Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

ELSEVIER

Journal of Biotechnology 83 (2000) 105–113

JOURNAL OF Biotechnology

www.elsevier.com/locate/jbiotec

Adenoviruses as vectors for delivering vaccines to mucosal surfaces

L.A. Babiuk *, S.K. Tikoo

Veterinary Infectious Disease Organization, University of Saskatchewan, 120 Veterinary Road, Saskatoon, Canada S7N 5E3

Received 9 August 1999; received in revised form 13 November 1999; accepted 9 December 1999

Abstract

Immunization of mucosal surfaces has become an attractive route of vaccine delivery because of its ability to induce mucosal immunity. Although various methods of inducing mucosal immunity are being developed, our laboratory has focused on developing adenoviruses as replication–competent and replication–incompetent vectors. The present report will summarize our progress in sequencing the entire bovine adenovirus-3 genome and identifying regions which can be deleted and subsequently used as insertion sites for foreign genes in developing recombinant viral vaccines. Using these recombinant viruses, we demonstrated the 'proof-of-principle' in developing mucosal immunity and, more importantly, inducing protection against bovine herpes virus in a natural host–cattle. Finally, we demonstrated that immunity and protection occurred even in animals that had pre-existing antibodies to the vector.

Keywords: Virus; Antibody; Immunization

1. Introduction

Vaccination continues to be one of the most important disease management tools for controlling infectious diseases in both livestock and humans. Indeed, with increasing resistance of bacteria to antibiotics and the continued paucity of antiviral drugs against many viruses, vaccination is the most attractive approach to infectious disease control. Although vaccination has been practised for over 200 years and there are many licensed vaccines used today, recent advances in molecular biology and immunology combined with our understanding of the pathogenesis of many infectious agents, stimulated renewed interest in developing safer and more efficacious vaccines. This is evident by the increased number of articles directly focusing on vaccines and the genesis of new journals dedicated specifically to vaccinology. These activities are focused on both improving conventional vaccines and their delivery to meet the needs of today's management systems as well as developing totally new approaches to vaccination.

Presently, novel vaccine technology is being used to develop: (1) subunit vaccines; (2) chimeric vaccines; (3) peptide vaccines; (4) gene-deleted

* Corresponding author.

0168-1656/00/$ - see front matter
PII: S0168-1656(00)00314-X

vaccines; (5) vectored vaccines; (6) DNA vaccines; and (7) plant-based vaccines. All of these novel approaches require a much more detailed characterization of the genetic organization of the pathogen and the genes responsible for virulence or induction of immunity. As a result of this increased knowledge and ability to manipulate the genomes of pathogens, we are in an unprecedented position to develop better live vectored vaccines that are not only safer, but are also able to induce a wide range of protective immune responses ranging from humoral to cellular immunity, as well as systemic and mucosal immunity. The generation of mucosal immunity is especially important if we hope to prevent infection. Although rarely will a vaccine induce sterile immunity, mucosal immunity should limit replication of the pathogen at the site of entry. Mucosal immunity reduces the rate of infection and the rate of replication by either: (1) preventing attachment of the pathogen to host cells; (2) interfering with transcription or proper assembly of the pathogen intracellularly; or (3) enhancing antigen uptake and clearance of the pathogen as well as improving presentation of the antigen to the immune system for increased immune responsiveness.

Presently, a number of strategies are being employed to enhance mucosal immunity including: (1) mucosal adjuvants such as cholera toxin (Isaka et al., 1999); (2) microparticles especially for oral delivery. These microparticles are generally biodegradable and can even be used for pulsatile release. Both polylactide–glycolide, and alginate particles have been shown to be effective, especially for oral delivery (McDermott et al., 1998; Bowersock et al., 1999); (3) DNA vaccines (Cox et al., 1993; Donnelly et al., 1993; Yokonama et al., 1995; Lewis et al., 1997); (4) transgenic plants (Arakawa et al., 1998); and (5) live vectors (Pastoret et al., 1988; Yilma et al., 1988). Live vectors are attractive because they can be engineered in such a way as to not only induce immunity to the vector, but if they are engineered correctly, they can induce immunity to an array of proteins encoded for by genes inserted into the vector. Indeed, one of the first licensed recombinant vaccines employed in vaccinology included vaccinia virus carrying the gene encoding rabies virus glycoproteins. This vaccine is presently licensed for immunization of wildlife against rabies. Not only has this vaccine reduced the spread of rabies virus in wildlife populations, but also reduced the spill-over of virus infection from wildlife to domestic animals and humans (Pastoret et al., 1988; Brochier and Pastoret, 1993; Mackowiak et al., 1999). Since the initial licensing of vaccinia-rabies glycoprotein recombinants, numerous other live viral and bacterial vectors have been investigated as potential vaccine vectors (Kit et al., 1992; Cirillo et al., 1995; Sheppard 1999). The present review will summarize the progress that has been made in using adenoviruses as potential vectors for induction of immunity in animals.

2. Adenovirus vectors

Adenoviruses were first isolated in the early 1950s and since then over 100 different serotypes have been isolated from humans and most mammalian and avian species (Ishibashi and Yasue, 1984). Since there are such a variety of different adenoviruses, it is not surprising that they can induce a variety of different diseases in their specific hosts. However, if one was to generalize, most of these pathogens enter via mucosal surfaces and, therefore, replicate, at least initially, in mucosal sites of the respiratory or gastrointestinal tracts. It is for this reason that adenoviruses are attractive as vectors for delivering vaccines to mucosal surfaces. Other important features of adenoviruses as vectors is that certain serotypes are often associated with only mild clinical diseases, the molecular biology and replication strategies of adenoviruses are well characterized, they replicate to extremely high titres (10^{10}–10^{11} pfu ml^{-1}), thereby reducing the cost of vaccine production and delivery and most recently, the cloning of the entire genome into a bacterial plasmid provides for rapid construction and isolation of recombinants (Chartier et al., 1996). Finally, a number of effective adenovirus vaccines have already been licensed for use in animals and humans, thereby, providing experience with adenovirus vaccines, both with regards to safety and

efficacy. All these factors are critical for successful development of live vectored vaccines.

Adenoviruses have a linear, double-stranded DNA genome which varies in size (30–45 kB) depending on the species from which the virus is isolated. The genome is packaged in a naked icosahedral capsid. Since naked viruses are generally more stable in the environment than are enveloped viruses, this is another attractive feature with regards to using adenoviruses as vectors for vaccines. Since the icosahedral capsid is rigid, the amount of DNA that can be packaged inside the capsid is strictly controlled by space limitations. In the case of human adenoviruses, it has been demonstrated that $\sim 105\%$ of the genome size can be effectively packaged (Ghosh-Choudhury et al., 1987). Since the addition of 5% of the genome does not provide much space for insertion of new genes, most recombinant adenovirus vaccines are developed by replacing existing viral sequences with genes of interest. Depending on the specific sequence replaced, the recombinant viruses can either be replication–competent or replication–defective.

Recent studies have also begun to develop helper-dependent (gutless) adenoviruses which contain very little adenovirus sequences. This helps to increase the cloning capacity of the vector, thus, allowing to insert large foreign DNA. In addition, it has the potential to remove any adenovirus gene sequences whose expression may have harmful effects on the desired application of the recombinant vector. Since a helper virus has to be used to propagate the desired gutless vector, one of the main problems associated with the production of such vectors is the final separation of helper and vector viruses during purification. However, constant improvements/modifications in the production of 'gutless' vectors has helped to obtain purified vectors which contain 0.1% helper virus (Parks et al., 1996).

For adenoviruses to be used successfully as vectors, it is important to fully characterize the genome in order to identify the specific regions of the virus that can be deleted or manipulated. This requires a thorough understanding of the replication strategy of the virus and the specific genes involved in these replication steps. Adenovirus genome replication is generally divided into two distinct chronological events. The early events, which can occur prior to replication of the viral genome, are controlled by the early regions (E). In contrast, the late events occur during or after viral genome replication begins and are primarily concerned with the production of most of the viral structural proteins and are turned late region genes (L).

The early region consists of four different regions distributed throughout the genome. Each early region contains different transcription regulatory genes. The E1 region polypeptides are the first to be expressed following infection of a cell with the virus and are important regulators of the transcription of viral and cellular genes. Since the gene products from the E1 region are critical for initiation of virus replication, deletion of these genes results in a replication-defective virus. However, it is still possible to use viruses deleted in the E1 region as vectors by providing the E1 gene products in a complementing cell line. These gene products must be expressed at sufficient levels to complement the E1 defective virus. Although the virus can be produced in these cell lines, once it is introduced into an animal, which does not produce these gene products, the virus infection results in an abortive infection (Graham and Prevec, 1992; Grunhaus and Horwitz, 1992). These replication-defective viruses can still express the inserted gene of interest and induce an immune response in animals, but since they are replication-defective, they are considered to be safe with little chance of environmental spread. Since these replication-defective viruses have a built-in suicide mechanism for biological control of the spread of the vector, it makes them attractive with regulatory agencies and environmentalists. Although the E1 deleted virus may pick up the E1 region from the transfected cell to produce replication competent viruses the chances of this occurring are remote since we use an E1 from human adenovirus rather than bovine adenovirus to transfect the cells.

The other region most often used for insertion of foreign genes is the E3 region. This region is not required for virus replication in vitro and, therefore, replacement of the E3 region with for-

eign genes does not result in a replication-defective virus. Even though this region is not essential for virus replication in vitro, its conservation in all adenoviruses studied to-date suggests that the virus has retained this region for a purpose. Studies primarily in human serotype 5 have shown that different proteins produced by the E3 region are involved in binding the MHC Class 1 molecules, in modulating lysis by tumor necrosis factor, and down-regulating epidermal growth factor receptors on host cells (Paabo et al., 1986; Gooding et al., 1988; Krajcsi et al., 1992). As a result, deletion of the E3 region may modulate the pathogenesis of E3-deleted viruses (Morin et al., 1987; Ginsberg et al., 1989). Our studies with E3-deleted bovine adenoviruses did not show any evidence of increased lymphocyte infiltration or pathogenesis so that increased pathogenesis seen with human adenovirus E3-deleted mutants may not be universal (Mittal et al., 1995b). However, before any E3-deleted mutants from any adenovirus are commercialized, it will be important to investigate the impact of E3-deleted viruses on viral pathogenesis.

The E4 region is another region that has been shown to be useful as an insertion site for foreign genes. The four gene products are involved in transition of the virus from early-to-late gene expression and shut-off of host cell gene expression, virus replication and assembly. In human adenoviruses, the E4 region is essential and E4-deleted viruses will only replicate in cells expressing E4 gene products. Whether E4 is essential in all adenoviruses has not yet been determined, but one would assume that it is. Therefore, as with E1-deleted viruses, cell lines expressing E4 will need to be developed before one can establish an E4-deleted recombinant virus. If one expects to develop an E1, E3, and E4-deleted virus, one will require a cell line expressing both E1 and E4 gene products at sufficient levels to complement the gene-deleted viruses, but not at such a level that these genes would be toxic to the cells. Thus, in addition to manipulating the virus, it is critical to develop the appropriate cell lines for expression of these viruses.

Although most of the preliminary 'proof-of-principle' was based on using human adenoviruses as vectors for studying immune responses in rodents, recent progress in analysis of the genomes of other animal viruses has provided a much broader experience in use of these viruses as vectors in various animal species. These include bovine adenoviruses, porcine adenoviruses, ovine adenoviruses, fowl adenoviruses, and canine adenoviruses (Mittal et al., 1995a; Klonjkowski et al., 1997; Xu et al., 1997; Sheppard et al., 1998; Reddy et al., 1999). Experience with these animal adenoviruses has clearly demonstrated commonality between all the adenoviruses studied to-date, but also demonstrates some very significant differences. Thus, one cannot directly translate the experiences with one adenovirus to another. For example, the genomic organization of most adenoviruses is similar but not identical. Even with this similarity in genomic organization, the transfection efficiency between human and animal adenoviruses may vary by one or two orders of magnitude. It was this low transfection efficiency that has hampered development of some of the animal adenoviruses as vectors. Unfortunately, the reasons for these differences are presently not well known. Fortunately, a novel approach using the homologous recombination system in *E. coli* has allowed us to overcome the impediment of low transfection efficiency with animal adenoviruses and to rapidly reconstruct recombinants expressing a number of different viral proteins (Chartier et al., 1996).

Our laboratory has focused primarily on bovine and porcine adenoviruses as vectors (Mittal et al., 1995b; Zakhartchouk et al., 1998; Reddy et al., 1999). However, before it was possible to develop these viruses as vectors, it required the complete sequencing of the respective genomes and characterization of the various regions of the viral genome that could be used for insertion of foreign genes. Secondly, it required the production of cell lines for isolation of the recombinants. To achieve these two goals, we needed to complete the entire transcriptional map and sequence the genome of BAV-3 (Reddy et al., 1998) Genbank Accession No. AF030154 (Fig. 1). From the analysis of the genome and its similarity to other adenoviruses, we focused on characterizing the E1, E3 and E4 regions specifically in order to identify the

boundaries of these regions for eventual removal to enhance the coding capacity of the virus for insertion of foreign genes. The combined coding capacity of these three regions in bovine adenovirus-3 is ~8 kB (E1, 3.5 kB; E3, 1.2 kB; E4, 3.2 kB). Following the removal of these three regions, it should be possible to insert a single large gene with essential promoters, etc. of ~8 kB or any combination of genes totalling 8 kB. Since most adenoviruses are thought to be able to package 105% of the genome, it should be possible to insert an additional 1.75 kB for a total of ~9.5 kB. These foreign genes could theoretically be inserted in each of the three different locations or could be combined in a single location. Presently, we have inserted genes into all three of these regions of BAV-3, but have not yet introduced genes into three different regions in the same construct. However, these possibilities are presently being investigated as are studies to incorporate a gene encoding for cytokines in combination with genes isolated from pathogens in an attempt to enhance immune responses at mucosal surfaces.

In addition to providing additional capacity for gene insertion by deleting specific genes, deletion of some genes also alters the replication capacity of these viruses, thereby making them potentially safer as vectors. Deletion of E1 and E4 is believed to result in the production of replication–incompetent viruses, therefore, if viral recombinants are to be isolated, these viral functions need to be provided in trans in a complementing cell line. Although this is an extra step in the production of recombinant viruses, it results in a safer vaccine since the recombinants can infect animals but undergo an abortive infection and, therefore, cannot spread in the environment. This is considered to be important by regulatory agencies whose concern is environmental release of potential recombinants. Since the virus only undergoes a single cycle replication step, the level of antigen expression in vivo is believed to be lower than with recombinants which can replicate in the host. For example, genes inserted into the E3 region can infect normal host cells and undergo a number of replication cycles in vivo. This additional replication is believed to increase the quantity of protein that is produced in vivo and recognized by the host's immune response. As a result, replication–competent viruses may induce higher levels of immunity than replication–incompetent viruses.

To test the feasibility of using bovine adenovirus as a live vector for the induction of systemic and mucosal immunity, we used bovine herpes virus-1 gD as a model gene for insertion into adenoviruses. We chose this gene because we had previously shown that this glycoprotein could induce protective immunity in cattle regardless of whether it was purified from virus-infected cells (Babiuk et al., 1987), produced by recombinant

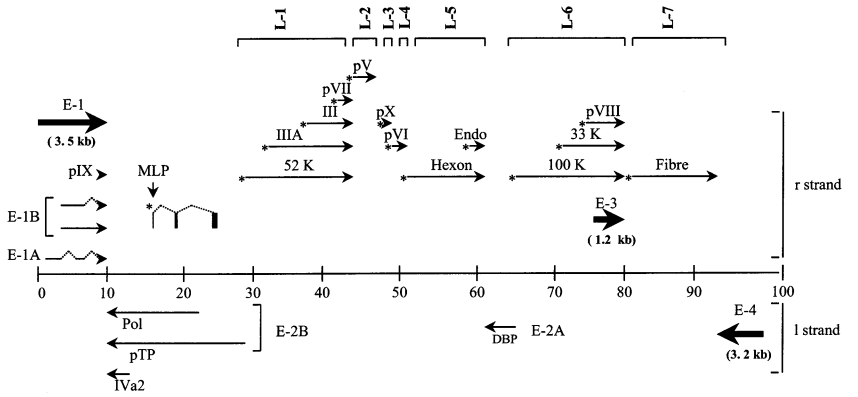

Fig. 1. Schematic representation of the bovine adenovirus-3 genome (adapted from Reddy et al., 1998). The location of the major regions of the genome are highlighted as are the main viral proteins. The E region codes for early proteins, whereas the L region codes for the late proteins.

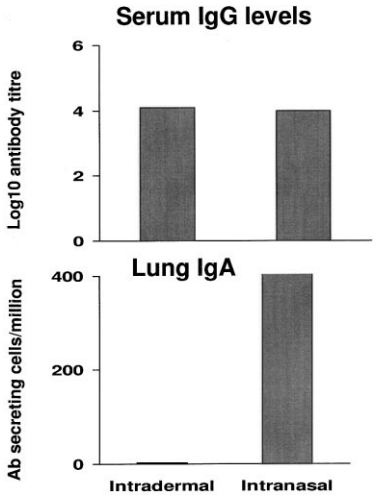

Fig. 2. Immunity to BHV-1 gD following immunization with an adenovirus vector expressing gD. Animals were vaccinated either intranasally or intradermally twice at a 4-week interval. A total of 3 weeks after the last immunization, serum antibody and antibody-secreting cells in the lung were assayed by ELISA and ELISPOT, respectively.

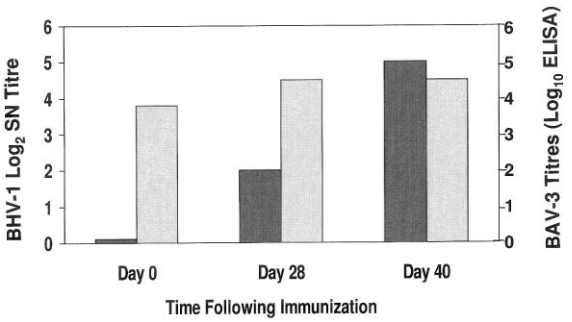

Fig. 3. Immune responses to BHV-1 gD following immunization of bovine adenovirus-3 seropositive animals. BAV-3 gD recombinants were administered intranasally on Day 0 and again on Day 28. Serum antibody responses to gD (dark bars) were measured by neutralization and to BAV-3 by ELISA (light-shaded bars).

DNA technology (van Drunen Littel-van den Hurk et al., 1994), or as a DNA vaccine (Cox et al., 1993). Indeed, gD is considered to be a major target for neutralizing antibody and the glycoprotein is essential for virus entry into cells.

To test BAV-3 as a potential vector, we inserted the entire gD gene which would function as an authentic viral-produced protein and be anchored in the host cell membrane. In parallel, we also inserted the truncated form of gD (TgD) lacking the transmembrane anchor which would allow secretion of gD into the extracellular environment. Regardless of whether the gD or TgD was used, the recombinants produced significant levels of immunity in both laboratory animals (cotton rats) and cattle (Mittal et al., 1996; Zakhartchouk et al., 1998). Delivery of these recombinants via systemic administration induced excellent levels of systemic immunity but no mucosal immunity. In contrast, delivery of the recombinants via the intranasal route lead to high mucosal lung antibody levels (both IgA and IgG) as well as systemic immunity. To demonstrate that the nasal or lung antibody was actually synthesized at the site of immunization, ELISPOT assays were performed. In all cases, animals immunized by the intranasal route showed evidence of antibody secreting cells in the mucosa, whereas systemically immunized animals did not (Fig. 2). These data supports previous studies which have lead to the concept of compartmentalization of immune system and a common mucosal immune system.

One of the major concerns with viral vectored vaccines is the fear that pre-existing antibody to the vector will reduce the immune responses to the transgene to such a level that they will be non-protective (Dong et al., 1996; Schulick et al., 1997; Papp et al., 1999). Although immunization of animals with no antibodies to adenoviruses produce slightly higher immune responses to the transgene, Fig. 3 clearly demonstrates that cattle possessing high levels of antibody to adenoviruses at the time of vaccination can still develop very significant immune responses to the transgene. Thus, animals that are seronegative to the transgene at the time of immunization, rapidly develop specific immunity to the transgene even after a single immunization and the immune response is boosted after a secondary immunization. Two weeks after boosting, calves were challenged with an intranasal challenge of 10^7 plaque-forming units of BHV-1 per animal. Each animal was clinically evaluated independently for temperature, depression, severity of nasal lesions, etc. Fig. 4 demonstrates the significant difference in clinical scores between animals immunized with a BAV-3

gD recombinant as compared to an equivalent BAV-3 lacking the gD gene. Although all animals did shed virus for the first 4 days, animals in the vaccinated group rapidly eliminated the virus such that by 5–6 days, very little virus was being shed from the nasal passages. In contrast, animals immunized with the BAV-3 were still shedding significant levels of virus. This rapid clearance of virus was associated with a rapid increase in IgA antibody in the nasal passages.

Adenovirus recombinants have also been made expressing the BHV-1 gD gene in the E1 or E4 region of bovine adenovirus-3. Although the levels of immunity in animals immunized with these recombinants was slightly lower than with the replication–competent recombinants, the levels of immunity induced by all recombinants was sufficient to limit virus replication and reduce the clinical signs.

To determine the broad utility of using adenoviruses as vectors, we tested a number of genes from different viruses for efficacy of expression in adenovirus. We were especially interested in genes from RNA viruses. Since RNA virus genes are rarely transcribed in the nucleus and others have experienced difficulty expressing genes from RNA viruses in recombinants which normally replicate in the nucleus, we tested whether adenoviruses could also express genes from an RNA virus. To-date, we have expressed the genes for bovine parainfluenza-3, bovine virus diarrhea gp53, and corona viruses HE. To enhance the level of expression of bovine coronavirus genes, we used a synthetic intron that possibly modifies the splicing

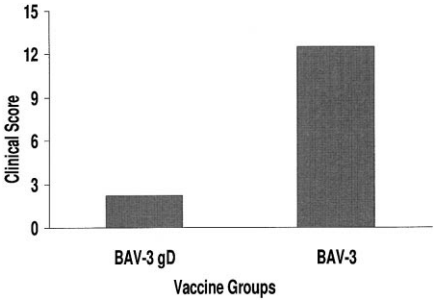

Fig. 4. Protection of animals from BHV-1 challenge. Animals in Fig. 3 were challenged on Day 40 and assessed for clinical protection.

of these genes as they are transported from the nucleus into the cytoplasm (Reddy et al., 1999). By using this synthetic intron, we were able to increase the gene expression by over 50-fold. Thus, we feel that this system should be applicable for expression of genes from the majority of pathogens of interest. The level of expression of certain genes may also be further increased by resynthesis of the gene to improve the codon biases or alter the secondary structure of the mRNA (Taylor et al., 1998).

3. Epilogue

Human adenoviruses have been used extensively to develop recombinant viruses and for testing in various animal model systems (Rosenthal et al., 1996). However, since human adenoviruses generally have a restricted host range and, therefore, do not infect or replicate well in non-human species, it appears appropriate to use the adenoviruses isolated from the species of interest as a vector. It is only after that these types of studies are done, will we be able to get a true appreciation of the potential benefit of using adenovirus vectors in different species. Our studies with bovine adenovirus clearly demonstrate 'proof-of-principle' that these vectors can be used in cattle. Even more important is the observation that seropositive cattle can be immunized with these vectors. The possible reason for this is that mucosal immunity levels are not often sustained as long as serum antibody levels, thereby allowing infection of mucosal cells of the upper respiratory tract even in the presence of high serum antibody levels. Once the virus enters these mucosal cells, it can produce sufficient levels of antigen to induce an immune response in the host. Our preliminary results suggest that intranasal administration is more effective than intratracheal administration of the recombinant adenoviruses in seropositive animals. These studies further support the contention that viruses attach to the nasal mucosa before they are neutralized by antibodies. Intratracheal administration probably leads to neutralization of the virus before the virus can initiate infection. Support for this contention comes from

the fact that serum antibody transudation occurs more efficiently in the lower lung than in the nasal mucosa.

Further support for delivery of adenovirus vectors to nasal passages comes from studies demonstrating that delivery of antigens to nasopharyngeal lymph node tissue is one of the most efficient methods for stimulating immune responses. These studies indicate that the route of administration will need to be an important consideration when comparing immunization data with adenovirus vectors and developing the most effective protocol for their delivery. Our rapidly expanding knowledge of host responses to antigens and the role of cytokines in polarizing or modulating these responses, we predict, will play an important role in further improving the potential of adenovirus vectors. For example, it should be possible to construct adenovirus vectors co-expressing antigens and cytokines of interest. Expressing cytokines that induce mucosal immunity should both enhance the efficacy of the recombinants and, more importantly, induce the desired type of immune response. Based on the recent progress, both in using adenoviruses as vaccine vectors for infectious diseases or cancer immunotherapy, we are confident that recombinant adenoviruses will become an important part of our armamentarium of vaccines for humans and animals.

Acknowledgements

We thank Michelle Balaski for typing the manuscript. Support for our research has been provided by the Medical Research Council of Canada, Natural Sciences and Engineering Research Council of Canada, Saskatchewan Department of Agriculture (ADF), Alberta Agricultural Research Institute, and the Saskatchewan Beef Development Fund.

References

Arakawa, T., Chong, D.K., Langridge, W.H., 1998. Efficacy of a food plant-based oral cholera toxin B subunit vaccine [published erratum appears in Natl. Biotechnol. 16 (5), 478]. Natl. Biotechnol. 16, 292–297.

Babiuk, L.A., L'Italien, J., van Drunen Littel-van den Hurk, S., Zamb, T., Lawman, M.J.P., Hughes, G., Gifford, G.A., 1987. Protection of cattle from bovine herpes virus type I (BHV-1) infection by immunization with individual viral glycoproteins. Virology 159, 57–66.

Bowersock, T.L., HogenEsch, H., Suckow, M., Guimond, P., Martin, S., Borie, D., Torregrosa, S., Park, H., Park, K., 1999. Oral vaccination of animals with antigens encapsulated in alginate microspheres. Vaccine 17, 1804–1811.

Brochier, B., Pastoret, P.P., 1993. Rabies eradication in Belgium by fox vaccination using vaccinia-rabies recombinant virus. Ondersterpoort J. Vet. Res. 601, 469–475.

Chartier, C., Degryse, E., Gantzer, M., Dieterle, A., Pavirani, A., Mehtali, M., 1996. Efficient generation of recombinant adenovirus vectors by homologous recombination in *Escherichia coli*. J. Virol. 70, 4805–4810.

Cirillo, J.D., Stover, C.K., Bloom, B.R., Jacobs, W.R., Barletta, R.G., 1995. Bacterial vaccine vectors and bacillus Calmette-Guerin. Clin. Infect. Dis. 20, 1001–1009.

Cox, G., Zamb, T., Babiuk, L.A., 1993. Bovine herpes virus-1: Immune responses in mice and cattle injected with plasmid DNA. J. Virol. 67, 5664–5667.

Dong, J.Y., Wang, D., Van Ginkel, F.W., Pascual, D.W., Frizzell, R.A., 1996. Systematic analysis of repeated gene delivery into animal lungs with a recombinant adenovirus vector. Hum. Gene Ther. 7, 319–331.

Donnelly, J.J., Ulmer, J.B., Liu, M.A., 1993. Immunization with polynucleotides: A novel approach to vaccination. Immunologist 2, 20–26.

Ghosh-Choudhury, G., Haj-Ahmad, Y., Graham, F.L., 1987. Protein IX, a minor component of the human adenovirus capsid, is essential for the packaging of full length genomes. Embo. J. 6, 1733–1739.

Ginsberg, H.S., Lundholm-Beauchamp, U., Horswood, R.L., Pernis, B., Wold, W.S., Chanock, R.M., Prince, G.A., 1989. Role of early region 3 (E3) in pathogenesis of adenovirus disease. Proc. Natl. Acad. Sci. USA 86, 3823–3827.

Gooding, L.R., Elmore, L.W., Tollefson, A.E., Brady, H.A., Wold, W.S., 1988. A 14 700 MW protein from the E3 region of adenovirus inhibits cytolysis by tumor necrosis factor. Cell 53, 341–346.

Graham, F.L., Prevec, L., 1992. Adenovirus-based expression vectors and recombinant vaccines. In: Ellis, R.W. (Ed.), Vaccines: New Approaches to Immunological Problems. Butterworth-Heinemann, Stoneham, MA. pp. 363–371.

Grunhaus, A., Horwitz, M.S., 1992. Adenoviruses as cloning vectors. Semin. Virol. 3, 237–245.

Isaka, M., Yasuda, Y., Kozuka, S., Taniguchi, T., Miura, Y., Matano, K., Goto, N., Tochikubo, K., 1999. Intranasal or subcutaneous co-administration of recombinant cholera toxin B subunit stimulates only a slight or no level of the specific IgE response in mice to tetanus toxoid. Vaccine 17, 944–948.

Ishibashi, M., Yasue, H., 1984. Adenoviruses of Animals. In: Ginsberg, H.S. (Ed.), The Adenoviruses. Plenum Press, New York, pp. 497–562.

Kit, S., Otsuka, H., Kit, M., 1992. Expression of porcine pseudorabies virus genes by a bovine herpes virus-1 (infectious bovine rhinotracheitis) vector. Arch. Virol. 124, 1–20.

Klonjkowski, B., Gilardi-Hebenstreit, P., Hadchouel, J., Randrianarison, V., Boutin, S., Yeh, P., Perricaudet, M., Kremer, E.J., 1997. A recombinant E1-deleted canine adenoviral vector capable of transduction and expression of a transgene in human-derived cells and in vivo. Hum. Gene Ther. 8, 2103–2115.

Krajcsi, P., Tollefson, A.E., Anderson, C.W., Wold, W.S., 1992. The adenovirus E3 14.5-kilodalton protein, which is required for down-regulation of the epidermal growth factor receptor and prevention of tumor necrosis factor cytolysis, is an integral membrane protein oriented with its C terminus in the cytoplasm. J. Virol. 66, 1665–1673.

Lewis, P.J., Cox, G.J.M., van Drunen Littel-van den Hurk, S., Babiuk, L., 1997. Polynucleotide vaccines in animals: Enhancing and modulating responses. Vaccine 15, 861–864.

Mackowiak, M., Maki, J., Motes-Kreimeyer, L., Harbin, T., van Kampen, K., 1999. Vaccination of wildlife against rabies: Successful use of a vectored vaccine obtained by recombinant technology. Adv. Vet. Med. 41, 571–583.

McDermott, M.R., Heritage, P.L., Bartzoka, V., Brook, M.A., 1998. Polymer-grafted starch microparticles for oral and nasal immunization. Immunol. Cell Biol. 76, 256–262.

Mittal, S.K., Middleton, D.M., Tikoo, S.K., Babiuk, L.A., 1995a. Pathogenesis and immunogenicity of bovine adenovirus type 3 in cotton rats (*Sigmodon hispidus*). Virology 213, 131–139.

Mittal, S.K., Papp, Z., Tikoo, S.K., Baca-Estrada, M.E., Yoo, D., Benko, M., 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 herpes virus type 1 glycoprotein gD. Virology 222, 299–309.

Mittal, S.K., Prevec, L., Graham, F.L., Babiuk, L.A., 1995b. Development of a bovine adenovirus type 3-based expression vector. J. Gen. Virol. 76, 93–102.

Morin, J.E., Lubeck, M.D., Barton, J.E., Conley, A.J., Davis, A.R., Hung, P.P., 1987. Recombinant adenovirus induces antibody response to hepatitis B virus surface antigen in hamsters. Proc. Natl. Acad. Sci. USA 84, 4626–4630.

Paabo, S., Nilsson, T., Peterson, P.A., 1986. Adenoviruses of subgenera B, C, D, and E modulate cell-surface expression of major histocompatibility complex Class I antigens. Proc. Natl. Acad. Sci USA 83, 9665–9669.

Papp, Z., Babiuk, L.A., Baca-Estrada, M., 1999. The effect of pre-existing adenovirus-specific immunity on immune responses induced by recombinant adenovirus expressing glycoprotein D of bovine herpes virus type 1. Vaccine 17, 933–943.

Parks, R.J., Chen, L., Anton, U., Sankar, M.A., Rudnicki, M.A., Graham, F.L., 1996. A helper-dependent adenovirus vector system: Removal of helper virus by Cre-mediated excision of the viral packaging signal. Proc. Natl. Acad. Sci. USA 93, 13565–13570.

Pastoret, P.P., Brochier, B., Languet, B., Thomas, I., Paguot, A., Bauduin, B., Kieny, M.P., Lecocq, J.P., DeBruyn, J., Costy, F., Antoine, H., et al., 1988. First field trial of fox vaccination against rabies using a vaccinia-rabies recombinant virus. Vet. Rec. 123, 481–483.

Reddy, P.S., Idamakanti, N., Hyun, B.-H., Tikoo, S.K., Babiuk, L.A., 1998. Development of porcine adenovirus-3 as an expression vector. J. Gen. Virol. 80, 563–570.

Reddy, P.S., Idamakanti, N., Chen, Y., Whale, T., Babiuk, L.A., Mehtali, M., Tikoo, S.K., 1999. Replication defective bovine adenovirus type 3 as an expression vector. J. Virol. 73, 9137–9144.

Rosenthal, K.L., Copeland, K.F.T., Gallichan, W.S., 1996. Recombinant adenoviruses as vectors for mucosal immunity. In: Kiyono, H., Ogra, P.L., McGhee, J.R. (Eds.), Mucosal Vaccines. Academic Press, San Diego, CA, pp. 147.

Schulick, A.H., Vassalli, G., Dunn, P.F., Dong, G., Rade, J.J., Zamarron, C., Dichek, D.A., 1997. Established immunity precludes adenovirus-mediated gene transfer in rat carotid arteries. Potential for immunosuppression and vector engineering to overcome barriers of immunity. J. Clin. Invest. 99, 209–219.

Sheppard, M., Werner, W., Tsatas, E., McCoy, R., Prowse, S., Johnson, S., 1998. Fowl adenovirus recombinant expressing VP2 of infectious bursal disease virus induces protective immunity against bursal disease. Arch. Virol. 143, 915–930.

Sheppard, M., 1999. Viral vectors for veterinary vaccines. Adv. Vet. Med. 41, 145–157.

Taylor, G., Rijsewijk, F.A.M., Thomas, L.H., Wyld, S.G., Gaddum, R.M., Cook, R.S., Morrison, W.I., Hensen, E., van Oirschot, J.T., Keil, G., 1998. Resistance to bovine respiratory syncytial virus (BRSV) induced in calves by a recombinant bovine herpes virus-1 expressing the attachment glycoprotein of BRSV. J. Gen. Virol. 79, 1759–1767.

van Drunen Littel-van den Hurk, S., van Donkersgoed, J., Kowalski, J., van den Hurk, J., Harland, R., Babiuk, L.A., Zamb, T., 1994. A subunit gIV vaccine produced by transfected mammalian cells in culture induces mucosal immunity against bovine herpes virus-1 in cattle. Vaccine 12, 1295–1302.

Xu, Z.Z., Hyatt, A., Boyle, D.B., Both, G.W., 1997. Construction of ovine adenovirus recombinants by gene insertion or deletion of related terminal region sequences. Virology 230, 62–71.

Yilma, T., Hsu, D., Jones, L., Owens, S., et al., 1988. Protection of cattle against rinderpest and vaccinia virus recombinants expressing the HA and F genes. Science 242, 1058–1061.

Yokonama, M., Zhang, J., Whitton, J.L., 1995. DNA immunization confers protection against lethal lymphocytic choriomeningitis virus infection. J. Virol. 69, 2684–2688.

Zakhartchouk, A.N., Reddy, P.S., Baxi, M.K., Baca-Estrada, M.E., Mehtali, M., Babiuk, L.A., Tikoo, S.K., 1998. Construction and characterization of E3 deleted bovine adenovirus type 3 expressing full length and truncated form of bovine herpes virus glycoprotein gD. Virology 250, 220–229.