Immunological strategies in vaccine development

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Abstract

One of the most pressing issues in animal and human medicine currently is the need to develop new and more effective vaccines, against an ever-increasing range of infectious diseases Most of the traditional vaccines have involved the use of killed microorganisms, live attenuated vaccines or antigenic extracts There have been very few newly developed vaccines for humans or animals, over the past 20 years The reasons for this are that it is extremely expensive to develop and evaluate new candidate vaccines, with acceptable levels of safety and efficacy

Recent developments in our understanding of the pathways of immunity required to produce protection against different infections allows us to apply immunological principles to design new and better vaccines This paper outlines the critical variables that need to be taken into consideration when developing a new vaccine

The three critical areas that must be considered are Choice of an appropriate animal model of infection, Identification of the immune markers for protection and; Design of optimal vaccine formulation and delivery systems

Choice of an animal model of infection

When choosing an animal model of infection it is important to first consider the relevance and appropriateness of the animal as a model, rather than its convenience and accessibility A variety of experimental animal models of infection have been used for research in infectious diseases and vaccination. With the current detailed knowledge of microbial pathogenesis and host immune responsiveness, it is now possible to apply more critical targeted planning in the development of animal models of infection and regimes to test vaccine efficacy, that are more relevant and cost-effective Development of new vaccines and testing of vaccine efficacy can now be carried out more effectively providing any confounding variables are factored into the design and testing protocols.

Inbred mice

Inbred mice are the most widely used animal in immunological research, and have been used to chart all the fundamental pathways of immunity. There are many advantages from the use of murine models in immunological research. Foremost amongst these is the wide range of laboratory reagents available to study immunity at the cellular or molecular level It is possible to transfer immune cells between donor and recipient mice from an inbred line, to study the role individual cells make to the integrated cellular functions within the immune system Genetically modified inbred mouse strains, which may have specific genes knocked out (G-KO), or newly introduced transgenes (Tg), are now commercially available.

However, inbred and genetically modified mice often produce atypical responses to specific infections, and are inappropriate to study infection or vaccine responses to a variety of important human and animal infectious diseases The limitations of murine models of infection become especially evident when studying intracellular infections, where the infectious pathogens usually have a very narrow host range (Smith and Wiegeshaus, 1989) Apart from the restricted host range of many infections, which limit the relevance of infectious disease studies in a murine model, inbreeding within a species introduces further constraints While genetically restricted animals provide a major advantage in dissecting fundamental immune processes, they express a limited number of homozygous major histocompatibility complex (MHC) genes Restricted MHC expression in inbred mice, results in a bias in the quality of the immune responsiveness which may produce atypical or unrepresentative responses compared with normal out-bred animals.

Out-bred laboratory animals

The main types of out-bred lab animals used in animal infectious disease research include guinea pigs, rabbits, ferrets and cats While there are a limited number of reagents available to critically study immunity in these animals, they have the advantage that they can produce natural responses to specific infections Different species of out-bred laboratory animals have been used to study selected diseases for which they are particularly appropriate Guinea pigs have been used extensively in tuberculosis research because they produce tubercles and granulomatous disease, similar to that found in domestic livestock and humans Selected breed lines of guinea pigs (Strain 2 & 13) (Wright and Lewis, 1921) and rabbits have been used to identify heritability of resistance to tuberculosis (Lurie, 1941) Ferrets have been used to study influenza (Haff *et al* 1966) and distemper (Ryland and Gorhan, 1978) infections, and cats are used to study feline immunodeficiency virus (FIV), which is the analogue for HIV infection in humans (Hartman, 1998) Rabbits have been used to study immunity to a variety of toxinogenic bacterial infections (Frenchs and Gray, 1975) which require neutralising antibody as the main pathway for protection. These latter types of infections, which require antibody mediated effector pathways for protection, provide far fewer constraints because those infections usually affect a broad range of target species

Large animals

New Zealand is especially well placed when considering the use of domestic ruminants as an experimental model system, to study infectious diseases and vaccine development Ready access to animals at a modest cost makes it feasible to carry out experimental work using cattle, sheep, deer or goats (Griffin *et al* 1995) The cost of carrying out experiments on these animals is not significantly higher, than experiments involving ferrets, guinea pigs or rabbits, overseas. This provides a unique opportunity for New Zealand based scientists to carry out extensive experiments involving a number of ruminant diseases. To date sheep, cattle and deer have been used extensively for such research. A major advantage in this approach is that the work can be carried out in a natural host species that will produce prototypic immune responses to infection and vaccination. Another advantage is that the responses found in one ruminant species are generic and can be extrapolated to other ruminants. As with out-bred animals a limitation is that there are few commercial immunological reagents available to study ruminant immunity This is offset somewhat by the availability of a set of specialised reagents produced by individual ruminant immunology researchers involved in a collaborative network, worldwide Also, it is now possible to produce recombinant peptides and monoclonal antibodies with relative ease, to generate customised reagents within individual laboratories. New Zealand biomedical scientists should recognise the unique resources that are available locally for fundamental research into infectious diseases of ruminants

Development of experimental animal models for infection

Conditions for experimental infection

The overriding requirement for the development of an experimental animal model of infection is to ensure that the experimental infection mimics the pattern found naturally (Griffin *et al* 1995) Infection should occur in organs typically affected naturally. Patterns of pathology, the location and severity of disease should also parallel patterns that occur in naturally infected animals Ideally, infection and disease should occur at a high prevalence, preferably >50%, so that studies involving vaccine efficacy may be carried out with relatively small treatment groups (10-20). Experimental infection should involve the route through which infection occurs naturally. Patterns of immune reactivity in experimentally infected animals should be similar to the patterns that are found in naturally infected animals (Griffin *et al* 2001) This provides evidence that the dynamics of the experimental infection is equivalent to natural infection

Ideally experimental infection should obey Koch's postulate that recovery of the infectious organism is possible, from tissues of the infected animal Recovery of microorganisms from experimentally challenged animals provides evidence that the animal has become *infected* The numbers of organisms recovered at fixed time points following infection may be used to define the severity of infection. Disease specific pathology can be monitored to determine the patterns of *disease* that occur in experimentally infected animals Severity of disease can be established by studying the severity of pathology and the spread to different organs

Clinical symptoms and morbidity may be used as measures of the establishment of infection/disease. Innapetance, weight loss, diarrhoea, respiratory symptoms or behavioural changes are indirect monitors of infection Whereas death has been used historically as the end point for infection, this is currently regarded as not only being ethically unacceptable, but also inappropriate Normally weight loss (>25%) provides a prognostic indicator for severe infection and individual animals should be treated, or electively slaughtered, when significant weight loss occurs

Whereas there are a wide range of endpoints that may be used to monitor infection, optimal use of experimental models should involve endpoints which allow the researcher to evaluate the presence and severity of both *infection* or *disease* This is especially important in vaccine efficacy testing where it is desirable to determine whether a given vaccine can protect against *infection* or *disease* or both.

Immune Pathways for Infection and Protection

The immune system can mount four distinct pathways of reactivity, each of which are appropriate for different types of infection (Table 1). Extracellular infections which are found in the interstitial fluids, blood or lymph usually require opsonophagocytic responses, where protection is mediated by serum antibody (IgG) and complement Extracellular infections, which occur at mucosal surfaces, may be contained non-specifically by a series of anti-microbial cationic-peptides produced by mucosal epithelial cells Specific acquired immune protection is mediated by secretory antibody (IgA), which normally neutralises infectious organisms on epithelial surfaces Intracellular infections are contained initially by Natural Killer (NK) cells, which are part of the non-specific innate resistance system. Persistence of intracellular infection in the face of innate resistance requires that acquired cellular immune pathways are activated. These involve cytotoxic T-cells (Tc), which belong to the cytotoxic CD8$^{(+)}$ subpopulation, or T-helper (CD4$^{(+)}$) cells which upregulate intracellular killing by macrophages

**Table 1** Broad categories of infection and targeted immune responses

Infection	Extracellular	Extracellular	Intracellular	Intracellular
Site	Interstitial, Blood & Lymph	Epithelial surfaces	Cytoplasmic	Vesicular
Organisms	Virus Bacteria Protozoa Parasites Fungi	Parasites Fungi	Virus Chlamydia Rickettsia Protozoa	Listeria Leishmania Histoplasma Cryptococcus
Innate resistance	Complement Phagoctyes	Cationic-peptides Lysozyme	NK-cells	NK-cells Macrophages
Acquired immunity	TH2 Cells Antibodies-IgG	TH2 Cells Antibodies-IgA	TH1 Cells	TH1 Cells T-cells (TH1)
Effector mechanism	Opsonisation	**Neutralisation**	**Cytotoxic**	Macrophages Containment

Immune markers of Infection and Protection

When developing a vaccine it is essential to first identify the relevant pathway of protection for each infection and design a vaccine which will activate the appropriate pathway As each pathway tends to exclude the others (Figure 1) it is essential that the quality of immune activation provides appropriate protection

Recently two distinct pathways (Mosmann *et al* 1986) of T-cell regulation (TH1 and TH2) have been discovered that regulate the development of humoral (antibody mediated) and cell mediated (T-cell) immunity The differential immune pathways are mediated by two types of regulatory T-cells which produce distinct immunoregulatory hormones (cytokines) The patterns of cytokines produced by TH1 and TH2 cells are mutually exclusive Interferon gamma (IFN-γ) produced by TH1 cells is inhibitory for TH2 cells, while IL-4, produced by TH2 cells suppresses TH1 cells

Figure 1 Immune parameters of infection
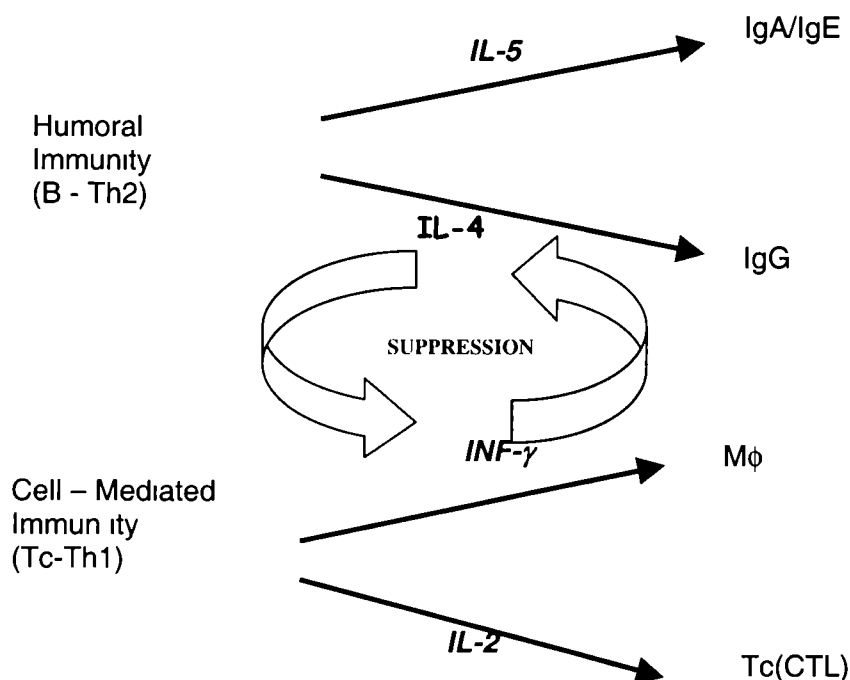
The extrinsic factors which influence differential activation of TH0 cells to become either TH1 or TH2 cells include, the mode of infection, the type and concentration of antigen, and the microenvironment in which the reaction occurs

Whereas activation of TH1 or TH2 cells represents the culmination of T-cell activation, these end cells are derived from naive precursor cells (pTH) which become activated following contact with novel antigens After activation of pTH cells by antigen, they differentiate into TH0 cells, which in turn differentiate further to become TH1 or TH2 cells

Th1 (Cell Mediated Immunity - CMI)

IFN-γ

pTH ──► TH0

IL-4

TH2 (Humoral Immunity - HI)
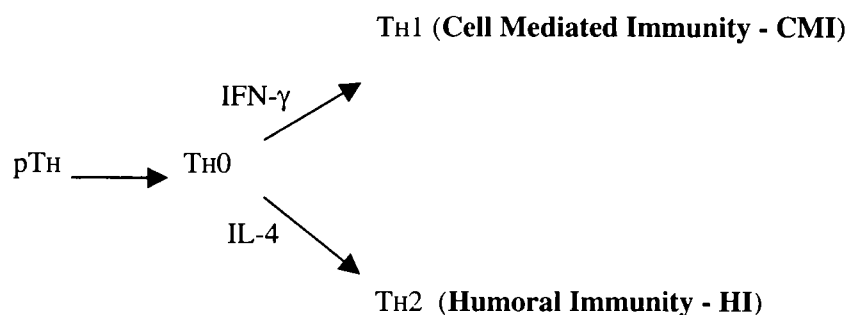
Not only is immune activation biased towards one pathway, but the memory response (TH1 or TH2) produced following activation of immunity will sustain the immune response in either, mutually exclusive pathway, in the long term It is imperative therefore that the initial exposure to a vaccine triggers the appropriate pathway of immunity Inappropriate vaccination may therefore produce a worse outcome than no vaccination Previous studies have established that exposure to a single dose of antigen may be insufficient to 'imprint' the immune system into the appropriate effector pathway. 'Prime-boost' vaccination is necessary to imprint cell mediated effector immune pathways, involving TH1 cells (Griffin *et al* 1993) By contrast, TH2 activation and antibody production can be achieved with a primary dose of vaccine, providing it is delivered in a slow release, depot, oil adjuvant

Types of vaccine

A wide range of different types of vaccine have been used in humans and animals since Pasteur first used live *attenuated* (non-virulent) cholera vaccine in chickens over a Century ago Concerns about vaccine safety have meant that most of the early vaccines involved *killed* microorganisms or chemically detoxified toxins *(toxoids)* from bacteria In recent years extensive research has been carried out to identify the antigens expressed on microorganisms that evoke a protective immune response. These are called protective immunogens It is possible to isolate the genes that code for protective immunogens and clone them to produce *recombinant peptides* An alternative approach is to insert the gene into a virus or bacterial vector to produce a *recombinant vector* vaccine. An even more fundamental approach to vaccine design has involved the used of naked *DNA*, that codes for protective immunogens Not only can microbial DNA code for antigens which are expresses *in vivo* within host cells but it also contains demethylated CpG motifs that act as adjuvants and are particularly effective in stimulating immune responses The main types of vaccines available currently are listed in Table 2 below.

Table 2: Types of vaccine and pathways of immunity

Type of vaccine	Target Microorganism	Type of Immunity
Killed Bacterial	Salmonella, Yersinia, Leptospires	IgG
Virus	Polio, Influenza	IgG
Attenuated Bacterial	Salmonella, Tuberculosis (BCG)	IgA, CMI
Virus	Mumps, Measles, Polio, Rubella	IgA, CMI
Subunit Bacterial	Clostridial,Fusiformis – Toxoids	IgG
Virus	FMDV - Capsid	IgG
Conjugate Bacterial	*Haemophilus influenzae*	IgG
(Carbohydrate-Protein)	*Neisseria meningitidis*	
Recombinant peptide	Hepatitis A, B	IgG
DNA . Bacterial	Tuberculosis	CMI
Virus	Hepatitis B, HIV	CMI

The data given in Table 2 show that the type of vaccine used influences the pathway of immunity activated As a general principle, extracellular antigens (*exogenous*) processed by phagocytic cells,

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stimulate TH2 cells and result in the activation of B-cells to produce antibody (IgG) Living vaccines which cause intracellular infections (*endogenous*) stimulate TH1 cells to produce cellular immunity (CMI), mediated by macrophages and Tc cells

Adjuvants and vaccines

A variety of chemical and biological additives may be added to vaccines to enhance their ability to evoke effective immune responses Alum adjuvant causes denaturation of protein antigens to enhance their uptake by antigen presenting phagocytic cells Oil based adjuvants create an antigen depot where slow release of antigen over a period of weeks evokes strong immune reactions. DEAE Dextran and bacterial toxins stimulate B cells to produce enhanced antibody responses Microbial CpG causes increased activity of TH1 cells resulting in enhanced cell mediated immunity (CMI)

Table 3 Adjuvants and their impact on vaccination

Adjuvant	Effect	Example
Alum	Denatures Protein	Aluminium hydroxide
Oil (Mineral)	Antigen depot	Freunds
DEAE dextran	B cell mitogen	Yersiniavax
Cholera/Enterotoxin	Mucosal stimulation	
Cytokines	IL-2, IL-12	
CpG	Activate CMI	

Routes of vaccination

The general principle is that the optimal route for vaccination is the route by which the individual becomes infected.

A number of different routes of vaccination may be used for vaccination. These include. Parenteral (id, sc or im), Transcutaneous, Mucosal [Oral, enteric, intranasal, respiratory, pulmonary and genital].

Parenteral vaccination produces systemic responses whereas localised vaccination usually generates immune responses within localised lymph nodes or follicular tissues (McGhee and Kinoyo, 1993) There is some autonomy between immune responses that occur at mucosal sites as there is an interchange of immunologically active cells within the mucosal tissues. Overall, while oral vaccination is very appealing, because of its simplicity, it has the disadvantage that the acidic conditions found in the stomach kill most live vaccines Exceptions to this rule occur with enteric microorganisms (eg Salmonella and Polio) that are adapted to resist passage through the gastrointestinal tract

Vaccine delivery systems

In recent years a considerable body of research has been carried out to develop vaccine delivery systems that enhance immunoprophylaxis to vaccines The most interesting of the delivery systems involve chemically synthesised liposomes, which contain a bilipid membrane equivalent to mammalian cell membranes Free antigen ban be incorporated into liposomes, and adjuvants such as cholera toxin (CT) or CpG motifs (Gursel *et al* 1999) . The advantage of these composite delivery systems is that they can be used to stimulate CMI responses using non-living vaccines. Plant extracts such as Quil A are added to liposomes to produce immune stimulating complexes (Iscoms) (Morein, 1990) Virus like particles (VLP – Virosomes (Gluck, 1992)) are also being developed where antigen is incorporated in viral capsids to produce systems where antigen can be delivered intracellularly in the cell cytosol This technique has the advantage that it can simulate intracellular infection and evoke CMI responses.

Restraints to the development of new vaccines

While the potential for vaccines to prevent infection cost-effectively is widely recognised, pharmaceutical companies in general have a low investment in the development of new vaccines. The

disincentive to invest in vaccine research relates to the difficulty in developing and accrediting new vaccines. The absence of definitive *in vitro* immune markers for protection for many infections require that extensive (and expensive) field studies are carried out to validate vaccine efficacy Any variation in efficacy found under different field conditions may threaten the registrability of a vaccine for use in humans or animals The limitations in the available experimental infection models mean that many of the efficacy studies involve extensive field trials. This is extremely expensive because field trials have to be carried out using large numbers of trial patients (> 100,000) in situations where disease prevalence is low (< 1%) A further constraint, especially in human vaccine development, involves the medico-legal liability should adverse reactions occur following vaccination

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