HENRY FRIESEN AWARD LECTURE

Lorne A. Babiuk

University of Alberta, Edmonton, Alberta,
Canada

Controlling Infectious Diseases through Vaccination

Infectious diseases continue to cause significant morbidity and mortality in both animals and humans. Indeed, every year infectious diseases cost the global economy billions of dollars in losses and are responsible for approximately one-third of all human deaths. These deaths occur from routine infections, hospital acquired infections (approximately 100,000 deaths occur annually in North America due to hospital-acquired infections), occasional pandemics or regional outbreaks. The most recent regional outbreak is Ebola in West Africa. This infection has caused significant challenges for the regional health care community and has had a global impact. The challenge in the control of infectious diseases is not only due to routine infections but also to the continued emergence and re-emergence of infectious diseases. These new threats occur on a regular basis with approximately thirty new emerging or re-emerging diseases recorded in the last thirty years. The majority of these emerging diseases are zoonotic (over 70%) causing even greater challenges to their control in humans and animals.

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Correspondence to:

Lorne A. Babiuk, O.C., SOM, PhD, DSc, FRSC
Vice-President (Research) University of Alberta
2-51 South Academic Building (SAB)
Edmonton, Alberta, Canada T6G 2G7
Email: lorne.babiuk@ualberta.ca

Infectious diseases continue to cause significant morbidity and mortality in both animals and humans. Indeed, every year infectious diseases cost the global economy billions of dollars in losses and are responsible for approximately one-third of all human deaths. These deaths occur from routine infections, hospital acquired infections (approximately 100,000 deaths occur annually in North America due to hospital-acquired infections), occasional pandemics or regional outbreaks. The most recent regional outbreak is Ebola in West Africa. This infection has caused significant challenges for the regional health care community and has had a global impact. The challenge in the control of infectious diseases is not only due to routine infections but also to the continued emergence and re-emergence of infectious diseases. These new threats occur on a regular basis with approximately thirty new emerging or re-emerging diseases recorded in the last thirty years. The majority of these emerging diseases are zoonotic (over 70%) causing even greater challenges to their control in humans and animals.

Current control of infectious diseases relies primarily on antimicrobial compounds or vaccinations. Although many infectious diseases have been controlled with antimicrobial treatment, the continued increase in resistance to antimicrobial agents is a constant challenge. The rapid increases in multi-drug resistant bacteria, including mycobacteria, are posing huge challenges to their control^(1, 2). The recent successes in using multiple drugs for controlling HIV⁽³⁾ and, more recently, success with controlling Hepatitis C⁽⁴⁾ are welcome developments but concerns continue regarding the development of drug resistance to viruses and bacteria. Unfortunately, the cost of these drugs can be prohibitive for use in all environments; including for patients in the developing world and for many animal species. Furthermore, in many cases, we do not have antimicrobial agents for many parasitic infections. The alternative to antimicrobials is the use of vaccines to both break the cycle of infection, by reducing the number of susceptible individuals in the environment (herd immunity) as well as protecting individuals should they be exposed to the infectious agents. These vaccines can be used to control outbreaks within a particular species, human to human transmission or be used in one species to prevent infection in another species. The best example of such a vaccine is where animals are immunized to prevent infection in humans from a zoonotic infection; for example, vaccinating cattle against brucellosis prevents transmission of this disease to humans⁽⁵⁾. Another example is vaccinating cattle against *E. coli* 0157:H7 to reduce shedding from animals into the environment. This vaccination dramatically reduces the opportunity for human infection, which is caused either by drinking contaminated water or eating contaminated food⁽⁶⁾. Recently,

the concept of “One Health” has been advocated to demonstrate the connection between animal infectious diseases and those in humans. Immunizing animals against West Nile virus reduces the level of West Nile virus circulating in the environment, thereby reducing the chances of infection of the vector (mosquito) and subsequent transmission to humans. A concerted effort to not only vaccinate humans but also develop a global strategy for linking animal vaccination with human vaccination is required. This coordinated effort has a great potential to reduce both animal as well as human infection.

Possibly the two best examples of the economic impact of vaccination on society is that of small pox and polio virus. Prior to the global eradication of small pox through vaccination, 2 million people died of the disease each year. The global effort to eradicate this disease has not only eliminated death due to this disease but dramatically reduced continuing health care costs. The second example is polio virus wherein it is estimated that over 8 million people have been spared from paralysis since the polio eradication program was initiated. This affects not only the individuals that would have been paralyzed but also their families and caregivers.

Historically, there were two types of vaccines: 1) killed vaccines; and, 2) live attenuated vaccines. Live attenuation is achieved by *in vitro* passage, which results in mutations in the pathogen such that it can still replicate *in vivo* but is crippled sufficiently that it cannot cause disease. Since the agent replicates *in vivo*, it produces all or most of the antigens, which are then recognized by the immune system. This induction of both humoral and cellular immunity is sufficient such that when the individual is later exposed to a field strain of the pathogen, the immune response will limit the degree of replication and provide protection. The advantage of these live attenuated vaccines is that they can be delivered either systemically or via the mucosal route, acting as a natural infection without inducing disease. The advantage of mucosal delivery is that delivery by this route induces both mucosal and systemic immunity. Since most pathogens enter via the mucosal route, the agent is neutralized even before it gets established if the individual has mucosal immunity. This is not the case with systemically-delivered killed vaccines that generally only induce systemic immunity. Examples of oral vaccines, which induce mucosal immunity, include rotavirus and polio virus vaccines^(7, 8). The disadvantage of live vaccines is that, since they are live, they carry the risk of being able to back mutate *in vivo*, resulting in the probability of shedding virulent pathogens into the environment. The best example is polio virus where attenuation occurs due to a single mutation in the virus. During replication in the intestine of the human, the virus back mutates resulting in the

shedding of a virulent virus into the environment ⁽⁸⁾. Furthermore, if this mutation occurs very quickly prior to induction of immunity in the vaccinated individual, the vaccinee may also suffer from effects of polio. It is for this reason that it is recommended that the live oral polio vaccines are only used in regions where there is a large amount of wild virus circulating. This approach provides rapid coverage of the population to reduce the viral load in the environment and then use killed virus to “mop up” any residual virus in the community.

More recently, using genetic engineering, it has been possible to delete virulence genes from various pathogens: this results in production of safer vaccines since it is almost impossible for a virus to regenerate a deleted gene during replication. Using such gene-deleted viruses not only capitalizes on the increased safety but it enables modulation of the degree of attenuation, which is more difficult with conventional attenuation. In addition, one can develop companion diagnostics to differentiate vaccinated individuals from those who have been exposed to virulent field strains of virus. This is especially useful in livestock vaccines used by countries trying to eradicate a pathogen from its borders. The best example is the vaccine for bovine herpes virus where a gene coding for a glycoprotein attenuates the virus ⁽⁹⁾. Animals treated with the vaccine do not induce antibodies to the gene-deleted component (gE). These animals can be differentiated from those that were exposed to field strains of virus and may be carrying the virus in a latent state in their bodies. If a country wants to eradicate the disease, they can cull the latent carrier animals and eventually establish herpes virus-free herds. These vaccines are called DIVA vaccines (DIVA = Differentiating Infected from Vaccinated Animals) and are useful for overcoming non-tariff trade barriers for livestock movement ⁽¹⁰⁾. An additional advantage of these gene-deleted vaccines is that one can use the deletion site to insert gene coding for protective antigens from other infectious agents. The best example of this is the insertion of the rabies virus glycoprotein into a pox virus ⁽¹¹⁾. This vaccine, which has the added advantage of thermal stability, has been incorporated into baits that are dropped from airplanes into areas where wildlife such as foxes eat the bait and are thus immunized against rabies virus. Such an approach not only eradicates rabies virus in wildlife but it eliminates spread to domestic animals and subsequently to people. We are currently developing a pox-based vaccine for cattle, sheep and goats to not only protect these animals from the devastating pox viruses that infect them but also to protect animals against other infections by incorporating other genes into the pox viruses. More specifically, we are inserting genes from Rift Valley fever into the ani-

mal pox viruses, which will reduce not only Rift Valley fever in animals but reduce transmission to humans.

A second approach to modulating virulence is to modify the virulence genes involved in virus replication. The best example is influenza virus. Since the hemagglutinin needs to be cleaved to allow infection of cells, it is possible to alter the cleavage site such that it can only be cleaved by specific cleavage enzymes. Normally, the hemagglutinin protein is cleaved by proteolytic enzymes such as trypsin, which are present in the respiratory tract. Changing the composition of the cleavage site so that it can no longer be recognized by trypsin will inhibit its replication in the natural host; however, if the cleavage site is changed to be cleaved by elastase, an enzyme not present in the normal animal respiratory tract, the virus can still be grown *in vitro* in the presence of elastase and then be used to vaccinate animals intra-nasally. The virus can infect cells in the respiratory tract, because the hemagglutinin has been cleaved with elastase and it can induce a full range of antigens, which induce mucosal and systemic immunity. Since the virus can only go through a single replication cycle, it is an abortive infection; this ensures vaccine safety. This vaccine has been shown to give much broader immunity and protection against homologous and even heterologous influenza virus, which is significantly broader than that provided by conventional kill vaccines containing only the hemagglutinin and neuraminidase ⁽¹²⁾.

Killed vaccines comprise the majority of vaccines used today. These can be whole organisms, which are inactivated through various means or individual components. The critical concern with inactivation is ensuring that the inactivation process does not alter the critical epitopes involved in inducing protective immunity. Alternatively, the vaccine may only contain individual purified components that are known to induce protective immunity. Such vaccines are often more expensive than live vaccines. Possibly the best example of a killed vaccine is influenza, which contains hemagglutinin and neuraminidase that are purified from the virus. As a third option, one can clone the protective components, as is done with Hepatitis B or human papilloma virus ^(13, 14). In these cases, the protective proteins are not only cloned but assembled into virus-like particles that present the epitopes in an extremely effective format for recognition by the immune system. These virus-like particles have been shown to be extremely effective since they are delivered to the immune system in a natural configuration.

Unfortunately, with all killed vaccines, for effective immunity to be induced, adjuvants are required. Until recently, the main adjuvant licensed for use in vaccines was alum. This adjuvant has a drawback in that it focuses the immune response to a Th2-like immune response, reducing the effectiveness of vac-

cines for pathogens requiring Th1 or cell mediated immunity for protection. More recently, as we become more knowledgeable regarding the constellation of events occurring during immune induction, people have begun using novel adjuvants and adjuvant combinations to drive the required immune effector cells. The advantage of these new adjuvant combinations is that some of them can be used to deliver vaccines not only systemically but also via mucosal routes. This overcomes some of the earlier limitations of killed vaccines where alum was the adjuvant of choice and the only delivery route was systemic.

The key to effective adjuvants is to create a cytokine micro-environment that attracts the correct cells to the site of vaccine delivery and to expand the appropriate cells to induce the correct immune response. One such combination adjuvant encompasses polyphosphazenes, which induce cytokines in their own right but also act as vehicles for mucosal delivery when formulated into micro particles, in combination with a toll-like receptor (CpG or poly IC) and a host defence peptide⁽¹⁵⁾. This triple adjuvant combination allows not only a dramatic increase in immune responses, both humoral and cell mediated, but also induces long-term memory, requiring only a single dose of the vaccine. This is extremely critical in the developing world, where multiple vaccinations for a single disease are often not feasible. In addition to providing a single shot vaccine, the ability to deliver the vaccine as micro-particles to mucosal services reduces both the trauma of vaccination via needles as well as the need for needles. Since needles are an expensive component of the delivery process, there have been reports of needle reuse to reduce costs, resulting in the spread of disease. An additional advantage of this adjuvant combination is the adjuvant induces a balanced immune response in all ages of individuals; ranging from neonates to elderly. This observation is critical since both age extremes – very young (neonates) and elderly – respond differently to vaccination than do young adults. This vaccine combination has recently been tested in respiratory syncytial (RSV) virus and in neonatal pertussis vaccines. Early attempts to deliver an RSV vaccine using alum resulted in a skewed Th2-like response, in that when vaccinated individuals were subsequently exposed to field strains of virus, they were not protected but actually suffered more severe disease and even death due to enhanced IgE responses. The triple adjuvant combination vaccine with RSV shifts the immune response to a more balanced response, eliminating the allergic IgE response and secretion of eotaxin components⁽¹⁶⁾. This results in very few clinical signs and in rapid clearance of the virus. This triple adjuvant can not only be used for development of new vaccines but has also shown to be effective when added to existing vaccines. The first vaccine in which this

adjuvant was used with was Hepatitis B⁽¹⁵⁾. Currently, Hepatitis B vaccination requires three doses for effective long-term immunity. The current cost of the three dose vaccine is over \$200. Unfortunately, this cost is prohibitive in the developing world where annual incomes in low and middle income countries can be in the \$1,000 per year range. Used in a triple adjuvant combination, excellent long-term immunity could be induced with a single dose. More importantly, the dose of antigen could be reduced by at least ten-fold (antigen sparing) thereby further reducing the cost of the vaccine. The low cost of production of the vaccine by regional vaccine manufacturers, such as the Serum Institute of India, and the requirement of only a single dose make this type of vaccine very economical to deliver to the world's most vulnerable populations.

Another advantage of using more effective adjuvants can be envisaged during new pandemics. When the H1N1 potential pandemic was feared, the vaccine companies could produce only approximately one tenth of the required vaccine. By reducing the amount of antigen required, the entire population could be immunized with the quantity of vaccine available. This approach might even be deployed in outbreaks such as Ebola, or other future outbreaks, where no licensed vaccine is available but the opportunity exists to curtail the rapid spread of the infection with limited quantities of potential vaccines. Clearly, in these situations, use of unlicensed vaccines would require significant discussions regarding the ethical considerations and potential risks and benefits before embarking on the roll out of the vaccine for emergency use. Hopefully, the lessons that have been learned, and are still being learned, from Ebola, Nepa and other unique outbreaks, will form the foundation for such discussions and decisions should these be required. These decisions must be made with a full appreciation of the potential benefits and risk of early introduction of such vaccines. This is especially important not only for the safety and wellbeing of the at-risk population but also as not to inflame the anti-vaccine debate. All the evidence to date demonstrates that the regulatory environment is exceptional for licensing safe vaccines and that the vaccines have saved millions of lives, yet the strong anti-vaccine lobby has captured the attention of many people including the media who continues to propagate the fear of vaccines. Any missteps in the introduction of such vaccines could add to this controversy and decisions about their use must be taken cautiously.

In summary, vaccination using conventional approaches has been extremely effective. Building on the hundred plus years of great success in vaccination, we now have additional tools to add to our armamentarium. Better understanding of the host immune system, the role of virulence genes and pro-

protective antigens from various pathogens, combined with molecular biology to attenuate pathogens or clone protective antigens for combination with novel delivery systems, provides confidence that safer and better vaccines will be developed in the future to further reduce morbidity and mortality due to infectious diseases. Although many challenges still exist in the development of vaccines against agents with complex life cycles and with host interactions, such as HIV, the majority of devastating diseases should be at least controlled by immunization in the future, even if eradication is not possible.

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