standard for identifying trichothecene mycotoxins in agricultural products and biomedical samples. As little as 1 ppb of T-2 toxin can be identified without extensive cleanup[132]; however, the method requires a time-consuming derivatization step. A high-performance liquid chromatography–mass spectrometry (HPLC–MS) procedure, described in 1991, provides a specific, reliable method for identifying trichothecene mycotoxins without derivatization,[134] achieving sensitivity at the 0.1-ppb level. HPLC-MS and GLC-MS are the best and most sensitive methods for detecting mycotoxins. Additionally, HPLC-MS can be used with diode array detection (DAD), which measures the ultraviolet spectrum of a sample. HPLC-DAD-MS limits of detection range from 1 pg to 3 ng.

Medical Management

Prexposure Treatment and Decontamination

The immediate use of protective clothing and mask at the first sign of a yellow-rain–like attack should protect an individual from the lethal effects of this mycotoxin. Because the area covered with agent is likely to be small, another helpful tactic is to simply leave the area. A lightweight face mask, outfitted with filters that block the penetration of aerosol particles 3 to 4 μm or larger, should provide respiratory protection against yellow rain. Only 1% to 2% of aerosolized T-2 toxin penetrated nuclear, biological, and chemical protective covers.[135] Regular military uniforms would offer some protection, depending on the age and condition of the fabric as well as the environmental conditions.

Skin exposure reduction paste against chemical warfare agents (SERPACWA), a Food and Drug Administration-approved preexposure skin treatment for use against chemical warfare agents and dermally active toxins, functions by forming a physical barrier on the skin. SERPACWA is designed for application at closure points of chemical over-garments—the neck, wrists, and ankles—as well as sweat-prone areas such as the armpits and groin. When SERPACWA was applied to anesthetized rabbits that were then exposed to a 6-hour challenge with T-2 mycotoxin, all signs of dermal irritation were blocked for 24 to 48 hours. However, SERPACWA must be applied before an attack; it is not effective after exposure.

As soon as individuals or units suspect exposure to a mycotoxin attack, they should remove their uniform, wash their contaminated skin with soap and water, and then rinse with water. Washing the contaminated skin area within 4 to 6 hours after exposure to T-2 toxin removes 80% to 98% of the toxin, thus preventing dermal lesions and death in laboratory animals.[13] Contaminated uniforms as well as wash waste from personnel decontamination should be exposed to household bleach (5% sodium hypochlorite) for 6 hours or more to inactivate any residual mycotoxin. The M291 decontamination kit for skin contains an XE-555 resin material as the active component, which is efficacious against most chemical warfare agents and presents no serious human safety problems. The XE-556 resin, a similar but different formulation, was effective in the physical removal of T-2 toxin from the skin of rabbits and guinea pigs.[136] The foregoing observations suggest that skin decontamination kits designed specifically for detoxification of chemical warfare agents could also provide protection by physically removing mycotoxins from the skin of exposed individuals.

Specific and Supportive Therapy

No specific therapy for trichothecene-induced mycotoxicosis is known or is presently under experimental evaluation. Several therapeutic approaches have been evaluated in animal models. Although experimental procedures for treating systemic exposure have successfully reduced mortality in animal models, they have not been tested in primates, and they are not available for field use in humans potentially exposed to trichothecene mycotoxins.

Individuals exposed to a yellow-rain–like attack should be treated with standardized clinical toxicology and emergency medicine practices for ingestion of toxic compounds. After an aerosol exposure, mycotoxins will be trapped in the nose, throat, and upper respiratory tract. The particles will be swallowed via ciliary action, resulting in a significant oral exposure. Superactive charcoal has a very high maximal binding capacity (0.48 mg of T-2 toxin per mg of charcoal), and treatment either immediately or 1 hour after oral or parenteral exposure to T-2 toxin significantly improves the survival of mice.[137]

Symptomatic measures for treating those exposed to trichothecene mycotoxins are modeled after casualty care for mustard poisoning. Irrigation of the eyes with large volumes of isotonic saline may assist in mechanically removing trichothecene mycotoxins, but such treatment would have limited useful therapeutic effects. Casualties with ocular involvement will likely need detailed ophthalmologic evaluation for corneal lesions and treatment to prevent vision loss, secondary infection, and the development of posterior synechie. After the skin has been decontaminated, some erythema may appear and accompany burning and itching sensations. Most casualties whose skin has

been treated with soap and water within 12 hours of exposure will have mild dermal effects, which can be relieved by calamine and other lotions or creams.

Limited data are available on the respiratory effects of inhaled trichothecene mycotoxins, although acute pulmonary edema was one of the serious, often lethal, consequences of a yellow rain attack. One of the major symptoms after the yellow rain attacks was an upper respiratory irritation consisting of sore throat, hoarseness, and nonproductive cough, which may be relieved by steam inhalation, codeine, cough suppressants, and other simple measures. A casualty who develops severe respiratory symptoms may require endotracheal intubation with positive pressure ventilation to maintain airway patency and oxygenation. A physician trained in pulmonary or intensive care medicine should conduct any required advanced airway management, with a focus upon maintaining ventilation and oxygenation, as well as preventing secondary infection. Theoretically, granuloctye-stimulating factors may be useful for patients who develop bone marrow suppression.

The early use of high doses of systemic glucocorticosteriods increases survival time by decreasing the primary injury and shock-like state that follows exposure to trichothecene mycotoxins.[138] Additionally, dosing before and after the exposure with diphenhydramine (an antihistamine) or naloxone (an opioid antagonist) prolonged the survival times of mice exposed subcutaneously or topically with lethal doses of T-2 toxin.[139]

Several bioregulators might mediate the shock-like state of trichothecene mycotoxicosis. Methylthiazolidine-4-carboxylate increased hepatic glutathione content and enhanced mouse survival after an acute intraperitoneal exposure to T-2 toxin.[140] The protective effects of this drug may result from increased detoxification and excretion of the glucuronide conjugate of T-2 toxin. A general therapeutic protocol that included combinations of metoclopramide, activated charcoal, magnesium sulfate, dexamethasone, sodium phosphate (which had very little effect), sodium bicarbonate, and normal saline was evaluated in swine given an intravenous LD_{50} dose of T-2 toxin.[141] All treatment groups showed improved survival times compared to survival of the nontreated controls.

Prophylaxis

To date, there is no licensed vaccine to protect against the mycotoxins. The mycotoxins are low–molecular-weight compounds that must be conjugated to a carrier protein to produce an effective antigen.[130] When T-2 toxin is conjugated to a protein, it elicits relatively low antibody titers and remains a marked skin irritant.[142] This would preclude the use of mycotoxins as immunogens in eliciting protective immunity. To circumvent such problems, a deoxy-verrucarol–protein conjugate was used to vaccinate rabbits.[143] Antibody titers developed rapidly after vaccination, but they were highly specific for the conjugate rather than for a common trichothecene backbone.

Another approach was to develop antibody-based (antiidiotype) vaccines against T-2 toxin. Protective monoclonal antibodies were generated and used to induce specific monoclonal antiidiotypic antibodies. When mice were vaccinated with these antibodies, they developed neutralizing titers that protected against challenge with a lethal dose of T-2 toxin.[144] Thus, an antiidiotypic antibody would be feasible as a vaccine candidate against T-2 toxin.

Several monoclonal antibodies against T-2 toxin will protect against the T-2–induced cytotoxicity in various cell lines. When a monoclonal antibody against T-2 toxin (15H6) was given to rats (250 mg / kg) 30 minutes before or 15 minutes after a lethal dose of mycotoxin, it protected 100% of them.[145] Thus, monoclonal antibodies do have some prophylactic and therapeutic value against T-2 toxicosis, but very large quantities are required for protection.

Prophylactic induction of enzymes involved in conjugating xenobiotics reduced or prevented the acute toxic effects of T-2 toxin in rats, whereas inhibition of these enzymes resulted in a higher toxicity.[146] Pretreatment with flavonoids, ascorbic acid, vitamin E, selenium, or chemoprotective compounds such as emetine that block trichothecene–cell association all reduce acute toxicity of these mycotoxins. However, none of these chemoprotective treatments has undergone extensive efficacy studies to evaluate their ability to protect against an aerosol or dermal exposure to trichothecene mycotoxins.

MARINE ALGAL TOXINS

History

Marine biotoxins are a problem of global distribution, estimated to cause more than 60,000 foodborne intoxications annually. In addition to human morbidity, some marine toxins may cause massive fish kills, such as those occurring during the Florida red tides, and others have been implicated in mass mortalities of birds and marine mammals. However, their presence in the environment is more often "silent," detectable only when contaminated foodstuffs are ingested. The long-term environmental and public health effects of

chronic exposure in humans are poorly understood, although questions are beginning to arise about whether chronic exposures to some marine toxins may increase the risk of cancer through their action as tumor promoters.

Ingesting seafood contaminated with marine biotoxins can cause six identifiable syndromes: (1) paralytic shellfish poisoning (PSP), (2) neurotoxic shellfish poisoning (NSP), (3) ciguatera fish poisoning, (4) diarrheic shellfish poisoning, (5) amnesic shellfish poisoning (ASP), and (6) azaspiracid poisoning. With the exception of ciguatera fish poisoning, which, as the name implies, is caused by eating contaminated finfish, all are caused by ingesting shellfish. With the exception of ASP, which is of diatom origin, the causative toxins all originate from marine dinoflagellates.

The toxin-producing algal species are a small fraction of the thousands of known phytoplankton. However, under the proper environmental conditions, they can proliferate to high cell densities known as blooms. During these blooms, they may be ingested in large quantities by zooplankton, filter-feeding shellfish, and grazing or filter-feeding fishes. Through these intermediates, toxins can be vectored to humans who consume the seafood.

In general, marine algal toxins are not viewed as important biological warfare threat agents for many reasons. Marine toxins occur naturally at low concentrations in wild resources, and extraction of large quantities is difficult. Most are nonproteinaceous and therefore not amenable to simple cloning and expression in microbial vectors. Although some toxins can be harvested from laboratory cultures of the toxic organism, yields are insufficient to supply the large amounts required for the development of traditional biological warfare weaponry.

Targeting food supplies as an act of biological terrorism is a much more likely scenario. The toxins occur naturally in seafood products in concentrations sufficient to cause incapacitation or death. The contaminated foodstuffs appear fresh and wholesome, and cannot be differentiated from nontoxic material except by chemical analysis. This negates the requirement for isolation of large quantities of pure toxins and subsequent adulteration of the food supply. In theory, the toxic seafood needs only to be harvested and then inserted into the food supply at the desired location. Regulatory testing, if any, is typically done only at the harvester and distributor levels.

In some cases, harvesting toxic seafood is difficult. In the case of ciguatoxin, contaminated fish are typically a small percentage of the catch, and levels of toxin within toxic fish tissues are low. In other cases, harvesting could be easy. The United States and other countries maintain monitoring programs at the state and local level to ensure consumer safety. On the US Gulf coast, concentrations of toxin-producing dinoflagellate *Karenia brevis* in the water column are closely monitored. When cell numbers increase to levels suggestive of an imminent bloom, harvesting of shellfish is officially halted. The shellfish are then monitored by chemical analysis or mouse bioassay until toxin concentrations in the edible tissues fall to safe levels, at which point harvesting is allowed to resume. During the period when shellfish are toxic, information is made available through the news media and regulatory agencies to discourage recreational harvesting, and anyone could conduct surreptitious harvesting during that time.

Of the six marine toxin syndromes, three—ciguatera fish poisoning, diarrheic shellfish poisoning, and azaspiracid poisoning—are unlikely to be a significant bioterrorism threat. Diarrheic shellfish and azaspiracid poisoning cause mild to moderate intoxications that are self-limiting and likely to be mistaken for common gastroenteritis or bacterial food poisoning; the syndromes are unlikely to cause the kind of turmoil sought by terrorists. Ciguatera fish poisoning can present a much more serious intoxication, but toxic fish are extremely difficult to procure. Acquiring sufficient material to launch a food-related bioterrorist attack of any magnitude is nearly impossible.

The three marine algal toxin syndromes with bioterrorism potential and the causative toxins (Table 17-1) are described in the following section. Some are a greater concern for homeland security than others. Issues that may impact or limit their potential use as weapons of bioterror will be discussed, followed by clinical aspects and treatment.

Paralytic Shellfish Poisoning

Description of the Toxin

PSP results from exposure to a family of heterocyclic guanidines called paralytic shellfish poisons, or gonyautoxins. STX was the first known member of this family, named for the giant butter clam, *Saxidoma giganteus*, from which it was first isolated.[147] Later it was learned that STX is the parent compound of over 20 derivatives of varying potency produced by marine dinoflagellates of the genera *Alexandrium* (previously *Gonyaulax*), *Pyrodinium*, and *Gymnodinium*, as well as several species of freshwater cyanobacteria. More recently, STX was isolated from bacterial species associated with dinoflagellate cells, suggesting the possibility of a bacterial origin for at least some dinoflagellates.[148] STX also occurs in other benthic marine

organisms, such as octopi and crabs, from which the ultimate source of toxin is unknown but assumed to be the food web.[149]

In humans, the greatest risk is associated with consumption of filter-feeding mollusks such as clams, mussels, and scallops that ingest dinoflagellate cells during bloom conditions or resting cysts from the sediment. The original toxin profiles in the dinoflagellate cells may be metabolically altered by the shellfish. Ingestion by humans results in signs and symptoms characteristic of PSP. Approximately 2,000 cases occur annually across regions of North and South America, Europe, Japan, Australia, Southeast Asia, and India. The overall mortality rate has been estimated at 15%,[150] although mortality is highly dependent upon the quality of medical care received.

Mechanism of Action

STX and its derivatives elicit their toxic effects by interacting with the voltage-dependent sodium channels in electrically excitable cells of heart, muscle, and neural tissue. High-affinity binding to a specific binding site (denoted neurotoxin binding site 1) on sodium channels blocks ionic conductance across the membranes, thereby inhibiting nerve polarization. Although voltage-dependent sodium channels in many tissues are susceptible to these toxins, pharmacokinetic considerations make the peripheral nervous system the primary target in seafood intoxications.

Clinical Signs and Symptoms

Ingestion. Ingestion of PSP toxins results in a rapid onset (minutes to hours) complex of paresthesias, including a circumoral prickling, burning, or tingling sensation that rapidly progresses to the extremities. At low doses, these sensations may disappear in a matter of hours with no sequelae. At higher doses, numbness can spread to the trunk, and weakness, ataxia, hypertension, loss of coordination, and impaired speech may follow.

A 20-year retrospective analysis of PSP documented by the Alaska Division of Public Health from 1973 to 1992 revealed 54 outbreaks involving 117 symptomatic patients. The most common symptom in these outbreaks was parasthesia, and 73% of patients had at least one other neurological symptom. Other documented symptoms in descending order of occurrence included perioral numbness, perioral tingling, nausea, extremity numbness, extremity tingling, vomiting, weakness, ataxia, shortness of breath, dizziness, floating sensation, dry mouth, diplopia, dysarthria, diarrhea, dysphagia, and limb paralysis.[151]

Approximately 10 outbreak-associated PSP cases are reported to the Centers for Disease Control and Prevention each year. In 2002 there were 13 cases of neurological illness associated with consumption of pufferfish containing STX caught near Titusville, Florida.[152] All 13 symptomatic patients reported tingling or numbness in the mouth or lips. Additionally, eight reported numbness or tingling of the face, ten

TABLE 17-1

COMPARISON OF SELECTED MARINE ALGAL TOXINS

	Paralytic Shellfish Poisoning	Neurotoxic Shellfish Poisoning	Amnesic Shellfish Poisoning
Toxin	Gonyautoxins (saxitoxin)	Brevetoxins	Domoic acid
Source	Marine dinoflagellates	*Karenia brevis*	*Pseudo-nitzschia* multiseries
Mechanism of action	Binds to site 1 of voltage-dependent sodium channels, leading to inhibition of nerve polarization.	Binds to site 5 of voltage-dependent sodium channels and prevents channel inactivation.	Binds to kainate and AMPA subtypes of glutamate receptors in the central nervous system, leading to excitotoxic effects and cell death.
Clinical manifestations	Circumoral parasthesias that may rapidly progress to the extremities. May result in diplopia, dysarthria, and dysphagia. Progression may lead to paralysis of extremities and respiratory musculature.	Symptoms similar to paralytic shellfish poisoning, but usually milder. Nausea, diarrhea, and abdominal pain. Neurological symptoms include oral parasthesias, ataxia, myalgia, and fatigue.	Vomiting, diarrhea, and abdominal cramps, which may be followed by confusion, disorientation, and memory loss. Severe intoxications may result in seizures, coma, or death.

AMPA: alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

reported these symptoms in the arms, seven reported these symptoms in the legs, and one reported these symptoms in the fingertips. Six of the 13 patients experienced nausea, and four reported vomiting. Symptoms began between 30 minutes and 8 hours after ingestion, with a median of 2 hours. The illness lasted from 10 hours to 45 days, with a median of 24 hours. All of these cases resolved.

At lethal doses, paralysis of the respiratory musculature results in respiratory failure. Intoxication of a 65-year-old female in the Titusville case series is illustrative. The patient experienced perioral tingling within minutes of meal ingestion. Her symptoms worsened over the next 2 hours, and she experienced vomiting and chest pain. Emergency department evaluation noted mild tachycardia and hypertension. Over the next 4 hours, she developed an ascending paralysis, carbon dioxide retention, and a decrease in vital capacity to less than 20% predicted for her age, which led to intubation and mechanical ventilation. She regained her reflexes and voluntary movement within 24 hours and was extubated in 72 hours.[153]

Children appear to be more susceptible than adults. The lethal dose for small children may be as low as 25 μg of STX equivalents, whereas that for adults may be 5 to 10 mg of STX equivalents.[144] In adults, clinical symptoms probably occur upon ingestion of 1- to 3-mg equivalents. Because shellfish can contain up to 10 to 20 mg equivalents per 100 grams of meat, ingestion of only a few shellfish can cause serious illness or death.[154,155]

Fortunately, clearance of toxin from the body is rapid. In one series of PSP outbreaks in Alaska resulting from the ingestion of mussels, serum half-life was estimated at less than 10 hours. In these victims, respiratory failure and hypertension resolved in 4 to 10 hours, and toxin was no longer detectable in the urine 20 hours postingestion.[155]

Inhalation. In mice, STX is significantly more toxic by inhalation (LD_{50} of 2 μg/kg) or by intraperitoneal injection (LD_{50} of 10 μg/kg) than by oral administration (LD_{50} of 400 μg/kg).[156] Unlike PSP in humans, which is an oral intoxication and has a lag time to toxicity resulting from absorption through the gastrointestinal tract, inhalation of STX can cause death in animals within minutes. At sublethal doses, symptoms in animals appear to parallel those of PSP, albeit with a more rapid onset reflective of rapid absorption through the pulmonary tissues.

Cause of Death

The cause of death in human cases of STX ingestion, as well as in experiments with animal models, is respiratory failure. Postmortem examination of STX victims reveals that the most notable effects are on the respiratory system, including pulmonary congestion and edema, without abnormalities of the heart, coronary arteries, or brain.[157,158] In vitro, STX does not directly affect the smooth muscle of airways or large blood vessels, but in vivo axonal blockade may lead to respiratory failure and hypotension.[159] Intoxication with large doses of STX may lead to metabolic acidosis, cardiac dysrhythmias, and cardiogenic shock, even with correction of ventilatory failure.[160]

Diagnosis

Clinicians should consider PSP in patients who present with rapid onset of neurological symptoms that are sensory, cerebellar, and motor in nature and occur shortly after consumption of seafood.

Confirmatory diagnosis should rely on analysis of body fluid samples, including serum and urine, as well as analysis of gastric contents or uneaten portions of recent meals. Animal studies have demonstrated that STX is excreted primarily in urine. After intravenous injection of STX in rats, 19% of the toxin was excreted 4 hours after injection. By 24 hours, 58% of the toxin was excreted, but small quantities of unmetabolized STX were still detected up to 144 hours after administration.

Postmortem examinations of fatally intoxicated humans have identified STX in gastric contents; body fluids including serum, urine, bile, and cerebrospinal fluid; and tissues including the liver, kidneys, lungs, stomach, spleen, heart, brain, adrenal glands, pancreas, and thyroid.[157,158] The largest concentrations of STX were in the gastric contents and urine.

Food or clinical samples can be evaluated by several methods. The traditional "gold-standard" method is the mouse bioassay, which is an official method of the Association of Official Analytical Chemists. HPLC can detect individual toxins but requires either precolumn or postcolumn derivatization of toxin mixtures for optimal detection.[161,162] Receptor-binding assays based on either rat brain membranes[163] or purified STX-binding proteins from frogs or snakes[164] measure total biological activity regardless of toxin profile. All of these have been used to detect paralytic shellfish poisons in the urine and serum of intoxicated victims.[155] Antibody-based assays can detect major toxins, but cross-reactivity among minor paralytic shellfish poisons is highly variable. Rapid-test kits are now commercially available.

Medical Management

Treatment for STX intoxication is supportive care. Patients who have recently ingested the toxin may benefit from gastric lavage to expedite removal of the

toxin from the gastrointestinal tract. Patients need to be monitored closely for at least 24 hours, and if signs of respiratory compromise occur, aggressive respiratory management should be instituted. Intravenous fluids should be used judiciously to maintain urine output and blood pressure. Intoxication with large doses of STX or intoxication in patients with underlying medical conditions may lead to cardiovascular abnormalities including hypotension, T-wave inversions, dysrhythmias, and cardiogenic shock. Sodium bicarbonate may be required for correction of severe metabolic acidosis. Vasopressor agents should be used to maintain blood pressure and perfusion of vital organs. Dobutamine may be the preferred agent; in experiments with high doses of STX given to cats intravenously, dobutamine improved recovery over dopamine.[160]

There is no specific therapy for patients with STX intoxication. Research into specific therapies has included use of anti-STX serum and antibodies as antidotes, and the use of pharmacologic agents to overcome inhibition of the voltage-dependent sodium channel.

Because of its high potency and relative stability, STX must be considered a potential bioterrorist threat agent. Toxins are easily isolated from laboratory cultures, but production constraints would limit the scope of an aerosol attack. The more likely threat is through the food supply, with the vector being naturally contaminated fresh shellfish. Blooms of the causative organisms occur annually on both the Atlantic and Pacific coasts of the United States and Canada, as well as elsewhere around the world, often in underdeveloped nations with poor screening programs. Toxins can easily reach lethal levels in filter-feeding shellfish. Threats to the water supply are minimal. Small-scale contamination (eg, of water coolers) is feasible, but large-scale contamination of reservoirs or even water towers is unlikely to be successful because of dilution effects and the reduced potency of the oral route.

Neurotoxic Shellfish Poisoning

Description of the Toxin

NSP results from exposure to brevetoxins, a group of cyclic polyether toxins produced by the marine dinoflagellate *K brevis* (formerly *Ptychodiscus brevis* or *Gymnodinium breve*). Blooms of *K brevis*, with the associated discolored water and mass mortalities of inshore fish, have been described in the Gulf of Mexico since 1844.[165] As are paralytic shellfish poisons, brevetoxins are typically vectored to humans through shellfish, although in the case of NSP, the proximal agents are actually molluscan metabolites of the parent brevetoxins.[166] In addition to causing NSP, annual blooms of *K brevis* in the Gulf of Mexico can cause significant revenue losses in the tourism and seafood industries. Beachgoers can be especially affected because the unarmored dinoflagellates are easily broken up by rough wave action, and the toxins become aerosolized into airborne water droplets, causing respiratory irritation and potentially severe bronchoconstriction in people with asthma.

Historically, NSP has been virtually nonexistent outside the Gulf of Mexico. However, in 1993 an outbreak was reported in New Zealand. In 2000 blooms of another dinoflagellate, *Chattonella verruculosa*, occurred in Rehoboth Beach, Delaware, and caused a series of localized fish kills.[167] Although no cases of NSP were reported, these events suggest a possible NSP range extension.

Mechanism of Action

Brevetoxins exert their physiological effects by binding with high affinity and specificity to neurotoxin receptor site 5 on the voltage-dependent sodium channel.[168] Unlike STX, which inhibits the sodium channel by binding to site 1, binding of brevetoxins to site 5 prevents channel inactivation. This shifting of the voltage-dependence of channel activation leads to channel opening at lower membrane potentials[169] and inappropriate ionic flux. Clinical effects are typically more centrally mediated than peripherally mediated.

Brevetoxin can cross the blood–brain barrier, and it hypothetically leads to injury and death of cerebellar neurons by stimulation of glutamate and aspartate release, activation of the N-methyl-D-aspartate receptor, and excitotoxic cell death.[170] A detailed review of the molecular pharmacology and toxicokinetics of brevetoxin can be found in Poli's *Recent Advances in Marine Biotechnology, Volume 7: Seafood Safety and Human Health*.[171]

Clinical Signs and Symptoms

Ingestion. Symptoms of NSP are similar to that of PSP, but are usually milder. Manifesting within hours after ingestion of contaminated seafood, symptoms include nausea, diarrhea, and abdominal pain. Typical neurological symptoms are oral paresthesia, ataxia, myalgia, and fatigue. In more severe cases, tachycardia, seizures, loss of consciousness, and respiratory failure can occur. During a 1987 outbreak, 48 cases of NSP occurred in the United States. Acute symptoms documented in the outbreak included gastrointestinal (23% of cases) and neurological (39% of cases) symptoms. Symptoms occurred quickly, with a median of 3

hours to onset, and lasted up to 72 hours. Most of the victims (94%) experienced multiple symptoms, and 71% reported more than one neurological symptom.[172] Although a fatal case of NSP has never been reported, children may be more susceptible, and a fatal dose must be considered a possibility.[166]

The toxic dose of brevetoxins in humans has not been established. However, important information has recently been gleaned from a clinical outbreak. In 1996 a father and two small children became ill after ingesting shellfish harvested in Sarasota Bay, Florida. Both children were hospitalized with severe symptoms, including seizures. Brevetoxin metabolites were detected in urine collected 3 hours postingestion. With supportive care, symptoms resolved in 48 to 72 hours, and no brevetoxin was detectable in the urine 4 days postingestion.[166] Mass chromatography of serum samples taken immediately after the family checked into the hospital demonstrated ion masses suggestive of brevetoxin metabolites, although these compounds were never isolated. The amount of toxin ingested was not determined, although the father, who had milder symptoms and was released from the hospital after treatment, reported eating "several" shellfish. The number eaten by the children (ages 2 and 3) were unknown.

The toxicity of brevetoxins in mice is well established. LD_{50} values range from 100 to 200 μg/kg after intravenous or intraperitoneal administration for PbTx-2 and PbTx-3, the two most common congeners. Oral toxicity is lower: 500 and 6600 μg/kg for PbTx-3 and PbTx-2, respectively.[173] Animal models indicate brevetoxin is excreted primarily in the bile, although urinary elimination is also significant. Toxin elimination is largely complete after 72 hours, although residues may remain in lipid-rich tissues for extended periods.[174]

Inhalation. Respiratory exposure may occur with brevetoxins associated with harmful algal blooms or "red tides." As the bloom progresses, the toxins are excreted and released by disruption of the dinoflagellate. Bubble-mediated transport of these toxins leads to accumulation on the sea surface; the toxins are released into the air by the bursting bubbles. The toxins are then incorporated into the marine aerosol by on-shore winds and breaking surf, leading to respiratory symptoms in humans and other animals. Sea foam may also serve as a source of toxin and result in symptoms if it is ingested or inhaled. During harmful algal blooms, the on-shore concentration of aerosolized toxins varies along beach locations by wind speed and direction, surf conditions, and exposure locations on the beach. Concentrations of the toxin are highest near the surf zone.[175]

Systemic toxicity from inhalation is a possibility. Distribution studies of intratracheal instillation of brevetoxin in rats have shown that the toxin is rapidly cleared from the lung, and more than 80% is distributed throughout the body. Twenty percent of the initial toxin concentration was present in several organs for 7 days.[176]

Diagnosis

Brevetoxin intoxication should be suspected clinically when patients present with gastrointestinal symptoms and neurological symptoms occurring shortly after ingesting shellfish. Although these symptoms may be similar to those of STX intoxication, they do not progress to paralysis. Epidemiological evaluation of cases may identify additional cases during an outbreak and allow for public health measures, including surveillance, to be put into place.

Human cases are typically self-limiting, with improvement in 1 to 3 days, but symptoms may be more severe in the young, the elderly, or those with underlying medical conditions. Evaluation of biological samples should include urine as well as any uneaten shellfish from the meal.

Toxins in clinical samples can be detected by liquid chromatography mass spectrometry receptor-binding assays, or immunoassay. Because metabolic conversion of parent toxins occurs in shellfish and the metabolites are apparently less active at the sodium channel, it appears that immunoassays are better screening tools. However, secondary metabolism in humans has yet to be fully investigated.

Medical Management

There is no specific therapy for NSP. If the ingestion is recent, treatment may include removal of unabsorbed material from the gastrointestinal tract or binding of residual unabsorbed toxin with activated charcoal. Supportive care, consisting of intravenous fluids, is the mainstay of therapy. Although brevetoxin has not been implicated in human fatalities, symptoms of NSP may overlap with symptoms of STX and thus warrant observation for developing paralysis and respiratory failure. Aggressive respiratory management may be required in severe cases.

Pulmonary symptoms resulting from inhalation of marine aerosols typically resolve upon removal from the environment, but may require treatment for reactive airway disease, including nebulized albuterol and anticholinergics to reverse bronchoconstriction. Mast cell release of histamine may be countered with the use of antihistamines. Mast cell stabilizers, such as cromolyn,

may be used prophylactically in susceptible persons exposed to marine aerosols during red tide events.

No antitoxins for NSP are available. However, experiments with an anti-brevetoxin IgG showed that treatment before exposure blocked nearly all neurological symptoms.[177] Additional research into pharmacologic agents should be pursued. Two brevetoxin derivatives that function as brevetoxin antagonists but do not exhibit pharmacologic properties have been identified. Other agents that compete with brevetoxin binding for the sodium channel include gambierol, gambieric acid, and brevenal.[178,179] Future research with these agents may assist in developing adequate therapeutics.

Brevetoxins are likely to have only moderate potential as agents of bioterror. Although unlikely to cause mortality in adults, oral intoxication can be severe and require hospitalization. Disruption of a local event, inundation of medical facilities by the "worried well," and societal overreaction possibly leading to economic disruption of local industry are the most likely repercussions. *K brevis* is easily cultured and produces toxins well in culture. Unpublished animal experiments suggest brevetoxins may be 10-fold to 100-fold more potent by aerosol, versus oral, exposure. Thus, small-scale aerosol attacks are technically feasible, although isolation and dissemination of toxins would be difficult for nonexperts.

Amnesic Shellfish Poisoning

Description of the Toxin

ASP was defined after an outbreak of mussel poisoning in Prince Edward Island, Canada, in 1987. Over 100 people became ill with an odd cluster of symptoms, and three died. Canadian researchers quickly isolated the causative agent and identified it as domoic acid.[180] Domoic acid was previously known as a compound tested and rejected as a potential insecticide and is a common ingredient in Japanese rural folk medicine. Domoic acid was originally isolated from a red alga, and researchers were surprised to discover that the diatom *Pseudo-nitzschia pungens f multiseries* (now *Pseudo-nitzschia multiseries)* was its causative organism. ASP remains the first and only known seafood toxin produced by a diatom.

Since the 1987 outbreak, toxic species of *Pseudo-nitzschia* have been found around the world and are now the subject of many regional monitoring programs. Domoic acid is seasonally widespread along the US Pacific coast and the Gulf of Mexico. It has also been found in New Zealand, Mexico, Denmark, Spain, Portugal, Scotland, Japan, and Korea. Although amounts of domoic acid in shellfish occasionally reach levels sufficient to stimulate harvesting bans, no further human cases have been reported, reflecting the efficacy of monitoring programs. However, the toxicity of domoic acid remains evident in biotic events.

In 1991 numerous cormorants and pelicans died after feeding on anchovies (a filter-feeding fish) during a bloom of *P australis* in Monterey Bay, California. High levels of domoic acid were detected in the gut contents of the anchovies. Later that year, after the bloom moved northward along the coast, razor clams and Dungeness crabs became toxic off the Washington and Oregon coasts. Several cases of human intoxication apparently followed ingestion of razor clams, although a definitive link was not found.[181] In 1998 over 400 sea lions died and numerous others became ill after ingesting anchovies feeding in a bloom of *P australis*, again in Monterey Bay.[182] Domoic acid was detected in both the anchovies and feces from the sea lions.[183] These events suggest that periodic blooms of domoic-acid–producing *Pseudo-nitzschia* on the western coast of the United States may cause significant toxicity in seafood items.

Mechanism of Action

Domoic acid is a neuroexcitatory amino acid structurally related to kainic acid. As such, it binds to the kainate and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid subtypes of the glutamate receptor in the central nervous system, which subsequently elicits nonsensitizing or very slowly sensitizing currents.[184] This causes a protracted influx of cations into the neurons and stimulates a variety of intracellular events leading to cell death.[185] This effect may be potentiated by synergism with the excitotoxic effects from high glutamate and aspartate levels found naturally in mussel tissue.[186] The kainate and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors are present in high densities in the hippocampus, a portion of the brain associated with learning and memory processing. Mice injected with domoic acid develop working memory deficits.[187] Neuropathological studies of four human fatalities revealed neuronal necrosis or loss with astrocytosis, mainly affecting the hippocampus and the amygdaloid nucleus.[188]

Clinical Signs and Symptoms

Ingestion. The 1987 Prince Edward Island outbreak provided information on the clinical effects of domoic acid ingestion in humans.[189] The outbreak occurred during November and December, with 250 reports of illness related to mussel consumption (107 of these

reports met classic case definition). All but seven of the patients reported gastrointestinal symptoms ranging from mild abdominal discomfort to severe emesis requiring intravenous hydration. Forty-three percent of patients reported headache, frequently characterized as incapacitating, and 25% reported memory loss, primarily affecting short-term memory.

At higher doses, confusion, disorientation, and memory loss can occur. Severe intoxications can produce seizures, coma, and death. Nineteen of the patients required hospitalization for between 4 and 101 days, with a median hospital stay of 37.5 days. Twelve patients required care in an intensive care unit. The intensive care patients displayed severe neurological dysfunction, including coma, mutism, seizures, and purposeless chewing and facial grimacing.[189] Severe neurological manifestations, more common in the elderly, included confusion, disorientation, altered states of arousal ranging from agitation to somnolence or coma, anterograde memory disorder, seizures, and myoclonus. Although mean verbal and performance IQ scores were in the average range and language tests did not reveal abnormalities, severe memory deficits included difficulty with initial learning of verbal and visuospatial material, with extremely poor recall. Some of the more severely affected patients also had retrograde amnesia that extended to several years before ingestion of the contaminated mussels.[188] Nine of the intensive care patients required intubation for airway control resulting from profuse secretions, and seven of them suffered unstable blood pressures or cardiac dysrhythmias. Three patients died during their hospitalization.[189]

Symptoms of intoxication occur after a latency period of a few hours. In the outbreak's mild cases, the gastrointestinal symptoms of vomiting, diarrhea, and abdominal cramps occurred within 24 hours. The time from ingestion of the mussels to symptom onset ranged from 15 minutes to 38 hours, with a median of 5.5 hours.[189] In a study of 14 patients who developed severe neurological manifestations, 13 developed gastrointestinal symptoms between 1 and 10 hours after ingestion of seafood, and all of the patients became confused and disoriented 1.5 to 48 hours postingestion. Maximal neurological deficits were seen 4 hours after mussel ingestion in the least affected patients and up to 72 hours postingestion in those patients who became unresponsive.[188] All the patients who developed severe neurological symptoms were older than 65 or had preexisting medical conditions such as diabetes or renal failure that altered their renal clearance.

Inhalation. There are no natural cases of domoic acid inhalation, and no experimental models have evaluated an aerosol exposure to this toxin. It may be assumed that the toxin would be absorbed through the pulmonary tissues leading to systemic symptoms comparable to that of other exposure routes, although no data are available to confirm this theory.

Diagnosis

Diagnosis should be suspected by the clinical presentation after ingestion of a seafood meal. Patients may have mild symptoms that resolve spontaneously or may present with more severe signs of neurotoxicity, including confusion, altered mental status, or seizures. Symptomatic patients typically are over the age of 65 or have underlying medical conditions that affect renal clearance.[189] Initial evaluation of these patients should include standard protocols for patients with altered mental status, including toxicological screens to rule out more common intoxicants, especially illicit substances. Other diagnostic tests that may be used to rule out other clinical causes of the symptoms include imaging with computed tomography scans, which do not show abnormality related to domoic acid intoxication, and monitoring of brain activity with electroencephalogram. Of the 12 patients that were admitted to the intensive care unit during the 1987 outbreak, electroencephalograms showed that nine had generalized slow-wave activity and two had localized epileptogenic activity.[189] Positron emission tomography scanning of four patients with varying degrees of illness revealed a correlation between glucose metabolism in the hippocampus and amygdala with memory scores.[188]

Based primarily on levels measured in Canadian shellfish after the 1987 outbreak, it is thought that mild symptoms in humans might appear after ingestion of approximately 1 mg/kg of domoic acid, and severe symptoms may follow ingestion of 2 to 4 mg/kg. The current regulatory limit for shellfish in Canada, the United States, and the European Union is 20 μg/g, although the European Union is revising this downward. The official regulatory testing method uses analytical HPLC, although both immunological methods and a simple, inexpensive TLC method are available.[190–192] There is no evidence of domoic acid metabolism by rodents or primates, as shown by recovery in an unchanged form from the urine or feces.[193] Samples to be included for definitive testing include serum, feces, urine, and any uneaten portions of the suspected meal.

Medical Management

Treatment for intoxication with domoic acid is supportive care. For patients who present early after ingesting the meal, gastric lavage or cathartics may

decrease toxin amounts absorbed systemically. A key issue with this intoxication is the maintenance of renal clearance; hydration or other measures may also be required. Additionally, severe intoxications may cause alterations in hemodynamic functions, requiring pharmacologic interventions to maintain perfusion. In the 1987 outbreak, some severely intoxicated patients developed substantial respiratory secretions requiring intubation. Patients should be monitored for seizure activity that may require anticonvulsants. Studies in mice have shown that sodium valproate, nimodipine, and pyridoxine suppress domoic-acid–induced spike and wave activity on electroencephalogram.[194]

There is no specific therapy for domoic acid intoxications. Research has revealed that competitive and noncompetitive N-methyl-D-aspartate receptor antagonists reduce the excitable amino acid cascade that leads to brain lesions.[170] Additionally, non–N-methyl-D-aspartate receptor antagonists have also been shown to antagonize domoic acid toxicity.[195]

Domoic acid should be considered a legitimate, if moderate, bioterrorist threat agent. Toxic shellfish are available, and ingestion elicits symptoms that can be life threatening. Although mass casualties are not likely, mortality can occur, and the frightening nature of the symptoms in survivors may cause the disruption sought by an aggressor.

CLOSTRIDIAL TOXINS

History

Clostridium perfringens is a gram-positive, spore-forming anaerobe commonly found throughout nature (eg, in soil, water, and the gastrointestinal tract). It is regarded as one of the most toxic bacteria known, with 17 different protein toxins described to date.[196] However, unlike several other bacterial pathogens (eg, *Listeria, Rickettsia, Salmonella, Shigella,* and *Yersinia* species), *C perfringens* pathogenesis is not generally thought to involve invasion of, and replication in, eukaryotic cells. By using technologies first developed in Robert Koch's laboratory at the Hygiene Institute of Berlin, William Welch and George Nuttall discovered the bacterium in 1892 at Johns Hopkins University in Baltimore. *C perfringens* has also been known in the literature as *Bacillus aerogenes capsulatus, Bacillus welchii,* or *Clostridium welchii*.

C perfringens consists of five toxin types (A, B, C, D, and E) as shown in Table 17-2, based upon the production of four major toxins (alpha, beta, epsilon, and iota). These toxins are lethal, dermonecrotic, and associated with a wide range of diseases and intoxications, including a rapid, life-threatening myonecrosis (gas gangrene) and various animal and human enterotoxemias (Table 17-3).

A major form of human food poisoning found worldwide is caused by another protein toxin, *C perfringens* enterotoxin, which is naturally synthesized during bacterial sporulation in the small intestine following ingestion of *C perfringens* in tainted food. Type A strains are most prevalent in the environment and most commonly linked with human disease. *C perfringens* (namely type A) has historically had a huge impact on those wounded during combat. Gangrene

TABLE 17-2

THE MAJOR TOXIN TYPES OF *CLOSTRIDIUM PERFRINGENS*

Toxin	A	B	C	D	E
Alpha	x	x	x	x	x
Beta		x	x		
Epsilon		x		x	
Iota					x

TABLE 17-3

***CLOSTRIDIUM PERFRINGENS* TOXIN TYPES AND DISEASES**

Toxin Type	Disease/Intoxication
A	Myonecrosis (gas gangrene) Necrotic enteritis of fowl and piglets Human food poisoning Antibiotic-associated diarrhea
B	Dysentery in lambs Hemorrhagic enteritis in calves, foals, and sheep
C	Necrotizing enteritis in humans (pigbel, darmbrand, or "fire-belly"), pigs, calves, goats, and foals Enterotoxemia in sheep (struck)
D	Enterotoxemia in lambs (pulpy kidney disease) and calves Enterocolitis in goats and cattle
E	Cattle and dog enteritis

from *C perfringens* (also known as clostridial myonecrosis) and other anaerobes resulting from wound contamination in the field or in nonsterile operating theaters (particularly prevalent before 1900) resulted in many amputations and deaths that would be unlikely to occur today. If administered soon after infection and the onset of disease, surgical debridement, various antibiotics (eg, beta-lactams, clindamycin, and metronidazole), and hyperbaric oxygen provide effective treatments for most cases of gangrene induced by *C perfringens*.

Protein toxins, considered the major virulence factors for *C perfringens*, have received considerable attention by various laboratories throughout the world. For example, progression of *C perfringens*-induced gangrene is linked to the alpha toxin (a zinc-dependent phospholipase C), which has profound effects upon endothelial cells, including (*a*) production of proinflammatory compounds; (*b*) aberrant binding of polymorphonuclear cells to endothelial cells in blood vessels around, but not in, the site of myonecrosis; and (*c*) enhanced vascular permeability.[197,198] Specific antibodies against alpha toxin have proven efficacious in preventing gangrene, as demonstrated by recent vaccination studies in a mouse model.[199] For many pathogens, toxins play important roles in survival, such as obtaining nutrients and thwarting the host's immune system. There are two primary modes of action described for the four major toxins produced by *C perfringens*: (1) "punching" holes in cell membranes (alpha, beta, and epsilon toxins), which causes ion imbalances and general leakiness; and (2) disruption of the actin cytoskeleton (iota toxin). In either scenario, the end result is the same: cell death. Studies of *C perfringens* from many laboratories show that the microorganism has evolved effective offensive (toxins) and defensive (toxins and spores) tools for surviving and thriving in diverse environments.

Because of recent national and international biodefense concerns, the epsilon toxin has been considered a potential problem for both civilians and the military.[200] As determined by LD_{50}, epsilon is the most potent of all *C perfringens* toxins, and ranks behind only the *C botulinum* and *C tetani* neurotoxins among all clostridial toxins. The Centers for Disease Control and Prevention have placed epsilon toxin on the category B list of select agents, along with bacterial diseases (eg, brucellosis, glanders, and typhus) and other protein toxins (eg, ricin, staphylococcal enterotoxin B). Epsilon toxin represents a potential agroterrorism threat, and is thus also deemed a select agent by the US Department of Agriculture (http://www.cdc.gov/od/sap/docs/salist.pdf).

Description of the Epsilon Toxin

Natural Occurrence

Naturally, epsilon toxin is produced by type B and D strains of *C perfringens* involved in animal (eg, cattle, goats, and sheep) enterotoxemias, which are often widespread, rapidly fatal, and economically damaging for the agriculture industry. Although *C perfringens* is considered normal intestinal flora in ruminants, types B and D cause life-threatening problems if introduced, respectively, into the digestive system in newborn animals or, after a diet change to higher carbohydrate levels (in particular starch), in older animals.[196] When there is little microbial competition, or a richer diet suddenly becomes available, resident *C perfringens* types B and D can rapidly proliferate in the intestines and produce a number of toxins, including epsilon. Epsilon toxin and *C perfringens* types B and D infections are linked to veterinary rather than human disease, which establishes an unusual scenario in the event of its use as a biological weapon against humans (possibly advantageous to the perpetrator). In such a situation, physicians would have difficulty diagnosing the resulting unusual syndrome. The following explanation of the biochemistry and biology of epsilon toxin in animals may provide useful information for a potential incident of epsilon intoxication within the general human population.

Chemical and Physical Properties

C perfringens epsilon toxin is synthesized from plasmid DNA as a 311–amino-acid "protoxin" that is subsequently activated extracellularly by proteolytic removal of small peptides at both the amino-terminal (13 residues) and carboxy-terminal (22 residues). In this sense, the toxin is resistant to inactivation by serine-type proteases commonly found throughout nature. The protoxin also contains a typical leader sequence (32 amino-terminal residues) that facilitates secretion from the bacterium into the environment. The crystal structure (Figure 17-2) reveals three domains and a shared conformation with another pore-forming toxin, aerolysin. Aerolysin is produced by *Aeromonas hydrophila* strains associated with ulcerative fish disease.[201] Proteolytic loss of the carboxy-terminus from epsilon toxin seems primarily responsible for activation and subsequent homoheptamer formation.[202] In epsilon toxin, proteolysis, a common method of activating bacterial toxins, induces conformational changes that facilitate oligomerization on the cell surface. In essence, proteolytic activation is a "protein priming" event that enables the protein toxin to act quickly after binding to a cell. Additionally, proteolysis of the amino-terminal

and carboxy-terminal on the epsilon protoxin leads to a more acidic isoelectric point, which may play a role in receptor interactions.[203] For enteric-produced toxins requiring proteolysis, the proteases synthesized by resident bacteria[204] and host[202] are bountiful.

Mechanism of Action

The mode of action for epsilon toxin involves pore formation in cell membranes facilitated by detergent-resistant membrane fractions (also known as lipid rafts) that concentrate toxin monomers into homoheptamers.[205,206] Epsilon toxin oligomers formed at 37ºC are more stable than oligomers formed at 4ºC, as shown by analysis of samples treated with detergent (sodium dodecyl sulfate) and heat before polyacrylamide gel electrophoresis.[207] Recent research suggests that these dynamic, cholesterol-rich membrane domains play important roles in many diseases elicited by bacteria (and associated toxins) and viruses.[208] Although largely unexplored, the burgeoning field of lipid rafts is apparently fertile for future therapeutic endeavors. Secondary effects of epsilon toxin involve cytoskeletal disruption,[209] which, in concert with the disrupted membrane integrity facilitating free passage of 1 kDa molecules,[210] inevitably proves lethal for an intoxicated cell. Additionally, the integrity of cell monolayers is readily disrupted by epsilon toxin,[205] which provides another clue to understanding edema involving the blood–brain barrier.[211]

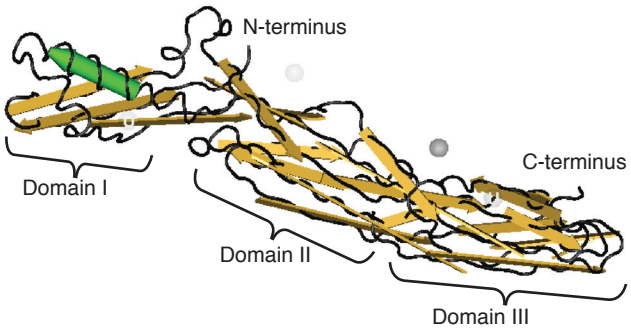

Fig. 17-2. Crystal structure of *Clostridium perfringens* epsilon protoxin. Based on analogous regions on other pore-forming toxins such as *Aeromonas hydrophila* aerolysin, there are three domains putatively involved in receptor binding (domain I), oligomerization (domain II), and membrane insertion (domain III).
Data sources: (1) Cole AR, Gibert M, Popoff MR, Moss DS, Titball RW, Basak AK. *Clostridium perfringens* epsilon-toxin shows structural similarity to the pore-forming toxin aerolysin. *Nat Struct Mol Biol*. 2004;11:797–798. (2) Chen J, Anderson JB, DeWeese-Scott C, et al. MMDB: Entrez's 3D-structure database. *Nucleic Acids Res*. 2003;31:474–477.

Clinical Signs and Symptoms

Although epsilon toxin is readily found in the heart, lungs, liver, and stomach following intoxication, it noticeably accumulates in kidneys, causing what veterinarians call "pulpy kidney disease."[196,212–214] Toxin accumulating in the kidney may represent a natural defense mechanism by the host to prevent lethal toxin concentrations in the brain.[214,215] The neurotropic and lethal aspects of *C perfringens* epsilon toxin are of utmost concern[212] (contributing to the toxin's listing as a category B select agent). Among neuronal cell populations, the neurons are most susceptible, followed by oligodendrocytes and astrocytes.[216] These neurotropic aspects cause profound effects in animals that succumb naturally to epsilon-toxin–producing *C perfringens*. Experimentally, the clinical signs attributed to epsilon toxin given intravenously to calves, lambs, and young goats occurred very quickly (in approximately 30 minutes).[217,218] The animals experienced labored breathing, excited or exaggerated movements, intermittent convulsions, loss of consciousness, and ultimately death. Results from another laboratory revealed that an intravenous injection of epsilon toxin (2–4 LD_{50}) into mice also yields seizures within 60 minutes. The intravenous LD_{50} for epsilon toxin in mice is low, at approximately 70 ng/kg.[215] Duodenal inoculation of goats with whole culture or supernatant of *C perfringens* type D led to diarrhea, respiratory distress, and central nervous system dysfunction (ie, recumbency and convulsions).[219] Similar symptoms were also evident in lambs, except for the diarrhea.[220] The mode of action for epsilon toxin in vivo likely involves ion imbalance, endothelial disruption, and edema. *C perfringens* epsilon toxin establishes a vicious cycle in the gut, with increased permeability of the intestinal tract leading to higher circulating levels of toxin.[216] It is clear in different animal models that the toxin is active when given intravenously or intraduodenally; however, the literature contains no data on either oral or aerosol routes of intoxication for epsilon toxin.

Medical Management

Partly because of its natural association with animal rather than human disease, there has been little study of therapy for *C perfringens* epsilon toxin. An effective vaccine against epsilon toxin (described below) is readily available for animal use, thus obviating the need for a therapeutic in susceptible animal populations. No therapeutic treatment or vaccine against epsilon toxin has been approved for human use. However, two studies, one in vivo and the other in vitro, suggest that therapy might be possible. One

endeavor by Miyamoto et al[215] showed that riluzole, a drug that prevents presynaptic glutamate release used for treating human amyotrophic lateral sclerosis, can minimize murine seizures induced by epsilon toxin. However, these results were derived from an injection of riluzole given 30 minutes before toxin, and the drug was evidently not used in subsequent experiments as a therapeutic (ie, administered after toxin injection).

The in-vitro study, recently reported by Beal et al,[221] showed that tolerance toward epsilon toxin occurs in various cell lines, especially Madin-Darby canine kidney cells, when incubated with increasing amounts of toxin over time. Concomitantly, a group of unknown acidic proteins was lost (or possibly shifted to a different isoelectric point) from the cells that become tolerant to epsilon toxin (vs untreated controls). Exactly how this mechanism works and how such findings can be exploited as a therapy are still unresolved.[221] Similar results with increased cell resistance (although possibly involving another mechanism) to the lethal toxin produced by *Bacillus anthracis*, the causative agent of anthrax, have also been discovered.[222]

Additional therapy and prophylaxis studies show that the epsilon protoxin affords protection (delayed time to death) in mice when given intravenously before activated toxin. This protective effect presumably occurs via competitive occupation of the cell-surface receptor by the protoxin, primarily localized within the brain.[212] In 1976 Buxton[223] discovered that a formalin toxoid of the protoxin affords protection (up to 100 minutes) after epsilon toxin exposure. Such data suggest that a receptor-targeted approach for prophylaxis is possible, and that a receptor antagonist (ie, receptor-binding domain or small molecular weight competitor) may be useful as an epsilon toxin prophylaxis or therapeutic. To date, the specific identity of the epsilon toxin receptor remains unknown. The receptor is perhaps a heat-labile sialoglycoprotein, because pretreatment of rat synaptosome membranes with heat (70–80°C for 10 minutes), neuraminidase, or pronase effectively reduced the binding of epsilon toxin.[224] Furthermore, the same study revealed that a snake presynaptic neurotoxin (beta-bungarotoxin) decreases epsilon toxin binding in a dose-dependent fashion, suggesting a common (unidentified) receptor. In contrast, the presynaptic neurotoxin produced by *C botulinum* type A had no effect upon binding of the epsilon toxin. Knowledge of the receptor and how it interacts with the epsilon toxin would be useful in formulating effective, receptor-based therapies.

Although they are readily available and commonly used in the field,[225] veterinary vaccines for *C perfringens* and associated toxins, like many other veterinary vaccines, are often formaldehyde toxoids consisting of various antigens from culture filtrates or even whole cell cultures. These vaccines are efficacious and cost-effective for animals but are generally considered too crude for human use. Any human epsilon toxin vaccine will likely be chemically (ie, formaldehyde) or recombinantly (ie, mutation of critical residues needed for receptor binding or heptamerization) detoxified versions of purified protein. The latter concept of recombinantly attenuating a toxin to generate a vaccine has been used successfully for other bacterial toxins, including the *S aureus* enterotoxins[226] such as staphylococcal enterotoxin B, which is on the category B list of select agents. The technique used by Ulrich et al[226] for generating recombinant vaccines against *S aureus* enterotoxins involved data from X-ray crystal structures of the toxin and major histocompatibility complex class II receptors, molecular modeling of toxin binding to the receptor, and the recombinant alteration of the specific toxin residues important for receptor interactions. This approach may prove useful for generating efficacious epsilon toxin vaccines pending the difficult process of receptor identification and crystallization.

In 1992 Hunter et al accomplished the cloning, sequencing, and expression of the gene, an important step toward a purified vaccine suitable for use in humans.[227] Earlier studies by Sakurai et al,[228] which showed through chemical modification that certain amino acids are essential for lethality, set the stage for subsequent alteration of select residues through recombinant technology. Oyston et al have taken another major step toward a recombinant vaccine for epsilon toxin by substituting a proline for the histidine at residue 106 of the toxin.[229] This recombinant molecule is nontoxic in vitro as well as in vivo, and affords protection as a vaccine in mice against a 100 LD_{50} of toxin given intravenously. X-ray crystallography of a toxin-receptor complex would also likely yield definitive, useful data for a better recombinant vaccine. Furthermore, it is evident that a single epitope on epsilon toxin can elicit protection against the toxin or the bacterium, as shown by immunization of mice or rabbits with a monoclonal antibody that generates antiidiotypic antibodies.[230] Clearly, a refined vaccine should ultimately provide a useful prophylaxis for humans against *C perfringens* epsilon toxin. With renewed interest in and funding opportunities for select agents such as *C perfringens* epsilon toxin, various researchers from around the world should quickly solve the protein's mysteries and generate more efficacious therapies as well as vaccines suitable for human use.

SUMMARY

Exposure to harmful biological toxins may occur via ingestion or delivery as an aerosol at the tactical level. Although the toxins may be highly lethal, extracting and weaponizing them is relatively difficult because of the small amounts of toxins typically produced by organisms. Biological toxins may be more suitable for causing incapacitation or death among small groups or for assassinations. The biological toxins presented in this chapter are diverse in structure and mode of action. Proper diagnosis and care represent a daunting challenge for physicians.

Trichothecene mycotoxins are toxic to humans and a host of other organisms by inhibiting DNA, RNA, and protein synthesis. Local route-specific effects include necrosis and inflammation. Systemic toxic responses are similar, regardless of the exposure route. Treatment relies on decontamination and symptom-based supportive care. There have been unconfirmed reports of trichothecene mycotoxins used as weapons in Southeast Asia.

STX, brevetoxins, and domoic acid are marine algal toxins associated with human illness in natural outbreaks related to harmful algal blooms. STX blocks ionic conductance of the voltage-dependent sodium channels, leading to neurological symptoms (parasthesias and paralysis), as well as respiratory distress and cardiovascular instability. Treatment includes respiratory support and intensive cardiovascular management. Anti-STX serum and antibodies have shown promise in animal models, but such reagents are unavailable for human use. Brevetoxins inhibit sodium channel inactivation, leading to depolarization of membranes. Brevetoxin symptoms are similar to those of STX but are usually milder and lack paralysis. Although naturally acquired cases typically resolve spontaneously in 1 to 3 days, patients should be carefully observed and may require aggressive airway management. Domoic acid is a neuroexcitatory amino acid that kills cells within the central nervous system, particularly in the hippocampus, which is associated with learning and memory. Patients with domoic acid intoxication develop gastrointestinal symptoms and neurological symptoms, including anterograde memory loss and myoclonus. Severe intoxications may lead to convulsions and death. Medical management of domoic acid intoxications includes monitoring of hemodynamic status and pharmacological treatment of seizures.

Epsilon toxin of *C perfringens*, a protein responsible for animal enterotoxemias, is rapidly fatal in various animal models. The toxin causes pore formation in cell membranes, ion imbalance, and cytoskeletal disruption, leading to cell death. Although it has not been implicated in human disease, epsiolon toxin causes severe symptoms in animals including diarrhea, respiratory distress, and convulsions. A vaccine exists for veterinary use, but there is no specific therapy for epsilon intoxication.

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