

Immunity and Practical Vaccine Development

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ABSTRACT — *The practical aspects of veterinary vaccine development and how vaccines may be designed better to protect animals against disease are reviewed. It is emphasized that vaccine development must be directed toward producing the type of immune response required to protect against a particular disease. In attempting to protect fish through vaccination, there are new opportunities and problems to be considered: The temperature dependent immune response, isolation problems, importance of group immunization VS individual protection, and the concept of breeding fish capable of producing a superior immune response.*

The work done by research and development groups in commercial organizations that prepare vaccines has much in common with the work done by laboratories of government and academic institutions in that some of their activities might be considered basic science. The scientific personnel of these commercial organizations are working at about the same level of competence as their colleagues in veterinary schools and the veterinary science departments. While vaccine development may not be a science, it is certainly sophisticated technology. The day is long past when it was possible to throw together the ingredients for a vaccine, test it in a few animals, demonstrate some level of protection, and call the result a vaccine. The public's expectations of effectiveness have increased; these expectations are reinforced and strengthened by appropriate government regulations. Although the public's expectations may be excessive, there is absolutely no question that

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vaccines today are much better than they were in 1950.

TYPES OF VACCINES

Basically, we might say that there are five kinds of veterinary vaccines: 1) Inactivated bacterial vaccines (referred to as bacterins); 2) modified live bacterial vaccines; 3) toxoids; 4) inactivated viral vaccines; and 5) modified live viral vaccines.

There are also vaccines against species of rickettsia, coccidia, hookworms, and other agents. Some antiserum is still used. In the past, combinations of virulent virus and antiserum were used for some diseases, but this practice has been discontinued.

TYPES OF IMMUNITY

In birds and mammals, the immune system may be conveniently divided into two components: Humoral immunity and cell mediated immunity. These two components serve to supplement each other and appear to naturally regulate each other. Humoral immunity involves a synthesis and release of free

antibody into the blood and other body fluids. Antibody acts by direct combination with the antigen. Humoral immunity is most effective in neutralizing toxins and by coating bacteria to enhance their phagocytosis. It also plays a part in neutralizing certain viruses. Cell mediated immunity involves a production of specifically sensitized cells and is expressed by such reactions as rejection of skin transplants, delayed type hypersensitivity, and the destruction of cells infected with viruses or bacteria. Cell mediated immunity is considered to be the body's primary response against cells such as cancer cells.

INACTIVATED VERSUS LIVE VACCINES

Before developing a vaccine, a clear-cut idea of the type of protection that is needed is helpful. If the disease is caused by a toxin, inoculation of toxoid or adjuvanted toxoid to stimulate humoral immunity is the route to follow. In this case fractionation and purification of bacterial antigens are inappropriate. If the disease is caused by a virus, inoculation of modified live virus which serves to stimulate both humoral and cell mediated immunity is probably the route to follow. Vaccines which have generally been most satisfactory fall into the two mentioned categories. There are exceptions but generally speaking, long-lasting and strong immunity has not been the strong point of inactivated bacterial vaccines

and inactivated viral vaccines. Veterinarians and physicians indicate their preference for modified live virus vaccines when they have a choice. Properly researched and prepared, I believe in modified live virus vaccines—there is less chance of the modified virus reverting to virulence by mutation than there is for inactivated viral vaccines to contain virulent virus because of failure to be fully inactivated. There have been some widely publicized examples of so-called inactivated viral vaccines which have not been inactivated. As capable a scientist as Jonas Salk failed to recognize all of the parameters involved in the inactivation of poliomyelitis virus. After the tragic results from use of improperly inactivated commercial vaccine, the entire procedure was reexamined by Sven Gard. He found that too rapid inactivation with formaldehyde served to denature the viral capsid protein. By not allowing the proper concentration of formaldehyde to enter into the interior of the virus, the viral nucleic acid was not inactivated. At this microlocation, the predetermined inactivation curve did not apply.

DEVELOPING AN INACTIVATED BACTERIAL VACCINE

We have much to consider when we start to develop a vaccine. I will present some of the points to be considered in developing an inactivated bacterial vaccine.

What is the etiological agent?

Which bacteria are important in the disease under study and how many strains are there? Is this a disease situation where four or five different strains are required for coverage or only one? Once identified it becomes necessary to select antigenic strains.

A production method retaining antigenicity must be developed.

A growth cycle has to be developed. This involves determining the time, the temperature, the culture medium, the oxygen content, whether a fermentor is feasible, and how the culture will be stored.

A method of inactivation has to be

developed, but not overinactivation to destroy the antigen. It must be determined whether inactivation is going to be a chemical procedure or a physical procedure. Often it will be a combination of both.

It must be determined if there will be a fractionation or concentration procedure and how to accomplish it.

It must be determined if an adjuvant is useful.

Control procedures to measure potency have to be developed.

At this point it is necessary to have the usual control procedures for safety, purity, and sterility involving *in vitro* and host animal and laboratory animal inoculation.

The best route of inoculation has to be determined. Not all routes of inoculation are equally effective for all vaccines.

Dosage has to be determined, not only the number of milliliters to be inoculated, but how much antigen per milliliter.

The number of doses required to stimulate what degree of immunity must be determined. Can effective immunization be achieved with one dose or will it take two doses, or three doses, and how are they to be spaced?

It is necessary to determine how rapidly it will be possible to develop immunity, the duration of immunity, the revaccination schedule, when that is going to be, a year following, two years following, and how often. More vaccination is not necessarily better.

Each inoculation is a medical procedure and not entirely free from risk. There are side reactions following vaccination. The dangers of hypersensitivity must be considered.

The practical considerations of production are necessary, such as: Single dose or multiple dose packaging; how the product should be protected against contamination from misuse; uniformity in appearance and packaging; ease of administration; respectable shelf life with retained potency; quality of bottles, stoppers, and seals; and documentation and support by host animal efficacy studies, and field trials, to uncover the unexpected.

DEVELOPING MODIFIED LIVE BACTERIAL VACCINES

Most of the same things have to be considered for developing modified live bacterial vaccines as for inactivated bacterial vaccines and, in addition, there are some other points to consider. Of foremost importance is the degree of attenuation required to achieve a balance between effectiveness and safety. We must be able to modify the bacteria so it will have no ability to produce disease nor tend toward reversion. Modified live bacterial vaccines are generally standardized by count. What that count will have to be has to be determined. The vaccine will probably have to be lyophilized to achieve useful shelf life, that is, vacuum dried from the frozen state. A stabilizer will have to be developed.

DEVELOPING VIRAL VACCINES

For viral vaccines, we have still further complications. The substrate on which the virus is grown is equally as important as the virus itself. Viruses only grow in living cells and if molecular biology has anything to teach us, it is that a virus-infected cell is a new biological entity different from the cell you started with and different from the virus. Originally, viral vaccines were produced from tissues or blood of infected animals. Normal, healthy animals were inoculated with virus. When they became sick, the tissues were removed, ground up, and inactivated. This was considered to be a virus vaccine. This was followed by the use of embryonated eggs—substituting them for animals. Following the discovery that low levels of antibiotics would control contamination in tissue culture, tissue culture gradually became the method of choice.

Originally, there was a great deal of difficulty in producing sterile and pure viral vaccines. This was because of latent infections in the cells as well as contamination being introduced in the process. While sterility and purity are not necessarily a problem with inactivated vaccines, it is a problem with

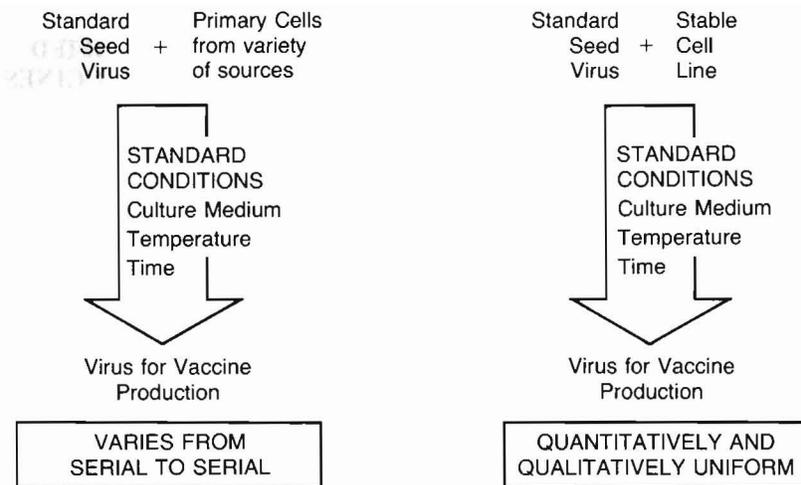


Figure 1.—Comparison of two procedures for obtaining virus for vaccine production.

modified live virus vaccines. Along with the attenuated virus, almost any other virus might be found in the tissues of the host being used as a source of cells. Examples of this may be found with all types of viral propagation. It is instructive that originally, the inactivated polio vaccine was contaminated with SV-40 virus. The SV-40 virus is found widely in stocks of rhesus monkeys and is latent in their kidney cells. How do you look for something that you do not even know about? Nobody looked for SV-40 virus in the earliest days of polio vaccine production. Furthermore, it was more difficult to inactivate than was poliomyelitis virus and consequently, some live SV-40 was inadvertently introduced into many lots of vaccine used to inoculate people. Sometime later it was discovered that SV-40 virus was capable of transforming human cells in tissue culture. So far, there is no evidence SV-40 virus has ever produced any transformations in people. Contamination of viral vaccine is not just peculiar to tissue culture. It is something that has been with us a long time. For example, the last foot and mouth disease outbreak in the United States was traced to importation of smallpox vaccine, which was used to inoculate a farmer, the farmer spread it to his calves. The calves used to produce the smallpox vaccine must have had foot and mouth disease.

STABLE CELL LINE DEVELOPMENT

It has become customary in the last few years to use pretested cells for tissue culture vaccine production. The checking of these cells is just about as complex as the development of a bacterial vaccine. I feel the best procedure is to use a stable cell line. By this procedure, it is possible to work with essentially the same cells over a long period of time. These cells are preserved by freezing away a master seed stock. Cells may be recovered from this source and used for many years to grow up cells for vaccine production. Figure 1 illustrates this point. From the beginning of virus vaccine production it has been customary to use a standard seed virus. There is no problem in maintaining such constants as time, temperature, and culture medium. The problem has been the inability to guarantee a good source of primary cells, whether we worked from animals, embryonated eggs, or primary tissue cultures. When unchecked cells are used as the source of virus, vaccine is going to vary from serial to serial. This whole situation can be greatly improved by using an established cell line. By adding an established cell line into the equation it is possible to produce virus for vaccine production which is quantitatively and qualitatively uniform from serial to serial.

The cells of an established cell line are all genetically related. Within the limits of biological variation, they all have the same biochemical characteristics, the same ability to absorb virus, the same response to the virus, and will produce virus quantitatively and qualitatively equal each time the procedure is followed in detail.

These details seem to be involved and complicated, but someplace in any vaccine development program they must be considered.

DEVELOPING FISH VACCINES

Temperature Dependent Immune Response

The immune response in fish is temperature dependent. This is a new complication. Generally speaking, mammals and birds all have a uniform body temperature. The safety of attenuated products has to be considered in terms of this temperature dependence.

Isolation Problems

Another problem I foresee in working with fish vaccines is the lack of isolation with animals living in water. With land animals we are not too concerned with spreading virus. Fresh air provides a big dilution factor. At Pirbright, England, where foot and mouth disease is studied, it is documented that virus escaped and was airborne to another place miles away but this is the exception. Fish are more intimately involved with their environment than land animals. Water can provide a medium for parasites and diseases that can spread to other species through very complex biological cycles.

Group (Not Individual) Vaccination Needed

In vaccinating dogs, it is necessary to protect every dog, or almost every dog, that is brought into the veterinarian's office. We have to be able to do this without regard to breed, age, sex, the health of the dog, and in spite of intercurrent infections, various medications and general husbandry. Some dogs, of

course, are unable to respond. Each dog is treated as an individual. Everyone expects 100 percent success. There are techniques and information which would permit dog breeders to breed dogs for resistance to canine distemper or away from the tendency to contract hepatitis, but these are problems on which dog breeders prefer not to work. Dog breeders are interested in conformation, temperament, hip dysplasia, trainability, and hunting instinct. They have a whole host of things to worry about other than immunization. The manufacturers of veterinary biologics are taking care of that for them.

With cattle we tend to consider them more in groups. There is still a great diversity in their backgrounds and it is expected vaccination will protect nearly 100 percent of the cattle. Although we suspect not all breeds of cattle immunize equally well, nobody is worried about breeding cattle for ability to immunize. In fact, some of our practices may actually work against selection of cattle able to produce a good immune response. However, people generally are concerned about cattle as individuals and expect to have a high degree of success. Nobody seems to worry about chickens as individuals. I see fish as being some place between poultry and field crops as far as being recognized as individuals. What we are concerned about in fish vaccination is the final result. Accordingly, I do not believe it should be necessary to protect virtually every individual providing the end result will produce a profit for the fish farmer. This is successful farming.

New Concept for Successful Fish Immunization

I can foresee with fish culture that if it is necessary to protect just any fish regardless of how they are raised or handled it is going to be a difficult situation. There are things in fish culture that can

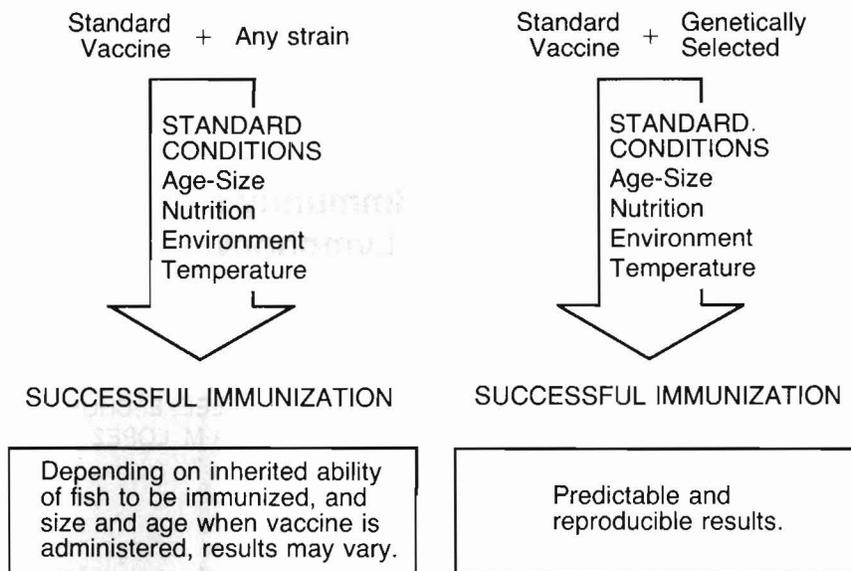


Figure 2.—Comparison of two procedures for immunization of fish.

be controlled, such as: The date of fertilization; physiological age; conditions of nutrition and environment; and genetics. If vaccine is to be administered in the water, the water can be adjusted for the chemical composition, temperature, and pH. It is possible to obtain many fish from one mating and I think the genetics of fish could be something that should be studied. It would be interesting to combine genetics with immunization and produce strains of fish which will produce a good immune response. We could either develop vaccines for the fish or the fish for the vaccine. Figure 2 shows how this fits into a scheme for obtaining more uniform response. By substituting stan-

dard vaccines for standard seed virus and substituting genetically selected fish for the stable cell line, it might be possible to move the concept of uniformly successful immunization one step further.

CONCLUSION

I would like to point out that researchers in commercial organizations preparing vaccines have a certain expertise and the aquatic scientists at this meeting have another. Perhaps, by pooling our scientific information and technology we might be able to progress more rapidly in the development of vaccines for fish.

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