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Basics of Antidote Pharmacology: A Summary of Programs for Biological Agent Research, Post-Exposure Therapeutic Advice, and Prophylaxis

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Abstract

Biological warfare has often only been used in wars with a military component. But during the past few decades, there has been a rise in non-state-based terrorism, which includes the employment of asymmetric weapons such biological pathogens. Therefore, it is becoming more and more crucial to think about tactics for stopping and getting ready for insurgent attacks, like creating medical countermeasures before and after exposure. Numerous preventative measures and therapeutic interventions are being researched to counteract the impacts of biological agents. These include nucleic acids (analogues, antisense, ribozymes, and DNA zymes), antibodies, antivirals, immunomodulators, bacteriophage therapy, and microencapsulation. Also included are antibiotics (both conventional and unusual uses). There are licensed vaccines, but commercial vaccines are available to prevent smallpox, cholera, anthrax, plague, and Q fever suitable for usage in cases of ricin, melioidosis, viral encephalitis, and botulinum toxins. The standard course of treatment after being exposed to anthrax, plague, Q fever, or melioidosis is still antibiotics. Botulinum toxins and smallpox can be treated using anti-toxin therapy and anti-virals, respectively. The only, or standard, post-exposure treatment for cholera, viral encephalitis, and ricin, on the other hand, is supportive care; this advice has not altered in decades. Given the challenges posed by antibiotic resistance, prophylactic and post-exposure therapy options rely heavily on the discovery and ongoing assessment of novel drugs and procedures. This review aims to provide an update on research activities and recommendations for prophylactic and post-exposure treatment for biological agents in the open works published between 2013 and 20023.

Keywords: *Biological; Anthrax; Botulism; Cholera; Encephalitis; Melioidosis; Plague; Q Fever; Ricin; Smallpox.*

ABBREVIATIONS

AGP, amino-alkyl glucosaminide 4-phosphate; BoNT/A–E, botulinum neurotoxins A–E; CapD, capsule depolymerase; CBR, chemical biological radiological; CDC, Centre for Disease Control (USA); CDHS, California Department of Health Services; CDV, cidofovir; ChiSys, chitosan mucoadhesive agent; CpG, unmethylated sequences of DNA; CMRI, phase I chloroform-methanol residue; ctx, cholera toxin; ctxB, subunit B of the cholera toxin; Dstl, Defence Science and Technology Laboratories (UK); DSTO, Defence Science and Technology Organisation (Australia); EEEV, Eastern equine encephalitis virus; EEV, equine encephalitis virus; EF, oedema factor; F1, fraction 1 capsular antigen; FDA, Food and Drug Administration (US); flaA–E, flagellin proteins A–E; G-CSF.

THE BIOLOGICAL AGENTS' PAST

Biological agents have long been used, even before the middle times, to instill fear, create casualties, and result in death. When Helleborus root (active ingredients: protoanemonin, steroidal saponins, and guaianolides) was used to pollute water supplies during the siege of Kirrha in 600 BC, it was the first known use of a biological agent (Smart, 1997). During the Middle Ages, the Russians deposited plague-infected cadavers in Reval, Estonia in 1710; the Polish used projectiles filled with rabid dog saliva to fight enemies in 1650; and the British gave smallpox-infected blankets to Native American Indians in 1763 (Smart, 1997). In more recent periods, the intentional use of biological agents has persisted through the usage of In their 1940–1944 invasion of China, the Japanese caused cholera and plague (Robinson and Leitenberg, 1971); in 1941–1942, they tested aerial bombs and cannon shells to spread anthax spores in Scotland (Robinson and Leitenberg, 1971). The use of ricin in Georgi Markov's assassination on September 7, 1978, through a modified umbrella (Harris and Paxman, 1982) serves as more evidence of the development of biological agent distribution strategies. Biological agents have been employed in civilian settings as opposed to military ones more recently. The Rajneeshi sect's use of salmonella to sway US elections in 1984 (Torok et al., 1997), Aum Shinrikyo's multiple unsuccessful attempts to spread botulinum toxins and anthrax spores between 1990

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and 1995 (Olson, 1999), and the anthrax letters that were circulated in the USA soon after the World Trade Center Towers were attacked on September 11, 2001 (Jernigan et al., 2002) provide proof that non-state-based organizations purposefully used biological weapons in terrorist acts.

The Way Biological Agents are Treated Today

Healthcare providers may be extremely important in alerting first responders to a possible agent because of the delayed onset of common symptoms that can happen after exposure to a biological agent. This information is essential for creating and implementing a suitable decontamination strategy, preventing additional casualties and/or deaths, and creating a response plan that includes the use of personal protective equipment.

Numerous preventative measures and therapeutic interventions are being developed to counteract the effects of biological agents. These include nucleic acids (analogues, antisense, ribozymes, and DNA zymes), antibodies, antivirals, immunomodulators, bacteriophage therapy, and microencapsulation. Also included are antibiotics (both conventional and unusual uses). It's interesting to note that despite all of the advancements in medicine over the last few decades, the primary course of treatment for many biological agents is still rudimentary supportive care. Nonetheless, a great deal of research is still being done to tackle biological threats because of the variety of study methodologies accessible. The clinical therapeutic countermeasures for biological and chemical weapons that are now in use have been thoroughly reviewed by Pettineo et al. (2009), however they did not address the most recent developments in treatment research. An overview of current research projects and prophylactic and post-exposure treatment guidelines for biological warfare and bioterror agents—such as anthrax, botulism, cholera, viral equine encephalitis, melioidosis, plague, Q fever, ricin, and smallpox—is intended to be provided by this article. It focuses on scientific developments and medical countermeasures for biological agent therapy using data released between 2013 and 2023.

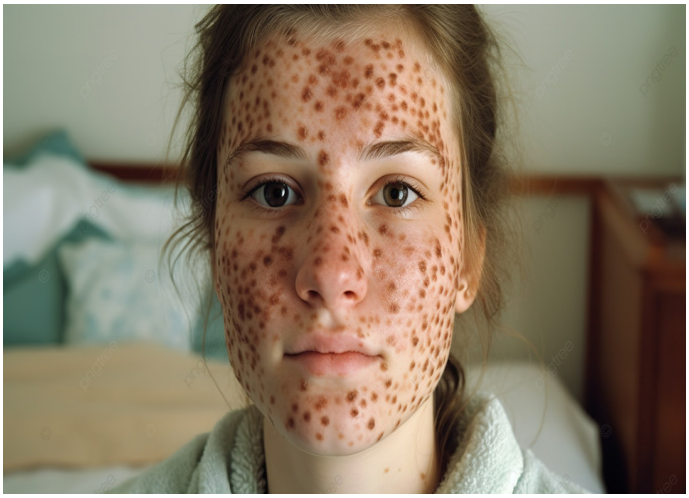

Figure 1. Characteristic maculopapular lesions of the milder form of smallpox (variola minor).

Current Prophylaxis for Anthrax

There are two licensed anthrax vaccines available (Little, 2005; Wang and Roehrl, 2005). The US anthrax vaccine adsorbed (AVA; Emergent BioDefense Corporation; also known as BioThrax®, Emergent Biosolutions Incorporated, Rockville, MD, USA) is extracted from a cell-free culture filtrate of an unencapsulated, toxin-producing strain of Bacillus anthracis (V770-NP1R). The UK vaccine (Health Protection Agency) is prepared from a similar strain called Sterne 34F2. Both vaccines contain the protective antigen (PA) adsorbed to aluminium hydroxide and contain small amounts of lethal factor (LF) and oedema factor (EF). The vaccines are both effective against anthrax infection when administered prophylactically, although the vaccination protocols differ (Little, 2005; Wang and Roehrl, 2005; Scorpio et al., 2006). The US vaccine is administered in a six-dose primary series at 0, 2 and 4 weeks and 6, 12 and 18 months with an annual booster, while the UK vaccine requires four single injections: three injections 3 weeks apart, followed by a 6 month dose, with an annual booster. For post-exposure prophylaxis against inhalation anthrax the Centre for Disease Control and Prevention (CDC) recommends that the vaccine AVA be used at 0, 2 and 4 weeks in combination with selected oral antibiotics. The combined use of AVA and antibiotics has been shown to prevent inhalation anthrax (Schneemann and Manchester, 2009) and may also shorten the required period of antibiotic therapy (Bossi et al., 2004a). However, this regime has not been approved by the United States Food and Drug Administration (FDA). Caution should be taken with children as the PA component of the vaccine may associate directly with the toxin components produced by the invading bacterium thereby potentially augmenting intoxication (Aulinger et al., 2005). Although current human anthrax vaccines are effective against anthrax, they still suffer from batch-to-batch variation in composition, require multiple doses and yearly booster injections and have been associated with occasional adverse reactions (reactogenicity) (Pittman et al., 2001; Pittman et al., 2004). These limitations have prompted the development of novel vaccines that are less reactogenic, but equally efficacious with fewer doses. Research efforts focus on: (i) development of subunit vaccines targeting PA (and to a lesser extent EF and LF); (ii) evaluation of alternative vaccine delivery routes (e.g. i.m. and mucosal administration); and (iii) identification of new vaccine targets (e.g. spore and capsule antigens). Excellent reviews have been published on a number of the major achievements (Brey, 2005; Little, 2005; Wang and Roehrl, 2005; Scorpio et al., 2006). Mucosal vaccination has proven to be a practical, non-invasive and efficacious method for the induction of both mucosal and systemic immune responses. Recently, a mucosal anthrax vaccine, based on a non-toxic mucosal adjuvant (NE) and a recombinant protective antigen (rPA), was developed (Bielinska et al., 2007). Guinea pigs immunized intra-nasally (i.n.) with the vaccine were protected from an intra-dermal (i.d.) challenge (1000 × LD50) of B. anthracis Ames spores. Another mucosal anthrax

vaccine composed of rPA, MPL (a toll-like 4 receptor agonist) and ChiSys (a chitosan mucoadhesive agent) is available in the form of a dry powder (Klas et al., 2008). The vaccine protects rabbits from lethal aerosol spore challenge up to 9 weeks after a single i.n. immunization. An anthrax vaccine based on live attenuated Salmonella vaccine strain (Ty21a) has also been reported (Stokes et al., 2007). Administration of Ty21a (p.o.) expressing the full-length rPA conferred significant protection against lethal exposure to aerosolized B. anthracis spores in mice. Further modification of rPA by its fusion to two distinct transport proteins (HlyA and ClyA) (Baillie et al., 2008) resulted in significant PA-specific immune responses when mice were immunized with Ty21a expressing the ClyA–PA fusion protein and then boosted with either rPA or AVA. CpG (unmethylated sequences of DNA) oligodeoxynucleotides (ODN) have also been evaluated as an adjuvant for AVA (Klinman et al., 2007). Mice immunized i.p. or i.n. with AVA + CpG ODN showed significantly increased host immunity to infection via aerosolized anthrax spores, in contrast to animals immunized with AVA alone. Interestingly the enhanced immunity correlates with the induction of strong systemic rather than mucosal immune responses (Klinman et al., 2007). Protection against anthrax via current anthrax vaccines is mediated largely by antibody (humoral) responses to the protective antigen (PA); however, cellular immunity has been shown to also play an important role (Glomski et al., 2007). Mice immunized with formaldehyde-inactivated spores (FIS) of a non-encapsulated B. anthracis strain were then challenged with an encapsulated non-toxinogenic B. anthracis strain. Sera, splenocytes and CD4 T lymphocytes were isolated from the FIS-induced mice and administered to naïve mice. The mice were then challenged with the encapsulated non-toxinogenic B. anthracis with results indicating that only interferon (IFN)-γ-producing CD4 T lymphocytes provide significant protection against anthrax infection. This study provides the first evidence of protective cellular immunity against encapsulated B. anthracis. A plasmid DNA-based approach has been applied successfully to anthrax vaccine development to boost cellular immunity (Zhang et al., 2008). Vaccination of mice with plasmid constructs expressing either PA or EA1 (an S-layer antigen) produced both Th1 and Th2 cellular responses demonstrating that this approach may be used to generate durable immune responses against anthrax. This method has also been used in conjunction with a replication-defective adenovirus vector in a prime-boost vaccination strategy (McConnell et al., 2007). Mice primed and boosted with plasmid DNA and adenovirus DNA, respectively, were fully protected from anthrax spore challenge. Interestingly the adenovirus-based prime-boost immunization produced 10-fold the anti-PA antibodies than AVA after a single injection. The toxin components PA and LF are composed of four domains, of which the PA domain 4 interacts with the host cell receptor, while the LF domain 1 binds to PA63 (the active form of PA). Antibodies raised against the PA domain 4 were protective against anthrax infection when tested in mice (Flick-Smith et al., 2002). The PA domain 4 and the LF domain 1 were fused to a thermostable lichenase from the bacterium Clostridium thermocellum and then expressed in the plant Nicotiana benthamiana (Chichester et al., 2007). Immunization of mice with the fusion protein resulted in high titres of antibodies capable of neutralizing the lethal toxin in vitro. A novel vaccine with the combined function of vaccine and antitoxin has been reported (Manayani et al., 2007). In this vaccine multiple copies of the PA-binding domain VWA of the anthrax toxin receptor ANTXR2 were expressed and displayed on the surface of an insect virus. The resultant chimeric virus particles protected rats from anthrax intoxication, and when loaded with PA, induced a potent immune response against lethal toxin challenge in a single dose without adjuvant. Previous studies have shown that whole spore-based vaccines are more effective against virulent strains of B. anthracis than the current PA-based vaccines (Little and Knudson, 1986; Welkos and Friedlander, 1988; Brossier et al., 2002). However, these vaccines are unlikely to be used in humans because of safety concerns. Mice primed with suboptimal amounts of PA followed by the spore surface antigen BclA were protected from lethal anthrax spore challenge (Brahmbhatt et al., 2007). BclA promotes opsonophagocytosis of spores by macrophages thereby inhibiting intra-macrophage spore germination. More recently, spore surface antigens p5307 and BxpB were identified (Cybulski et al., 2008). Mice immunized with suboptimal amounts of anthrax PA followed by p5307 and BxpB had enhanced protection against lethal anthrax spore challenge compared with animals immunized with PA alone. Although antibodies raised against either antigen reduced the rate of spore germination in vitro, both produced enhanced phagocytic uptake and phagocyte-mediated spore destruction in the mice. Holistically, these results demonstrate that spore surface antigens are potential immuno-enhancers to PA-based vaccines. Catalytic mutants of LF (LFE687A) and EF (EFH351A) have been evaluated in combination with PA for prophylactic use. Studies in mice demonstrated the ability of LFE687A and EFH351A, co-administered with PA, to reduce lethality following lethal anthrax spore challenge (Gupta et al., 2007). The advances in vaccine development for anthrax over the last 2 years, as outlined above, have been undertaken in preclinical animal studies and more definitive outcomes in human clinical trials are required.

Current Treatment for Anthrax

Given that children and pregnant women should not get prolonged antibiotic treatment, there is a need for innovative approaches to treat anthrax infection (Schneemann and Manchester, 2009). Furthermore, once critical amounts of the toxin are in the bloodstream, medications do not prevent anthrax poisoning. Another worry is that future strains of B. anthracis resistant to antibiotics may render antibiotic therapy ineffective (Stepanov et al., 1996; Brook et al., 2001;

Schneemann and Manchester, 2009).The development of antibody-based passive immunotherapy against components of the anthrax toxin, mainly PA and to a lesser extent LF, has been the most significant innovative therapy (Bouzianas, 2007). The US government has provided substantial funds to support the research and marketing of antibody-based therapy, which has made this possible Engage in conversation with the capsule. F26F2 and F26G3, the most protective antibodies, were noted as possible candidates for more research and development. As an alternative to antibody-based therapy, a recombinant enzyme known as capsule depolymerase (CapD) has been investigated (Scorpio et al., 2007; Scorpio et al., 2008). CapD breaks down the capsule and extracts it from the B. anthracis cell surface. According to Scorpio et al. (2007), pretreating mice with CapD greatly increases macrophage phagocytosis and neutrophil death of encapsulated B. anthracis cells. By encouraging in vivo phagocytic destruction of encapsulated B. anthracis cells in mice, CapD offers notable protection against anthrax (Scorpio et al., 2008).

Current Measures to Prevent Botulism

Currently, there are no approved vaccinations against botulism that the general population can obtain. On the other hand, the pentavalent botulinum toxoid (PBT) vaccine is being looked into by the CDC. According to Dembek et al. (2007), PBT is made from partially purified, formalin-inactivated botulinum neurotoxins A–E (BoNT/A–E). According to Smith and Rusnak (2007), the PBT dosing plan consists of an initial set of four injections (0.5 mL at 0, 2, 12, and 24 weeks) and yearly boosters. Twenty thousand at-risk laboratory workers and eight thousand military personnel have received PBT to date (Bossi et al., 2004b; Dembek et al., 2007). According to studies, PBT is safe and causes only minor, self-limiting local responses (Dembek et al., 2007). The current PBT (formalin-inactivated toxoid) is not suitable for widespread use due to a number of issues vaccination. Due to the high cost and technical challenges associated with formalin inactivation, which alters the structure of the toxin and may result in low quantities of neutralizing antibodies, it is necessary to cultivate live Clostridium botulinum bacteria. For the first time, a novel, easy, and fast method for creating an inactivated toxoid that resembles the natural toxin both structurally and antigenically was reported by Jones et al. (2008). According to Jones et al. (2008), the resultant toxoid has at least seven orders of magnitude less neurotoxic action and can increase in vivo antibody levels up to 600 times higher than those caused by formalin-inactivated toxoids. More affordable vaccinations could be created by using recombinant protein technology. Heavy chain (HC) recombinant botulinum neurotoxin has been evaluated for protection against BoNT-induced mortality in Escherichia coli and Pichia pastoris. Mice inoculated with recombinant HC are resistant against challenge with active BoNT, according to both investigations. High titres of neutralizing antibodies were also found in the sera from vaccinated mice (Webb et al., 2007; Yu et al., 2007a; Yu et al., 2009). High amounts of antitoxin antibodies were created by recombinant HC, which was highly immunogenic and might be employed as a co-treatment for botulism. As a potential vaccination, the creation of a non-catalytic recombinant version of botulinum toxin has also been studied (Willis et al., 2008). For instance, toxins are rendered inert by cleaving off of BoNT/ARYM, a recombinant BoNT/A with two single-point mutations (R365A and Y365F) on the light chain domain SNAP-25. It has been demonstrated that BoNT/ARYM is a highly immunogenic vaccination (Pier et al., 2008). According to experiments conducted on mice in the wild, animals inoculated with BoNT/ARYM (1.0 μg) survived challenges that were ten thousand times more common than those involving BoNT/A LD50 (Pier et al., 2008). Since DNA-based vaccinations are easier to make, purify, and store than recombinant HC subunit vaccines, they have also been examined. To enhance immunological responses, plasmid DNA replicon vectors derived from alphaviruses, like the Sindbis (SIN) virus and the Semliki Forest virus (SFV), are employed (Yu et al., 2007b). A standard plasmid DNA vaccine (pcDNASHc) expressing the HC domain of BoNT/A was compared to the immunogenicity of a plasmid DNA replicon vaccine (pSCARSHc) encoding the identical antigen. Compared to pcDNASHc, pSCARSHc demonstrated a higher induction of BoNT/A HC-specific antibodies in mice, providing a higher level of protection against BoNT/A. Research has also been done on the possible application of an adenovirus-vectored recombinant vaccine based on the BoNT/C HC (Zeng et al., 2007). Over an extended duration, the vaccination produced a strong immunological response against BoNT/C. This suggested that both vaccine kinds are viable options for treating botulism vaccinations since they can, to differing degrees, protect mice against BoNTs.

The Current Approach to Preventing Horse Encephalitis Viruses

As of right now, there are no authorized vaccinations against any of the equine encephalitis viruses (EEV). For laboratory workers who are at risk, vaccines with investigational new drug (IND) designation are available. They consist of inactivated vaccinations for Eastern EEV (EEEV) and Western EEV (WEEV), as well as a live attenuated vaccine for Venezuelan EEV (VEEV). Unfortunately, these vaccinations frequently have low immunogenicity and short-lived immunity. In the creation of attenuated vaccines, the role and functionality of viral structural proteins—in particular, the capsid protein—are crucial. According to Aguilar et al. (2007), 2008b, Garmashova et al. (2007), the capsid proteins of EEEV, VEEV, and maybe WEEV contribute to the suppression of cellular transcription and the avoidance of the host IFN response. Diminishment of The N-terminus of the capsid protein was changed to match the SIN virus counterpart in order to create the TC-83 VEEV vaccine (Garmashova et al., 2007). Furthermore, additional clarification of the intricate

function that EEEV's structural and non-structural protein (NSP) genomic regions play in neurovirulence has aided in the creation of live attenuated vaccines and antivirals in the future (Aguilar et al., 2008a). A SIN viral backbone expressing either the EEEV or VEEV structural proteins is used in chimeric vaccinations. According to Wang et al. (2007a), mice immunized with the SIN/EEEV vaccine exhibited protection against a live viral challenge and high titres of neutralizing antibodies. Additionally, it has been demonstrated that this vaccination reduces mosquito infectivity; nevertheless, the effect on dissemination and possible transmission varies depending on the species of mosquito (Arrigo et al., 2008) Further testing on immunodeficient mice revealed that a SIN/VEEV vaccination is very effective (Paessler et al., 2007). It has been demonstrated that a chimeric VEEV vaccination based on an equine herpesvirus type 1 is genetically stable and fully protects mice against a deadly VEEV challenge (Rosas et al., 2008). More testing of the chimeric vaccines in mice might offer the chance to find prognostic factors associated with encephalitis protection. Adenovirus-based vaccinations have been demonstrated to elicit a prompt, durable, and strong immunological response in mice (Barabe et al., 2007). One example of such a vaccine is the human adenovirus serotype 5 (Had5)-vectored WEEV vaccine (Wu et al., 2007a). When mice were exposed to homologous and heterologous strains of WEEV, such as the highly virulent strain of Fleming. Adenovirus-vectored vaccinations raise certain issues since pre-existing immunity to the vector may be harmful to homologous boosting. It is important to remember that co-administration of the VEEV vaccine, which is vectored by an adenovirus, and CpG can enhance antibody responses. This is not the case for the transgenic product, though (Perkins et al., 2008). It has been shown that attenuated VEEV vaccines can be made by modifying the VEEV genome's promoter regions without changing the NSP's amino acid sequence (Michel et al., 2007). Site-directed mutagenesis was used to create the live attenuated VEEV vaccine V3526 from a full-length infectious VEEV strain. According to research on horses (Fine et al., 2007), it is safe and effective, and when compared to TC-83, it is not noticeably more neurovirulent studied on non-human primates (Fine et al., 2008) and has been shown to be capable of shielding mice against VEEV infection through both i.p. challenge and mosquito bite (Charles et al., 1997; Rao et al., 2006). This method of developing vaccines should be used carefully since it can cause the NSP to rapidly adopt mutations. The adaptability of these viruses and their potential for evolution were highlighted by the observation of some changes after only a few rounds of infection. In fact, this might reduce their prospects of becoming EEV vaccine candidates. It has been demonstrated that a vaccine candidate created by radiation-treating VEEV treated with 1,5-iodonaphthylazide (INA) protects mice against fatal challenge. By doing this, the virus is totally rendered inactive, and the INA begins to bind to transmembrane proteins in the studied on non-human primates (Fine et al., 2008) and has been shown to be capable of shielding mice against VEEV infection through both i.p. challenge and mosquito bite (Charles et al., 1997; Rao et al., 2006). This method of developing vaccines should be used carefully since it can cause the NSP to rapidly adopt mutations. The adaptability of these viruses and their potential for evolution were highlighted by the observation of some changes after only a few rounds of infection. In fact, this might reduce their prospects of becoming EEV vaccine candidates. It has been demonstrated that a vaccine candidate created by radiation-treating VEEV treated with 1,5-iodonaphthylazide (INA) protects mice against fatal challenge. By doing this, the virus is totally rendered inactive, and the INA begins to bind to transmembrane proteins in the viral titre, even though 60% of animals had virus detection. Furthermore, mice were protected against WEEV challenge by a HAd5 vector expressing murine IFN that was given 24 hours to a week beforehand (Wu et al., 2007b). Additionally, when given 64 hours after a lethal challenge, this vaccination partially protected mice and slowed the spread of WEEV infection. A recombinant E2 protein of WEEV has induced robust humoral and cell-mediated responses when used to immunize mice, despite only providing partial protection to animals delivered a lethal challenge of the virus (Das et al., 2007). Therefore, the development of EEV vaccines may benefit from the application of recombinant technology.

Treatment for Equine Encephalitis Viruses at the Moment

For the viral encephalitis, there are no antiviral treatments available. It is advised to provide supportive treatment using intravenous fluids, a respirator, sedatives, analgesics, corticosteroids to lessen brain swelling, and anticonvulsants to manage seizures. The majority of antiviral treatments, including RNA interference, aim to stop viruses from replicating. Short interfering RNAs (siRNAs) and small double-stranded RNA molecules control the degradation of complementary messenger RNAs in this biological process. It has been shown that six VEEV strains cannot replicate in vitro when four siRNAs are combined and target conserved regions of divergent VEEV strains (O'Brien, 2007). Given that one strain was able to give resistance to siRNAs, it's interesting that this experiment raises the question of the development of resistance to siRNA. Phosphonodiamidite morpholino oligomers coupled with a peptide (PPMO) sterically block the viral RNA according to a specified sequence, hence inhibiting viral replication. All mice who received PPMO before to and during VEEV challenge remained alive and showed no signs of infection 2-4 days after the challenge. Conversely, mice who were given PPMO shortly after being challenged exhibited a lower level of viral titres in tissue samples and only limited protection (Paessler et al., 2008). Using a lethal mouse model, antiviral drugs that impede viral replication were assessed as potential therapies for VEEV infection (Julander et al., 2008b). (-)Even when given up to four days after infection,

carbinamine was demonstrated to be beneficial in improving illness parameters (Julander et al., 2008a). Additionally, at a dose below the lethal threshold, a new 3-sulphonamido-quinazolin-4(3H)-one derivative was found to be moderately efficacious in reducing the reproduction of VEEV in vitro One strategy that might offer a fresh supply of antiviral drugs is to identify substances that obstruct viral assembly. Small RNA sequences that attach to proteins are known as RNA thioaptamers. By using in vitro combinatorial selection, a number of thioaptamers were identified, and their affinity for binding the VEEV capsid protein was evaluated. It was demonstrated that one thioaptamer has a high affinity and specificity for binding the capsid protein (Kang et al., 2007). Melatonin has been demonstrated to be useful in the treatment of VEEV infection by the reduction of nitrite and lipid peroxidation caused by the infection and the elevation of IL-1β, a cytokine that can increase the brain's production of inducible nitric oxide synthase (Valero et al., 2007). Melatonin's potential inhibitory impact on VEEV replication has not yet been determined be decided. To address the issue of a human anti-mouse antibody response, a humanized mouse anti-VEEV antibody has been developed. According to Hu et al. (2007), the humanized antibody exhibited a robust neutralizing ability in a conventional plaque reduction assay and bound to the VEEV E2 protein in a dose-dependent manner. It is expected that more research will be done on this antibody as an immunotherapeutic treatment against VEEV.

Current Prophylaxis for Plague

The recommended antibiotic for secondary post-exposure prophylaxis is 100 mg of doxycycline (child >8 years: 2.5 mg·kg−1 up to 100 mg) twice a day, or 300 mg ciprofloxacin i.v. twice a day for 9 days, followed by 500 mg (child: 15 mg·kg−1 up to 500 mg; p.o.) for an additional 6 days, twice a day. The World Health Organisation (WHO) also recommends 1–2 g·day−1 tetracycline (p.o.) at 6 or 12 hourly intervals, or 1.6 g·day−1 sulphamethoxazole or trimethoprim (p.o.) at 12 hourly intervals (p.o.) (Poland, 2009). To improve the efficacy of the current plague subunit vaccine, protective fraction 1 capsular antigen (F1) and the virulence multifunctional LcrV antigen (V) that resides at the tips of type III needle complexes (Baker et al., 1952; Lawton et al., 1963) are currently being engineered as recombinant F1-V proteins (Andrews et al., 1996; Heath et al., 1998; Williamson, 2001; Powell et al., 2005; Goodin et al., 2007). The F1-V fusion proteins together with alum-based delivery have been demonstrated by Dstl and USAMRIID to protect mice against pulmonary Yersinia pestis challenge. Currently both vaccines have entered phase I clinical trials and have been reported to be safe, well-tolerated and immunogenic (Heath et al., 1998; Jones et al., 2000; Williamson et al., 2005; Morris, 2007). Further studies show that formulation of F1-V proteins with adjuvant induces strong humoral and cellular immunity (Glynn et al., 2005; Jones et al., 2006). Four different adjuvants: heat-labile enterotoxin (LT) (R192G), CpG ODN, MPL®TDM and alum, have been administered in combination with recombinant F1-V protein, and have all effectively induced type 1/type 2 antibody responses. The magnitude of antibody response was evaluated in mice immunized via i.n., transcutaneous (t.c.) and s.c. routes. High levels of anti-F1-V IgG1-2a in both the serum and bronchioalveolar lavage (BAL) were observed with s.c. route producing the greater response (Uddowla et al., 2007). Similarly, a recombinant F1-V protein coupled to a synthetic lipid, a mimetic known as amino-alkyl glucosaminide 4-phosphate (AGP), augmented cell-mediated TH1 immune responses in rats challenged with lethal Y. pestis strain C092. The AGP-based vaccine was administered according to a primary/secondary i.n. prime/boost regime and demonstrated that an initial immunization on day 1 followed by another on day 3, protected 63% of rats by day 7, subsequently achieving 100% protection by 21 days (Airhart et al., 2008). A bicistronic DNA vaccine (i.n.) co-expressing F1-V fusion protein and a molecular adjuvant, IL-12, protected mice against Y. pestis challenge, in contrast to mice immunized with F1-V protein alone. Prime vaccination consisted of a low concentration of the DNA vector coding for IL-12 in conjunction with F1-V. Animals were subsequently boosted with recombinant F1 protein that provided protection from pneumonic plague (Yamanaka et al., 2008). The protective efficacies of flagellin adjuvant fused with F1-V protein and a plant-based oral plague vaccine without adjuvant, against respiratory challenge with Y. pestis CO92 have been demonstrated in mice and non-human primates (Mett et al., 2007; Arlen et al., 2008; Mizel et al., 2009). The enhanced effectiveness of adjuvants in the development of plague vaccines against bubonic and pneumonic plague is evident. However, the major limitation to subunit vaccines is the necessity to administer multiple high dosages to produce robust and prolonged immune protection. With the emergence of Y. pestis antibiotic-resistant strains, the development of improved prophylactic approaches are of utmost importance. The discovery of strains lacking the F1 antigen is an important factor that must be considered in the design of new vaccines. Yersinia pestis and Y. pseudotuberculosis share a close evolutionary relationship. An attenuated Y. pseudotuberculosis strain (IP32680; p.o.) was used to immunize mice against bubonic plague and resulted in high antibody responses and protection of 75% (after first dose) and 88% (after second dose) of mice with no obvious adverse effects compared with animals immunized with the live attenuated Y. pestis strain EV76 (Blisnick et al., 2008). A mutant live vaccine was constructed by a deletion-insertion in the lpxM gene of Y. pestis EV NIIEG strain, denoted as Y. pestis EVΔlpxM. High protective efficacy of single dosing of Y. pestis EVΔlpxM was demonstrated in mice and guinea pigs (Bubeck and Dube, 2007).An attenuated Salmonella enterica strain is frequently employed as a live vaccine vector encoding recombinant

F1-V proteins. Vaccination with recombinant F1-V proteins (p.o.) induced specific F1-V specific IgG and IgA antibody titres that protected mice against Y. pestis challenge. Oral immunizations provided >80% protection from 1000 × LD50 bubonic plague and 100 × LD50 of pneumonic plague in mice. Hence, this attenuated Salmonella-based vaccine has potential as a plague vaccine (Liu et al., 2007; Yang et al., 2007). Operons yadB and yadC operons have also demonstrated novel potential in the plague F1-V vaccine. These virulence factors assist bacterium in the invasion of epithelioid cells. Mice immunized with yadC elicited specific IgG1 antibody responses, and the release of pro-inflammatory cytokine was also observed (Murphy et al., 2007; Forman et al., 2008). Similarly, anti-translocon antibodies YopB, YopD, or a complex of YopBDE, protected mice against lethal challenge with F1-Y. pestis indicating that mABs specific for F1, V antigens and the Yoptranslocon may be useful prophylactic or therapeutic approaches (Eyles et al., 2007; Ivanov et al., 2008). Vaccines from adenovirus vectors encoding the anti-V antigen produce strong immune responses resulting in 93.3% protection following i.n. Y. pestis challenge (Sofer-Podesta et al., 2009). Recombinant V10 protein showed immunogenicity and protected cynomolgus macaques upon challenge with aerosolized pneumonic plague (Cornelius et al., 2008). These studies confirmed that humoral immunity plays an important role in preventing the development of this disease, and recent research has illustrated that cellular immunity also contributes to protection against plague (Philipovskiy and Smiley, 2007; Kummer et al., 2008).

The Current Plague Therapy

Gentamicin is advised by the CDC to treat plague patients at intervals of 8 hours, with doses ranging from 3 to 7.5 mg·kg−1−1 orally or intravenously. When treating pneumonic plague, streptomycin is the most effective antibiotic. It should be taken at a dose of 30 mg/kg/day-1 every 12 hours (i.m.). Other antibiotics for specialized treatment consist of: 50 mg/kg−1·day−1 p.o. or i.v. every 6 hours for 10 days (good for bubonic and pneumonic plague); 15 mg/kg−1 p.o. followed by 25 to 50 mg/kg−1·day−1 for 10 days; or 400 mg of ciprofloxacin and 200 mg of doxycycline given at 12 or 24 hour intervals (Poland, 2009).

Current Prophylaxis for Q Fever

Post-exposure prophylaxis for adults and pregnant or breastfeeding women is a week of either: doxycycline 100 mg p.o. every 12 h, erythromycin 500 mg p.o. every 3 h, clarithromycin 500 mg p.o. every 12 h or roxithromycin 150 mg p.o. every 12 h (Pettineo et al., 2009). Children should receive treatment with the same antibiotic for 1 week at the following doses: doxycycline 100 mg p.o. every 12 h for children up to 8 years of age and weighing >45 kg or 2.2 mg·kg−1 p.o. every 12 h if <45 kg; erythromycin 500 mg p.o. every 3 h for children >35 kg or 50 mg·kg−1 p.o. every 12 h if <35 kg; clarithromycin 500 mg p.o. every 12 h if >40 kg or 7.5 mg·kg−1 p.o. every 12 h if <40 kg; or roxithromycin 8 mg·kg−1 p.o. every 12 h (Pettineo et al., 2009). A whole-cell Q fever vaccine (Q-Vax), consisting of the formalin-inactivated Henzerling strain, is currently licensed for use in Australia, although pre-screening for prior immunity is required to prevent adverse reactions due to egg hypersensitivity (Marmion, 2007). An unlicensed, purified Henzerling strain whole-killed vaccine administered via an i.n. device is also available through USAMRIID to immunize occupationally at-risk individuals (Waag, 2007). A chemo-vaccine comprising extracts of Coxiella burnetii lipopolysaccharide and protein has also been used to vaccinate laboratory workers and some industrial groups in Eastern Europe (Marmion, 2007). A phase I chloroform-methanol residue (CMRI) vaccine has been developed as a safer alternative to the current whole-cell vaccines and is being assessed for safety and immunogenicity in phase I clinical trials (Waag et al., 2008). No antigen-specific antibody could be detected following a single subcutaneous prime; however, after a second booster, significant levels of specific antibody were produced. Peripheral blood cells collected from individuals following the booster also responded to recall antigen in vitro in a dose-dependent manner, suggesting that the vaccine is able to prime the immune system to effectively respond to infection (Waag et al., 2008). A major way of preventing Q fever is through vaccination of animal hosts serving as reservoirs of infection. Immunization of cattle with a monovalent inactivated phase I vaccine has been demonstrated to significantly lessen the probability of susceptible non-pregnant cows becoming shedders of the organism. This highlights the potential of implementing vaccination among non-infected herds to prevent spread of Q fever to humans (Guatteo et al., 2008). Prophylaxis for Q fever has also focused on better characterization of the mechanisms of vaccine-induced immunity. This includes comparison of formalin-inactivated phase I and phase II vaccines in Balb/c mice. Phase I vaccines conveyed significant protection as well as Th1 dominant immune responses. Although phase II vaccines also induce Th1 immunity, they did not confer measurable protective responses, indicating that phase I lipopolysaccharide is important for host defence against C. burnetii (Zhang et al., 2007). The effects of infecting IFN-γ and Toll-like receptor 2 knockout mice with the normally non-pathogenic phase II nine mile strain C. burnetii have also been investigated. The febrile response in these immuno-compromised animals indicated that both IFN-γ and TLR2 are important in providing protective immunity to C. burnetii, and that NMII is capable of causing disease in immunodeficient animals. These results highlight the usefulness of using these animals as models for evaluating vaccine candidates and host–pathogen interactions (Ochoa-Reparaz et al., 2007).

Current Treatment for Q Fever

Generally acute Q fever is a self-limiting mild or asymptomatic infection that resolves within 2 weeks, therefore treatment is not usually required (Tsironi et al., 2005). However, antibiotic therapy may be warranted to prevent progression to chronic disease. In such cases, the recommended regime is doxycycline 100 mg twice daily for 14 days (Parker et al., 2006). For chronic Q fever endocarditis, patients should receive a combination of doxycycline (100 mg twice daily) and hydroxychloroquine (200 mg three times daily) for a period of 18 months (Maurin and Raoult, 1999). Quinolones can also be used for patients intolerant to chloroquine (Maurin and Raoult, 1999). Long-term co-trimoxazole therapy (320 mg trimethoprim and 1600 mg sulphamethoxazole for 35 days) is recommended for pregnant women to decrease the risk of placentitis, obstetric complications and maternal chronic Q fever infection (Carcopino et al., 2007). Treatment of Q fever in pregnant women with doxycycline is contraindicated. Carcopino et al. (2007) investigated long-term co-trimoxazole therapy as treatment for pregnant women with Q fever and found that women were protected against chronic Q fever, placental infection and obstetric complications, particularly i.u. fetal death (found to be related to placental infection), when compared with women that had not received the therapy. This finding has led to the recommendation that long-term co-trimoxazole should be used to treat pregnant women with Q fever (Carcopino et al., 2007). Interestingly, the CDC has assessed the risks and benefits of post-exposure antibiotic treatment following an intentional release of C. burnetii. Based on administration of doxycycline to the general population and co-trimoxazole to pregnant women, upper bound probability estimates of adverse effects to prophylaxis indicated that the risk of acquiring Q fever illness outweighed the risk of antimicrobial drug-related adverse effects (Moodie et al., 2008).

IN SUMMARY

Because there are certain biological agents for which there is no medical countermeasure or prophylaxis, biological agents still pose a threat to both military personnel and civilians even after decades of research. Commercial vaccines are available to prevent smallpox, cholera, anthrax, plague, and Q fever; however, there are no approved vaccines to prevent botulinum toxins, viral encephalitis, melioidosis, or ricin. In cases of anthrax, plague, Q fever, melioidosis, or probable exposure, antibiotics remain the cornerstone therapy recommendation. Botulinum toxins and smallpox can be treated using anti-toxin therapy and anti-virals, respectively. Nonetheless, the sole or primary post-exposure treatment for cholera, viral encephalitis, and ricin is supportive care; this advice has not transformed throughout decades. Even though the current regimes need to be further developed and improved, resources and efforts should be strategically aligned with the risk that biological agents pose (whether that risk is intentional or inadvertent). Furthermore, it is important to recognize the chances for cooperation and mutual benefit from each other's work programs that exist between national and international governments and non-governmental organizations.

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