Cell. Mol. Life Sci. 63 (2006) 2193–2195
1420-682X/06/202193-3
DOI 10.1007/s00018-006-6307-0

Cellular and Molecular Life Sciences

Biological weapons

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Online First 9 September 2006

Bioterrorism is defined as the intentional or threatened use of microorganisms or toxins derived from living organisms to cause death or diseases in humans, animals or plants on which we depend. The premeditated use of infectious agents as weapons has been attempted throughout history, killing thousands of people and animals. The first biological attack was reported in 1346 during the siege of Kaffa, where Tatars catapulted the bodies of their plague-infected soldiers over the city walls that initiated the second major outbreak of the plague (the Black Death).The last one was perpetrated in 2001, shortly after the terrorist attacks of September 11, with *Bacillus anthracis* was deliberately released in the United States.
Although the Geneva Protocol (1972), also known as the Biological and Toxin Weapons Convention or BWC, prohibits the development, production and stockpiling of toxins and biological weapons, there is no effective international mechanism for challenging either the development of biological weapons or their use. More than 140 nations have ratified this convention, but it seems that the number of countries known or suspected of having biological weapons capability has doubled since the convention went into force in March1975.
The events of 2001 in the United States had worldwide repercussions on the awareness of bioterrorism as well as on the development of plans to counteract bioterrorism amongst many countries. Individual nation states have developed plans to fight against bioterrorism and have united in order to resist this threat [1–3]. The list of agents that could pose the greatest public health risk in the event of a bioterrorist attack is long. More than 180 pathogens have been reported as potential agents for bioterrorism. This list includes agents that, if acquired and properly disseminated, could inflict a serious public health crisis in terms of our ability to limit the number of casualties and control damage to our cities and nations.

Most of the biological agents that could be used as weapons are poorly (if at all) known by most clinicians. Therefore, the first part of this review deals with the clinical management of the most frequent and dangerous infectious agents that could be used as biological weapons: *Bacillus anthracis*, smallpox, *Yersinia pestis*, *Francisella tularensis* and the toxin produced by *Clostridium botulinum*. These agents could be easily disseminated, transmitted person-to-person (e.g. smallpox and plague pneumonia) and cause high mortality. Most of these agents have been studied or used in biological warfare in the past. All could be used as biological weapons in the form of aerosols, which is the most effective route of contamination in a bioterrorist attack.
It appears difficult to predict when, where or how a deliberate release of a biological agent will occur, and equally impossible to predict which emergent infection will next threaten global public health. However, it seems easier to predict that infectious disease emergencies will continue to occur regularly. Appropriate planning will ensure that these emergencies disrupt society as little as possible. As reviewed by Ippolito and colleagues, in the last 2 decades successive outbreaks have been caused by new, newly recognised and resurgent pathogens [4–6]. The risk that high-consequence pathogens might be used as bioterrorism agents have amply demonstrated the need to enhance capacity in clinical and public health management of highly infectious diseases. They discuss some of the components of hospital preparedness for infectious disease emergencies. These include clinical awareness and education; initial investigation and management; surge capacity; communication; and caring for those affected by the emergency. Furthermore, emphasis is given to the importance of improving the every-day practice of infection control by healthcare professionals and of taking a generic, 'all-hazards' approach to hospital preparedness. This requires integrated planning for response to infectious disease emergencies, whether naturally occurring or caused by accidental or intentional release.

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Adequate public health preparedness for bioterrorism includes the elaboration of an agreed list of biological and chemical agents that might be used in an attack or as threats of deliberate release [3, 7–9]. Tegnell et al. describe a logical method called 'matrix' and the characteristics of the variables to be brought in a weighting process to reach a priority list for preparedness. A matrix is defined as a rectangular array of mathematical elements (as the coefficients of simultaneous linear equations) that can be combined to form sums and products with similar arrays having an appropriate number of rows and columns but also as something from which something else originates, develops or takes form. This matrix should help in prioritising the public health needs to prevent and manage the impact of the agent used in a bioterror setting. The members of the European Commission Task Force on Biological and Chemical Agent Threats (TF BICHAT) developed a matrix that can function as a tool for epidemiologists, microbiologists and public health policy makers to establish the need for further interventions or other activities to improve preparedness for bioterror events. Initially, the matrix was set up to assist the European Union to prepare for deliberate release. Today, the approach has evolved into a tool that could serve public health authorities in identifying priorities for activities to limit infectious disease threats, including deliberate release, taking into account the specific national environment for which they are planning.

Many biological agents are zoonotic and could have a considerable impact on agriculture, animal and human health. The review by Pappas et al. reveals that brucellosis is an ancient disease which remains the most common anthropozoonosis worldwide, inducing an often chronic and incapacitating disease with low mortality [10, 11]. The significance as a potential agent of bioterrorism was acknowledged early: *Brucella* was initially attractive as biowarfare partly due to its ability to induce chronic disease. The pathogenesis of brucellosis is unique, and animal models often cannot accurately reproduce events that evolve during human infection. Brucellae have a propensity for invading the reticuloendothelial system, hiding inside macrophages and non-specialised phagocytes. In the phagocytes, they reside in specialised compartments with an acidic environment and multiply using parts of the cytoskeleton without interrupting cellular cycle and function. Furthermore, they are apoptosis inhibitors, creating a frame for eternal survival and replication. Immune response is partly muted by certain *Brucella* factors; inhibition of tumor necrosis factor-α being a prominent event. It has long been postulated that the outcome of the disease reflects the equilibrium developed between the bacterium and the human immune response, and that relapses and chronic disease should be also viewed in this context. The optimal treatment has to be a combination regimen, since monotherapy has traditionally been associated with an increased percentage of treatment failure and relapse. Future options may incorporate adjuvants aimed at altering the acidic intracellular environment or new antibiotics. The development of a vaccine for brucellosis suitable for humans would be an ideal solution to the problems of inadequate veterinary control of animal disease demonstrated by the lack of epidemiological study of human disease and antibiotic treatment data. Numerous vaccines have been tested in the past; none has gained wide acceptance.

In their anthrax vaccines review, Chabot et al. describe historical achievements and new developments in anthrax vaccine research. Due to its exceptional virulence, ease of preparation and ability to form stable and environmentally resistant spores, *B. anthracis* has been developed as a biological weapon [12]. In addition to the anthrax toxins, capsule, and their regulators, a number of genes/proteins that make a measurable contribution to virulence have been identified [13]. Anthrax has been a major cause of death in grazing animals and an occasional cause of death in humans for thousands of years. Due to animal vaccinations, the rate of infection has decreased dramatically. Anthrax vaccines have progressed from uncharacterised whole-cell vaccines in 1881 to pX02-negative spores in the 1930s. In 1970 culture filtrates bonded to aluminum hydroxide were developed, and in the near future the use of recombinant protective antigen is probable. The threat of genetically engineered, antibiotic- and vaccine-resistant strains of *B. anthracis* is driving hypothesis-driven research and global techniques, including genomics, proteomics and transposon site hybridization, to facilitate the discovery of novel vaccine targets.

Smallpox virus eradication has been one of the greatest successes of the 20th century [14]. However, with the intent to overcome the fear of its use in biological warfare, progress in understanding residual immune memory and development of new animal models were rapidly sought by the scientific community. This subsequently provided a great opportunity to increase our knowledge regarding humoral and cellular memory to non-persistent pathogens and to study factors that might influence further vaccination strategies in humans. This is in spite of the many publications of animal studies of vaccinia virus infection. Puissant and Combadière provide further insights on the role of the persistence of immune responses during the two eras in the presence and absence of circulating smallpox. Although recent studies have thoroughly discussed T cell immunity because of notable technical advances in this field, neutralising antibodies against vaccinia virus remain the principal route to controlling and eradicating infection. They also discuss immunological results concerning vaccinia-specific effector/memory T cells (by using interferon γ, enzyme-linked immunospot assay and intracellular cytokine staining) and central memory T cells (lymphoproliferation assay) [15]. The potent im-

muno-stimulatory activities of poxvirus vectors, have led to growth in the development and evaluation of new-generation vaccine candidates that are discussed in this review.

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