General Sessions

Moderator - Eddie Richey, DVM

The Immunologic Basis for Effective Vaccines

James A. Roth, *DVM, PhD*
College of Veterinary Medicine
Iowa State University
Ames, Iowa 50011

Abstract

The protective immune response to a vaccine may be due to the presence of circulating antibody (humoral immunity), the actions of sensitized T-lymphocytes (cell-mediated immunity), the presence of antibody on mucosa surfaces (mucosal immunity); or a combination of these factors. When attenuated or killed viruses or bacteria are injected into an animal, they will induce an immunologic response. However, this immunologic response may not confer protective immunity because the vaccine may not have contained certain important antigens or it may not induce an immune response with the characteristics needed for protection. The immunologic basis for factors that influence the efficacy of vaccines will be discussed in this presentation. These factors include: the type of immune response induced, characteristics if viral and bacterial antigens, route and timing of vaccine administration, vaccine adjuvants, and the physiologic status of the animal receiving the vaccine.

Introduction

For nearly 100 years scientists have known that animals may develop immunity to diseases if exposed to either the killed infectious agent or a live strain of the agent which has been modified so it does not cause disease. This approach led to the development of many successful vaccines in the early 1900s. However, it soon became apparent that for certain diseases this simple approach was not effective. An animal, for example, might produce antibody in response to vaccination, but still come down with the disease. These are diseases for which circulating antibody alone is not protective or for which the vaccines do not induce antibody against the important antigens of the pathogen. The challenge for these diseases is to understand the basis for successful immunity, then to develop vaccines which induce this type of immunity.

The basic types of immune defense mechanisms against infectious agents are:

1) Native defense mechanisms, the first line of defense and already operational, even in the non-vaccinated animal.
2) Humoral immunity, due to the presence of antibodies in the bloodstream.
3) Cell-mediated immunity, caused by the action of various types of white blood cells and orchestrated by T lymphocytes.
4) The secretory IgA system, important for resistance to diseases at mucosal surfaces such as the gut, the respiratory tract, the mammary gland, and the reproductive tract.

It became apparent that different diseases required different types of immunity for protection and that the type of vaccine (modified live versus killed), route of administration, and type of adjuvant made a difference in the type of immune response. Some vaccines can prevent disease, but fail to prevent infection. The vaccinated animal may be infected with the organism and not become ill; however, they may serve as a reservoir of infection for the rest of the herd. The basic types of immunity and the characteristics of vaccines for inducing these types of immunity will be discussed here. Special problems associated with inducing immunity in young animals will also be discussed.

Types of Immunity

Native Defense Mechanisms

The native defense mechanisms include enzymes in the saliva and tears, acids in the stomach, fatty acids in the skin, and normal flora at mucosal surfaces. Native defense mechanisms also include the complement system and phagocytic white blood cells that are capable of killing some bacteria and viruses. These native defense mechanisms are functional immediately when an infectious agent enters the body even if an animal is not vaccinated. However, the complement system and phagocytic cells work more efficiently in a vaccinated

animal. Those bacteria and viruses which are capable of producing disease have evolved ways to avoid being killed by the native defense mechanisms. In order for an animal to be protected from economically important infectious diseases, it must have either been previously exposed to the disease or vaccinated against the disease so it developed humoral immunity, cell-mediated immunity, and/or mucosal immunity.

Humoral Immunity

Humoral immunity is due to the presence of either IgG or IgM in the bloodstream. When an animal is vaccinated, B lymphocytes will respond to the vaccine by producing IgM and IgG class antibodies. These antibodies are proteins which will circulate in the bloodstream and can attach to the infectious agent when it is encountered in the blood or in the tissues. Antibody alone is not capable of killing infectious agents. The presence of circulating IgG and IgM may help to control disease by:

1) Agglutinating infectious agents thereby reducing the number of infectious particles (for viruses) and facilitating removal by phagocytosis.
2) Binding to and neutralizing toxins.
3) Binding to the infectious agent and blocking attachment to cell surfaces.
4) Binding to the infectious agent and initiating the classical pathway of complement activation.
5) Opsonizing infectious agents and facilitating phagocytosis.
6) Mediating attachment of cytotoxic cells to the surface of infected cells so the infected cells may be destroyed by antibody-dependent cell-mediated cytotoxicity.

Some disease-causing organisms, however, are resistant to control by these activities of circulating antibody. These organisms must be attacked and destroyed by the cell-mediated immune system or the secretory IgA system.

Cell-Mediated Immunity

Cell-mediated immunity is brought about by the interaction of different types of white blood cells and is orchestrated by T lymphocytes which specifically recognize antigen. When an animal is exposed to a disease or is vaccinated with a vaccine that induces cell-mediated immunity, the T lymphocytes which recognize the antigen will respond by reproducing themselves through mitosis. When that infectious agent enters the body through natural exposure sometime later, a large number of T lymphocytes will recognize it. The T lymphocytes will attempt to destroy the infectious agent by either directly attacking it or by secreting protein molecules, called lymphokines, which direct and encourage other white blood cells to attack and destroy the agent.

Mucosal Immunity

Protecting the animal from infection on mucosal surfaces such as the intestinal tract, respiratory tract, mammary glands, and reproductive tract is especially difficult for the immune system. The antibodies responsible for humoral immunity and the white blood cells responsible for cell-mediated immunity are found in the blood stream and in the tissues to some extent, including submucosal surfaces. However, they are not found on some mucosal surfaces. Therefore, they can help to prevent invasion through the mucosal surface, but are not very effective at controlling infection on the mucosal surface. Even in the lung and the mammary gland, where IgG and white blood cells are found in relative abundance, they are not able to function as effectively as in the blood stream and tissues. Protection on mucosal surfaces is due in large part to a special class of antibody called secretory IgA. Secretory IgA is secreted onto mucosal surfaces where it may bind to mucus and be present in fairly high concentration. Secretory IgA is resistant to destruction by the proteolytic enzymes on mucosal surfaces that are capable of breaking down IgG and IgM.

Characteristics of Protective Antigens

There are many antigens associated with a viral or bacterial pathogen. Some of these antigens may induce a protective immune response, while other antigens may induce an immune response which is not related to protection from disease. The antigens associated with bacterial pathogens can be roughly grouped into external antigens, internal antigens, and secreted antigens. The external antigens are accessible to antibody on the living bacteria and include flagella, pili, capsular antigens, and some cell wall antigens. The internal antigens may induce an immune response, but in the living bacteria are not accessible to antibodies. These include cytoplasmic antigens, and some cell membrane and cell wall antigens. The soluble antigens would include exotoxins secreted by the living organism and endotoxins which may be released upon the death of the bacteria and which may be important in the pathogenesis of disease. Antibodies to the external antigens may confer protection from disease through agglutination, opsonization of the bacteria for phagocytosis, initiation of the complement cascade, or blocking of attachment to cell surfaces. Antibodies to the internal antigens are not likely to be protective. Antibodies to exotoxins and endotoxins can neutralize the activity of these toxins and contribute to protection. In general, antibodies alone to any of these antigens are not capable of conferring protection against facultative intracellular bacterial pathogens.

A cell-mediated immune (CMI) response could po-

tentially be induced by internal, external, or secreted bacterial antigens. Since antigen processing and presentation by an antigen-presenting cell is needed for both induction of a CMI response and for the T cells to respond to the presence of infection, the protective antigens for a CMI response do not necessarily have to be on the surface of the bacterial organism. Cell-mediated immunity is particularly important in protection from facultative intracellular bacterial pathogens. This immunity is primarily due to the secretion of lymphokines which activate phagocytic cells and enhance their ability to kill bacteria. The CMI response cannot directly neutralize toxins, and cytotoxic T lymphocytes cannot directly attack bacteria. Cytotoxic T lymphocytes may be capable of killing cells that harbor intracellular bacteria. This will result in cell death and release of the intracellular bacteria.

Care must be taken in selection of bacterial strains for biological products, in methods of growing the bacteria for production, and in processing bacterins so that important antigens are not denatured. There may be considerable antigenic variation between isolates of disease causing organisms. The method of growing the bacteria *in vitro* for inclusion in a vaccine can influence the antigens expressed by that bacteria. Some bacteria will not express important virulence factors when grown *in vitro* but will express them *in vivo* where they are required for survival. Other important antigens, for example exotoxins, may be expressed only during log phase growth in vitro and may be destroyed by the time the bacterial culture reaches the stationary phase. Certain labile antigens may also be destroyed by the processes used for inactivating or preserving bacterins.

Important antigens for protection against viral infections may be found on the viral particle itself or on the membrane of virus infected cells. Antigens of the virus particle may be classified as external (i.e., accessible to antibody in the intact virion) or internal (i.e., not accessible to antibody). External antigens include: envelop antigens and some antigens associated with the viral capsid. The core antigens are internal antigens. As discussed for bacteria above, antibodies against external antigens but not internal antigens have the potential for providing protective immunity from viruses. Because of the necessity for antigen processing for cell mediated immunity, both external and internal viral antigens have the potential to contribute to the cell mediated immune response.

Antigens on the surface of a virus-infected cell may be antigens remaining on the cell surface after viral attachment and penetration, viral antigens synthesized by the cell and expressed on the cell surface, or processed antigens associated with major histocompatibility complex (MHC) class 1 molecules. Antibody against the cell surface antigens may mediate killing of the infected cell through activation of complement or interaction with cytotoxic cells (neutrophils, macrophages, or killer lymphocytes).

Cytotoxic T lymphocytes recognize antigens associated with the MHC class 1 molecule. Cytotoxic T lymphocyte destruction of cells expressing viral antigens associated with MHC class 1 molecules is thought to be important in protective immunity from many viral diseases. Therefore, the antigens associated with the MHC class 1 molecules on cell membranes may be important protective antigens. These antigens may be internal or external antigens associated with the viral particle, or they may be viral coded proteins that are not found in the virus particle but are synthesized in the infected cell. The antigens associated with MHC class 1 molecules are only portions of whole proteins and are usually 10 to 20 amino acids in size. T helper lymphocytes responding to antigens associated with MHC class 2 molecules may mediate protection from viral disease through secretion of lymphokines which may make cells resistant to viral infection (e.g. gamma interferon); may damage viral infected cells (e.g. tumor necrosis factor); and may enhance the activity of cytotoxic cells including macrophages, neutrophils, and NK cells.

Characterization of the Protective Immune Response

The protective immune response to a vaccine may be due to the presence of circulating antibody (humoral immunity), the actions of sensitized T lymphocytes (cell mediated immunity), the presence of antibody on mucosal surfaces (mucosal immunity usually due to secretory IgA), or a combination of these factors. In general, humoral immunity is felt to be particularly important in protection against extracellular phases of systemic viral and bacterial infections and in protection against toxin induced diseases. Cell mediated immunity is particularly important in protection from facultative intracellular bacterial pathogens, intracellular phases of viral infections, fungal diseases, and protozoal diseases. Antibody on mucosal surfaces is important in protecting against those bacterial and viral diseases where the organism must attach to epithelial surfaces in order to produce disease and against diseases induced by toxins produced at mucosal surfaces. In order to determine which aspects of the immune response are responsible for protection from disease, one must be able to measure each aspect of the specific immune response and demonstrate that it contributes to protective immunity.

There are many techniques for measuring serum antibody. Some of the assays for antibody, such as ELISA, passive hemagglutination, precipitin reactions and agglutination tests, detect presence of antibody only. Other assays imply that the antibodies detected

may be functionally significant in immunity. These assays include virus neutralization tests, detection of antibody in antibody-dependent cell-mediated cytotoxicity assays, bactericidal activity of antibody plus complement, bactericidal activity of antibody plus phagocytic cells, and toxin neutralization tests. If the important virulence factors to be neutralized by antibody are known, then detecting the presence of that antibody with an assay like an ELISA using purified antigen would also imply protective immunity.

The presence of antibody on a mucosal surface can be detected by collecting mucosal secretions and testing them by using assays that detect only the presence of antibody or assays which imply functional significance of the antibody detected. Assays which may be particularly useful for detecting protective antibody on a mucosal surface would include virus neutralization assays, assays which demonstrate inhibition of attachment to receptors on mucosal surfaces, assays which demonstrate inhibition of penetration, and toxin neutralization assays. If the important antigens associated with virulence of a pathogen on a mucosal surface are known, then antibody in mucosal secretions against those antigens can be detected using an ELISA with purified antigen. The ELISA can be structured to determine the class of antibody involved by using isotype specific secondary antibodies.

The presence of a CMI response after vaccination may be detected by demonstrating the presence of expanded clones of lymphocytes that recognize specific pathogen-associated antigens. Assays that can be used include delayed type hypersensitivity skin test responses to specific antigens, lymphocyte blastogenic responsiveness in vitro to specific antigens, induction of lymphokine secretion in vitro by specific antigens and detection of the presence of antigen specific cytotoxic T lymphocyte activity.

Detection of the presence of any of the components of the immune response in the assays mentioned above does not demonstrate that these components are responsible for protective immunity. Some of the assays are in vitro correlates of in vivo protective mechanisms, but in order to demonstrate that a particular aspect of the immune response is an important protective mechanism, it is necessary to demonstrate in vivo that aspect of the immune response to a specific antigen confers a degree of protection.

It may be difficult to determine if a particular component of the immune response contributes to protective immunity. Demonstrating that some aspect of the immune response can be measured in animals that have recovered from disease does not prove that that particular aspect contributed to recovery or protection. In some cases, it may be possible to passively transfer antibody or immune lymphocytes to an animal and thereby demonstrate that the passively transferred component alone is capable of providing immunity. It may also be possible to selectively induce individual components of the immune response in an animal without inducing other components and then demonstrate that that individual component contributes to protective immunity.

It is probably easiest to determine whether circulating antibody to a specific antigen is capable of providing protective immunity. It is relatively easy to passively transfer antibody for a specific antigen to the plasma of a susceptible animal and then determine if that animal is protected from a clinically relevant challenge. It is also relatively easy to selectively induce a serum antibody response to a specific antigen in an animal without also inducing mucosal antibody or cell mediated immunity. If the animal is then protected from a clinically relevant challenge, then one can be relatively certain that circulating antibody alone will provide protection.

It may be more difficult to demonstrate that mucosal antibody to a particular antigen is capable of providing protection depending upon the mucosal surface involved and the age of the animal. This should ideally be done in an animal that has mucosal antibody to the specific antigen but does not have circulating antibody or cell mediated immunity to the pathogen. For example, if antibody passively transferred from the milk to the intestinal mucosal surface of the young animal provides protection from a clinically relevant challenge, one can be relatively certain that antibody to that specific antigen on the mucosal surface provided protection. It is probably not possible to specifically induce a mucosal IgA response in an animal without also inducing humoral immunity and perhaps cell mediated immunity. If an animal that has a mucosal IgA response plus humoral and/or cell mediated immunity is protected whereas an animal that has an essentially equivalent humoral and/or cell mediated response without mucosal antibody is not protected, then this is a strong indication that the mucosal antibody significantly contributed to protection.

It may also be relatively difficult to prove that some aspect of a cell mediated immune response to a specific antigen is responsible for protection from disease. Immune lymphocytes can only be passively transferred between syngeneic animals. It is probably not possible to induce a specific cell mediated response in an animal without also inducing humoral antibody and perhaps mucosal antibody. If an animal with a cell mediated immune response to a specific antigen along with humoral and/or mucosal immunity is protected from a clinically relevant disease challenge whereas an animal with humoral and/or mucosal immunity and no cell mediated immune response is not protected, then the

cell mediated immune response is likely to be an important factor in protection from disease.

Lymphokine secretion by T helper lymphocytes is an important component of cell mediated immunity. Since the lymphokines are not antigen specific, one can passively transfer lymphokines to a susceptible animal or induce lymphokine production in an animal to determine if this aspect of cell mediated immunity is capable of providing protection. This approach will not give any information about the identity of the protective antigens on a particular pathogen, but will give an indication if the lymphokine aspect of the cell mediated immune response can protect from the disease process. If an animal that has no antibodies to a particular pathogen is protected from a clinically relevant disease challenge by treatment with either lymphokines or a lymphokine inducer, then this is an indication that cell mediated immunity can provide protection from this disease.

Selective Induction of Different Types of Immunity

It is relatively easy to develop a vaccine which will cause the production of IgG and IgM antibody in the bloodstream. However, it is more difficult to develop a vaccine which induces cell-mediated or mucosal immunity. The nature of the vaccine and the route of administration are important. Subcutaneous or intramuscular injection of a bacterin or killed-virus vaccine will stimulate the immune system to produce IgM and IgG classes of antibody. However, there is very little production of IgA to protect the mucosal surfaces and the killed products are not very effective at inducing cell-mediated immunity.

The induction of cell-mediated immunity either requires a modified live vaccine capable of replicating in the animal or a killed vaccine with a highly effective adjuvant. Adjuvants which have traditionally been used in animal vaccines are not very effective at inducing cell-mediated immunity. New adjuvants are being developed which show promise for inducing cell-mediated immunity using killed vaccines. There are some killed vaccines that have been available for many years and have been effective in controlling certain systemic type diseases. These are diseases that can be controlled by the presence of IgG in the circulation.

The route of vaccine administration is important when attempting to induce mucosal immunity. To get secretory IgA produced at mucosal surfaces, it is best for the vaccine to enter the body through exposure to a mucosal surface. This can be accomplished by feeding the vaccine to the animal, aerosolizing the vaccine so the animal will inhale it, eye drops, or intramammary exposure. If a dam is exposed to a vaccine or infectious agent in her intestinal tract, she may respond by producing secretory IgA not only in her own intestinal tract, but also in her mammary gland. The dam passes the IgA against the infectious agent to the neonate when it suckles. Therefore, the secretory IgA in the dam's milk can protect the neonate from infectious agents present in the dam's intestine. Enteric infections by many organisms are not controlled by the presence of IgG and IgM in the bloodstream or by cell-mediated immunity. If a modified live vaccine is given by injection, but goes to a mucosal surface to replicate, it may also induce a secretory IgA response.

Vaccination Failure

There are many reasons why animals may develop disease even though they have been vaccinated. Disease may occur because:

1) The animal may have been incubating the disease when it was vaccinated;
2) Something may have happened to the vaccine to make it ineffective;
3) The physiologic status of the host may make it unresponsive or hyporesponsive to the vaccine; or
4) The host may be exposed to an overwhelming challenge dose of infectious agent.

By being aware of these factors, veterinarians and owners can help to minimize the occurrence of vaccine failures.

Occurrence of Disease Shortly After Vaccination

The host requires several days after vaccination before an effective immune response will develop. If the animal encounters an infectious agent shortly before or after vaccination, the vaccine will not have had time to induce immunity, and the animal will come down with clinical disease resulting in an apparent vaccination failure. This may occur in animals from a contaminated environment or if susceptible healthy animals are exposed to infected animals when they arrive at the veterinary clinic for vaccination. In this situation, disease symptoms will appear shortly after vaccination and may be mistakenly attributed to vaccine virus causing the disease. Modified live vaccine viruses have been attenuated to be of reduced virulence. The attenuation must be shown to be stable, therefore, reversion to virulence is thought to be a rare event. However, the attenuated vaccine strains may be capable of producing disease in immunosuppressed animals (to be discussed later).

Alterations in the Vaccine

Improperly handled and administered vaccines may fail to induce the expected immune response in

normal, healthy animals. Modified live bacterial and viral vaccines are only effective if the agent in the vaccine is viable and able to replicate in the vaccinated animal. Observing proper storage conditions and proper methods of administration are very important for maintaining vaccine viability. Failure to store the vaccine at refrigerator temperatures or exposure to light may inactivate the vaccine. Even when stored under appropriate conditions, the vaccine loses viability over time. Therefore, vaccines that are past their expiration date should not be used. The use of chemical disinfectants on syringes and needles can inactivate modified live vaccines if there is any residual disinfectant. The use of improper diluent or the mixing of vaccines in a single syringe may also inactivate modified live vaccines. Diluent for lyophilized vaccines are formulated specifically for each vaccine. A diluent which is appropriate for one vaccine may inactivate a different vaccine. Some vaccines and diluents contain preservatives which may inactivate other modified live vaccines. For this reason, multiple vaccines should not be mixed in a single syringe unless that particular combination has been adequately tested to insure there is no interference.

Host Factors Contributing to Vaccine Failure

Most vaccine failures probably occur because a vaccinated animal is not able to respond appropriately to the vaccine. Vaccine failure in young animals may be due to the presence of maternal antibody which prevents adequate response to vaccination. It can also be due to immunosuppression from a variety of causes.

Maternal antibodies are a well known cause of vaccine failure. This is a reason for frequent re-immunization of puppies and kittens between approximately 6- and 18-weeks of age. By immunizing young animals every 3 weeks, the period of susceptibility to disease (which occurs between the loss of maternal antibody and the response to vaccination) is shortened but not eliminated. Typically, virulent infectious agents are capable of breaking through maternal immunity earlier than modified live or killed vaccines. This means that even if young animals are immunized every three weeks, there still is a period when they are vulnerable to infection. Vulnerability occurs between the time that young animals lose their maternal antibody and before they develop their own active immune response. This period can be shortened by the use of less-attenuated modified live vaccines or the use of killed vaccines with high antigenic mass and strong adjuvants. A high challenge dose of infectious agents will break through maternal immunity sooner than low exposure to infectious agents. Therefore, overcrowding and poor sanitation exacerbate the problem of inducing immunity in young animals before they come down with clinical disease.

Veterinarians commonly recommend that puppies and kittens be vaccinated every 3 weeks between approximately 6- and 18-weeks of age. However, for large domestic animals, a single vaccination is commonly recommended to induce immunity during the first few months of life. There is no inherent difference between large and small domestic animals in their response to vaccination in the face of maternal immunity. The frequent vaccinations recommended in puppies and kittens minimizes the period of vulnerability to infectious diseases.

Because only one vaccination is recommended for large domestic animals, the timing of the vaccination is important. If the vaccine is administered too soon, it will be ineffective because of the presence of maternal antibody. If the vaccine is administered after all maternal antibodies are gone from animals in the herd, there will be a prolonged period of vulnerability before they develop their own immune response. Most veterinarians and producers decide that because of time and expense considerations it is impractical to vaccinate young food-producing animals frequently. However, frequent vaccination should be considered in cases of unusually high disease incidence in young food-producing animals. Conversely, owners of puppies and kittens should, perhaps, be offered the option of a reduced number of immunizations if their animals are not likely to be exposed to infected animals. This would reduce the expense to the owner, however, they must be aware that their young animal will have an increased period of vulnerability to infectious disease.

Immunosuppression

Immunosuppression due to a variety of factors including stress, malnutrition, concurrent infection, or immaturity or senescence of the immune system may also lead to vaccination failure. If the immunosuppression occurs at the time of vaccination, the vaccine may fail to induce an adequate immune response. If the immunosuppression occurs sometime after vaccination, then disease may occur due to reduced immunity in spite of an adequate response to the original vaccine. Therapy with immunosuppressive drugs (e.g., glucocorticoids) may also cause this to occur.

A second concern is that some modified live vaccines are capable of inducing disease in the immunosuppressed animal. Modified live vaccines are tested for safety in normal, healthy animals. They are not recommended for use in animals with compromised immune systems. Therefore, these vaccines should not be used in animals that are immunosuppressed for any reason, including animals in the first few weeks of life. When it is necessary to vaccinate animals under these conditions, killed vaccines should be used.

Overwhelming Challenge Dose

Most vaccines do not produce complete immunity to disease. They provide an increased ability to resist

challenge by infectious agents. If a high challenge dose of organisms is present due to overcrowding or poor sanitation, the immune system may be overwhelmed resulting in clinical disease.

Vaccine Efficacy

Vaccines that are federally licensed have been tested to determine that they are safe and effective. However, "effective" is a relative term. It does not mean that the vaccine must be able to induce complete immunity under all conditions which may be found in the field. This would not be realistic since the immune system is not capable of such potent protection under adverse conditions.

To be federally licensed, the vaccine must have been tested under controlled experimental conditions. The vaccinated group must have had significantly less disease than the non-vaccinated control group. This testing is typically done on healthy, non-stressed animals under good environmental conditions and with a controlled exposure to a single infectious agent. Vaccines may be much less effective when used in animals that are under stress, incubating other infectious diseases, or exposed to a high dose of infectious agents due to overcrowding or poor sanitation.

It is important to remember that for most diseases the relationship between the infectious agent and the host is sufficiently complicated that vaccination cannot be expected to provide complete protection. The vaccine can increase the animals resistance to disease, but that resistance to disease can be overwhelmed if good management practices are not followed.

References

Abbas, A.K., Lichtman, A.H. and Pober, J.S., 1991. Immunity to Microbes. In: Cellular and Molecular Immunology. W.B. Saunders Company, Philadelphia, pp. 301-316. Bittle, J. L., and F. A. Murphy (eds): Vaccine Biotechnology. Advances in Veterinary Science and Comparative Medicine. (Vol 33), 444 pages, 1989. Heingel, F.P. and Root R.K., 1990. Antibodies. In: G.L. Mandell, R.G. Douglas and J.E. Bennett (Editors), Principles and Practices of Infectious Diseases. Churchill Livingstone, New York, pp. 41-61. Szakal, A.K., Burton, G.F., Smith, J.P. and Tew, J.G., 1991. Antigen processing and presentation in vivo. In: D.R. Spriggs and W.C. Koff (Editors), Topics in Vaccine Adjuvant Research. CRC Press, Boca Raton, pp. 11-24. Tizard, I: General principles of vaccination and vaccines. In: I. Tizard: Veterinary Immunology: An Introduction. (4th Edition). W. B. Saunders Co. 1992, pp 261-276. Tyler, K.L. and Fields, B.N., 1990. Introduction to viruses and viral diseases. In: G.L. Mandell, R.G. Douglas and J.E. Bennett (Editors), Principles and Practices of Infectious Diseases. Churchill Livingstone, New York, pp. 1124-1134. Volk, W.A., Benjamin, D.C., Kadner, R.J. and Parsons, J.T., 1991. Bacterial cell structures. In: J.P. Lippincott Co. Essentials of Medical Microbiology, Philadelphia, pp. 235-256. Wilson, C.B., 1990. The cellular immune system and its role in host defense. In: G.L. Mandell, R.G. Douglas and J.E. Bennett (Editors), Principles and Practices of Infectious Diseases. Churchill Livingstone, New York, pp. 101-138.