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Advanced Technology to Promote Productivity, Efficiency and Disease Prevention

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Advances in Viral Vaccine Technology

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Abstract

Although the era of vaccination has seen major advances in controlling many important diseases of man and animals, only one viral disease, small pox, has been eliminated globally. Other infectious diseases have proven more resilient to control and therefore, a need exists for the development of better, more effective vaccines. The advent of various genetic and biochemical techniques, combined with the understanding of microbial pathogenesis and host responses of these infections during the last decade has led to the emergence of a variety of new approaches to development of better vaccines, as well as potential methods of delivery of these vaccines. Based on these developments, an attempt will be made at summarizing some of the unique strategies that are being used to combat viral infections. Approaches used to develop synthetic peptide vaccines, recombinant hybrid vaccines, deletion mutants and subunit viral vaccines will be discussed. Furthermore, the advantages and limitations of these approaches will be addressed, as will some of the potential ways of overcoming these limitations.

Introduction

At present there are a number of methods available for controlling infectious diseases of humans and animals. These include passive immunity which can be obtained either in utero, via antibody secreted in milk or passively administered either as monoclonal antibodies or polyclonal antibodies. The disadvantages of passive immunity lie mainly in the short duration of protection, thus, active immunization has become the more accepted method of immunizing animals to protect them from infections. The third method of control is by chemotherapy using either antibacterials, antivirals or immunomodulators. The major emphasis in this summary will be devoted to viral vaccines presently being used and those that we anticipate will become common place within the next decade.

Conventional Viruses

The majority of licensed vaccines for humans and animals presently in use are produced by conventional methods. These include live attenuated or inactivated vaccines. At present, a large number of viral vaccines are of the killed variety. One of the major advantages of such vaccines is that they are relatively stable under environmental conditions, therefore, it is not as crucial to maintain a cold chain to ensure efficacy of the vaccines. Some other advantages are that in specific disease situations such as rabies virus, clinicians are often reluctant to use live viral vaccines, because of the fear that they may inject themselves with the vaccine and there may be some adverse side effects. Although this possibility is extremely remote, the psychological trauma of injection with a virus such as rabies is sufficiently great to discourage some clinicians from using live virus vaccines. The disadvantages of killed vaccines is that they do not replicate within the host and therefore, large amounts of antigen are required for injection before immunity will be induced. Since these vaccines are often produced in foreign tissue there is also the possibility of reactions developing against foreign proteins. The possibility of developing reactions to foreign proteins is further exacerbated by the fact that immunity is usually short-lived with killed virus vaccines, therefore, multiple doses are required. Since the vaccines are killed, they generally are injected intramuscularly. If the specific disease being vaccinated against is one that causes a local infection in the respiratory tract or in the gastrointestinal tract killed vaccines will not be very effective since they do not induce immunity at these sites. Therefore, a killed vaccine will be more effective against systemic viruses than against viruses which replicate in local mucosal sites. These latter disadvantages have lead to the development of a large number of attenuated vaccines.

The main advantage of attenuated vaccines results primarily from their ability to replicate in the host. Since their mode of action is similar to natural infections,

immunity is generally of a broader spectrum than it is with killed virus vaccines. Furthermore, they induce a balanced immune response, ranging from humoral to cellular including local as well as systemic immunity. Furthermore, immunity is usually broader with more cross reactivity to related strains, and of longer duration with attenuated vaccines than with killed virus vaccines. Finally, since the virus replicates in the host and produces large quantities of proteins to which the host responds to, the possibility of injecting foreign proteins is dramatically reduced with attenuated virus vaccines. However, these conventional attenuated vaccines are not without certain disadvantages. Since the vaccines are produced by passage in culture, to induce random mutations or mutated with a specific agent and thereby reduce virulence, it is possible that passage in the natural host may result in reversion back to virulence. One of the best examples of such reversion is in the case of attenuated polio virus. In the case of polio, reversion can occur within a few days of oral immunization.[1,2] If the individual is unable to mount a rapid immune response disease can occur. Since reverted virulent virus is shed into the environment, there is a danger of contacts getting infected with the virus. One other very important disadvantage is that the viruses are grown in culture and it is possible to have other contaminating viruses present. One very common occurrence is the presence of BVD virus in viral vaccines grown for immunizing cattle. This virus is ubiquitous and is present in many of the cell lines and fetal bovine sera that are used for growing bovine viruses.[3] Interference is also a potential problem when animals are immunized with a number of different vaccines at the same time, or if animals are suffering from a subclinical virus infection at the time of vaccination. This scenario may result in reduced replication of the attenuated virus vaccine and thus, reduced immunity. Live attenuated virus vaccines are also extremely susceptible to environmental factors which may reduce their efficacy upon storage. Finally, the attenuated virus vaccines can induce latent infections, congenital defects and abortions if not administered properly or if administered at the wrong time in the individual's life.[4-7]

Why New Vaccines?

Since conventional vaccines have not eliminated viral diseases, with the exception of small pox, there continues to be a need to produce better vaccines that may be more efficacious and safer for use in human and animal medicine. In addition to the above discussed limitations of present vaccines, it should be emphasized that there are a number of viruses for which we do not have vaccines due to the inability to grow virus in culture or in other economically acceptable culturing media. Some viruses may be of suppressive nature or impossible to attenuate by *in vitro* passage. In North America there are a number of exotic diseases for which we do not want to introduce the virus, therefore, it makes it impossible to produce virus vaccines against agents by conventional methods. In order to develop vaccines against these exotic viruses would require excessive laboratory containment or would restrict the use and application of such vaccines. Some of the newer technologies available would greatly eliminate some of these restrictions.

Methodologies for Producing New Vaccines

Table 1 summarizes some of the newer technologies that are available and presently being used to produce new virus vaccines. Although some of these technologies are based on classical approaches (reassortants, temperature sensitive or cold adapted, heterologous vaccines), many of them are based on the ability to manipulate the genetic material of the viruses in such a way as to either reduce the virulence of an individual virus in a specific way or identify the specific protective proteins and express them in a foreign host. Although there is at present some controversy regarding the reductionist approach to vaccine production versus the conventional approach, many examples are available whereby subunit vaccines have been very efficacious in reducing viral induced disease at least in experimental models. A number of these have now been licensed for use in animals and humans.

Table 1—Technologies for Producing New Vaccines

Methodology	Example
1) Recombinant DNA—Expression of genes in foreign hosts	
—viruses (baculovirus, herpesvirus adenovirus, vaccinia)	Influenza, AIDS, VSV
—bacteria (Salmonella)	Hepatitis B
—yeast	Hepatitis B
—mammalian cells	Herpes
2) Reassortants	Influenza
3) Heterologous viruses	Rota
4) Genetic deletions	Herpes
5) Mutations	Polio
6) Antiidiotypes	Rabies
7) Synthetic peptides	Hepatitis B

For the production of subunit vaccines a number of specific steps are required. 1) Identify protective proteins or epitopes on the proteins. Once this is done an individual can either produce a subunit vaccine by recombinant DNA technology or by synthetic peptide technology. 2) Identify gene coding for the protein. 3) Clone the gene coding for the specific protein and express it in a suitable expression system. 4) Purify the protective protein to homogeneity. Using bovine herpesvirus-1 as an example, the application of the subunit vaccine approach to protect cattle against bovine respiratory disease will be demonstrated. BHV-1 has four major glycoproteins: GVP I, GVP II, GVP III

and GVP IV.[8] Using monoclonal antibodies, which we have developed against the individual glycoproteins, immunosorbent columns were prepared and used for purification of large quantities of the BHV-1 glycoproteins.

These glycoproteins were then mixed with the adjuvant avridine and used to immunize animals against BHV-1 virus. Following immunization, animals responded by producing serum neutralizing antibody titers far in excess of that produced by a commercial killed conventionally produced vaccine.[9] In addition to producing higher levels of serum neutralizing antibodies, the animals also exhibited much lower clinical involvement following challenge with a virulent BHV-1/P. haemolytica challenge.[9] The mortality rates for the placebo vaccinated animals was 3/5, animals immunized with the conventional commercial vaccine 2/5, whereas 0/35 animals immunized with the subunit vaccine died. These studies clearly indicate the potential for application of subunit vaccines to infections of man and animals.

An advantage of using the subunit vaccine approach, especially for viruses such as herpesviruses which can induce latency is the possibility of developing tests to differentiate animals which are latent carriers of the virus from those which are immunized with a subunit vaccine and are protected from subsequent challenge, but are not latent carriers of the virus. This type of approach has a tremendous amount of appeal to breeders of pedigree stock who may want to export or sell carrier free animals. Therefore, it is possible to maintain and develop specific pathogen free herds but still provide protection to accidental introduction of virus into the herd. This approach is also compatible with an eradiation program for diseases such as pseudorabies and bovine herpesvirus.

Expression Systems

Once the specific protective proteins are identified, it is important to develop expression systems to produce large quantities of the specific protein in an economical fashion. At present, there are four different expression systems: 1) prokaryotic, 2) viruses, 3) eukaryotic and 4) mammalian. Although prokaryotic expression systems were among the first to be used and very efficient production of viral proteins occurs in *Escherichia coli*[10,11] it does not appear to be very useful for production of vaccines and therefore, will not be discussed in detail in the present report. The main reason for the lack of efficacy of vaccines produced by bacterial expression systems is the viral protein produced in bacteria are often not folded properly for induction of the desired immune response. Thus gene products which do not require post-translational modification, phosphorylation, cleavage, etc. are the only ones that could be expressed in proteolytic systems. A considerable amount of activity is being directed towards using other viruses such as vaccinia, herpesviruses or adenoviruses to express specific viral proteins in mammalian systems.[12-18] Another very popular expression system is the application of an insect virus, baculovirus, to produce high quantities of animal virus proteins in insect cells.[19]

Viral Expression Systems

Vaccinia virus will be used as an example wherein a number of different viral proteins have been introduced into the vaccinia virus and are being used by a variety of different delivery systems to induce both local as well as systemic immunity. The advantages of vaccinia expression are that both a humoral, as well as a cellular immune response is ellicited. Even more attractive is that the vaccinia genome is very large and it is possible to delete large quantities of its genome and still maintain a viable virus. The non-essential vaccinia genes can be replaced with a number of genes coding for other proteins from other viruses. Therefore, it would be possible to introduce a cassette of up to 5 or 6 different genes into vaccinia virus and immunity could be induced simultaneously towards all these proteins. These genes could either be under the control of vaccinia promotors or more efficient promotors such as the T7 RNA polymerase promotors[20] which can increase the level of expression 10-fold over that produced by vaccinia promotors. Expression of a number of genes in one virus would be much more economical to do than to culture each individual vaccine independently. An even more attractive possibility of using vaccinia virus expression systems is the thermal stability of the virus and the international experience with immunization of large numbers of individuals with vaccinia. Finally, vaccinia can replicate in a wide variety of hosts, making it attractive for controlling infectious diseases in veterinary medicine and in human medicine. Furthermore, the thermal stability and its ability to replicate in a wide variety of hosts provide the opportunity to immunize wildlife against infectious diseases that can be transmitted to domestic livestock. One example of such an approach is that recently being used is the case of wildlife rabies, where vaccinia virus containing the rabies virus glycoproteins might be incorporated in bait and seeded in rural areas by dropping the bait from planes. Foxes and raccoons, which can be carriers of rabies virus, would eat the bait and be immunized against rabies virus. Using this approach, the number of animals that can be immunized is greatly increased, thereby reducing the epidemiological spread of virus in the environment. One further way of reducing the chance of transmission is to clone the genes into Avianpox virus. This virus does not replicate in mammalian species thereby preventing spread of the virus. This approach also limits the possibility of generalized infections which may occur occasionally as a result of vaccinia virus infection of immunocompromised hosts. Recently, considerable progress has been made in ensuring that generalized spread of vaccinia is prevented

by identifying potential virulent genes and deleting them from vaccinia.[22] In addition, insertion of a gene for interleukin-2 (IL-2) greatly reduces the virulence of vaccinia in immunocompromised hosts.[23] Thus, it appears highly likely that vaccinia virus will be engineered to be an effective vector for a large number of different viral antigen to prevent acute, chronic or leukemia virus infections.

The recent discovery that other viruses such as herpes[14] and adenovirus[13] also have regions within the genome which are non-essential for either *in vitro* or *in vivo* replication has prompted investigations into their use as potential viral vectors for a number of antigens. Since both of these viruses are considerably smaller than vaccinia there is probably less potential for inserting as many genes into these two viruses but they may offer other advantages regarding delivery into the oral or respiratory tract. For example, adenoviruses may persist in the respiratory tract and thereby continue to stimulate local immunity. Experience with adenovirus[24] and its use as an oral vaccine is well established with over 1 x 10^6 military recruits being immunized with no adverse effect.

Eukaryotic Expression Systems

Expression of viral genes in eukaroytic cells is often toted as the most natural method of producing non-infectious viral vaccines. The reason for the attractiveness of eukaroytic cells is that the proper level and degree of glycosylation and folding is more natural than in prokaryotic systems. At present a number of viral genes have been successfully expressed in yeast,[25] mammalian cells,[26] and more recently in filamentous water fungi and green algae.[27,28] These latter two systems should provide large quantities of cheap proteins with the correct post-translational modification required for proper recognition of the host's immune system. The advantages of yeast as an expression system are that extensive experience is already available with the yeast *S. cerevisiae*, and since animals already have antibodies to yeast, there would not be the needed expense to ensure that all yeast proteins are removed from the vaccine thereby reducing costs of the final product. Finally, yeasts do not have any oncogenes. This makes vaccines expressed in yeast potentially safer than vaccines produced in mammalian cells. Unfortunately, in some cases yeast may over-glycosylate proteins, which may influence immune response to that specific protein. Thus, the degree of glycosylation of highly glycosylated proteins may preclude its use in vaccine development.

The ultimate eukaroytic expression system is the use of mammalian cells for the continuous production of and secretion of viral proteins and glycoproteins. An advantage of mammalian cells is that cloned eukaroytic genes are often expressed as fully functional and processed proteins. However, the level of expression is relatively low and the high cost of cell cultures are serious disadvantages in the use of mammalian cells for production of vaccines for veterinary use. For mammalian cells to be an economically viable vehicle the development of micro-carriers to produce large quantities of mammalian cells in a very concentrated environment as well as strong promotors is needed.

Extensive progress is being made in developing microcarriers to culture mammalian cells in a continuous fashion. In parallel with the development of microcarrier systems is the requirement for new media and profusion of the bioreactors so that cells can grow continuously with minimal manipulations.[29]

Synthetic Peptide Vaccines

Rather than introducing the specific protein into various expression systems, it is possible to identify the specific epitopes involved in inducing protective immunity and synthesize the peptide. As an example of these approaches, the identification and characterization of the major neutralizing antigen of bovine rotavirus will be described. Using monoclonal antibodies against different proteins of bovine rotavirus, we have identified a immunodominant neutralizing epitope on the outer coat glycoprotein of bovine rotavirus. This epitope was identified by the ability of monoclonal antibody directed against it to neutralize virus *in vitro*[30] as well as prevent diarrhea in animals *in vivo*. Using this epitope we further found that a common conserved sequences were present between different serotypes of rotavirus of animals and humans. Using an animal model, developed to study bovine rotavirus, we clearly demonstrated that immunization with a synthetic peptide could be used to protect animals against virulent challenge with field strains of virus.[31] Although this has been demonstrated to be efficacious against bovine rotavirus, it must be emphasized that it is imperative to find a stable conserved region within the virus before such approaches can be used. In the case of foot and mouth disease virus, where antigenic drift is a common feature, such approaches have been less than satisfactory. However, the recent finding that many serotypes of rhinovirus share conserved regions responsible for interacting with the host cell receptor renew hope that even for antigenically labile viruses, it may be possible to identify biologically important conserved epitopes.[32]

The advantage of peptide vaccines is that it is possible to identify crucial epitopes on all viruses. Once these are identified, a chemically defined vaccine which is stable can be developed. Furthermore, the requirements for large production plants and downstream processing are minimal. In addition, peptides can be designed in such a way as to develop the appropriate immune responses (both T and B cell responses if epitopes responsible for stimulating T and B cell responses are incorporated into the peptide).

Finally, these vaccines can be formulated into delayed release and delivery systems which can continue to stimulate the immune system over an extended period of time, thereby removing the requirement for multiple injections.

Synthetic peptides generally are not very immunogenic unless they are linked to specific carriers and incorporated in strong adjuvants. The present identification of synthetic adjuvants is greatly improving the potential for using synthetic peptides as vaccines.[33] In addition to incorporating adjuvants into peptide vaccines, the peptides can be engineered in such a way that they are linked to specific carriers which can act as ideal delivery systems for presenting the important epitopes on the peptide.[34] The recent incorporation of peptides into self-assembling viruses such as hepatitis B and Tobacco mosaic virus (TMV) which can be produced by recombinant DNA technology provides a convenient one-step method of both producing the peptide as well as the carrier.[34,35] Experience with virus carriers for synthetic peptides clearly indicates that the immunogenicity of these peptides linked to virus carriers approaches that of whole virus.

Deletion Mutants

Using recombinant DNA technology it has been possible to construct deletion mutants against a number of animal herpesviruses, including pseudorabies and bovine herpesvirus-1, whose virulence and ability to induce latency is dramatically reduced following deletion of some genes.[36] Thus, some of the advantages of attenuated virus vaccines are preserved, but some of the disadvantages are deleted. Although a number of genes have been associated with virulence, the thymidine kinase gene has received the most attention.[36] Deletion of these gene not only reduces neurovirulence dramatically but it also reduces the viruses' ability to induce latency.[37] Based on these observations a number of pseudorabies virus vaccines are either licensed already or are in the process of being licensed. The next logical step is to insert genes from other viruses into the region of the deleted TK genes or other non-essential herpes glycoprotein genes.[38]

Epilogue

Regardless of the specific approach used to develop new viral vaccines it must be emphasized that no single approach will be ideal for all pathogens since the pattern of pathogenesis between different agents is extremely variable. Therefore, a tremendous amount of basic knowledge is required regarding whether infection provides protective immunity for subsequent infections, what type of immunity is required whether it be cellular or humoral, local or systemic, etc. before effective vaccines can be engineered. However, with the tremendous amount of activity both in the area of understanding the viruses and their proteins, the host immune responses, pathogenesis of different viruses as well as methods to regulate the immune response using lymphokines,[39] the future for developing effective vaccines against the major economically important infections of domestic animals is imminent.

References

1. Almond, J.W., The attenuation of poliovirus neurovirulence. Annu. Rev. Microbiol., 1987, 41, 153-180. 2. Kew, O.M., Nottay, B.K., Hatch, M.H., Nakano, J.H., and Obijeski, J.F., Multiple genetic changes can occur in the oral polio vaccines upon replication in humans. J. Gen. Virol., 1981, 56, 337-347. 3. Bielefeldt Ohmann, H. Ronsholt, L. and Bloch, B., Demonstrations of bovine viral diarrhoea virus in peripheral blood mononuclear cells of persistently infected clinicly normal cattle. J. Gen. Virol., 1987, 60, 1971-1982. 4. Pastoret, P.P., Babiuk, L.A., Misra, V. and Griebel, O., Reactivation of temperature sensitive and non-temperature sensitive infectious bovine rhinotracheitis vaccine virus with Dexamethasone. Infect. Immun., 1980, 29, 483-488. 5. Wyatt, H.V., Poliomyelitis in hypogrammaglobulinemic. J. Infect. Dis., 1973, 128, 802-806. 6. Chantler, J.K. Ford, D.K., and Tingle, A.J., Persistent rubella infection associated arthritis. Lancet, 1982, 1, 1323-1325. 7. Chantler, J.K., Ford, D.K., and Tingle, A.J., Rubella associated arthritis: Rescue of rubella virus from peripheral blood lymphocytes two years post-vaccination. Infect. Immun., 1981, 32, 1274-1280. 8. van Drunen Littel-van den Hurk, S. and Babiuk, L.A. Synthesis and processing of bovine herpesvirus-1 (BHV-1) glycoproteins. J. Virol., 1986, 59, 401-410. 9. Babiuk, L.A., L'Italien, J., van Drunen Littel-van den Hurk, S., Zamb, T., Lawman, M.J.P., Hughes, G. and Gifford, G.A., Protection of cattle from bovine herpesvirus-1 (BHV-1) infection by immunization with individual viral glycoproteins. Virol., 1987, 159, 57-66. 10. Kleid, D.G., Yansura, D., Small, B. Dowbenko, D., Moore, D.M., Grubman, M.J., McKercher, P.D., Morgan, D.O., Robertson, B.H., and Backrack, H.L., Cloned viral protein for foot and mouth disease: response in cattle and swine. Science, 1981, 214, 1125-1129. 11. Malek, L.T., Soostmeyer, G., Garvin, R.T., and James, E., The rabies glycoprotein gene is expressed in *E. coli* as renatured polypeptide. In Modern Approached to Vaccines. ed. R.A. Lerner and R.M. Chanock. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 1984, pp. 203-208. 12. Brown, F., Schild, G.C. and Ada, G.L., Recombinant vaccinia viruses as vaccines. Nature, 1986, 319, 549-550. 13. Morin, J.E., Lubeck, M.D., Barton, J.E., Conley, A.J., Davis, A.R. and Hung, P.P., Recombinant adenovirus induces antibody responses to hepatitis B virus surface antigen in hamsters. Proc. Natl. Acad. Sci. USA, 1987, 84, 4626-4630. 14. Sauer, B. Whealy, M., Robbins, A and Enquist, L., Site for specific insertion of DNA into a pseudorabies virus vector. Proc. Natl Acad. Sci. Usa, 1987, 84, 9108-9112. 15. Gilbert, J.H., Pedersen, N.O., Nunberg, J.H., Feline leukemia virus envelope protein expression encoded by a recombinant vaccinia virus: apparent lack of immunogenicity in vaccinated animals. Virus Research, 1987, 7, 49-67. 16. Roizman, B., Meigner, B., Norrild, B., and Wagner, J.L., Bioengineering of herpes simplex virus variants for potential use as live vaccines. In Modern Approaches to Vaccines., ed, R.A. Lerner and R.M. Chanock. Cold Spring Hargor Laboratory, Cold Spring Harbor, New York, 1984. 17. Nayak, D.P., Davis, A.R., Ueda, M. Bos, T.J., and Sivasubramanian, N., Expression of influenza virus glycoproteins. In Modern Approaches to Vaccines. ed. R.A. Lerner and R.M. Chanock. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1984, pp. 165-172. 18. Berman, P.W., Gregory, T., Dowbenko, D., and Lasky, L., Production of viral glycoproteins in genetically engineered mammalian cell lines for use as vaccines against immune deficiency retrovirus. Applied Virol. Res., 1988, 1, 17-24. 19. Possee, R.D., Cell surface expression of influenza virus hemagglutinatinin in insect cells using a baculovirus vector. Virus Res., 1986, 5, 43-59. 20. Li, Y. Luo, L., Snyder, R.M. and Wagner, R.R., Expression of the M gene of vesicular stomatitis virus

cloned in various vaccinia virus vector. J. Virol., 1988, 62, 776-782. 21. Rupprecht, C.E., Wiktor, T.J., Johnston, D.H., Hamir, A.N., Dietzchold, B., Wunner, W.H., Glickman, L.T. and Koprowski, H., Oral immunization and protection of raccoons. (Porcyonloter), with a vaccinia-rabies glycoprotein recombinant virus vaccine. Proc. Natl. Acad. Sci. USA, 1986, 83, 7947-7950. 22. Buller, R.M.L., Chaksabarti, S., Cooper, J.A., Twardzik, D.R. and Moss, B., Deletion of vaccinia virus growth factor gene reduces virus virulence. J. Virol., 1988, 62, 866-874. 23. Flexner, C., Hugin, A. and Moss, B., Prevention of vaccinia virus infection in immunodeficient mice by vector-directed IL-2 expression. Nature, 1987, 330, 259-262. 24. Smith, T.J., Buescher, E.L., Top, F.H., Altemeier, W.A., and McCown, J.M., Experimental respiratory infection with type 4 adenovirus vaccine in volunteers: clinical and immunological responses. J. Infect. Dis., 1970, 122, 239-248. 25. Valenzuela, P., Coit, O., Medina-Selky, M.A., Kuo, C.H., van Nest, G., Burke, R.L., Ball, P., Urdea, M.S. and Graves, P.V., Antigen engineering in yeast: Synthesis and assembly of hybrid hepatitis B surface antigen—herpes simplex 1 gD particles. Biotechnology, 1985, 3, 323-326. 26. Gething, M.J., Sambrook, J.F., Braciale, T.J. and Brand, C.M., Comparison of different eukaryotic hemagglutinin glycoprotein of influencing a virus. In Modern Approaches to Vaccines, ed. R.M. Chanock and R.A. Lerner. Colst Spring Harbor, New York, 1984, pp. 263-268. 27, Hasnain, S.E., Manavathu, E.K, and Leung, W.C., DNA mediated transormation of Chlamydomonas dreinhandi cells: Use of aminoglycoside 3′-phosphotrasferase as a selectable marker. Mol. CCell Biol., 1985, 5, 3647-3650. 28. Leung, W.C., Manavathu, E.K., Zwaagstra, J., Surunarayana, K., Hasnain, S.E. and Leung, M.F.K., Development of fungal and algal cells for expression of foreign genes. In Applied Virol., ed. E. Kurstak, Academic Press, 1986. pp. 29. Microcarrier cell culture. Principle and Methods, Pharmacia, Uppsala, Sweden, 1986, pp. 1-127. 30. Sabara, M., Barrington, A. and Babiuk, L.A., Immunogenicity of a bovine rotavirus glycoprotein fragment. J. Virol., 1985, 36, 1037-1040. 31. Ijaz, M.K., Sabara, M.I., Frenchick, P.J. and Babiuk, LA.A, Synthetic peptide vaccine confers passive protection against experimental rotavirus infection in neonatal mice. J. Gen. Virol. 1988, Submitted. 32. McCray, J. and Werner, G., Different rhinovirus serotypes neutralized by antipeptide antibodies. Nature, 1987, 329, 736-739. 33. Allison, A. and Byars, N., An adjuvant formulation that selectively elicits the formation of antibodies of protective isotypes and of cell medicated immunity. J. Immunol. Meth., 1986, 95, 157-168. 34. Clarke, B.E., Newton, S.E., Carroll, A.R., Francis, M.J., Appleyard, G., Syred, A.D., Highfield, P.E., Rowlands, D.J. and Brown, F., Improved immunogenicity of a peptide epitope after fusion to hepatitis B core protein. Nature, 1987, 330, 381-384. 35. Haynes, J.R., Cunningham, J., von Seefried, A., Lennick, M., Garvin, R.T. and Shen, S.H., Development of a genetically-engineered candidate polio vaccine employing the self assembly properties of the tobacco mosaic virus coat protein. Biotechol., 1986, 4, 637-641. 36. Stanberry, L.R., Kit, S. and Myers, M.G., Thymidine kinase-deficient herpes simplex virus type 2 genital infection in guinea pigs. J. Virol., 1985, 55, 322-328. 37. Kit, S., Sheppart, M., Incimura, H. and Kit, M., Second generation pseudorabies virus vaccine with deletions in thymidine kinase and glycoprotine genes. Am J. Vet. Res., 1987, 48, 780-793. 38, Thomson, D.R., Marchioli, C.C., Yancey, R.J. and Post, L.E., Replication and virulence of pseudorabies virus mutants lacking gx. J. Virol, 1987, 61, 229-232. 39. Anderson, G., Urban, O. and Fedorka-Cray, P. Interleukin--2 and protective immunity in Haemophilus pleuropneumonia: Preliminary studies. In Vaccines 87, Modern Approaches to New Vaccines, ed. R. Chanockk. R., Lerner, F. Brown and H. Ginsberg. Coldspring Harbor Laboratories, N.Y., 1987, pp. 22-25.