Vaccine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Vaccine development: From concept to early clinical testing

Anthony L. Cunningham [a,1], Nathalie Garçon [b,1], Oberdan Leo [c], Leonard R. Friedland [d], Richard Strugnell [e], Béatrice Laupèze [f], Mark Doherty [f,*], Peter Stern [g]

[a] Westmead Institute, The Centre for Virus Research, 176 Hawkesbury Road, NSW 2145, Australia
[b] Bioaster, 321 Avenue Jean Jaurès, 69007 Lyon, France
[c] Université Libre de Bruxelles, CP300, Rue des Professeurs Jeener et Brachet 12, 6041 Charleroi, Belgium
[d] GSK Vaccines, 5 Crescent Drive, Philadelphia, PA 19112, United States
[e] Department of Microbiology & Immunology, University of Melbourne, Doherty Institute, Melbourne, VIC 3000, Australia
[f] GSK Vaccines, Avenue Fleming 20, Parc de la Noire Epine, B-1300 Wavre, Belgium
[g] Institute of Cancer Sciences, The University of Manchester, Manchester M20 4BX, United Kingdom

ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Vaccine
Innate immunity
Adaptive immunity
Adjuvants
Antigen
Clinical development

ABSTRACT

In the 21st century, an array of microbiological and molecular allow antigens for new vaccines to be specifically identified, designed, produced and delivered with the aim of optimising the induction of a protective immune response against a well-defined immunogen. New knowledge about the functioning of the immune system and host pathogen interactions has stimulated the rational design of vaccines. The design toolbox includes vaccines made from whole pathogens, protein subunits, polysaccharides, pathogen-like particles, use of viral/bacterial vectors, plus adjuvants and conjugation technology to increase and broaden the immune response. Processes such as recombinant DNA technology can simplify the complexity of manufacturing and facilitate consistent production of large quantities of antigen. Any new vaccine development is greatly enhanced by, and requires integration of information concerning:

1. Pathogen life-cycle & epidemiology. Knowledge of pathogen structure, route of entry, interaction with cellular receptors, subsequent replication sites and disease-causing mechanisms are all important to identify antigens suitable for disease prevention. The demographics of infection, specific risk groups and age-specific infection rates determine which population to immunise, and at what age.

2. Immune control & escape. Interactions between the host and pathogen are explored, with determination of the relative importance of antibodies, T-cells of different types and innate immunity, immune escape strategies during infection, and possible immune correlates of protection. This information guides identification and selection of antigen and the specific immune response required for protection.

3. Antigen selection & vaccine formulation. The selected antigen is formulated to remain suitably immunogenic and stable over time, induce an immune response that is likely to be protective, plus be amenable to eventual scale-up to commercial production.

4. Vaccine preclinical & clinical testing. The candidate vaccine must be tested for immunogenicity, safety and efficacy in preclinical and appropriately designed clinical trials.

This review considers these processes using examples of differing pathogenic challenges, including human papillomavirus, malaria, and ebola.

1. Introduction

Natural immunity against a pathogen derives from the integrated activation of the innate and adaptive immune systems (Table 1) [1]. Innate immunity arises after detection of specific pathogen-associated molecular patterns (PAMPs) through a variety of pattern recognition receptors (PRRs) [2]. The PRRs are able to detect common structural and functional features associated with different classes of microorganisms, and depending on the type of PAMP, activate specialised Antigen Presenting Cells (APCs) e.g., dendritic cells. Activation of innate immunity induces expansion

Abbreviations: APC, antigen presenting cells; CSP, circumsporozoite protein; DHF, dengue haemorrhagic fever; DSS, dengue shock syndrome; GMP, good manufacturing practices; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HIV, human immunodeficiency virus; HPV, human papillomavirus; HSV, herpes simplex virus; MPL, 3-O-de-acylated-4-mono-phosphoryl lipid A; PAMPS, pathogen-associated molecular patterns; PRR, pattern recognition receptors; VLP, virus-like particles; VSV, vesicular stomatitis virus; VZV, varicella zoster virus.

* Corresponding author.
E-mail addresses: tony.cunningham@sydney.edu.au (A.L. Cunningham), nathalie.garcon@bioaster.org (N. Garçon), oleo@ulb.ac.be (O. Leo), leonard.r.friedland@gsk.com (L.R. Friedland), rastru@unimelb.edu.au (R. Strugnell), beatrice.n.laupeze@gsk.com (B. Laupèze), mark.x.doherty@gsk.com (M. Doherty), peter.stern@cruk.manchester.ac.uk (P. Stern).
[1] Co-lead authors.

http://dx.doi.org/10.1016/j.vaccine.2016.10.016
0264-410X/

Table 1
Key characteristics and effectors cells of the innate and adaptive immune response.

Innate immunity: first line of defence	Adaptive immunity: second line of defence
• Triggered by damage or threat (recognition of PAMPs) • Rapid response (hours) • Usually No development of immune memory • Pathogen destruction via phagocytosis, killing and release of bioactive mediators • Triggers tissue repair • Stereotypical response • Triggers downstream adaptive responses via antigen-presenting cells • Effector cells: Granulocytes (basophils, neutrophils, eosinophils), Mast cells, Macrophages, Monocytes, Natural killer cells, Dendritic cells	• Activated by pathogen encounter • Slower response (days or weeks) • Large repertoire of effector molecules • Antibody mediated and T-cell mediated destruction • Development of memory • Highly specific and adaptable • Effector cells: CD4+ T-cells, CD8+ T-cells, B-cells, Plasma cells

PAMPS = pathogen-associated molecular patterns.

of adaptive immune cells targeted to the particular threat through antigen-specific T-cell effector and antibody mechanisms. The immunological memory derived from this antigen-specific response persists and can react more rapidly upon subsequent infection [3].

Immunisation is the strategy of stimulating the host's defence against a specific pathogen to establish immunological memory and thus protect against the consequences of infection. Some vaccines are made of whole viruses or bacteria which contain the microbial elements (PAMPs) that trigger the innate immune response required to initiate a suitable adaptive response. However, a whole-pathogen approach may not be feasible, practically or from a safety perspective, or desirable, especially if the agent is very reactogenic or tumorogenic. In such cases, partial fractionation may reduce reactogenicity or tumorgenicity by removing some pathogen components. Alternatively, recombinant DNA technology and biotechnology, or chemical purification can be used to produce a subunit of the pathogen as the vaccine antigen. The latter approaches require in-depth knowledge of the biology of the pathogen to identify the immunologically-relevant vaccine component(s). Since purified proteins usually demonstrate poor immunogenicity by themselves, adjuvants are used to enhance and modulate the immune responses by providing innate/PAMP triggers, thereby driving a protective response to the pathogenic threat. Combining the correct antigens and adjuvants to optimise the subsequent downstream adaptive immune response is a crucial task in the development of any new vaccine. Here we discuss the key principles and challenges faced in the development of vaccines targeting a diverse set of pathogens from concept to clinical trial in humans.

2. Pathogen life-cycle and epidemiology

Detailed knowledge of the biology and structure of the pathogen, its interaction with cellular receptors and its disease-causing mechanisms is important in order to identify antigens suitable for disease prevention. For some microorganisms, characteristics that differentiate commensal from pathogenic forms may need to be identified. Where the key subunit immunogens, e.g., capsule polysaccharides or virus surface proteins, are not conserved, or broad cross-reactive immunity cannot be generated (e.g., pneumococcus or human papilloma virus [HPV], Box 1 [3–5]), it may be necessary to prioritise the most common or the most medically important strains or serogroups. These often vary geographically or temporally: understanding the epidemiology of the disease is crucial to identifying the target antigens. The selection of serotypes is based on complex modelling involving serotype distribution, value and reimbursement, and the number of different subunits the vaccine may realistically contain based on costs and complexity of manufacture [6]. Where the epidemiology indicates a constrained distribution, a vaccine providing relevant – but less broad – strain coverage may be preferred on the grounds of cost or availability [7]. In some circumstances, such as for seasonal influenza, variability of the key antigens is unavoidable and a new vaccine is made each year.

Knowledge of the route of entry and subsequent replication sites of the pathogen is essential. This is because protection against pathogens entering via the respiratory (influenza, pneumococcus), gastrointestinal (Salmonella) or genital tracts (Herpes simplex virus [HSV] or human immunodeficiency virus [HIV]), or entering the bloodstream by injury/injection (hepatitis B/C) or mosquito bite (Malaria, Box 2 [8–10]), may require different vaccination strategies. To take one example, the immune response after natural malaria infection is considered to be predominantly directed against the blood stage of the pathogen but some vaccines have shown that it is possible to induce effective immunity by targeting the pre-erythrocytic stage, e.g. during sporozoite stage and the liver stage of the pathogen [8–10]. Similarly, prevention of the reactivation of infection may require different strategies to preventing primary infection. Special cases in vulnerable populations include postpartum infections such as group B streptococcus, and antenatal/perinatal infection such as hepatitis B (HBV) and HSV, as well as persistent virus such as Varicella zoster virus (VZV) and cytomegalovirus (CMV).

The clinical manifestations of the disease of interest and potential outcomes in the natural setting will also influence the vaccine requirements. For example, some pathogens, such as pneumococcus, can cause multiple clinical syndromes (invasive disease, pneumonia and otitis media), while dengue virus-associated diseases are substantially more serious when antibodies to one of the four types are already present [11].

Knowledge of the demographics of infection (poverty, overcrowding versus delayed exposure in wealthier countries), specific risk groups and age-specific infection rates determine which population to immunise, and at what age. Having clear diagnostic criteria is fundamental and increasingly these diagnostic approaches include the capacity to identify both pathogen and serotype. Partner diagnostics are now being developed to support new vaccines. If the accuracy of diagnosis is poor, then the frequency of infection may be grossly under- or over-estimated, which has implications for understanding the disease burden to be prevented, and the impact of the vaccine after it is used.

The breadth of challenge for successful vaccine development is illustrated by comparison of the diversity of structure, polymorphism, natural history of infection and the consequence for human health of oncogenic HPV (Box 1), *Plasmodium falciparum* (responsible for the most aggressive malaria) (Box 2), and haemorrhagic Ebolavirus (Box 3 [12–14]).

3. Natural immune control & escape

Human pathogens show enormous diversity in their biology, differing in the type of infection they induce (acute, chronic, latent), tissue target (skin or mucosal infections of the gastroin-

Please cite this article in press as: Cunningham AL et al. Vaccine development: From concept to early clinical testing. Vaccine (2016), http://dx.doi.org/10.1016/j.vaccine.2016.10.016