Applications and Optimization of Immunization Procedures

Michael K. Schunk and G. Eileen Macallum

Abstract

Classical immunization protocols have produced an antibody-based humoral response that is very effective against susceptible infectious diseases. Immunization introduces an external substance to induce the host immune system to respond specifically. Typically an antigen is used, but DNA, or a primed, pre-existing leukocyte or antigen-presenting cell, can also be used. Immunization is currently being used or investigated for the prevention and treatment of infectious diseases, cancer, addictions, allergies, pregnancy, and autoimmune diseases. It is also being used to produce biologically active materials such as polyclonal and monoclonal antibodies, antivenins, and antitoxins for treating a wide range of conditions. Animals have been integral to the development of immunization techniques, as producers of toxoids and antitoxins, as models (e.g., to validate materials and protocols used for immunization, to understand the impact of immunization itself on the immune system, and to help investigators devise methods for determining the efficacy of vaccines) and as beneficiaries themselves of vaccines and antitoxins. The choice of immunization protocols is complex, and results may be affected by many factors such as dose and concentration of antigen, choice of adjuvants, time between inoculation and response measurement, and method of detection. The immune system responses to an antigen are also complex and continue to develop with advancing age. Anatomical, physiological, and immune system differences between species influence responses to immunization, as do the purity and presentation of the antigens and adjuvants. When directly comparing results, animals should be sourced from the same supplier. This review highlights the many uses of immunization techniques and introduces important considerations for the choice of protocols and animal models.

Key Words: adjuvant; animal research; antibody; disease; immunity; immunization; vaccine

Introduction

Immunization has been one of the great technical developments contributing to the freedom from life-threatening infectious diseases that we currently enjoy. Even before the days of Jenner's first vaccine published in 1798 as "An inquiry into the causes and effects of the variolæ vaccinæ," inoculation of material from patients with mild cases of smallpox was used to induce a local response and prevent more severe and often fatal systemic disease. We have for many years profited from the benefits of immunization without fully understanding how those benefits occurred. Animals have been integral to the development of immunization techniques, as producers of antitoxins, as models (to validate materials and protocols used for immunization, to understand the impact of immunization on the immune system, and to determine the efficacy of vaccines), and as beneficiaries themselves of vaccines and antitoxins.

Classical immunization protocols produced an antibody-based humoral response that is very effective against susceptible infectious diseases. The ability of the first immunizations to induce protection against viruses, bacteria, and bacterial toxins is the basis of current vaccination protocols. Since then, additional applications of immunization have been used to advance medical science. The targeted impact of immunization procedures in blocking peptide and protein functions has greatly contributed to the understanding of how our immune systems and physiological processes are controlled. This knowledge is being used to adapt new technologies to prevent or control health conditions (e.g., cancers and addictions) that were previously unable to benefit from immunization.

Immunization introduces an external substance to induce the host immune system to respond specifically. Typically this substance is an antigen, and can include DNA or a primed, pre-existing leukocyte or antigen-presenting cell. Currently, immunization is used or is being investigated for the prevention and treatment of an extensive number of conditions ranging from infectious diseases, cancer, addictions, allergies, and pregnancy to autoimmune diseases. It is also being used to produce biologically active materials such as polyclonal and monoclonal antibodies, antivenins, and antitoxins for treating a wide range of conditions. In this article, we highlight the many uses of immunization techniques and discuss important considerations for the choice

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Purposes of Immunization

Infectious Disease Prevention

Traditional vaccines have used immunization processes to protect an individual against potential future exposure to an infectious agent. Even when the process is not 100% efficacious, a sufficient proportion of the population is protected and "herd immunity" is created. In such a case, the population is protected against subsequent infections of individuals and endemic persistence of the infectious agent. When an infectious agent infects one individual, another susceptible host is not encountered in the immediate environment and transmission of the organism cannot occur before the transmissible stage of the life cycle of the infection is passed.

A large number of diseases exist for which there is no effective preventive vaccine, usually because the traditional protocol of immunization—with a live attenuated organism, an inactivated organism, or antigenic components of an organism combined with aluminium-based adjuvants—has not been demonstrated to be safe and effective. In many cases, complex immune interactions with the infectious agents may suppress or moderate the host response. In these situations, a simple antibody response against primary surface antigens is not effective. Other strategies used to evade elimination by the host immune system include the following: antigenic variation, latency of infection, ability to replicate intracellularly or within immunoprivileged sites, immune suppression induced through mediators or infection and destruction of crucial immune cells, and integration of viral DNA into the host cell genome. The process of inducing responses against poorly immunogenic antigens has improved in recent years, particularly for polysaccharide antigens such as *Streptococcus pneumoniae* conjugate vaccines, wherein lack of immunogenicity has been overcome by conjugation with larger, more immunogenic molecules (Moreau 1999).

The efficacy of standard vaccines has typically been measured by the degree of humoral response. Protection against disease for which many next-generation vaccines are currently being developed depends on cell-mediated immunity (CMI[1]) and specific responses in tissues such as mucosa of the lung, gut, or reproductive tract. Tuberculosis is a typical example for which new protocols add adjuvants to induce type 1 responses, and new routes (e.g., oral vaccination) are used to induce a lung mucosal CMI response by targeting the gut mucosa (Doherty et al. 2002).

Several additional strategies are currently being used to produce vaccines for infections against which traditional vaccines have not been successful. Examples of strategies used to induce effective immunity without adverse consequences include subcomponent vaccines, peptide-based vaccines, recombinant DNA vaccines, chimeric vectors, and alternative routes of administration. These strategies are described further in the respective sections of the text below.

Maternal and Fetal Immunization

Neonates are particularly susceptible to infectious disease and are very dependent on maternal antibodies for protection until they can develop and mount their own immune responses. Neonatal vaccination is complicated by the maternal antibodies and the immaturity of the immune system, but the advent of conjugate vaccines and greater understanding of the immune system are providing opportunities for its application (Marchant and Newport 2001). Routine prophylactic immunization has focused to date on protecting neonates after the maternal antibodies wane. Maternal immunization, in which the dam is immunized against a specific disease to develop high levels of antibodies to pass on to her newborn, has been practiced for many years in veterinary medicine and is being seriously investigated as an approach in human medicine (Lehmann et al. 2003). Placental anatomy and the process of antibody transfer vary greatly between species. Nonhuman primate species have a placental anatomy and physiology most closely linked to humans, which makes them appropriate for this type of research.

The concept of fetal immunization has been demonstrated successfully in various animal models. Fetal immunization with a protein antigen has been studied in baboons, and a single DNA immunization against a truncated form of glycoprotein D of bovine herpesvirus-1 into the amniotic fluid of the oral cavity has resulted in high serum antibody titers and cell-mediated immune response in lambs (Gerdts et al. 2002).

Production of Antitoxin

Immunization has been used to produce antiserum since the 1890s. The antiserum produced is rich in antibodies against the specific antigen inoculated. These processed antisera or extracted immunoglobulins are used to treat life-threatening conditions such as rabies infection, diphtheria, tetanus, botulinum intoxication, and venomous snakebites. Antitoxin and antivenom are generally manufactured from antibody-rich serum produced by horses or other large

[1]Abbreviations used in this article : ALVAC-FIV, canarypox virus-based feline immunodeficiency virus; BCG, Bacille Calmette-Guérin; CMI, cell-mediated immunity; CpG motifs, sections of olgonuceotide with a high concentration of cytosine-guanine dinucleotides, more prevalent in prokaryotic cells; CTL, cytotoxic T lymphocyte; DTH, delayed-type hypersensitivity; ELISA, enzyme-linked immunosorbent assay; FIV, feline immunodeficiency virus; HIV, human immunodeficiency virus; HLA, human lymphocyte antigen; Ig, immunoglobulin; IL, interleukin; KLH, keyhole limpet hemocyanin; LT, heat-labile enterotoxin; MAb, monoclonal antibody; MHC, major histocompatibility complex; SCID, severe combined immunodeficient; SPF, specific pathogen-free; TCR, T cell receptor; TLR, toll-like receptor; TNF, tumor necrosis factor; YAC, yeast artificial chromosome.

animals. Multiple booster inoculations of small quantities of toxin or venom are used to induce the production of a hyperimmune response. The booster inoculations produce a large quantity of antibodies that also have a greater affinity for the inciting antigen.

Cancer Treatment

The hypothesis of using vaccines to stimulate the immune system to prevent and treat cancer has been considered for more than a century. In Table 1, the applications of vaccines in cancer therapy are listed. Bacille Calmette-Guérin (BCG[1]) is used around the world to treat bladder cancer (Kassouf and Kamat 2004). Vaccines may also be used to prevent infection by pathogens known to predispose individuals to cancer such as hepatitis B virus or *Chlamydia* spp. (Moingeon 2001).

Several immunological vaccines have been tested in clinical trials for treatment of melanoma. Whole cell vaccines (allogeneic and autologous cellular vaccines) comprise a broad spectrum of antigenic targets. Ganglioside vaccines have been prepared with defined purified antigens that may allow for a specific type of immune response (Guthman et al. 2004). Other types of cancer vaccines under development include DNA vaccines, heat shock protein-based vaccines, peptide vaccines, and dendritic cell vaccines (Wolochok and Livingston 2001).

The majority of current research on cancer vaccines is focused on tumor-associated vaccines. In theory, cancer vaccines should provide a specific immune response against the primary tumor and result in strong immune memory to prevent recurrence. Antigenic differences between normal and malignant cells provide the basis of cancer vaccines to stimulate tumor-specific immune responses. Advances in molecular characterization of tumors have identified tumor-associated antigens that are potential targets for use in cancer immunization protocols (Conroy et al. 1996; Moingeon 2001).

Immunological approaches in cancer vaccines are varied. The normal immune response is not sufficient to eradicate tumor cells, and cancer cells may escape detection via secretion of immunosuppressive factors, down regulation of antigen expression, major histocompatibility complex molecules, or lack of costimulation. Vaccines may be developed that transfer genes of immune-stimulant cells and produce appropriate cytokines or that manipulate antigen-presenting cells such as dendritic cells. Direct injection of immature dendritic cells into tumors is a novel approach being used to induce an immune response based on the processing and presenting of existing antigens from apoptotic cells (Kim et al. 2004a,b). DNA vaccines may also be used after conventional treatments to eliminate metastasis (El-Aneed 2004).

Other Therapeutic Vaccines

Vaccines are under development for a number of chronic infectious and degenerative diseases. Human immunodeficiency virus (HIV[1]) is one example. Whole virus, killed virus, and recombinant vaccines have been examined for this purpose but have not shown sufficient efficacy. Vaccines using recombinant live virus vectors appear to have promise, demonstrating both safety and a cytotoxic lymphocyte response (Rocha et al. 2004).

A highly effective vaccine exists that can prevent hepatitis B, and research is now focused on a therapeutic vaccine. Inducing Th1 responses to the hepatitis B core antigen, has been demonstrated to be important in recovery from disease and infection. Peptide vaccines including B cell- and T cell-inducing peptide epitopes have been tested in animal models and clinical trials (Arnon and Ben-Yedidia 2003). Core antigen loaded nanoparticles are also being developed as a strategy to induce an appropriate therapeutic immune response (Chong et al. 2005).

Vaccines are being developed to treat other disease conditions, including tuberculosis, parasitic disease, and gastric ulcers (Arnon and Ben-Yedidia 2003; Sela et al. 2002). Neurodegenerative conditions such as Huntington's and Alzheimer's diseases, in which there is an abnormal accumulation of protein aggregates, are also potential candidates for vaccine treatment (Sela et al. 2002). Nonspecific methods of treating these and other neurodegenerative disorders

Table 1 Possible application of vaccines against cancer[a]

Modality	Status	Comments
Vaccines (prophylactic or therapeutic against pathogens predisposing to specific cancers	Ongoing clinical studies—hepatitis B vaccine available	Other targets include oncogenic papilloma viruses, hepatitis C, *Helicobacter pylori*
Therapeutic (adjuvant setting)	Ongoing clinical trials	Aim is to prevent recurrence after surgical removal
Therapeutic (metastatic disease)	Ongoing clinical trials	Aim is to control and maintain quality of life
Prophylactic	Theoretical	Aim is to prevent high-risk healthy people from developing cancer

include using vaccines to boost the aging immune system (Schwartz and Kipnis 2004).

Therapeutic Antibodies

Monoclonal antibodies (MAbs[1]) were originally proposed for use in a variety of chronic inflammatory diseases and for preventing transplant organ rejection. MAb therapy has been used to induce immunosuppression and prevent organ rejection. OKT3, the first MAb approved for this indication, inhibits T cell responses by targeted binding of the pan-T cell marker CD3. More recently approved humanized neutralizing MAbs target the interleukin (IL[1])-2 receptor α-chain.

Antibodies protect organisms by binding and neutralizing active molecules, enabling phagocytosis, and activating complement. By capitalizing on some of these functions to inhibit the activity of proinflammatory molecules, they can serve as a useful tool in the potential treatment of chronic inflammatory disease. Antitumor necrosis factor α (TNFα[1]) MAbs have been used in the treatment of rheumatoid arthritis. Anti-TNFα MAbs developed from a mouse:human chimerized antibody is currently licensed as Inflixmab. Clinical studies have confirmed efficacy with improved clinical responses and arresting of joint degeneration. Studies are also ongoing to investigate anti-IL-1 and IL-6 MAb in rheumatoid arthritis (Andreakos et al. 2002).

Anti-TNFα therapy has also been studied in other chronic immune and inflammatory conditions such as Crohn's disease, spondyloarthropathies, juvenile arthritis, and psoriasis. Clinical trials have shown promising response in these diseases. Studies investigating the role of antibodies in the treatment and prevention of prion diseases have also shown promise. In vitro studies demonstrate that cultures appear to be rid of the agent, but animal studies are difficult to conduct due to the long incubation of the disease (Sela et al. 2002).

Murine MAbs, although effective, may be poorly tolerated in humans as multidose therapeutics. Chimeric antibody technology followed by humanization may provide antibodies that are still immunogenic. Fully human antibodies are preferred, but to date, there has been limited success in developing human B cell hybridomas. Transgenic mouse technologies have allowed the introduction of transgenes on yeast artificial chromosomes (YACs[1]) into the mouse germline, generating mice with larger portions of the human immunoglobulin (Ig[1]) loci. XenoMouse® contain large base-sized YACs from which IgG MAbs have a diverse human adult-like repertoire with the CDR3 regions more similar in length to human than to mouse (Houdebine 2002; Kellermann and Green 2002).

Diagnostic Reagents

Immunization for antibodies has been used to produce reagents for diagnostic tests that depend on antibodies as part of the detection systems, such as radioimmune assays and enzyme-linked immunosorbent assays (ELISAs[1]). Many of the diagnostic tools used to identify proteins or examine the immune system depend on monoclonal or polyclonal antibodies to bind a specific molecule. Detection of the bound complex occurs through light scattering or tagging of the antibody with radioisotopes, fluorescent molecules, or enzymes to elicit a color change.

Treatment of Allergies

Immunization with food proteins at an appropriate level can sensitize animals and cause conditions that mimic human food allergies. One example is a protocol successfully used in dogs to induce peanut and other nut allergies by 6 mo of age. Animals are inoculated s.c. with 1 μg of protein extract in alum, first at birth and then at 3, 7, and 11 wk of age, immediately after modified live virus vaccinations (Teuber et al. 2002). Specific allergen immunotherapy has been effective in treating rhinitis and anaphylaxis. Immunostimulatory DNA has been studied in models of allergen-induced airway inflammation and has shown promising results in mice (Silverman and Drazen 2003; Walker and Zuany-Amorium 2001).

Research Models

As a technique, immunization continues to be used extensively and has benefited from the large number of currently available animal models with well-defined immune cell deficiencies and from the increasing availability of immune modulators such as interleukins. These tools have helped to define more completely how the immune response is controlled. Initially, mice deficient in specific lymphocyte populations (e.g., natural killer cell-deficient mice, nude athymic mice, and severe combined immunodeficient (SCID[1]) mice) became available and were widely used. More recently, transgenic mice have provided opportunities to study the immune system in even greater detail. For example, the ability of an adjuvant to activate toll-like receptors (TLRs[1]) is derived from a lack of effect on mice deficient in TLR-4.

In addition to the use of immunization techniques to study the immune system itself, immunization is frequently used to block biological reactions using antibodies. This application has been frequent in reproductive research in which neutralizing particular peptides, proteins, or cell surface antigens are used to study reproductive physiology and the etiology of specific diseases.

Autoimmunity and Degenerative Disease Models

Immunization is used to generate models of diseases that have an autoimmune basis. A list of examples is included in Table 2. Many degenerative inflammatory conditions,

from diabetes to multiple sclerosis, are understood to have a misdirected immune response that induces pathology. Combinations of immunizations to mimic the conditions and animal models with similar immune alterations are used to understand and develop therapies for these conditions. For example, a systemic lupus erythematosus-like syndrome has been established in mice by inoculation with active chromatin (Li et al. 2004). Multiple sclerosis-like experimental allergic encephalitis has also been established using immunization.

Immunization protocols are also used to investigate potential therapies. To treat multiple sclerosis-like inflammatory disease in a mouse model, one successful protocol uses autologous attenuated autoreactive T cells to induce an immune response specifically against autoreactive cells in order to attenuate the condition (Stinissen et al. 1996). Beta crystalline autoantibodies can contribute to the development of cataracts. In mice, oral administration of lens homogenate combined with immunization against beta-crystallins in adjuvant has been shown to suppress anti-beta crystalline antibody production (Sueno et al. 1997).

Screening Potential Vaccines and Adjuvants

As part of the initiative to find and introduce new vaccines and new adjuvants, it is necessary to develop methods to determine and optimize the in vivo response to new candidate agents. Bringing a potential agent from discovery to clinical use is an exercise than can take many years and consume many millions of dollars. Screening is the part of the process that can differentiate between many potential antigens, multiple combinations of antigens, and different adjuvant matrices, in an effort to choose those agents with the most potential for success. Immunization protocols in animals are useful for this process because they incorporate the biological complexities of the immune system that may be predictive of the result in the final host as well as being predictive of adverse secondary affects. For example, a foreign peptide that is nonimmunogenic in a mouse may be more likely to be nonimmunogenic in a human. The predictive ability of one animal species for another is not complete. The mouse response to DNA vaccination has been very successful, but such success has not translated as predictably in other mammalian species such as dogs and humans (Kutzler and Weiner 2004).

With an allowance for the constraints of interpretation, animal models have been very useful for screening. Proteins can present with many different antigenic sites. Screening is used to determine which sites will most likely provide the wanted immune response. The human lymphocyte antigen (HLA^1) transgenic mouse expresses human major histocompatibility complex (MHC^1) molecules and is an example of a transgenic mouse strain that has been used for screening a battery of antigen candidates for potential human CMI responses (Firat et al. 1999).

Quality Control/Testing

Animal immunization is required for testing and quality control of vaccines before release to the market. Testing is

Table 2 Examples of autoimmune degenerative diseases with immunization-induced models

Disease	Model	Animal	Reference (see text)
Multiple sclerosis	Experimental autoimmune encephalitis induced by multiple antigen peptide myelin oligodendticyte glycoprotein 35-55 (MOG35-55)	C57BL6/J mouse	Costa et al. 2003
Systemic lupus erythematosus	Inoculation with active chromatin	Mouse	Li et al. 2004
Rheumatoid arthritis	Rat adjuvant arthritis	Rat	Ku et al. 1993
Autoimmune disease	Collagen-induced arthritis	Mouse	Chiocchia et al. 1993; Nagler-Anderson et al. 1986
Ulcerative colitis	Carageenan model enhanced by immunization with *Bacteroides vulgatus*	Guinea pig	Breeling et al. 1988
Interstitial cystitis	Bladder homogenate and complete Freund's adjuvant immunization	Rat	Luber-Narod et al. 1996
Autoimmune uveitis	Interphotoreceptor retinoid binding protein	C57BL/6 mouse	Willbanks et al. 1997
Atheloscleroses	Immunization with heat shock protein 65	Low-density lipoprotein receptor-deficient (LDL-RD) mice	Afek et al. 2000

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