Defence Research and Development Canada
Recherche et développement pour la défense Canada

DEFENCE R&D DÉFENSE

Adenovirus and its vector for developing vaccines against biological warfare agents

Qiaohua (Josh) Wu
Defence R&D Canada – Suffield

Technical Memorandum
DRDC Suffield TM 2004-249
November 2004

Canada

Adenovirus and its vector for developing vaccines against biological warfare agents

Qiaohua (Josh) Wu
DRDC Suffield

Defence R&D Canada – Suffield

Technical Memorandum

DRDC Suffield TM 2004-249

November 2004

Author

Dr J. Q. Wu

Approved by

Dr L. P. Nagata

Head, Chemical & Biological Defence Section

Approved for release by

~~Dr R. Bide~~ P.A. D'Agostino

Chair, DRDC Suffield Document Review Panel

Abstract

This memorandum reviews a new platform for the development of vaccines against biological warfare (BW) agents. The noted platform uses adenovirus as a vector to deliver DNA that encodes for key antigens of BW agents. Once inside the mammalian cell, this adenovirus vectored vaccine induces rapid immune responses. This memorandum describes the basic virology of adenovirus, methods used to modify adenovirus as vaccination vector, advantages of adenovirus vector for developing vaccines against BW agents such as Ebola and anthrax, and the potential obstacles, with proposed solutions, for the use of adenovirus vector.

Résumé

Ce mémorandum examine une nouvelle plate-forme de mise au point de vaccins contre les agents de guerre biologiques (BW). La plate-forme en question utilise l'adénovirus comme un vecteur pourvoyeur d'ADN codant les antigènes clés des agents BW. Une fois à l'intérieur de la cellule mammalienne, ce vaccin à vecteur adénoviral induit des réponses immunitaires rapides. Ce mémorandum décrit la virologie fondamentale de l'adénovirus, des méthodes utilisées pour modifier l'adénovirus en vecteur de vaccination, de l'avantage du vecteur adénoviral développant des vaccins contre les agents BW, tels qu'Ébola et le charbon bactéridien et des obstacles pouvant se présenter aux solutions proposées, en ce qui concerne l'utilisation du vecteur adénoviral.

This page intentionally left blank.

Executive summary

Vaccines remain the most cost-effective medical countermeasure to protect the Canadian Forces (CF) against BW agents. With only a few vaccines covering a small proportion of BW agents, current biodefence against attacks of BW agents is inadequate. In addition, current vaccines suffer poor safety profiles and usually require multiple injections to achieve protection. To address these deficiencies, this memorandum introduces adenovirus vector for developing vaccines against BW agents.

Adenovirus and adenovirus vector

Adenovirus is a non-enveloped, double-stranded DNA virus, which can be found in a wide variety of mammalian and avian species. Human adenoviruses represent a large group within the adenovirus family; So far, 51 serotypes have been identified. The virus causes outbreaks of acute respiratory disease (ARD) in military trainees. A live adenovirus vaccine was developed in the early 1970s and used to successfully control outbreaks of ARD.

Because of the success and safe use of live adenovirus vaccines in military trainees, adenovirus has been proposed to be used as a delivery vector for making genetic vaccines against other pathogens. To modify adenovirus as a vaccine vector, part of adenovirus genome is deleted allowing insertion of foreign DNA into the vector. In addition, the deletion cripples virus replication, which enhances the safety of adenovirus vector. After vaccination, adenovirus vectors carry the foreign DNA into cells, express the antigens. and induce immune responses.

Advantages of adenovirus vectored vaccine against BW agents

There are several advantages for the use of adenovirus vectored vaccine against BW agents: 1) Adenovirus vectored vaccines offer immediate protection against BW agents after single dose injection; 2) Adenovirus vectored vaccine induces immune responses on mucosal surface of respiratory tract where most BW agents enter and initiate infections; 3) Adenovirus vectored vaccines are easy to produce in large quantities and can be formulated as oral vaccines, which simplify the vaccination process; and 4) Adenovirus vector acts as an adjuvant for the encoding antigens, eliciting strong humoral and cellular immune responses.

Future development

Vaccines made from adenovirus vector have been shown to rapidly protect animals from challenges of BW agents such as Ebola and anthrax. To use these vaccines in humans, obstacle of preexisting immunity against adenovirus vector in human population needs to be overcome. The preexisting immune response neutralizes the adenovirus vector and prevents it from expressing foreign antigens. Moreover, preexisting immunity increases the toxicity of adenovirus vector. Solutions to circumvent the preexisting immunity is to use other nonpathogenic adenovirus to which the majority of humans do not have neutralizing antibodies or to encapsulate adenovirus vectored vaccine with cationic liposomes or alginate microparticles.

Conclusion

The increased threat of bioterrorism highlights the need to improve existing vaccines against BW agents and to develop new ones. An ideal vaccine against BW agents should be safe and easy to deliver, provide long-lasting protection, and require only one or a few doses. Adenovirus vector meets most of these requirements. Adenovirus vectored vaccines are promising candidate for the protection of animals against BW agents; however, obstacle of preexisting immunity to adenovirus vector in the human population needs to be overcome before these vaccines can be applied to civilians or the military.

Wu, Q.J. (2004). Adenovirus and its vector for developing vaccines against biological warfare agents. (DRDC Suffield TM 2004-249). Defence R&D Canada – Suffield.

Sommaire

Les vaccins demeurent les contre-mesures médicales protégeant les Forces canadiennes (FC) contre les agents BW qui sont les plus économiques. Étant donné qu'il n'existe que quelques vaccins contre un petit nombre d'agents BW, la défense biochimique actuelle contre les attaques d'agents BW n'est pas suffisante. De plus, les vaccins actuels ont des profils d'innocuité peu satisfaisants et requièrent des injections multiples pour atteindre un niveau de protection adéquat. Ce mémorandum aborde le problème de ces faiblesses en introduisant le vecteur adénoviral pour la mise au point des vaccins contre les agents BW.

L'adénovirus et le vecteur adénoviral

Un adénovirus est un virus ADN double chaîne, dépourvu d'enveloppe que l'on peut trouver chez une grande variété d'espèces mammaliennes et aviaires. Les adénovirus humains constituent un groupe important de la famille adénovirus ; 51 sérotypes ont été identifiés jusqu'à présent. Ce virus cause une épidémie de maladies respiratoires graves chez les militaires durant leur entraînement. Un vaccin adénovirus vivant a été mis au point au début des années 1970 et a réussi à maîtriser l'épidémie de maladies respiratoires aiguës.

Les vaccins adénovirus vivant étant sécuritaires et efficaces chez les militaires durant leur entraînement, on a proposé d'utiliser l'adénovirus comme vecteur pourvoyeur pour fabriquer des vaccins génétiques contre d'autres pathogènes. Pour modifier l'adénovirus en vecteur vaccinal, une partie du génome adénovirus est supprimé, ce qui permet l'insertion de DNA étranger dans le vecteur. Ceci paralyse aussi la réplication du virus et améliore la sécurité du vecteur adénoviral. Après la vaccination, les vecteurs adénoviraux transportent du DNA étranger dans les cellules, expriment les antigènes et induisent des réponses immunitaires.

Les avantages des vaccins à vecteur adénoviral contre les agents BW

Il existe plusieurs avantages à utiliser les vaccins à vecteur adénoviral contre les agents BW : 1) une seule dose d'injection des vaccins à vecteur adénoviral offre une protection immédiate contre les agents BW ; 2) le vaccin à vecteur adénoviral induit des réponses immunitaires à la surface muqueuse des voies respiratoires où la plupart des agents BW s'introduisent et initient des infections ; 3) les vaccins à vecteur adénoviral sont faciles à produire en grandes quantités et peuvent être formulés en vaccins oraux, ce qui simplifie le processus de vaccination et 4) un vecteur adénoviral agit comme adjuvant pour l'antigène de codage, ce qui suscite de fortes réponses immunitaires humorales et cellulaires.

Développements futurs

Les vaccins fabriqués à partir de vecteur adénoviral ont démontré qu'ils protègent rapidement les animaux contre les agents BW tels qu'Ébola ou le charbon bactéridien. Pour utiliser ces vaccins chez les humains, il faut surmonter les obstacles liés à une immunité préexistante contre le vecteur adénoviral. La réponse immunitaire préexistante neutralise le vecteur

adénoviral et l'empêche d'exprimer les antigènes étrangers. De plus, l'immunité préexistante augmente la toxicité du vecteur adénoviral. La solution contournant l'immunité préexistante consiste à utiliser d'autres vecteurs adénoviraux non pathogéniques, non existants chez la majorité des humains, qui neutralisent les anticorps ou bien d'encapsuler le vaccin à vecteur adénoviral avec des liposomes cationiques ou des microparticules d'alginate.

Conclusion

La menace croissante du terrorisme biologique met en évidence le besoin d'améliorer les vaccins existants contre les agents BW et d'en mettre au point de nouveaux. Un vaccin idéal contre les agents BW devrait être sécuritaire et facile à livrer ; il devrait procurer une protection de longue durée et ne requérir que quelques doses. Le vecteur adénoviral répond à ces besoins. Les vaccins à vecteur adénoviral sont des candidats prometteurs à la protection des animaux contre les agents BW ; l'obstacle causé par l'immunité préexistante au vecteur adénoviral chez les humains doit cependant être surmonté avant que ces vaccins puissent être appliqués aux civils et aux militaires.

Wu, Q.J. (2004). Adenovirus and its vector for developing vaccines against biological warfare agents. (DRDC Suffield TM 2004-249). R & D pour la défense Canada – Suffield.

Table of contents

List of figures

List of tables

This page intentionally left blank.

Introduction

By the 1960s, adenovirus had caused outbreaks of ARD in military trainees. In 1971, live adenovirus vaccine was developed and used successfully to control the outbreaks of ARD [1, 2]. The safe and effective adenovirus vaccine has stimulated the idea of using adenovirus as a vector to develop vaccines. Vaccines made from adenovirus vector overcome some problems facing the conventional vaccines. Like other live viral vaccines, adenovirus vectored vaccines induce strong immune responses against pathogens. In this memorandum, background information about adenovirus and adenovirus vaccine to control ARD in military trainees will be provided. Other topics will be how to generate adenovirus vectored vaccine, the advantages of adenovirus vector for developing vaccines against BW agents, and the obstacle facing adenovirus vectored vaccine and solutions to overcome these limitations.

Overview of adenovirus

Adenovirus was isolated in the early 1950s from human tonsils and adenoidal tissue [3], as well as respiratory secretions obtained from military recruits with ARD [4]. The virus was subsequently named *adenovirus* based on the original tissue (adenoids) in which the virus was isolated [5]. Adenovirus belongs to the *Adenoviridae* family, which includes two genera: the *Aviadenovirus* genus infects only birds, whereas the *Mastadenovirus* genus infects a wide range of mammalian species. Human adenovirus represents a large group within the *Adenoviridae* family. So far, 51 serotypes have been identified by neutralization assays with reference horse antisera. These serotypes can be classified into six subgroups based on the ability to agglutinate red blood cells and percentage of guanine/cytosine in viral DNA (Table 1).

Table 1. *Classification of human adenoviruses*

SUBGROUP	SEROTYPE	HEMAGGLUTINATION
A	12, 18, 31	Very weak to none
B	3, 7, 11, 14, 16, 21, 34, 35, 50	Monkey erythrocytes (complete)
C	1, 2, 5, 6	Rat erythrocytes (partial)
D	8, 9, 10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-49, 51	Rat erythrocytes (complete)
E	4	Rat erythrocytes (partial)
F	40, 41	Rat erythrocytes (atypical)

Among human adenoviruses, serotype 5 (HAd5) is well characterized. When other human adenoviruses have been studied, their properties have proved similar to those of HAd5. Thus, the following information about adenovirus is mainly based on study of HAd5.

Virion structure

Human adenovirus is a non-enveloped virus. The viral particle (virion) is 70 to 90 nm in diameter and contains 13% DNA, 87% protein, and small amounts of carbohydrate. A single molecule of linear, double-stranded DNA is surrounded by an icosahedral (20 faces) protein shell known as capsid. The capsid is composed of 252 subunits, of which 240 are hexons and 12 are pentons. Each penton contains a base, which forms part of the surface of the capsid, and a projecting fiber whose length varies among different serotypes. The hexons primarily induce group-specific complement-fixing antibodies, whereas the pentons are especially active in hemagglutination. Besides hexon and penton, adenovirus virion contains 7 other structural proteins.

The virion of adenovirus is stable in the presence of physical and chemical agents, as well as adverse pH condition. This allows it to survive for long periods of time outside the body and makes them available for transmission to others. For example, adenovirus type 3 survived up to 10 days on paper under ambient condition and adenovirus type 2 survived from 3-8 weeks at room temperature [6]. The virion is susceptible to heat at 56^0C for 30 minutes, ultraviolet irradiation, 0.25% sodium dodecyl sulfate, 1% sodium hypochlorite, and 2% glutaraldehyde, but are resistant to ether and chloroform.

Viral genome

The size of human adenovirus DNA genome is 36 kilobases (kb) with molecular weight of $35x10^6$. All human adenovirus genomes identified so far have the same organization. The linear DNA genome contains two identical origins for DNA replication, present in left and right ends of the genome. There is a cis-acting packaging sequence within several hundred base pairs of the left end of the viral genome to direct the encapsidation of the viral DNA. At least 30 different mRNA species are transcribed from human adenovirus genome. Based upon the onset of viral DNA replication, these mRNA can be divided into three classes: early transcripts (E1A, E1B, E2, E3, and E4), delayed early transcripts (IX and IVa2), and late transcripts (L1 to L5) [6]. These transcripts are made from both strands of the viral DNA with the rightward reading strand coding for the E1A, E1B, E3, IX, and late transcripts and the leftward reading strand coding the E4, E2 and IVa2 transcripts (Figure 1).

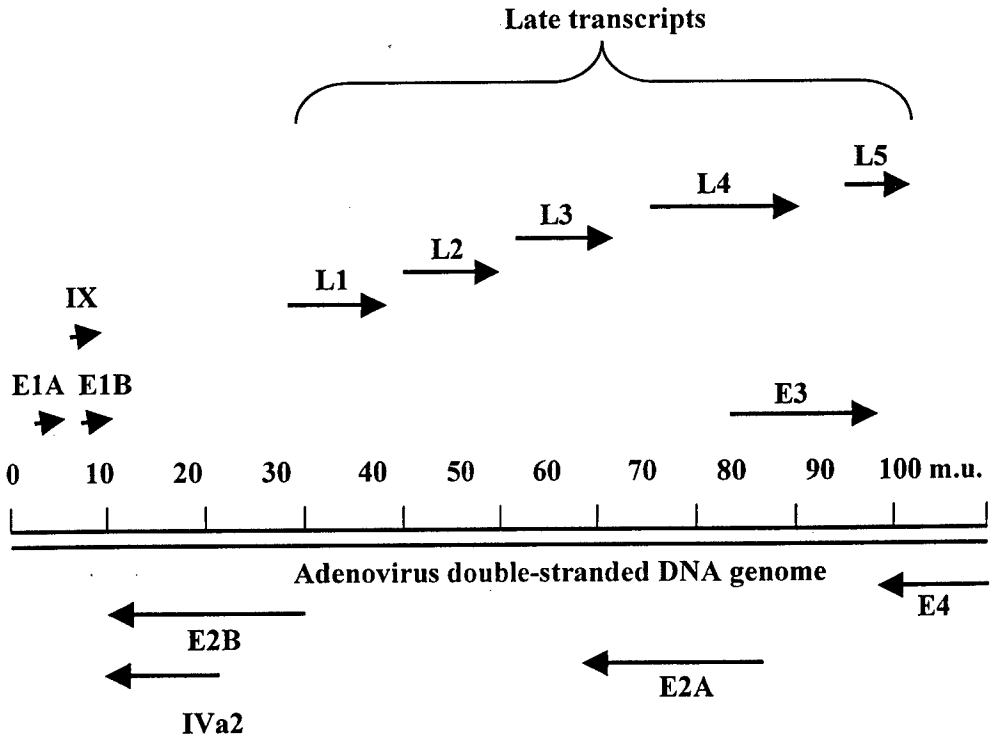

Figure 1. *Schematic diagram of human adenovirus genome and transcription map. The length of genome is about 36 kb and is divided into 100 map units. Arrows above and below the central double lines indicate the direction of transcription with capital E for early transcripts and L for late transcripts.*

Replication cycle

The replication cycle of human adenovirus starts with an attachment of fiber protein to a cell surface molecule known as the coxsackievirus group B and adenovirus receptor (CAR; [7]) (Figure 2). The interaction between fiber and the CAR allows another viral structural protein, the penton base, to bind to a second cell surface molecule, the integrin [8]. Binding to integrins promotes internalization of the adenoviral particle via receptor mediated endocytosis. Once inside the cell, a sequential disassembly of the capsid proteins allows the virus to escape from the endosome and enter the cytoplasm. The virion is subsequently transported to the nuclear pore complex and releases its DNA into the nucleus to initiate viral gene expressions [9].

The expression of adenovirus proteins is divided into early, delayed early, and late phases. The E1A and E1B proteins are the first early proteins to be expressed from viral DNA and these are absolutely required for virus replication. These proteins engage in a number of regulatory functions including: 1) induction of DNA synthesis in quiescent cells, 2) immortalization of primary cells, 3) activation of other early and late genes, and 4) induction of apoptosis. Early proteins encoded by E3 transcripts are not required for virus replication; however, these proteins play a role in the invasion of host immune system and may be related to pathogenesis of adenovirus. For instance, E3-encoded proteins (10.4K, 14.5K, and 14.7K) inhibit tumor necrosis factor (TNF), a key inflammatory cytokine to prevent viral replication [10]. Gp19K, another E3-encoded protein, binds to class I antigen of the major histocompatibility complex, preventing cells from signaling cytotoxic T lymphocytes to eliminate virus-infected cells [11].

The replication of adenovirus DNA begins at about 7 hours after infection [6]. Viral DNA replication is initiated by a set of early proteins (terminal protein, DNA polymerase, and DNA-binding protein) encoded by E2 transcripts. Replication starts at either end of the double-stranded DNA through the displacement of one of the parental strands and subsequently replicating the displaced strand. After onset of viral DNA replication, the late genes are expressed by alternative splicing and polyadenylation of a large pre-mRNA generated at the single major late promoter (Figure 1). All late mRNA transcripts have a tripartite leader sequence at their 5' termini that enhance translation of viral structural proteins. The accumulation of viral structural proteins and newly synthesized viral DNA in nucleus triggers virus assembly at 20 to 24 hours after infection. Virions are released from the cells after 2 to 3 days of infection.

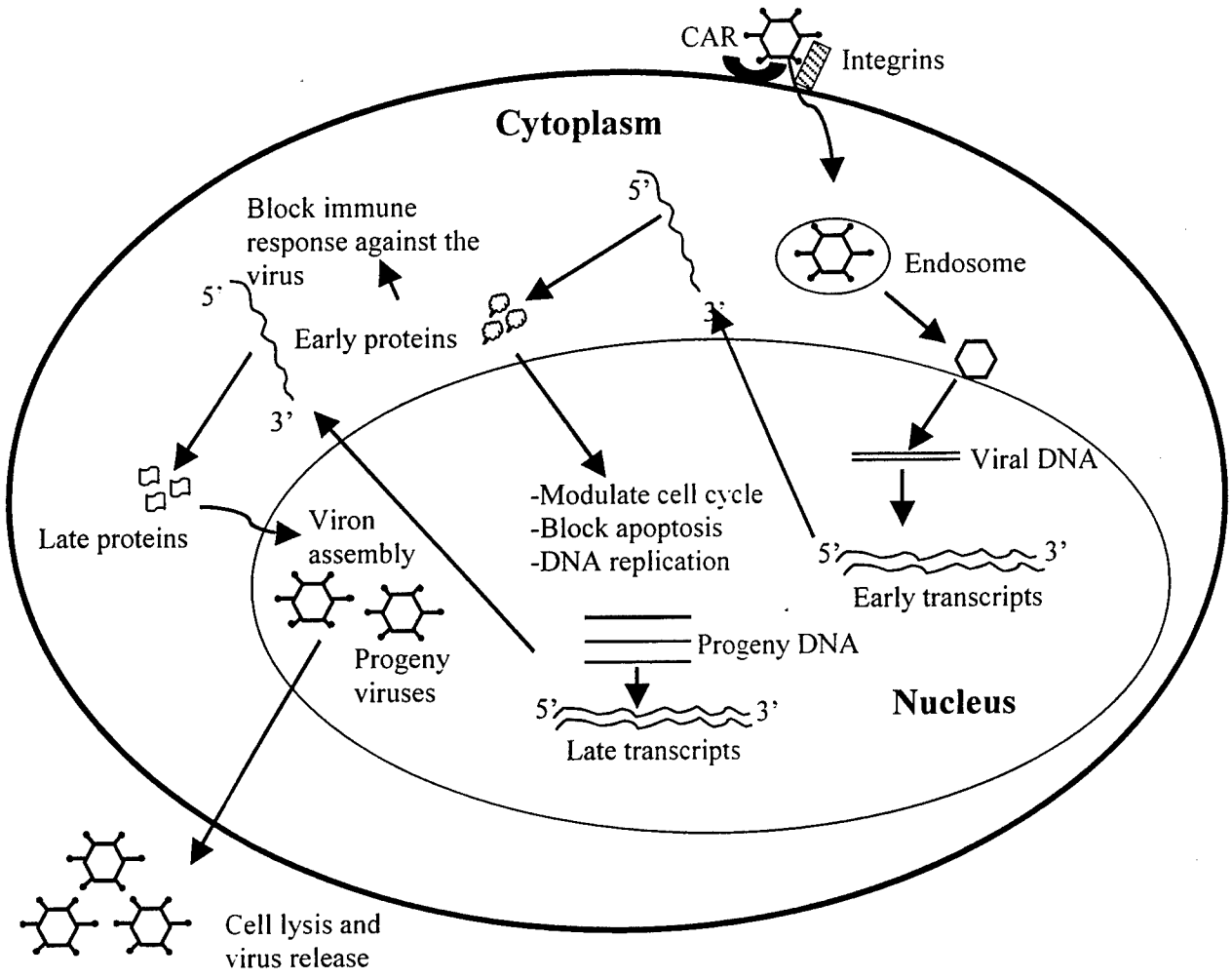

Figure 2. *The replication cycle of human adenovirus*

Acute respiratory disease and adenovirus vaccine

Human adenoviruses cause varieties of diseases in the respiratory tract, the eye and the gastrointestinal tract (Table 2). Among them is ARD, a febrile respiratory illness with symptoms similar to influenza. The disease usually occurs in young adults in a closed setting such as military training camp [12]. ARD is transmitted through airborne or aerosolized virus inhaled into the lung. The incubation time of the disease is 4 to 5 days. The disease is usually self-limited, lasting only 3 to 10 days. However, some military trainees have developed pneumonia and died due to ARD [13].

***Table 2.** Human adenovirus – associated infections* *

INFECTION	SEROTYPE	RISK GROUP
Acute pharyngitis	1-3, 5-7	Infants, young children
Pharyngoconjunctival fever	3, 7, 14	School-aged children
Acute respiratory disease	3, 4, 7, 14, 21	Military trainees
Pneumonia	1-3, 7	Infants, young children
Pneumonia	4, 7	Military trainees
Epidemic keratoconjunctivitis	8, 11, 19, 37	All age group
Gastroenteritis	40, 41	Infants, young children

* Adapted from Fields Virology, 4th edition, Editors: D.M Knipe and P.M. Howley, 2001.

Adenovirus vaccine plays a major role for the control of outbreaks of ARD among military recruits. The development of adenovirus vaccine is inspired by the observation that human adenovirus serotype 4 and 7, the main pathogens of ARD, infect the gastrointestinal tract without causing clinical disease [14]. The vaccine was developed in late 1960s by formulating live human adenovirus serotype 4 or 7 into a tablet, which consists of three layers: a central core of lyophilized adenovirus, a middle layer of inert material, and an outer layer of protective enteric coating [15]. The tablet is swallowed quickly without chewing. Oral administration of the vaccine produces an asymptomatic, intestinal infection while protecting the individual against ARD. The mechanism of induction of respiratory immunity through oral vaccination is unclear. This might involve the generation of serum neutralizing antibody against adenovirus. Alternatively, the virus might spread from the gut to the respiratory tract to induce local immunity since adenovirus could be isolated from pharynx secretions following oral administration [16].

The military began to routinely vaccinate recruits reporting to training centers in the 1970s. To obtain quick protection, the recruits were given the vaccine within hours of arrival at their training centres. Initially, the vaccine was only given during the high-risk winter season. Outbreaks of adenovirus-associated ARD during late spring and early fall prompted the military to administrate vaccine through the year. The vaccine is safe and reduced adenovirus-associated ARD by over 90% and ARD-related hospitalization by 50% [17]. Adenovirus vaccine is also cost-effective, saving the U.S. military $7.53 million during 1970 to 1971 [18].

Unfortunately, in the early 1990s, Wyeth-Ayerst Laboratories (the sole manufacturer for adenovirus vaccine) was informed by the US Department of Defense that the Food and Drug Administration required a new facility to manufacture the vaccine. The lack of funds to build such facility at that time forced Wyeth-Ayerst to end vaccine production in 1996. Since the cessation of vaccination in 1999, approximately 10%-12% of all trainees have become ill with adenovirus-associated ARD and two deaths have been reported [13, 19, 20]. This has prompted the military to resume the production of adenovirus vaccine. It is expected that the vaccine will be available in 2008.

Adenovirus vector for developing vaccines against BW agents

Currently, only a few of vaccines are available for protecting the CF from BW agents [21] (Table 3). These vaccines are either inactivated or attenuated live pathogens. Several drawbacks are related to the production and use of these vaccines (Table 4). To be effective, vaccines based on inactivated pathogens require relatively large doses (usually a few micrograms) and multiple injections. Because of the use of human pathogens as the source material, the production of killed vaccines needs costly containment facilities to minimize risk of infection of process workers and extensive quality control to ensure complete inactivation. In addition, the presence of adventitious agents in vaccine preparations could lead to side effects. Compared to inactivated vaccines, live attenuated vaccines often offer strong immunity because these vaccines closely mimic the natural infection of pathogen. Live attenuated vaccines, however, are vulnerable to genetic reversion to virulent phenotypes, which would be catastrophic for some diseases such as Ebola. The event of September 11, 2001 and the incidents of inhalation anthrax bioterrorism underscore new approaches required for improving current vaccines and developing new ones.

Successful use of the adenovirus vaccine to protect military trainees from ARD has aroused the idea of modifying adenovirus as a vector for vaccine delivery [22-24]. Through genetic engineering, DNA encoding protective antigen can be isolated from BW agents and cloned into adenovirus vector to make vaccine, avoiding work with live human pathogens in the production of conventional vaccines. As effective as other live virus vaccines, adenovirus vectored vaccine induces strong immune responses against the encoded antigen.

Table 3. *Current status of vaccines against BW agents* [a]

Disease	Vaccine Type
Anthrax	Licensed Subunit
Brucellosis	No vaccine
Botulism	Pentavalent toxoid [b]
Tularemia	Live, attenuated [b]
Plague	Licensed inactivated
Q fever	Inactivated [b]
Smallpox	Licensed live, attenuated
Venezuelan, eastern, and western equine encephalitis	Live, attenuated or inactivated [b]
Viral hemorrhagic fevers such as Ebola	No vaccine

a. Adapted from Vaccine, 4th edition, Editors: S.A Plotkin and W.A. Orenstein, 2004.
b. Investigational new drug, limited-use vaccines given only under protocol and written informed consent.

Table 4. *Drawbacks of current vaccines against BW agents*

Live attenuated

1. Reversion to virulent forms that may causes clinical disease
2. Side effects because of adventitious agents in the cells and medium used for growth
3. Low temperature required for storage and transport
4. Limited shelf life

Inactivated

1. Multiple injections usually required for protection
2. Hazard to personnel working with large amounts of human pathogens
3. Need to ensure complete inactivation of pathogens
4. Side effects because of large amounts of cellular material
5. Low temperatures required for storage and transport
6. Limited shelf life

Modification of adenovirus as vaccine vector

HAd5 has been used as a prototype for vaccine development because of its low pathogenesis, ease to grow in cell culture, and well-known molecular biology. Since the size of the capsid of adenovirus is fixed, the amount of foreign DNA that can be packaged inside the capsid is limited. For example, HAd5 capsid can only accommodate up to 1.2 kb of extra foreign DNA without affecting its stability and infectivity [25]. To insert larger size of foreign DNA, most adenovirus vectored vaccines are developed by replacing existing viral sequences with genes of interest. To expand the cloning capacity of HAd5 vector, the E1 and E3 coding regions of HAd5 genome have been deleted, which allows for the insertion of foreign DNA up to 7.5 kb [26].

Because of the large size of HAd5 vector (36 kb), it is difficult to directly stitch foreign DNA into the vector. To overcome this problem, Graham and his colleagues at the McMaster University developed a cloning system based on homologous recombination in mammalian cells [27]. The system involves construction of a transfer plasmid containing the left and right end of HAd5 genome sequence and an expression cassette, consisting of a foreign gene of interest, a promoter, and a poly A signal, flanked by the HAd5 sequence. Cells are then co-transfected with the transfer plasmid and the plasmid containing HAd5 genome with deletions of the E1 and E3 region. Homologous recombination in cells allows cloning the expression cassette into HAd5 vector.

Although Graham's method for inserting foreign gene into HAd5 vector has proven extremely useful, the major limitations are the low efficiency of homologous recombination and the need for screening individual plaques for the desired recombinants. To simplify HAd5 vector technology, a procedure based on the homologous recombination in bacteria was developed [28, 29] (Figure 3). Because of the high efficiency of homologous recombination in bacteria and deleting the step for plaque isolation, the procedure speeds up the production of HAd5 vectored vaccines.

To produce adenovirus expressing foreign gene, the plasmid, containing the expression cassette and HAd5 genome with deletions of the E1 and E3 region, is transfected into a cell line that provides E1 proteins essential for the vector replication

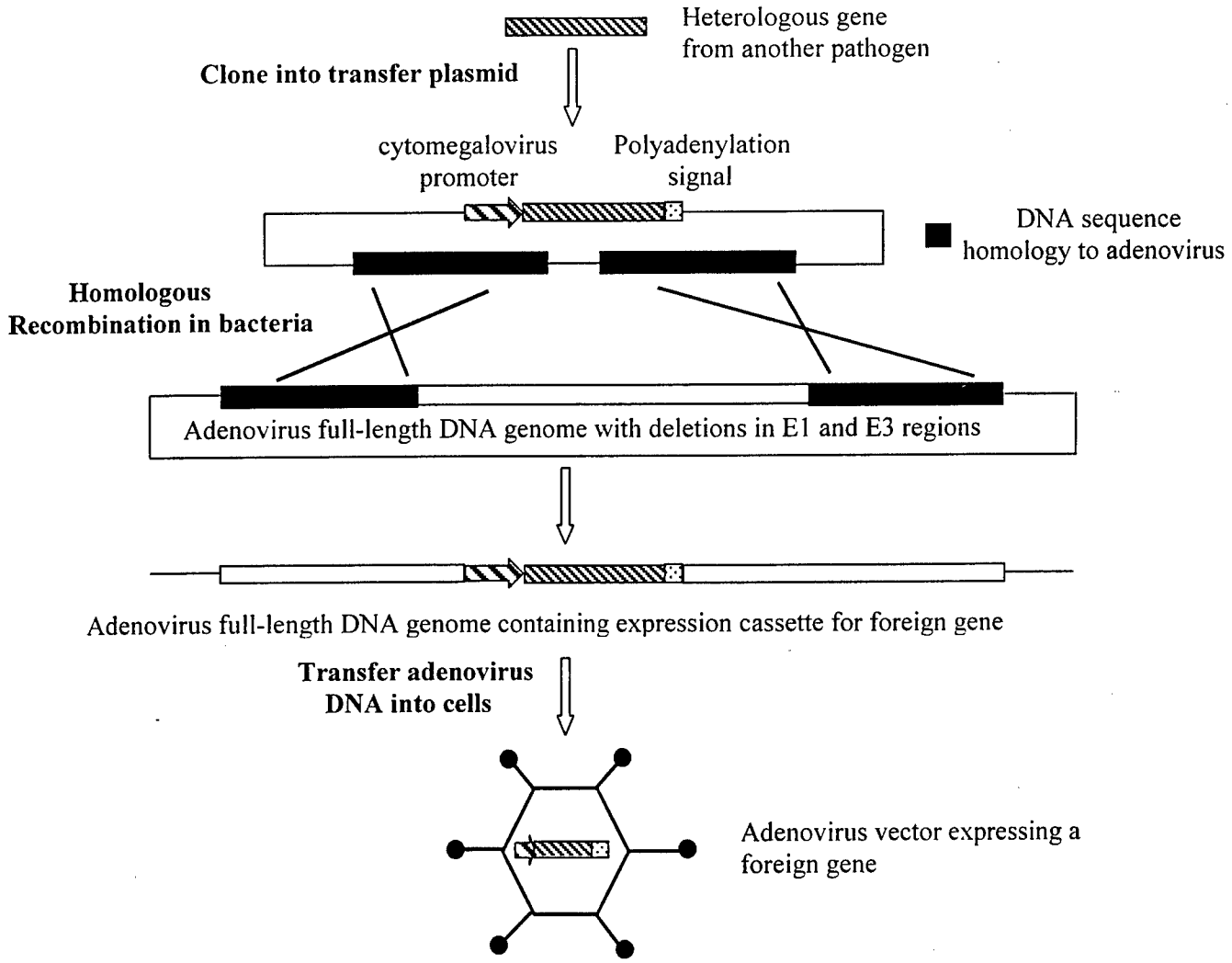

Figure 3. *Generation of human adenovirus vector carrying a foreign gene*

Production of adenovirus vectored vaccine

HEK 293 cell line [30], derived from human embryonic kidney cells containing the E1 coding region of adenovirus, is the main cell line used for large scale production of adenovirus vectored vaccine [31]. Two methods can be used for the production of adenovirus vectors: adherent cell culture and suspension cell culture. Usually the productivity of the vector is slightly higher in adherent HEK 293 cell culture (7,000-12,000 virus/cell) as compared to the cells cultured in suspension (5,000 virus/cell) [32]. However, for manufacturing purpose, suspension cell culture is more convenient for large scale production in which $1X10^{11}$ virus/ml media has been obtained in a HEK 293 suspension culture [33].

HEK 293 cells can grow in serum free media free of any bovine-derived additives. In addition, the cells are not patented and can be accessed without licensing agreement. A drawback of HEK 293 cell lines is the generation of wild-type adenoviruses during the production of the vaccines. These viruses are generated by recombination between sequences in adenovirus vector and homologous adenovirus sequences in HEK 293 cells, resulting in the acquisition of E1 region by the adenovirus vector. The present of wild-type adenovirus in vaccine preparation raises safe issue of the vaccine. To avoid generation of wild-type adenovirus, PER.C6 and 911 cell lines were developed in which all the sequences homologous to adenovirus vector are deleted [34].

Safety of adenovirus vectored vaccine

Adenovirus vector has been widely used in gene therapy clinical trials to treat diseases such as cancer, peripheral artery disease, and cystic fibrosis. For instance, in 2001, there were 331 clinical trials worldwide using recombinant viruses as gene delivery vectors, 40% of which used adenovirus vectors [35]. Oral administration of live adenovirus, the backbone of adenovirus vector, has proven safe to vaccinate military trainees against ARD. To make the vector safer, the less virulent serotype of human adenovirus such as HAd5 is selected and the gene such as E1 region essential for virus replication is deleted, rendering the vector replication defective. Additionally, adenovirus vector do not integrate its DNA into host chromosomes. This feature avoids the possibility of disturbing vital cellular genes or inducing cancer as seen occasionally in studies using the retrovirus vector [36].

Advantages of adenovirus vector for developing vaccines against BW agents

Immediate protection by adenovirus vectored vaccine

Current vaccines against BW agents, such as anthrax and viral encephalitis, require multiple doses for priming and frequent periodic boosting to reach acceptable levels of protection. Animal study has shown that after a single dose of inoculation, adenovirus vectored vaccine elicited a fast (15 days after vaccination) and a long-lasting (6 months after vaccination) immune responses [37]. The magnitude and kinetics of immune responses induced by single dose administration were similar to those obtained by multiple immunizations.

Recently, a single-dose, fast-acting vaccine against Ebola virus was developed using adenovirus vector expressing Ebola glycoprotein and nucleoprotein [38]. Macaques became immune to Ebola 28 days after a single dose injection. Protection was highly effective and correlated with the generation of Ebola-specific cytotoxic T cell and antibody responses. Adenovirus vector was also used to make fast-acting vaccine against anthrax [39]. When compared to the vaccine made from recombinant protective antigen (PA)/Alhydrogel, the adenovirus vector encoding PA generated anti-PA antibodies more rapidly and at a higher level. At 11 days after a single vaccination, the adenovirus vectored vaccine was able to provide mice some protection against *B. anthracis* lethal toxin, whereas the recombinant PA/Alhydrogel vaccine provided none. Four weeks after a single vaccination, mice injected with the adenovirus vectored vaccine had rendered approximately 2.7-fold more protection than the recombinant PA vaccine.

Therefore, immediate protection after single dose administration of adenovirus vectored vaccines allows these to be used at short notice. These will also avoid routinely vaccinating military populations against BW agents in the absence of any clear indication of an impending attack.

Induction of mucosal immunity by adenovirus vectored vaccine

The majority of BW agents enter the body through the mucosal surfaces of respiratory or gastrointestinal tract, biodefence vaccines eliciting mucosal immunity should limit the spread and replication of BW agents at the site of entry. Like skin, mucosa which covers the respiratory, intestinal, and urogenital tracts is the first line of defence against the invasion of microorganisms. One of major weapons in the mucosal defence system is the secretory IgA antibody. It is estimated that up to three grams of IgA is made and secreted daily in a normal 70 kg adult, which accounts for 60 to 70 percent of the total output of antibodies [40]. Microorganisms produce virulence factors that allow them to adhere, colonize or invade mucosal surfaces. Secretory IgA prevents invasion of these microorganisms by blocking their attachment while these are still at the external side of the mucosal barrier.

Adenoviurses are pathogens that infect mucosal cells of respiratory and gastrointestinal tracts. Therefore, vaccine vectors made from these viruses can deliver antigens to mucosal surface and induce mucosal immunity. For instance, cotton rats intranasally immunized with HAd5 vectors expressing the bovine herpesvirus glycoprotein induced a strong mucosal IgA antibody response against this protein [41]. These animals were protected from challenge of bovine herpesvirus and no infectious virions were isolated from the trachea of the animals vaccinated with HAd5 vectors. Similarly, when mice were intranasally immunized with an adenovirus vector expressing the rabies virus glycoprotein. these developed both serum antibody against rabies virus and secreted specific IgA antibody in the genital and intestinal tracts [42]. The induction of mucosal immunity by adenovirus vectored vaccines also demonstrated the protection of animals from HIV and rotavirus-associated infection.

Oral delivery of adenovirus vectored vaccine

Similar to adenovirus vaccine used for control of outbreaks of ARD in military trainees, adenovirus vectored vaccines can be formulated as an oral vaccine. Oral vaccination of animals with adenovirus vectored vaccines induced both serum and mucosal antibodies and protected animals from viral infections. Current vaccines against BW agents are usually administrated by needle injections. The effectiveness of vaccine programs would be enhanced by oral administration of adenovirus vectored vaccine that allows rapid vaccination of large numbers of individuals and induction of mucosal immunity against BW agents.

Adenovirus vector as an adjuvant for the encoding antigens

Adjuvants induce innate immunity by increasing the expression of costimulators and cytokines. Innate immunity not only confers early defence against pathogens, but it also sends out warning signals to trigger adaptive immunity, which specifically eliminates pathogens and enables us to ward off re-infection. Innate immunity influences the type of the adaptive immunity that subsequently develops. For example, macrophages, the major component of innate immunity, often secrete specific cytokines after ingestion of

microorganisms [40] These cytokines in turn promote the activation of lymphocytes specific for killing the microorganisms inside macrophages. Because of their ability to induce innate immunity, adjuvants have been used to enhance vaccine efficacy. In addition, adjuvants have been explored for use as a broad-spectrum therapeutics against BW [43].

Animal and clinical studies have shown that adenovirus vector can induce a strong innate immune response [44-46] and this response is associated with the adjuvant effect of adenovirus capsid protein [47]. Further analysis demonstrated that the hexon of adenovirus vector is a potent adjuvant [47]. When mice were immunized with a suboptimal dose of lipopeptides as a model immunogen along with hexon, the immunogenecity of lipopeptide was enhanced even in the absence of booster immunization. Therefore, adenovirus vector fulfills double duties, one as a vaccine carrier and anther as an adjuvant for the encoding antigen.

Induction of humoral and cellular immune response by adenovirus vectored vaccine

Humoral and cellular immunities are two major components of the adaptive immune system. Humoral immunity produces antibodies that kill microorganisms growing outside cells. Cellular immunity eradicates pathogens that colonize within cells by the use of specific cytotoxic T cells. When cells are infected by a pathogen such as a virus, these display bits of viral proteins on their surface. Cytotoxic T cells recognize these protein markers and destroy both the cells and the viruses within. Thus, activation of cellular immunity requires *do novo* expression of microorganism proteins within cells and presentation of these proteins onto cell surface.

Vaccines based on inactivated killed pathogens or on antigens isolated from pathogens usually require multiply shots to the recipient to boost the protection that often fades with time. On the other hand, vaccines based on attenuated live microorganisms, such as smallpox, often render lifelong immunity after a single shot; therefore, live attenuated vaccines have become the gold standard of existing vaccines. Live attenuated pathogens infect cells and make antigens that are presented by the infected cells. These thus elicit an attack by the cytotoxic T cells as well as by antibodies. The induction of humoral and cellular immunity is essential for blocking viral infection.

Like live attenuated vaccines, adenovirus vectored vaccine has been shown to induce both humoral and cellular immunities [48]. The induction of both humoral and cellular immunities by adenovirus vectored vaccine is associated with the ability of adenovirus vector to deliver large amounts of antigens into cells of the lymphoid tissues [49, 50]. In these cells, foreign proteins expressed by adenovirus vector are processed and presented on the cell surface to effectively produce cytotoxic T cells responses. Additionally, the expressed proteins secreted outside the cell are engulfed by antigen-presenting cells. These cells then stimulate T lymphocytes to produce cytokines for antigen-responsive B-cells to induce humoral immunity (Figure 4).

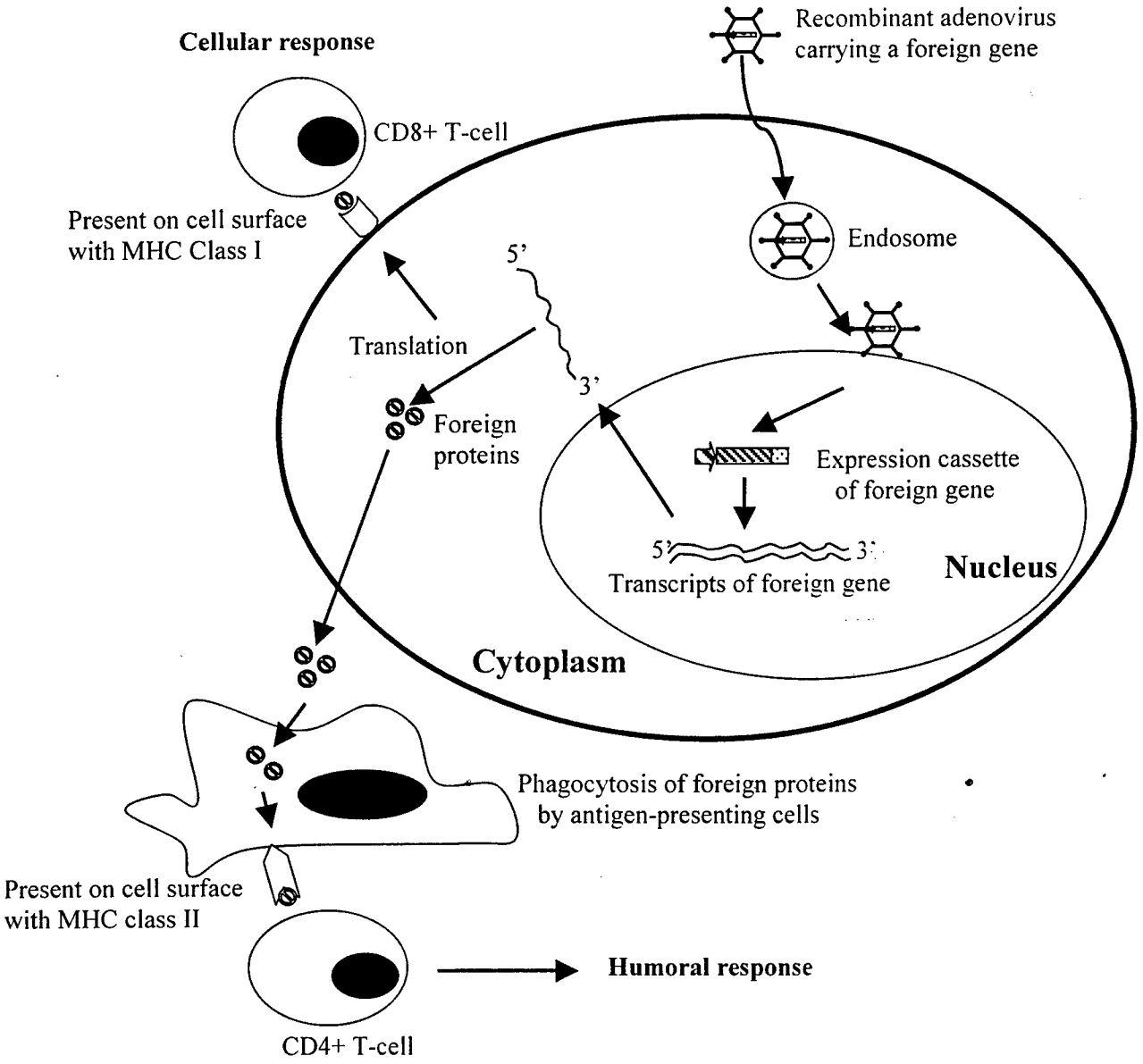

Figure 4. *Adenovirus-mediated delivery of foreign proteins and induction of humoral and cellular immune responses. MHC: major histocompatibility complex.*

Future development

Animal studies have shown promising new applications for adenovirus vectored vaccines against BW agents such as Ebola and anthrax. However, some obstacles need to be overcome before these vaccines can be used in humans. One of these obstacles is the preexisting immunity to adenoviruses which may neutralize adenovirus vectors and prevent these from inducing a protective immune response. Adenoviruses are ubiquitous human viruses. Many adenovirus infections present no obvious clinical symptoms and signs, but these still induce an immune response against re-infection by the same adenovirus serotype. One survey showed that 97% of tested individuals had antibodies specific to HAd5, the most used serotype for vaccine vector. More than 50% of people carry preexisting cellular immunity to adenoviruses through naturally acquired infections [51]. Anti-adenovirus neutralizing antibodies induced by initial administration of adenovirus vector would also prevent using the same serotype of adenovirus for booster. Moreover, preexisting immunity to adenoviruses significantly increases vector-mediated liver toxicity in mice upon re-exposure [52].

One solution to circumvent this preexisting immunity is to use other nonpathogenic serotypes of human or animal adenoviruses as alternative to HAd5. For example, human adenovirus serotype 35 is a virus with low prevalence in the human population. Transduction with the vector based on this virus is not hampered by preexisting anti-HAd5 immunity [53]. A replication defective adenoviral vector based on a chimpanzee adenovirus was also developed to express the rabies virus glycoprotein [54]. Mice immunized with this construct developed antibodies to rabies virus, which was not impaired by preexisting immunity to common human adenovirus serotypes, such as 2, 4, 5, 7, and 12. Another solution to ensure that adenoviral vectors avoid neutralization by preexisting immunity is through encapsulation. Yotnda and co-workers encapsulated HAd5 vectors using bilamellar cationic liposomes [55]. Unlike other liposomal systems, HAd5 vector encapsulated by bilamellar cationic liposomes resisted the neutralizing effects of human anti-adenoviral antibodies. Encapsulation of adenovirus vectors into biodegradable alginate microparticles also circumvented the vector-specific immune response [56].

Conclusion

Vaccines are the cornerstone to protect the CF from the attack of BW agents. The increased threat of bioterrorism highlights the need to improve existing biodefence vaccines and to develop new ones. An ideal vaccine against BW agents should be safe, easy to deliver, provide long-lasting protection, and require only one or a few doses. To this end, a vaccine platform based on adenovirus vector is recommended. Adenovirus vector is relatively safe. Vaccines delivered by adenovirus vector induce mucosal immunities that prevent replication of BW agents at the site of entry. Finally adenovirus vectored vaccines are easy to produce and manufacture as an oral vaccine. Vaccines derived from adenovirus vector protect animals from infections of BW agents. However, problems of preexisting immunity to adenovirus vector need to be overcome before these vaccines can be used in humans. The use of adenoviruses of different serotypes than those already used or present in the human population is recommended.

References

1. Chaloner-Larsson, G., Contreras. G., Furesz, J., Boucher, D.W., Krepps. D., Humphreys, G.R., and Mohanna. S.M. (1986). Immunization of Canadian Armed Forces personnel with live types 4 and 7 adenovirus vaccines. *Canadian Journal of Public Health*, 77(5), 367-370.

2. Gaydos, C.A. and Gaydos, J.C. (1995). Adenovirus vaccines in the U.S. military. *Military Medicine*, 160(6), 300-304.

3. Rowe, W.P., Huebner, R.J., Gilmore, L.K., Parrott, R.H. and Ward, T.G. (1953). Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. *Proceeding Society of Experimental Biological Medicine,* 84(3), 570-573.

4. Hilleman, M.R. and Werner, J.H. (1954). Recovery of new agent from patients with acute respiratory illness. *Proceeding Society of Experimental Biological Medicine,* 85(1), 183-188.

5. Enders, J.F., Bell, J.A., Dingle, J.H., Francis, T., Jr., Hilleman, M.R., Huebner, R.J., and Payne, A.M. (1956). Adenoviruses: group name proposed for new respiratory-tract viruses. *Science,*124(3212). 119-120.

6. Shenk, T.E. (2001). Adenoviridae: The viruses and their replication, in *Fields Virology*, D.M. Knipe and P.M. Howley, Editors., Lippincott Williams & Wilkins: Philadelphia. p. 2265-2300.

7. Bergelson, J.M., Cunningham. J.A., Droguett, G., Kurt-Jones, E.A., Krithivas, A., Hong, J.S., Horwitz, M.S., Crowell, R.L., and Finberg, R.W. (1997) Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. *Science,* 275(5304), 1320-1323.

8. Wickham, T.J., Mathias, P., Cheresh, D.A., and Nemerow, G.R. (1993). Integrins alpha v beta 3 and alpha v beta 5 promote adenovirus internalization but not virus attachment. *Cell,* 73(2), 309-319.

9. Greber, U.F., Willetts, M., Webster, P., and Helenius, A. (1993). Stepwise dismantling of adenovirus 2 during entry into cells. *Cell,* 75(3), 477-486.

10. Wold, W.S.(1993). Adenovirus genes that modulate the sensitivity of virus-infected cells to lysis by TNF. *Journal of Cell Biochemistry*, 53(4), 329-335.

11. Ginsberg, H.S., Lundholm-Beauchamp, U., Horswood, R.L., Pernis, B., Wold, W.S., Chanock, R.M., and Prince, G.A. (1989). Role of early region 3 (E3) in pathogenesis of adenovirus disease. *Proceeding National Academy of Science U S A,* 86(10), 3823-7.

12. Top, F.H., Jr. (1975). Control of adenovirus acute respiratory disease in U.S. Army trainees. *Yale Journal of Biological Medicine,* 48(3), 185-195.

13. Ryan, M.A.K., Gray, G.C., Malasig, M.D., Binn, L.N., Asher, L.V., Cute, D., Kehl, S.C., Dunn, B.E., and Yund, A.Y. (2000). Two fatal cases of adenovirus-related illness in previously healthy young adults--Illinois, 2000. *Morbidity Mortality Weekly Report,* 50(26), 553-555.

14. Couch, R.B., Chanock, R.M., Cate, T.R., Lang, D.J., Knight, V., and Huebner, R.J. (1963). Immunization with Types 4 and 7 Adenovirus by Selective Infection of the Intestinal Tract. *American Review of Respiratory Disease,* 88, Supplement, 394-403.

15. Gaydos, C.A. and Gaydos, J.C. (2004). Adenovirus vaccine, in *Vaccines*, S.A. Plotkin and W.A. Orenstein, Editors., Saunders: Philadelphia., 863-885.

16. Schwartz, A.R., Togo, Y. and Hornick, R.B. (1974). Clinical evaluation of live, oral types 1, 2, and 5 adenovirus vaccines. *American Review of Respiratory Disease,* 109(2), 233-239.

17. Peckinpaugh, R.O., Pierce, W.E.. Rosenbaum, M.J., Edwards, E.A., and Jackson, G.G. (1968). Mass enteric live adenovirus vaccination during epidemic ARD. *JAMA,* 205(1), 75-80.

18. Collis, P.B., Dudding, B.A., Winter, P.E., Russell, P.K., and Buescher, E.L. (1973). Adenovirus vaccines in military recruit populations: a cost-benefit analysis. *Journal of Infectious Diseases,* 128(6), 745-752.

19. Kolavic-Gray, S.A., Binn, L.N., Sanchez, J.L., Cersovsky, S.B., Polyak, C.S., Mitchell-Raymundo, F., Asher, L.V., Vaughn, D.W., Feighner, B.H., and Innis, B.L. (2002). Large epidemic of adenovirus type 4 infection among military trainees: epidemiological, clinical, and laboratory studies. *Clinical Infectious Diseases,* 35(7), 808-818.

20. Barraza, E.M., Ludwig, S.L., Gaydos. J.C., and Brundage, J.F. (1999). Reemergence of adenovirus type 4 acute respiratory disease in military trainees: report of an outbreak during a lapse in vaccination. *Journal of Infect ious Diseases,* 179(6), 1531-1533.

21. Cohen, J. and Marshall, E.(2001). Vaccines for biodefense: a system in distress. *Science,* 294(5542), 498-4501.

22. Tacket, C.O., Losonsky, G., Lubeck. M.D., Davis, A.R., Mizutani, S., Horwith, G., Hung, P., Edelman, R., and Levine, M.M. (1992). Initial safety and immunogenicity studies of an oral recombinant adenohepatitis B vaccine. *Vaccine,* 10(10), 673-676.

23. Babiuk, L.A. and Tikoo, S.K. (2000). Adenoviruses as vectors for delivering vaccines to mucosal surfaces. *Journal of Biotechnology*, 83(1-2), 105-113.

24. Imler, J.L.(1995). Adenovirus vectors as recombinant viral vaccines. *Vaccine,* 13(13), 1143-1151.

25. Bett, A.J., Prevec, L. and Graham, F.L. (1993). Packaging capacity and stability of human adenovirus type 5 vectors. *Journal of Viroogy,* 67(10), 5911-5921.

26. Graham, F.L. and Prevec, L. (1991). Manipulation of adenovirus vectors, in *Methods in Molecular Biology*, E.J. Murray and N.J. Clifton, Editors, Humana Press: Totowa, NJ., 109-128.

27. Bett, A.J., Haddara, W., Prevec, L., and Graham, F.L. (1994). An efficient and flexible system for construction of adenovirus vectors with insertions or deletions in early regions 1 and 3. *Proceeding National Academy of Science U S A,* 91(19), 8802-8806.

28. He, T.C., Zhou, S., da Costa, L.T., Yu, J., Kinzler, K.W., and Vogelstein, B. (1998). A simplified system for generating recombinant adenoviruses. *Proceeding National Academy of Science U S A,* 95(5), 2509-2514.

29. Chartier, C., Degryse, E., Gantzer, M., Dieterle, A., Pavirani, A., and Mehtali, M. (1996). Efficient generation of recombinant adenovirus vectors by homologous recombination in Escherichia coli. *Journal of Virology,* 70(7), 4805-4810.

30. Graham, F.L., Smiley, J., Russell, W.C., and Nairn, R. (1977). Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *Journal of General of Viroogy,* 36(1), 59-74.

31. Nadeau, I. and Kamen, A. (2003). Production of adenovirus vector for gene therapy. *Biotechnology Advance,* 20(7-8), 475-489.

32. Nadeau, I., Seanez, G. and Wu, F. (2001). Adenovirus production in 293 cells: a comparative study between a suspension cell and an adherent cell process. in *The 17th ESACT Meeting, Tylosand, Sweden, June 10-14, 2001*. Tylosand, Sweden.

33. Chaubard, J.(2000). Serum-free media and bioreactor strategies for manufacturing adenoviral gene therapy vectors. in *Viral Vectors and Vaccines, Lake Tahoe, NV, Nov. 6-9, 2000.*

34. Fallaux, F.J., Bout, A., van der Velde, I., van den Wollenberg, D.J., Hehir, K.M., Keegan, J., Auger, C., Cramer, S.J., van Ormondt, H., van der Eb, A.J., Valerio, D., and Hoeben, R.C. (1998). New helper cells and matched early region 1-deleted adenovirus vectors prevent generation of replication-competent adenoviruses. *Human Gene Therapy,* 9(13), 1909-1917.

35. Ferber, D.(2001). Gene therapy. Safer and virus-free? *Science,* 294(5547), 1638-1642.

36. Dave, U.P., Jenkins, N.A. and Copeland, N.G. (2004). Gene therapy insertional mutagenesis insights. *Science,* 303(5656), 333.

37. Juillard, V., Villefroy, P., Godfrin, D., Pavirani, A., Venet, A., and Guillet, J.G. (1995). Long-term humoral and cellular immunity induced by a single immunization with replication-defective adenovirus recombinant vector. *European Journal of Immunology,* 25(12), 3467-373.

38. Sullivan, N.J., Geisbert, T.W., Geisbert, J.B., Xu, L., Yang, Z.Y., Roederer, M., Koup, R.A., Jahrling, P.B., and Nabel, G.J. (2003). Accelerated vaccination for Ebola virus haemorrhagic fever in non-human primates. *Nature,* 424(6949), 681-684.

39. Tan, Y., Hackett, N.R., Boyer, J.L., and Crystal, R.G. (2003). Protective immunity evoked against anthrax lethal toxin after a single intramuscular administration of an adenovirus-based vaccine encoding humanized protective antigen. *Human Gene Therapy,* 14(17), 1673-1682.

40. Abbas, A.K., Lichtman, A.H. and Pober, J.S. (1997). *Cellular and Molecular Immunology*. 3rd ed., Philadelphia, PA: W.B. Saunders Company.

41. Mittal, S.K., Papp, Z., Tikoo, S.K., Baca-Estrada, M.E., Yoo, D., Benko, M., and Babiuk, L.A. (1996). Induction of systemic and mucosal immune responses in cotton rats immunized with human adenovirus type 5 recombinants expressing the full and truncated forms of bovine herpesvirus type 1 glycoprotein gD. *Virology,* 222(2), 299-309.

42. Xiang, Z. and Ertl, H.C(1999). Induction of mucosal immunity with a replication-defective adenoviral recombinant. *Vaccine,* 17(15-16), 2003-2008.

43. Valiante, N.M., O'Hagan, D.T. and Ulmer, J.B. (2003). Innate immunity and biodefence vaccines. *Cell Microbiology,* 5(11), 755-760.

44. Gahery-Segard, H., Molinier-Frenkel, V., Le Boulaire, C., Saulnier, P., Opolon, P., Lengagne, R., Gautier, E., Le Cesne, A., Zitvogel, L., Venet, A., Schatz, C., Courtney, M., Le Chevalier, T., Tursz, T., Guillet, J.G., and Farace, F. (1997). Phase I trial of recombinant adenovirus gene transfer in lung cancer. Longitudinal study of the immune responses to transgene and viral products. *Journal of Clinical Investigation,* 100(9), 2218-2226.

45. Yang, Y., Li, Q., Ertl, H.C., and Wilson, J.M. (1995). Cellular and humoral immune responses to viral antigens create barriers to lung-directed gene therapy with recombinant adenoviruses. *Journal of Virology,* 69(4), 2004-2015.

46. Yang, Y., Nunes, F.A., Berencsi, K., Furth, E.E., Gonczol, E., and Wilson, J.M. (1994). Cellular immunity to viral antigens limits E1-deleted adenoviruses for gene therapy. *Proceeding National Academy of Science U S A,* 91(10), 4407-4411.

47. Molinier-Frenkel, V., Lengagne, R., Gaden, F., Hong, S.S., Choppin, J., Gahery-Segard, H., Boulanger, P., and Guillet, J.G. (2002) Adenovirus hexon protein is a potent adjuvant for activation of a cellular immune response. *Journal of Virology,* 76(1), 127-135.

48. Xiang, Z.Q., Yang, Y., Wilson, J.M., and Ertl, H.C. (1996). A replication-defective human adenovirus recombinant serves as a highly efficacious vaccine carrier. *Virology,* 219(1), 220-227.

49. Yang, T.C., Dayball, K., Wan, Y.H., and Bramson, J. (2003). Detailed analysis of the CD8+ T-cell response following adenovirus vaccination. *Journal of Virology,* 77(240, 13407-13411.

50. Labow, D., Lee, S., Ginsberg, R.J., Crystal, R.G., and Korst, R.J. (2000). Adenovirus vector-mediated gene transfer to regional lymph nodes. *Human Gene Therapy,* 11(5), 759-769.

51. Chirmule, N., Propert, K., Magosin, S., Qian, Y., Qian, R., and Wilson, J. (1999). Immune responses to adenovirus and adeno-associated virus in humans. *Gene Therapy,* 6(9), 1574-1583.

52. Vlachaki, M.T., Hernandez-Garcia, A., Ittmann, M., Chhikara, M., Aguilar, L.K., Zhu, X., Teh, B.S., Butler, E.B., Woo, S., Thompson, T.C., Barrera-Saldana, H., Aguilar-Cordova, E., and The, B.S. (2002). Impact of preimmunization on adenoviral vector expression and toxicity in a subcutaneous mouse cancer model. *Molecular Therapy,* 6(3), 342-348.

53. Vogels, R., Zuijdgeest, D., van Rijnsoever, R., Hartkoorn, E., Damen, I., de Bethune, M.P., Kostense, S., Penders, G., Helmus, N., Koudstaal, W., Cecchini, M., Wetterwald, A., Sprangers, M., Lemckert, A., Ophorst, O., Koel, B., van Meerendonk, M., Quax, P., Panitti, L., Grimbergen, J., Bout, A., Goudsmit, J., and Havenga, M. (2003). Replication-deficient human adenovirus type 35 vectors for gene transfer and vaccination: efficient human cell infection and bypass of preexisting adenovirus immunity. *Journal of Virology,* 77(15), 8263-8271.

54. Xiang, Z., ., Gao, G., Reyes-Sandoval, A., Cohen, C.J., Li, Y., Bergelson, J.M., Wilson, J.M., and Ertl, H.C. (2002). Novel, chimpanzee serotype 68-based adenoviral vaccine carrier for induction of antibodies to a transgene product. *Journal of Virology,* 76(6), 2667-2675.

55. Yotnda, P., Chen, D.H., Chiu, W., Piedra, P.A., Davis, A., Templeton, N.S., and Brenner, M.K. (2002) Bilamellar cationic liposomes protect adenovectors from preexisting humoral immune responses. *Molecular Therapy,* 5(3), 233-241.

56. Sailaja, G., HogenEsch, H., North, A., Hays, J., and Mittal. S.K. (2002). Encapsulation of recombinant adenovirus into alginate microspheres circumvents vector-specific immune response. *Gene Therapy,* 9(24), 1722-1729.

List of symbols/abbreviations/acronyms/initialisms

ARD	Acute Respiratory Disease
BW	Biological Warfare
CAR	Coxsackievirus Group B and Adenovirus Receptor
CF	Canadian Forces
HAd5	Human Adenovirus Serotype 5
kb	kilobases
TNF	Tumour Necrosis Factor

DOCUMENT CONTROL DATA

(Security classification of title, body of abstract and indexing annotation must be entered when the overall document is classified)

1. ORIGINATOR (the name and address of the organization preparing the document. Organizations for who the document was prepared, e.g. Establishment sponsoring a contractor's report, or tasking agency, are entered in Section 8.)

   Defence R&D Canada – Suffield

2. SECURITY CLASSIFICATION (overall security classification of the document, including special warning terms if applicable)

   UNCLASSIFIED

3. TITLE (the complete document title as indicated on the title page. Its classification should be indicated by the appropriate abbreviation (S, C or U) in parentheses after the title).

   Adenovirus and its vector for developing vaccines against biological warfare agents (U)

4. AUTHORS (Last name, first name, middle initial. If military, show rank, e.g. Doe, Maj. John E.)

   Wu, Qiaohua (Josh)

5. DATE OF PUBLICATION (month and year of publication of document)

   November 2004

6a. NO. OF PAGES (total containing information, include Annexes, Appendices, etc) 35

6b. NO. OF REFS (total cited in document) 56

7. DESCRIPTIVE NOTES (the category of the document, e.g. technical report, technical note or memorandum. If appropriate, enter the type of report, e.g. interim, progress, summary, annual or final. Give the inclusive dates when a specific reporting period is covered.)

   Technical Memorandum

8. SPONSORING ACTIVITY (the name of the department project office or laboratory sponsoring the research and development. Include the address.)

   Defence R&D Canada – Suffield

9a. PROJECT OR GRANT NO. (If appropriate, the applicable research and development project or grant number under which the document was written. Please specify whether project or grant.)

   WBE 16qb32

9b. CONTRACT NO. (If appropriate, the applicable number under which the document was written.)

10a. ORIGINATOR'S DOCUMENT NUMBER (the official document number by which the document is identified by the originating activity. This number must be unique to this document.)

   DRDC Suffield TM 2004-249

10b. OTHER DOCUMENT NOs. (Any other numbers which may be assigned this document either by the originator or by the sponsor.)

11. DOCUMENT AVAILABILITY (any limitations on further dissemination of the document, other than those imposed by security classification)

   (x) Unlimited distribution
   ( ) Distribution limited to defence departments and defence contractors; further distribution only as approved
   ( ) Distribution limited to defence departments and Canadian defence contractors; further distribution only as approved
   ( ) Distribution limited to government departments and agencies; further distribution only as approved
   ( ) Distribution limited to defence departments; further distribution only as approved
   ( ) Other (please specify):

12. DOCUMENT ANNOUNCEMENT (any limitation to the bibliographic announcement of this document. This will normally corresponded to the Document Availability (11). However, where further distribution (beyond the audience specified in 11) is possible, a wider announcement audience may be selected).

   Unlimited

13. ABSTRACT (a brief and factual summary of the document. It may also appear elsewhere in the body of the document itself. It is highly desirable that the abstract of classified documents be unclassified. Each paragraph of the abstract shall begin with an indication of the security classification of the information in the paragraph (unless the document itself is unclassified) represented as (S), (C) or (U). It is not necessary to include here abstracts in both official languages unless the text is bilingual).

This memorandum reviews a new platform for the development of vaccines against biological warfare (BW) agents. The noted platform uses adenovirus as a vector to deliver DNA that encodes for key antigens of BW agents. Once inside the mammalian cell, this adenovirus vectored vaccine induces rapid immune responses. This memorandum describes the basic virology of adenovirus, methods used to modify adenovirus as vaccination vector, advantages of adenovirus vector for developing vaccines against BW agents such as Ebola and anthrax, and the potential obstacles, with proposed solutions, for the use of adenovirus vector.

14. KEYWORDS, DESCRIPTORS or IDENTIFIERS (technically meaningful terms or short phrases that characterize a document and could be helpful in cataloguing the document. They should be selected so that no security classification is required. Identifies, such as equipment model designation, trade name, military project code name, geographic location may also be included. If possible keywords should be selected from a published thesaurus, e.g. Thesaurus of Engineering and Scientific Terms (TEST) and that thesaurus-identified. If it is not possible to select indexing terms which are Unclassified, the classification of each should be indicated as with the title.)

Vaccine development, adenovirus infection, adenovirus vector, biological warfare agent