Medical management of biological warfare and bioterrorism: place of the immunoprevention and the immunotherapy

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Abstract

Biological weapons are considered as mass destruction and terror weapons. Terrorism including bioterrorism is the major threat in the future conflicts for our nations. The aim of bioterrorism is more related to the potential disorganisation of the society than to the lethal effects of the agents used. The dramatic consequences cannot be discarded, especially if contagious agents such viral are used. The preparation of specific defence measures is a major challenge for our countries.

The knowledge acquired from the struggle against natural infectious diseases and recent events are essential to improve behaviours to face the biological weapon threats. The defence attitude is based on the anticipation of the threat, the management of the victims, and the restoration of the operational capabilities. This global defence attitude implies six important functions: (i) alert, (ii) detection and diagnosis, (iii) availability of pharmaceutical countermeasures such as vaccine, sera and anti-infectious medicine and products, (iv) medical management of victims, (v) training and information, (vi) research and development.

Passive and active immunoprevention and immunotherapy belong to the approaches discussed in the context of bioterrorism countermeasures. Further researches might be focused on these topics.

Keywords: Biological weapon; Bioterrorism; Countermeasures; Vaccine; Sera
Résumé

Les armes biologiques appartiennent à ce que l’on appelle les ‘armes de destruction massives’ ou ‘armes de terreur’. Le terrorisme, notamment le bioterrorisme, ne peut plus être exclu des scénarios de conflits auxquels nos pays se trouveront confrontés. C’est une menace majeure. Le nombre de victimes consécutives à une attaque biologique reste très incertain, mais le potentiel de désorganisation de nos sociétés est indéniable. Des conséquences graves d’un acte terroriste ne peuvent être écartées, particulièrement si l’agent dispersé, par exemple un virus, est très contagieux. Une posture de défense réaliste doit donc être adoptée. La mise en place de mesures générales et spécifiques à cet effet est l’un des challenges à relever pour nos pays.

Les connaissances acquises dans la lutte contre les maladies infectieuses naturelles et le retour d’expérience des événements récents sont essentiels pour améliorer les comportements face aux menaces d’agression par armes biologiques. Une posture de défense réaliste est basée sur l’anticipation de la menace, la prise en charge rationnelle des victimes, et la restauration des capacités opérationnelles. Cette posture de défense est générale et s’appuie sur six grandes fonctions: (i) l’alerte, (ii) la détection et le diagnostic, (iii) la disponibilité des produits pharmaceutiques destinés aux contre-mesures tel que les vaccins, sérums et produits anti-infectieux, (iv) la prise en charge des victimes, (v) l’entraînement des acteurs et la communication de l’information, (vi) la recherche et le développement.

La place de l’immunoprévention et de l’immunothérapie passive et active est discutée dans le contexte particulier des contre-mesures aux risques biterroristes. Il s’agit de sujets sur lesquels la recherche doit être développée.

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Mots-clé: Les armes biologiques; Le bioterrorisme; Contre-mesures; Les vaccins; Sérums

1. Introduction

The infectious diseases hazards are permanent, and particularly in poor hygiene conditions. Military operations are increasing the natural risk and imply the knowledge of the local epidemiological situation. Two recent military deployment are illustrative: peace keeping troops in Timor (1999) were immunised against Japanese encephalitis, and reinforcement of measures were carried out in Kosovo in 2001, to avoid tick born infections as Crimea-Congo encephalitis. Despite the 1972 Biological convention, proliferation of biological weapons is today a reality and beside the natural threats, the possible use of biological weapon by enemy or by terrorist cannot be discarded. The mailing of *Bacillus anthracis* contaminated envelops is one of the first case of a concrete bioterrorism action. This event has pointed that the psychological impact on the population was much more important than the number of victims. This corresponds perfectly to the main objectives of the bio-terrorists: fear, disorganisation and panic. In France more than 4000 false alerts of anthrax contamination were registered during the three last month of 2001. More than 2500 analyses were conducted in two military laboratories with expertise for detection of *B. anthracis*. Their normal activity was completely stopped during this period. The States security services were reactive. Major disorganisation was avoided but some defects were pointed out in the National Security...
Plan named BIOTOX (for bioterrorism) particularly in the organisation of the laboratory network of the seven French National Defence Zones [1]. The lessons of the crisis have been fruitful for improving BIOTOX but also the other national plans: PIRATOME (for radiological and nuclear terrorism) and the PIRATOX (for chemical terrorism).

Among the countermeasures against biological weapon and bioterrorism, medical countermeasures are critical. The response for aggressive biological threat is identical to the response against natural biological events. The most important measures are: (i) epidemiological surveillance and strategy; it is critical for the alert, (ii) a laboratory network for detection and rapid biological diagnostic including national reference laboratories, (iii) availability of pharmaceutical products such as vaccine, sera and anti-infectious products for prevention and therapeutic, (iv) medical management of victims: restriction of movement and quarantine are among possible measures imposed in case of declaration of contagious diseases as smallpox; special organisation of hospitals and sites for housing and for cares are needed, (v) training courses for responsible and a communication policy, (vi) a research and development policy.

Today vaccination against biological weapons agents is controversial. Availability of useful vaccines, side effects of vaccines and the immune response time are among the key questions that might be solved. Specific immunoglobulins could be a useful alternative in some cases.

2. Historical overview of recent biological threats

Un combat sanglant est préférable à un marais malsain (A bloody battle is preferable to any pernicious marsh). By this meaning, Napoleon the first referred to natural infectious diseases like malaria, cholera, typhus and other fevers which were frequently responsible for a higher mortality than weapons for soldiers before the XX century.

It is probably the dysentery, due to *Shigella dysenteriae*, who helped the forces of the young French Republic to defeat the Prussian troops at the Valmy battle in 1792. Infectious diseases are still they are the first enemy of military forces during wars and troops must pay attention to hygiene.

The sentence of Napoleon could translate the fear of militaries for unknown threats and biological weapons are surely included among them.

Historical aspects of development and use of biological weapons were previously described in some publications. Today weapons using classical agents remain the most hazardous, but new concepts merging from genetic and molecular biology could be the source of new terrifying biological weapons [2–4].

In April 1979 a major outbreak of anthrax occurred in the Ural town of Sverdlovsk. More than forty people were officially declared died. The authorities of the former Soviet claimed that this outbreak was the consequence of accidental infected meat eating. They denied vigorously the existence of secret military biological plans in this area. For more than 10 years, Meselson and Guillemin drove on site investigations [5]. Their conclusions associated with the pathological descriptions of Abramova [6] finally convinced the scientific international community that the origin of this anthrax outbreak was the consequence of an accidental release of *B. anthracis*. This event was only recognised by
Boris Eltsine in February 1992. The Sverdlovsk’s factory was part of a huge organisation ‘Biopreparat’ covering a military biological program under civilian activities. This programme was revealed when high responsible of this organisation: Vladimir Pasechnik, and the Colonel Ken Alibek, defected Soviet Union at the beginning of 90 years [7]. This programme was developed when Soviet Union, United Kingdom and United States of America were the warrants states of the Biological convention signed in 1972.

During the same period Iraqis developed their own secret biological weapon program. The United Nations special commission (UNSCOM) was established in 1991 after the Gulf War to investigate the mass destruction weapon programme. It carried out on site inspections during 4 years before being able to demonstrate the level of the Iraqi biological weapon programme. Information about non-justified importation of media for bacteria fermentation and the declarations of Hussein Kamal, the son-in-law of Saddam Hussein, were the main reasons, which conducted Iraqi authorities to recognise their effort to weaponise biological agents. In less than 10 years an imposing biological arsenal was settled. According to UNSCOM information, 20,000 l of botulinum toxin 20 fold concentrated, 8000 l of B. anthracis 10 fold concentrated, kilograms of ricin, and of aflatoxin were produced and weaponised in 250 kg R-400 bombs, 155 mm shells and 122 mm rockets. Modified F1 tank for aircraft dispersion and drones were also built and Iraqis have been deploying 25 heads of Al Hussein missile [8]. UNSCOM inspectors have not been able to detect them for 4 years because the sites of Al Hakam and Salman Pak, and their productions, were meticulously cleaned, destroyed or hidden. Without convincing information the United Nations were only suspicious.

During the China war, the Japanese Unit 731 leaded by the general Ishii Iro achieved a huge military programme of biological weapon including human trials and use of weapons at against civilian Chinese populations [9]. The real operational efficacy of these weapons was not established but the adverse effect of these weapons lead to a cholera epidemic in Japanese troops: thousands of their soldiers were ill and died consecutively.

Biological agents were also used for sabotage or as antipersonnel weapons [10]. During the First World War German Special Forces tried unsuccessfully to contaminate the horses of both French and US armies with glanders (Burkholderia mallei). During World War II, Check patriots killed Reinhardt Heydrich, head of the Gestapo in Bohemia, with a grenade equipped with a small glass tube containing botulinum toxin.

In 1978 the Bulgarian dissident Gregory Markov died in London after the injection of ricin performed with an umbrella containing this toxin. Ten years after, in 1984, Rajneesh sect used Salmonella to contaminate Food in ‘Salad bars’ in Oregon. More than 750 peoples were ill including forty hospitalised.

The objective of the programmes of the 7th medical battalion of the former Afrikaner army is also instructive. During the 80s, under the command of the doctor Wouter Basson, this special force was in charge to develop biological and chemical weapons to fight against the African National Congress.

Sarin’s terrorist attack of March 1995 by Aum sect and the anthrax attacks of September 2001 when B. anthracis spores were sent in at least five letters to Florida, New
York City and Washington have been the major recent events underlining the reality of the chemical and biological terrorism threat.

3. Clinical aspects of biological threats

3.1. Key criteria for biological weapon threats

The most discriminating criteria for the selection of putative bioterrorism warfare agents are: their intrinsic virulence, the facility of production, conservation and dissemination. The psychological impact of the threat and the potential disorganisation are also in first place for the choice of a terrorist group [11]. The agents corresponding to these criteria belong essentially to the Group A of the classification of the Centres for Diseases Control (CDC). The threat can be bacterial (B. anthracis, Yersinia pestis, Francisella tularensis), viral (smallpox, viruses of hemorrhagic fever as filoviridae, arenaviridae, bunyaviridae) or toxinic (Botulinum toxins) [http://www.cdc.gov]. Some agents of the Group B of the CDC can be also included (ricin, staphylococcal enterotoxin B, B. mallei and B. pseudomallei).

Despite their particular characteristics, the majority of the agents have common points to underline:

- Aerosolisation is the most probable route of contamination [12] and results in a short incubation period (1–5 days for anthrax or plague) associated with respiratory syndromes, fast evolution and high lethality rate.
- An intracellular phase (macrophage-cells and/or intra-tissular multiplication). For example, B. anthracis infection can be reactivated until 60 days after inhalation. This imposes a long stage of prophylactic treatment.
- In some cases, a risk of spread in the community (pulmonary plague, smallpox, viral haemorrhagic fevers).

The bacterial infectious diseases due to B.anthracis [13,14], Y. pestis [15], F. tularensis [16] as well as viral infectious diseases like smallpox [17,18] or viral encephalitis and the intoxication by ricin [19] or by botulinum toxins [20] are today the higher potential hazards.

The possibility of genetic modification related to virulence or antibiotic resistance increases the risk related to these agents.

3.2. Clinical features: syndrome approach

On-time detecting devices of specific bacteria, viruses or toxins in the environment are not operational. Some are in development, but we cannot expect reliable systems in next future. Early clinical and biological diagnostic remain the basement of the medical countermeasures. Symptoms support the clinical diagnostic but are not really specific. A syndrome approach could be promoted to be reactive for alarm and to guide biological investigations. Table 1 summarise the major symptoms encountered in biological hazard.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Incubation in days</th>
<th>Febrile syndrome</th>
<th>Respiratory syndrome</th>
<th>Cutaneous and mucosal syndrome</th>
<th>Neurological syndrome</th>
<th>Gastrointestinal syndrome</th>
<th>Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary anthrax</td>
<td>1–5</td>
<td>First stage: febrile illness undifferentiated, headache, chills, weakness</td>
<td>Early: absence of bronchopneumonia but cough, chest pain,</td>
<td>No cutaneous manifestation</td>
<td>Second stage:</td>
<td>Abdominal pain</td>
<td>Death: 90% non contagious antibiotic therapy reducing mortality</td>
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<tr>
<td>(Bacillus anthracis)</td>
<td></td>
<td></td>
<td>Second stage: dyspnea, ‘stridor’, radiography: symmetric mediastinal widening</td>
<td>at the first stage</td>
<td>hemorrhagic meningitis</td>
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<tr>
<td>Pulmonary plague</td>
<td>1–3</td>
<td>Firstly: sudden onset of fever, hemoptysis, and severe sepsis</td>
<td>Severe pneumonia, cough, dyspnea, purulent sputum, chest pain, radiography: bilateral infiltrates</td>
<td>No bubo</td>
<td>–</td>
<td>Nausea, vomiting, pain and diarrhea</td>
<td>Disseminated intravascular coagulation necrosis. Death: 100% very contagious Death: 30–60%</td>
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<tr>
<td>(Yersinia pestis)</td>
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<tr>
<td>Pulmonary tularemia</td>
<td>3–10</td>
<td>Onhaling fever undifferentiated, chills, weakness pharyngitis</td>
<td>Slower progression of bronchopneumonia, bronchiolitis, pleuritis, radiography: peribronchial infiltrates, enlargement of hilar nodes</td>
<td>No cutaneous manifestation</td>
<td>–</td>
<td>–</td>
<td></td>
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<tr>
<td>(F. tularensis)</td>
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<tr>
<td>Glanders</td>
<td>10–14</td>
<td>Fever</td>
<td>Pleurodynia radiography: 0.5–1 cm disseminated nodules, bilateral pneumonia...</td>
<td>Rash like smallpox</td>
<td>–</td>
<td>Diarrhoea</td>
<td>Photophobia, splenomegalia asthenia Death: 30% Very contagious by airway (7th day). Differential diagnostic: varicella</td>
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<tr>
<td>(B. mallei)</td>
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<tr>
<td>Smallpox</td>
<td>10–17</td>
<td>Fever</td>
<td>–</td>
<td>Centrifugal rash on face and arms; whole lesions at a same site evolving at the same rate</td>
<td>–</td>
<td>–</td>
<td>Death: 30% Very contagious by airway (7th day). Differential diagnostic: varicella</td>
</tr>
<tr>
<td>Botulism</td>
<td>1–5 (function of doses)</td>
<td>No fever</td>
<td>Result of paralysis of respiratory muscle</td>
<td>–</td>
<td>Acute and flaccid paralysis Ocular symptoms: enlarged or sluggishly reactive pupils, 4Ds: Diplopia, Dysarthria, Dysphonia, Dysphagia</td>
<td>–</td>
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</tr>
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</table>

Febrile syndrome is not specific: fever, headache, chills, weakness but its intensity is often function of the aetiology. Four other syndromes can also individualized after an aerosolization:

- A severe respiratory syndrome can occur in the natural form of inhalation anthrax [12, 13] and in pulmonary plague [15]. This syndrome is present, but less perceptible in tularaemia [16] and for other agents (e.g. bacterial, viral or toxins).
- A cutaneous and mucosal syndrome is highly meaningful in smallpox [17]. Bubo of bubonic plague and typical lesions of cutaneous anthrax are not present in case of airway infection.
- A neurological syndrome without fever could be due to botulinum toxin but if fever is noticed, diagnostic could be oriented to viral encephalitis, brucellosis or Q fever. Differential diagnosis between botulism and organophosphorous intoxication is usually simple and based on the rapid apparition of symptoms after chemical intoxication and ocular and paralysis symptoms (flaccid paralysis and enlarged pupils) in botulism [20]. In case of encephalitis, symptom could vary from simple headache to convulsion and loss of conscience in a context of fever.
- A gastrointestinal syndrome with abdominal pain, nausea, vomiting and diarrhoea can be observed. Food poisoning could be used in case of bioterrorism aggression with some agents as *V. Cholerae*, *Salmonella* spp. or staphylococcal enterotoxin.

4. Global approach for medical countermeasures

4.1. Medical and epidemiological surveillance

Detection of BW agent prior inhalation is theoretically the best way to reduce casualties. Detecting devices however, are not fully efficient, in insufficient number, and cannot be permanently deployed. Efficient intelligence is needed in order to implement a defense against biological threat. The first indication of a biological attack in an unprotected population is the emergence of a rare or unexpected disease and/or the observation of a dramatic increasing number of cases. In front of a hypothetical B attack, the main challenge is to identify the first case of the disease, and to detect the concomitant or secondary cases (i.e. epidemiological approach). The epidemiological aspect of detection needs medical intelligence (before the attack) and epidemiological surveillance\(^1\) (before and after the attack). Medical intelligence collects data about potential risks and threats and abilities of terrorist organisations, or rogue states, to develop and use B agents. Epidemiological surveillance may be defined as the ongoing systematic collection, analysis, and interpretation of outcome-specific data for use in the planning, implementation and evaluation of public health practice [21]. It can be used to point an unexpected event occurring in a population, to detect an abnormal increase of cases and to identify exposed persons.

\(^1\) North American authors had rather use of the term Public Health Surveillance than Epidemiologic Surveillance. Medical intelligence belongs to the medical surveillance.
It can also retrospectively compare patients (victims) and controls in order to identify a putative exposition linked with the disease occurrence.

When medical intelligence is not available or accurate, epidemiological surveillance is, and remains, the corner stone for alert and detection of a bioterrorism attack.

The following examples highlight the importance of such policy in detection of B attacks.

Numerous infectious diseases are ubiquitous, but some of as anthrax or haemorrhagic fevers have geographic distribution. For instance the emergence of anthrax could be considered as natural in an African rural area but will be probably not in Paris or London. The large outbreak of Crimean Congo Hemorrhagic Fever in central Balkans during spring and summer 2001 was considered as a tragic resurgence of an endemic disease, whereas even a small outbreak in France would be most surprising and suspicious.

Other infectious diseases have seasonal distribution. In the North Hemisphere an outbreak of influenza (more after an antigenic drift) in summer might not be easily explained by a natural occurrence. The Q-Fever agent is commonly transmitted via the placental secretions of parturient sheep and is usually seen in Southern France in spring or early in the summer after lambing season. A winter outbreak in an industrial Rhine-land will not be considered as a natural occurrence. For a given infectious disease, in each sex and age group, attack rates are often comparable in every urban area of a given country. An outbreak of severe pneumonia affecting especially (significantly higher attack rate) young adults in only one city, few days after a First League football match or a ‘Rock Star live show’ or any other great popular manifestation, may be a natural event but should justify higher levels of suspicion.

All these examples illustrate how we need epidemiological surveillance.

To be efficient epidemiological surveillance needs to be implemented previously of aggression. In fact it must be included in a permanent and routine policy in all surveillance systems of western countries. The surveillance may be reinforced during time crisis e.g. political crisis, wartime, sport events, religious pilgrims, and main events. Reactivity can be improved by adding sentinel syndromes in the scope of noticeable diseases (pneumonia, acute respiratory syndrome, rash, gastro-enteritis, etc.). The main difficulty is to mobilise the medical community for the implementation or the surveillance recommendations. Finally, if routinely implemented and efficient, epidemiological surveillance is the fastest and best tool in hands of epidemiologists to alert Public Health decision-makers after occurrence of abnormal or numerous cases potentially linked with B threat.

4.2. Organisation of laboratories for rapid diagnosis

The biological diagnosis is based on microbiologic and serological investigations. Early diagnosis requires a high index of suspicion following the first clinical symptoms in absence of warning systems. Microbiologic studies are important to recommend or to adapt earlier treatments.

Identification and characterisation of pathogens could request non-routinely disposable techniques. Unusual samples from different origin might be analysed in safety conditions. All these reasons justify a specialized laboratory network including environmental,
veterinarian and hospital laboratories as well as highest biological safety level (BSL), research laboratories (BSL 3 and BSL 4) and national or World Health Organisation (WHO) reference laboratories.

At this time in France, only two laboratories are able to process unknown, nuclear, radiological, chemical or biological suspicious samples. These laboratories belongs to the French ministry of Defence and are:

- the Centre d’études du Bouchet (CEB) of the Délegation générale pour l’armement (DGA), Vert le Petit, near Paris;
- the Centre de recherche du Service de santé des Armées «Emile Pardé» (CRSSA), Grenoble.

In the governmental plan BIOTOX, a small laboratory network including civilians and military laboratories performs the rapid detection of the BW agents in suspicious things and environmental samples. The absence of explosive as well as chemical and radiological hazards can be previously carried out if requested. Human samples (essentially nasal swabs) were processed in hospital laboratories. To face the flow of samples, veterinary laboratories and university hospital laboratories can rapidly reinforced this network.

The major problem encountered during the post eleventh September envelope crisis was the absence of standardisation of the procedures used by the participating laboratories. Standardization and decision to take according to the different situations encountered, has not found a clear answer at this time. The lack of previous identification of reference centres was also an important problem. These structures are indispensable for confirmation of identification, typing and for sharing information. They could be also of great interest for the definition of reference techniques, development and validation of new ones.

If, the positivity of human nasal swabs is considered as a good marker of contamination, their negativity is not clinically relevant to stop the antibiotic prophylactic treatment. This treatment, however, can be stopped according to the negativity of the putative contamination source.

The systematic surveillance of the environment is not recommended, but sampling can be used for monitoring significant potential target zones. After attack, environmental samples can be also used for the isolation of the strain(s) used and for monitoring the efficacy of decontamination.

After several weeks, it has been decided that only the people who have been directly exposed to the powder had to be hospitalised. Medical personnel were managing the other ‘victims’ before leaving hospital.

Communication with press must also be clarified. It seems important to give a single official point of contact to all the media in order to avoid confusion and to limit the spread of false information.

According to the recent experience, the main countermeasures to take are: the identification of reference centres for each agent, the standardisation of procedures and of quality controls, and the adaptation of specific structures dedicated to the process of environmental samples and especially of putative contaminated letters.
At this time, the efforts were targeted on *B. anthracis* but procedures must be enlarged to other BW agents in order to face the threat.

4.3. Medical measures and pharmaceutical means for prevention, therapeutic and decontamination

Good standard of hygiene is critical for the control of biological environment. It is the first preventive measure. The reinforcement of the biological environmental control using simple rules of hygiene is the first efficient corpus of measures to put in place in case of bioterrorism or biological warfare threat [22]. These measures are the following:

- Food and beverages control,
- Individual and clothing hygiene,
- Hospital and housing organisation, cleaning and waste control,
- Reinforcement of biological control of air and water supply facilities,
- Insects, arthropods and rodents control,
- Reinforcement of hygiene and nursing good practices in hospitals
- Decontamination of ill or exposed peoples.

The available medical means are drugs and procedures for prevention (vaccination, chimioprophylaxis, and passive immunoprophylaxis), treatment, and decontamination or disinfection.

4.3.1. Vaccinations

The scheme of military vaccinations is combining regular vaccinations and occasional vaccinations adapted to the biological environment of the military operations. All vaccines require a license from the national health authority: in France it is the AMM (‘autorisation de mise sur le marché’/authorization for sell on the national market) or an ATU (‘autorisation temporaire d’utilisation’/temporary authorisation of use) [23].

Concerning vaccinations against biological warfare agents, including bioterrorism threat, the systematic vaccinations do not appear as the best solution in absence of a direct threat clearly identified. Each situation must be evaluated in a cost/benefit balance [24]. Storage of available vaccines to face these threats seems to be justified. The best vaccines are those with a quick and high-level protective efficacy and a therapeutic ability. Procedures for use in case of emergence are identical for military and civilian populations. They must be clearly established by the health authorities [25]. Today very few number of efficacy vaccines are available. Anthrax vaccine was the first vaccine successful used by Pasteur at Pouilly-le-Fort in 1881. Since this time vaccines based on alum adsorbed Protective antigen (PA) of *B. anthracis* were licensed in US and in United Kingdom in seventies years. PA is produced using acid precipitation of a culture supernatant of a non-virulent strain of *B. anthracis*. The protocol of vaccination recommends six shots during eighteen months and annual boosts. Side effects were described and real efficacy of these vaccines against pulmonary anthrax is controversial. These reasons are not pushing to use largely these vaccines to prevent a hypothetical bioterrorism event. In return a vaccine
based on purified PA could be very useful in the real time prevention and treatment of pulmonary anthrax combined with antibiotics.

A great international effort to prevent re-emergence of smallpox with new vaccine stockpile is carried out by health authorities [17,18]. That is in part because even a single case could be a harbinger of a larger outbreak and in part because even one case would undoubtedly spark panic and a clamour for vaccine. One case of smallpox might result in a nationwide program of voluntary vaccinations. According to official strategies, a ring procedure around cases might be applied. Moreover, the first to be vaccinated would include infectious disease specialists and emergency room personnel, including doctors, nurses, technicians, even security officers working at hospitals and clinics. The shots carry a risk of serious side effects, including generalised vaccine, encephalitis and death. There are a number of different types of vaccines, and all were used by the World Health Organisation to eradicate smallpox around the world by 1979. Some government has opted for the so-called Lister strain vaccine. This has been used extensively since the 1950s. However, authorities in the US have decided to stockpile another type of smallpox vaccine. Their version, developed by the New York State Health Department (NY strain vaccine), has been shown to be effective in fighting smallpox in India. According to Promed information exchange on the Net, the Health Ministry of Israel has begun vaccinating about 1500 health workers against smallpox in July 2002. More than 1,50,000 people might be selected to extend the vaccinations to ‘first responders’: police officers, soldiers, emergency medical personnel, and hospital workers who would be involved in an immediate response to a biological attack. Vaccinated persons could also provide vaccinated plasma, so called ‘vaccine immune globulin’, which can be used to protect people who cannot take the regular vaccine, like pregnant women and people with compromised immune systems. Procedures and training of vaccinators are on duty. A policy of research, development, fabrication and stockpile of vaccines is ambitious and costly. Cooperation programmes must be promoted and the needs justify the requested efforts.

Botulinum vaccines exist. Pentavalent vaccine (A–E) is under restricted license in US and a so-called Pentanatoxin was performed by the former Soviet Union. Inhibitory antibodies are found at protective level in animals. More than thousand people were vaccinated using the recommended protocol including three shots and annual boosts. A recombinant heptavalent vaccine is under development in US army. The major restrictions of the use of botulinum vaccine are the level of the botulism hazards regarding the potential use of botulinum toxin for medical treatment of some diseases such as blepharospasm or spasmodic stiff neck.

Living plague vaccine (EV76 strain) or inactivated plague vaccines (EV 40 strains) were discontinued. Tentative of vaccine using proteins from Yersinia enterocolitica (YOPS) and Y. pseudotuberculosis have been discontinued in France. Killed vaccines as well as subunit plague vaccines are usually effective for preventing or ameliorating bubonic but not pneumonic plague. It is a challenge for recombinant vaccine candidates that are on researches.

Tularamia killed strains as well as subunits vaccines are not protective. Living attenuated vaccine is sometime used by scarification. Beside the side effects of these
Vaccines, the immunity is not well done and the risk for these vaccine strains to return as virulent strains might not be neglected.

Vaccine against Q-fever exists in Australia (Q-Vacc). Efficacy of this vaccine is around 90% during 5 years. Useful for individuals, this vaccine cannot be use for mass vaccination due to possible severe side effect in natural immunised people. New vaccines are on researches.

Attenuated venezuelian equine encephalitis viruses produced in cell culture (TC-83) were used to vaccinate thousands exposed professionals. Side effects as febrile reactions headheaches; myalgies are noticed in about 18% of cases. This vaccine is licensed in US for animal (horses) vaccination. Other vaccines are under developments in US.

For the other weaponisable agents such as Burkholderiae, ricine, mycotoxins, vaccine does not exist. The priority of researches on vaccine solutions to prevent the use of these agents by bioterrorists is questionable. Researches and development of vaccines as bioterrorism countermeasures is fully justified if these vaccines are useful in combination with other pharmaceutical products as antibiotics or antiviral drugs to prevent a short noticed threat or for the treatment of victims. Today positive arguments to promote a health policy firstly based on long-term vaccination to face bioterrorism threat are weak. Such policy is not justified regarding the knowledge and the level of the risks. A countermeasure policy based on short noticed prevention using anti-infectious drugs, antidotes and passive immunoprophylaxis seems to be a more realistic behaviour.

Priorities for research and development might be coherent with this objective.

4.3.2. Passive immunoprophylaxis

Passive immunisation, through the administration of specific antibodies (antibody-based therapy), may provide medical protection against the main biological warfare (BW) agents [26–35]. Antibodies are highly versatile defence molecules and can be produced against any foreign molecule. They can directly neutralise the pathogen (inhibition of binding to a target receptor) and/or invoke its destruction by other effectors of the immune system.

As antimicrobial chemotherapy, the antibody-based therapy is immediately active and confers a rapid protection. This is especially important in the context of a bioterrorism threat where: (i) the preventive vaccination of large populations would be difficult, and (ii) vaccination procedures would be of little use during a bioterrorism crisis because immunization needs several weeks-to-months delays for protection.

Antibody-based therapy may be used in pre-exposure or post-exposure prophylaxis as well as in curative therapy. It could be useful when chemotherapy is not available or insufficient, such as for toxins, some viruses, and antibiotics-resistant bacterial strains.

Botulinum toxin is of primary concern because of its extreme toxicity and ease of production. Botulism would be one of the best indications for antibody-based therapy in the context of bioterrorism [20]. There is no effective chemotherapy against botulism, and patient management requires intensive monitoring and care including mechanical ventilation that, in a large outbreak, would rapidly overwhelm the available capacities. Botulinum antitoxins (polyclonal antibodies purified from animals or humans hyper immunised against botulinum toxin) provide effective prophylaxis both pre- and post-exposure (before the onset of clinical signs), and reduce extension and severity of
the disease when given as a curative therapy of clinical botulism. The only existing botulinum antitoxins are the American licensed trivalent (ABE) and heptavalent (ABCDEFG) preparations under development (seven serotypes of botulinum toxin exist, defined by their absence of cross-neutralisation), both of them are of equine origin. Unfortunately, only very scarce amounts are available. The vaccine is on investigation and will not be available in a next future.

This underlines the urgent needs to develop and produce new antibody-based therapeutic preparations against botulinum toxins and other BW agents. Although serum polyclonal antibodies are at closer reach, they suffer a number of limitation [36], including batch-to-batch variation, low content of specific antibodies, infectious risk of plasma-derived products and immunogenicity of non-human (equine) antibodies. These limitations should be overcome in the next future by the development of therapeutic humanised antibodies (if possible use is shown) or human recombinant antibodies [37]. Passive local immunoprevention or immunotherapy might be promoted [37–39].

4.3.3. Drugs for treatment and decontamination or disinfection

Drugs usually used for treatment such as antibiotics can be useful for prophylaxis [40]. Antibiotherapy could be occasionally justified in case of specified threat like bioterrorism threat or biological warfare threat. Antibiotics (fluoroquinolone antibiotics, or doxycycline) were stockpiled for this purpose. Some antiviral drugs as Ribavirin could be also recommended. In first line-prophylactic treatment, these drugs are administered in probabilistic manner.

Procedures for the treatment of the most frequent biological agents have been published in France by the Agence Française de Sécurité des Produits de Santé (AFSSaPS). These recommendations are part of the governmental security plan BIOTOX. They are available for anthrax, plague, tularaemia, brucellosis, haemorrhagic fevers, smallpox and botulism. The European agency (EMEA) has also published recommendations. All these recommendations are available on their websites.

4.3.3.1. Antibacterial drugs (antibiotics). The lack of safe and effective vaccines places the antibiotics as the first defense line for the treatment and prevention of bacterial infections. As antibiotherapy, antibio prophylaxis is given in a curative manner after exposition to a biological warfare agent. Prescriptions are done according to the recommendations of the different national medical authorities. Within the framework of the biological aggressive risk, the medical authorities consider that the traditional restrictions of the use of the antibiotics can be adapted for the benefits of the patients. Each situation and each prescription must be evaluated in term of balance benefit/risk in order to render the therapeutic decision useful for the community and the individuals.

Interdependent criteria have to be considered for the choice of the antibiotics. The natural susceptibility of the various classes of antibiotics must be evaluated on a wide panel including reference strains and epidemiological relevant ones. For each isolate, susceptibility testing must be systematic in order to detect acquired resistance. A broad spectrum, a good bactericidal activity and a long post antibiotic effect are undeniable advantages for the choice of an antibiotic. The other criteria are: a good tissue and intracellular diffusion, a good level of absorption, a long half-life, a good biodisponibility
and a low toxicity, especially when prophylactic treatment will be administered during a long period (as for anthrax). The antibiotics, which answer best to these different criteria for curative and prophylactic treatments, are the fluoroquinolone antibiotics (ciprofloxacin), and doxycycline. For curative treatments, aminoglycosides as gentamicin are of great interest (e.g. for plague or tularemia). Doxycycline, fluoroquinolone antibiotics and aminoglycosides are not usually recommended in infants and in pregnant women. The recommendations have to be adapted. Other families as beta-lactams, chloramphenicol, or rifampicin can be used only after susceptibility testing. In France, the recommendations are regularly up-to-dated and available on the website of the AFSSaPS agency (http://www.afssaps.sante.fr).

4.3.3.2. Antiviral drugs. Antiviral drug such as Ribavirin and Cidofovir can be of interest. These two antiviral compounds are effective in vitro against smallpox and have been tested in a mouse model of cowpox infection. Cidofovir (100 mg/kg bid 4 days J0–J4) protected all the animals and Ribavirin has been found able to increase the survival time when used at the regimen of 100 mg/kg bid 5 days [41]. Anyway these treatment or prophylaxes have not yet been validated for smallpox in humans. Ribavirin can be recommended against Arenaviridae (Lassa fever) [42] and Bunyaviridae (Crimea Congo) but has poor activity against Filoviruses (Ebola, Marburg) [43].

4.3.3.3. Decontamination and disinfection procedures. The aim of decontamination of humans is to eliminate or reduce the number of microorganisms on the surface of the body. It also protects against a secondary contamination due to the re-aerosolisation of the agents. For example the deposit of B. anthracis spores after a single exposure to an aerosol of 1,25,000 particules/m³ is estimated to only 120 particles. Clothe removal, hand washing, a single shower with soft shampoo, water, and soap is able to eliminate 99.99% of these bacteria. In case of direct cutaneous exposition, the contaminated zone can be washed with a 0.5% chlorine solution (time of contact 5 min). The eyes must be rinsed with a physiological solution.

Disinfection of surfaces can be performed with an active chlorine solution (3 or 5%) or with formaldehyde. Hydrogen peroxide and glutaraldehyde can be also used, but only for re-usable medical materials. The US experience demonstrates that the procedures used for decontamination were simply able to decrease the level of contamination. In certain buildings three successive sequences of decontamination have been used before the negativation of the environmental samples. The decontamination of the air-conditioning circuits is at this time extremely difficult and there is no really satisfactory procedure currently available.

4.4. Medical management of victims: restriction of movement and housing of victims and exposed people in hospital or dedicated sites

Quarantine and restriction of movement are critical parts of this question. Housing strategy in hospital or in dedicated sites is largely depending of the threat. Two situations can be encountered:
4.4.1. Military operations

Capability, logistic of health support and doctrine must be appreciated and adapted to face the threat and preserve the military capabilities.

Mobile hospitals and dedicated non-permanent home might be equipped to maintain the best level of hygiene. All professionals would be trained to face a biological attack in poor medical conditions. The preferable sites for the implementation of these housing structures are located in the immediate vicinity of the contaminated area. The precise location is largely dependent of the wind, the climate and the local geography.

The following equipments are proposed in the army:

- Collective modular units with special air filtration treatment (MOSTHOM®);
- Individual protective equipment including protective mask and garments. Specific equipment for biological agents is not available but chemical suits and gas mask are suitable for a first level of protection in case of biological attack;
- Enlargement and reinforcement of the military medical health organisation with trained people and adapted devices for sampling and laboratory analysis;
- Quickly accessible stockpiles of dedicated means and drugs for prevention, treatment, disinfection or decontamination;
- Operational transport equipments and vehicles for rapid evacuation of hazardous areas.

Procedure and organisation would be closely related to the concept of the ‘Bioforce’ which is operable in case of major outbreak or health crisis in requesting countries. The French ministry of foreign affairs is the point of contact and the, ministry of defence is in charge of logistic. Industry (Merieux foundation especially) supplies drugs and vaccines. Professional staffs belongs can belong both to Military Medical Service and civilian organisation.

4.4.2. National security preparedness

A hospital network must be organised in the governmental security plans. This network might be able to take care of victims and exposed people. In case of bioterrorism action involving a large number of victims or exposed people, quarantine and restriction of movement would be surely a huge problem implying a political decision. Apart from the potential simultaneous number of victims (particularly in case of contagious diseases), the implementation of measures doesn’t differ from those usually used for the natural infectious diseases. Critical point will be the management of people to avoid panic and disorganisation. As armed forces, civilian security an emergency teams must be trained.

This hospital network must be clearly identified by professionals and equipped with a minimum of means for housing peoples during a limited period. Drug, vaccine and other mean storage might be sized and managed to be less costly. Mobile and easily operable decontamination and disinfection facilities might be disposable.

Military hospitals and are participating to the governmental security plant and have for mission to reinforce the civilian organisation but not to replace it.
4.5. Training courses of experts and responsible, public information

The training of professionals is fundamental for the credibility of the security plans and for the improvement of their performances. This training must be focused on the knowledge of threats and hazards the use practice of security equipments and the implementation of procedures. Information and communication strategy of authorities is critical in these circumstances to avoid panic and disorganisation.

The increasing concern about bioterrorism and biological warfare during the last decades pointed the need for training of military and civilian medical health professionals for the medical management of biological casualties. Some years ago, in a report about the biological threat, the French army health services established that the training for military medical professionals has to be extended largely. Physicians, pharmacists and veterinarians of the armies will have both an initial theory and practice training on BW protection at the military medical school in the Val de Grâce academy in Paris from 2003. A specific Nuclear Radiological Biological Chemical (NRBC) training course managed by the research center of the Military Medical Service (CRSSA) in Grenoble (France) is in force. This course is similar to those existing in other countries as the medical management of biological casualties course of the United States Army Medical Research Institute of Infectious diseases (USAMRID) in Fort-Detrick (Maryland). NATO promotes relevant individual and collective NBC defense training and especially training of medical personnel for NRBC operations [44–47].

The NRBC course of CRSSA is scheduled four times a year. The participants are professionals from military units, from military hospitals and from civilian emergency services. The duration is 5 days with classroom lectures and practical exercises. Lectures include: NRBC threats, general concepts of the headquarters in NRBC defense, environmental problems, city and civilian aspects and psychological aspects. Practical exercises include: individual and collective protection devices utilization, decontamination and medical management. The specific part concerning BW and bioterrorism includes general aspects on bacteria, viruses and toxins, samples collection, prophylactic and treatment. CRSSA experts have been occasionally implied in the chemical and biological part of the United Nations Monitoring Verification Inspection Commission (UNMOVIC) training courses for disarmament inspectors. These training courses are intended to give the future inspectors a best knowledge in biosafety and prevention against the biological hazards possibly encountered during site inspections. It appears necessary to enlarge the training on bioterrorism prevention and protection on the basic and advanced levels in order to emphasize the practical training and the integration of NRBC aspects.

4.6. Research and development: priorities for the next future

In order to face present and future hazards, a research and development strategy combining fundamental researches and applied development in microbiology and biotechnology must be implemented. Different national organizations, including Ministry of Defence in France, largely support initiatives for research programmes on infectious diseases. The projects combining both applied and fundamental approaches and linking different complementary research groups should be treated as a priority. There is however
an important need of support for national and international research programmes on medical defence against bioterrorism.

4.6.1. French national first priorities

Beside alert strategy based on medical intelligence and epidemiological surveillance, authorities claim alarms systems and rapid detection for air and water control. Advisory strategy based on a pertinent analysis of information and credible mathematics models is a key for the operational decision. Today it is one of the first priorities of researches. Development of technology useful for liable devices and systems for rapid detection is at the same level of priority.

Concerning weaponisable agents and biomedical researches, the priorities are firstly rapid diagnostic and short-term prevention and treatment countermeasures, including passive immunoprophylaxis and immunotherapy, as it was described above. Three first targets have been identified:

- Pulmonary anthrax: rapid diagnosis, new strategy for antibiotherapy and vaccine [48, 49],
- Smallpox: animal model, rapid diagnosis, antiviral drugs and new vaccines [17,18],
- Botulinum toxins and ricin: rapid detection, antidote and specific immunoglobulins for short-noticed prevention and treatment [50].

Despite these priorities, the other biological threats must not be discarded specially when they present a risk for the community as: plague, tularaemia, glanders and melioidosis, viral encephalitis, haemorrhagic fevers. Improvement of: (i) diagnosis methods, (ii) antiviral products and strategies, (iii) passive immunoprophylaxis and immunotherapy including vaccine therapy, are probably the critical challenges for the next future in the matter [51].

National, European and international research cooperation must be promoted on these objectives.

4.6.2. Others transversal technologic priorities for the military medical service

Beside above priorities the French Ministry of Defence had identified three other specific areas of biomedical researches concerning military operations under biological threat: (i) need for a reactive epidemiological surveillance and modelization of epidemiological data; it is important in order to support operational decisions [52], (ii) simple biosafety devices for the transport and analysis of biological samples in the field (sanitary campaign units) in order to perform early adapted prevention and treatments, (iii) drugs and regimens adapted to mass prevention, curative treatments and disinfection according to the local hostile environmental conditions.

4.6.3. Long term researches priorities

An active scientific and technological surveillance is requested to anticipate the future vulnerabilities. They must be particularly performed on potential biohazards due to the rapid evolution of biotechnology including genetic and biomedical potential applications. The following areas must be firstly targeted.
Physiopathology of diseases and evaluation of combined infectious diseases including influenza [53]. Knowledge of pathogenic mechanisms and specific or non-specific immune response during these infections could be major information to identify molecular targets and to improve prevention and treatment strategies. Genomic and post-genomic researches are one of the keys for this purpose, not only to determine the structure of potential targets but also to identify their functional role and their interactions.

Design and screening of new active substances. For example, the design of potential inhibitor of neurotoxic effects of tetanus neurotoxin (TeNT) and botulinum neurotoxin (BoTNT) is a good illustration of such capabilities. The toxicity of these toxins is related to their selective zinc-metallopeptidase nature. They cleave small proteins involved in the neurotransmitters exocytosis such as synaptobrevin [50]. This natural and/or modified peptide used as a substrate has shown the allosteric nature of the above enzymes. Some inhibitors candidates were synthesized by solid phase method within combinatorial chemistry strategy and were successfully tested in vitro [54].

Molecular engineering could chemically or genetically modify these toxins in order to shift their target or to increase their activity. These toxins could be cloned in bacteria such as Escherichia coli or in influenza viruses to improve their production or their activity on humans. The development of these technologies must not be discarded [55].

Gene therapy vectors and viral shuttles such as adenoviruses are commercially accessible. Viral targeting is one of the most efficient methods to transfer expressing transgene according to the therapeutic needs especially to turn off the side effects [56]. These vectors could be potential recombinant biological weapons. Strategies for adapted countermeasures might be researched.

Vaccines and drugs delivery systems using particle compounds and liposome are of concern. Many data have demonstrated the interest of drug targeting. The objective of these techniques is the improvement of the efficacy associated with a reduction of doses. This way was successful in mice for delivering of nanoparticles of antibiotics by oral route against salmonellas. Challenge for the future could be the development of nanoparticles capable to protect and to deliver selectively peptides and proteins avoiding proteolysis and adverse reactions of the immune system. These particles could modify pharmacokinetic of the compounds and could delay and control the sequence of drug delivery [57].

5. Conclusion

The international Convention banning biological weapons is in force since 1975 and has been ratified by more than 140 states. Due to the absence of a control policy, the strength of this convention was not well established. Programs developed by the former Soviet Union, the apartheid regime in South Africa or Iraq has largely weakened its credibility. The tentative to reinforce the Convention by an additional Verification Protocol failed in December 2001. To face the reality of the threat, strategies for the prevention of Biological warfare have been proposed since 10 years [58].
The anthrax attack in September 2001 had clearly demonstrated the reality and the capabilities of bioterrorism. All the experience acquired from the struggle against natural emergent or re-emergent infectious diseases is useful to face biological warfare or bioterrorism threats. Training of health professionals to face biological hazards is a realistic defence position and has to be managed as a national public health programme priority.

Biomedical defence against biological warfare requests complementarily of civilian and military experts, not only for the management of procedures, but also for professional training, drug or device equipment strategies, research and development programmes.

A short list of natural pathogens represents the main hazards. All efforts must be focused on medical countermeasure in order to prevent these diseases and to reduce the panic, source of dramatic disorganisation. For a long time, the French Ministry of Defence and the French Military Medical Service have conducted a continuous research and development strategy [59]. Immunological domains belong to the first line among researches, not only for vaccine design but also to provide short-noticed specific countermeasures and treatments. The forthcoming hazards due to new biological agents and molecular or genomic technologies might not be underestimated [60]. It is a challenge in term of future concerns for both military and civilian responsible as well as for the whole society. The research policy must anticipate the future threats through a continuous effort in fundamental research. This research is the foundation of the knowledge of the hazards and the implementation of the appropriate responses.

References


