

IMMUNE PEPTIDE ENHANCEMENT OF PEPTIDE BASED VACCINES

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1. ABSTRACT

Vaccines optimize the presentation of an immunogen to the immune system, oftentimes enhancing or replacing the natural activators of antigen presenting cells in order to promote the delivery and the response of T and B lymphocytes to the immunogen. The purpose of this series is to describe new technologies which allow vaccine design, based on our understanding of the immune response, using different approaches to immune peptide enhancement of peptide based vaccines. In this introduction to the series entitled, "Immune Peptide Enhancement of Peptide Based Vaccines", some of the immunological concepts relevant to vaccine design are presented.

2. INTRODUCTION

Vaccination is the single most efficient way to prevent infectious diseases in the population and is one of the most beneficial treatments that a physician can provide for a patient. Diseases such as smallpox and wild type polio have completely or nearly been eliminated and cases of measles, mumps, and rubella have become rare in all but developing countries, due to the efficacy of vaccines and vaccination programs. Successful vaccines and vaccination programs reduce incidence of disease, health care costs and worker absenteeism. In the past, vaccines were developed by the trial, luck and error method but with newer technology, vaccines can be designed. This series entitled, "Immune Peptide Enhancement of Peptide Based Vaccines" presents new technologies which allow vaccine design, based on our understanding of the immune response, to generate and optimize the most helpful, protective immune responses. This report introduces some of the immunological concepts relevant to vaccine design and three approaches to immune peptide enhancement of peptide based vaccines that will be discussed in this series.

The goal of immunization is to elicit a protective immune response and immune memory without side effects. Antibody may be a sufficient protective response for toxins, some bacteria and viruses which spread extracellularly by viremia. For intracellular bacteria, most viruses, fungi and many parasites, a combination of antibody and cell mediated immune responses is required to limit the spread of the agent in the body and resolve the infection.

Vaccines optimize the functional presentation of the immunogen to the immune system, oftentimes enhancing or replacing the natural activators of antigen presenting cells in order to promote the delivery and the response of T and B lymphocytes to the immunogen. Initiation of the immune response to a protein usually requires proteolytic processing of the protein into specific peptides by a dendritic cell followed by presentation of the peptides on major histocompatibility antigen (MHC) class I or II molecules to T cells. Upon recognition and stimulation, these T cells activate other T cells, B cells and other cells by means of soluble cytokine proteins and by cell-cell interactions. Cytokines produced by cells of the immune system are sometimes referred to as lymphokines to distinguish them from cytokines acting upon, and produced by other cells. Dendritic cells present these antigenic peptides by an exogenous pathway, an endogenous pathway and a cross-presentation pathway.

The exogenous pathway acquires proteins, bacteria, viruses and cell debris by phagocytosis and pinocytosis. The phagocytic or pinocytic vesicle merges with lysosomes, and the proteins are proteolyzed in these intracellular vesicles (phagosomes) to produce peptides of 10 to 12 amino acids. The ultimate carriers of these peptides, the MHC II molecules are synthesized in the endoplasmic reticulum. Upon acquisition of the invariant chain, they progress through the Golgi apparatus to phagosomes where the invariant chain is degraded and the empty groove can bind antigenic peptides. The MHC II-peptide complexes are then transported to the cell surface for display (1,2). Many of these peptides contain minimal immune recognition structures, termed epitopes. The ultimate antigen presenting cells for the exogenous pathway are the dendritic cells (DC). Macrophage and B lymphocytes also present antigen and can stimulate secondary (booster) immune responses. The MHCII molecule associates with epitope specific T cell receptors (TCR) and the CD4 molecule on the surface of a subset of T cells. The CD4⁺ T cells, also known as helper T cells, initiate an antigen specific immune response with the release of cytokines, to promote and direct the growth and differentiation of other T cells, B cells, and other lymphoid and myeloid cells.

Endogenous (those found within the cell) proteins are presented to CD8⁺ T cells on MHC I proteins which are

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present on the surface of most nucleated cells. Dendritic cells initiate the response in naïve CD8⁺ T cells. Cellular proteins are processed by the proteasome into 8 or 9 amino acid peptides, transported into the endoplasmic reticulum by the TAP (transporter associated with presentation), and then they bind to MHC I molecules. Occupancy of the antigen binding cleft allows completion of MHC I assembly and movement to the cell surface. MHC I presents the antigen to CD8⁺ T cells, which can also produce cytokines similar to those produced by CD4⁺ T cells. CD8⁺ T cells can also be directed to kill virus infected and tumor cells. These processes are well described in the following reviews (1,2).

Cross presentation of antigenic peptides follows the phagocytosis or macropinocytosis of cellular debris from cells which have undergone apoptosis, lysis due to virus infection, including tumor cell debris and other proteins by a dendritic cell or rare macrophage (3). Leakage of proteins from the pinocytic vesicle or phagolysosome to the cytoplasm allows the proteins to be digested by the proteasome, and enter the endoplasmic reticulum. Alternatively, the ER membrane may transiently fuse with the plasma membrane to allow direct access to the ER where the peptide can bind to MHC I molecules and subsequently, be presented to CD8 T cells.

In addition to presenting the antigen to T cells, the antigen presenting cell also releases cytokines which determine the nature of the subsequent response of the T cells. The types of immune response can be classified as a Th1 or Th2 type of response (4-9). The Th1(CD4 T cell)/Tc1(CD8 T cell) responses are usually initial, early and activate local and inflammatory responses, consisting primarily of cell mediated responses and activation of B cells for specific IgG subtype production (IgG2a in the mouse). The Th1 response is characterized by the production of interferon gamma (IFN-gamma) (also referred to as macrophage activation factor (MAF)), interleukin 2 (IL-2) and lymphotoxin (LT). These responses are important for control of viruses, intracellular bacteria, fungi and some parasites. The Th2(CD4⁺)/Tc2(CD8⁺) responses generally occur later in a natural immune response and involve systemic (total body), clean-up(mop-up) responses to infection which are predominantly antibody mediated. The Th2 cytokine profile is characterized by the production of IL4, IL5, IL6, IL 10, and IL13 cytokines which promote the activation and production of antibody responses. IFN-gamma promotes the expansion of Th1 responses and inhibits Th2 – associated responses (8). Similarly, IL4 and IL10 promote Th2-associated responses and inhibit Th1 responses. Interestingly, IL-10 may also regulate both Th1 and Th2 responses (10,11).

The nature of the immune response can also be determined by the characteristics and concentration of the immunogen. These and other factors influence the DC which secrete cytokines capable of promoting a Th1 response (DC1 cells) or a Th2 response (DC2 cells (12-17). The Th1 responses are promoted by IL12 and IL18 and pro-inflammatory cytokines such as TNF-alpha, IL1 and

IL6 produced by DC1 cells and also monocytes and macrophages and is reinforced by IFN(produced by natural killer cells and T cells. The response is often triggered by the binding of molecules with pathogen associated molecular patterns (PAMP), such as lipopolysaccharide, other bacterial and microbial structures, guanosine-cytosine rich (CpG) oligodeoxynucleotides, and certain drugs (e.g. imiquimod), to Toll Like Receptors (TLR). TLRs were initially identified in drosophila as important to anti-fungal protective responses. There are at least 9 different TLR molecules which interact with different stimuli and then initiate the production of specific cytokines (18-21). Stimulation of a Th2 response often follows immunization with soluble immunogens, high doses of immunogen or exposure to continuous low doses of immunogen, especially when present in the absence of the aforementioned activators (9).

Vaccines used for artificial immunization can take the form of a live attenuated microbe, inactivated microbe, or the protein, peptide or carbohydrate subunit of the microbe that elicits protective immunity presented in a manner optimized to elicit protective immunity. The ideal immunogen is a live attenuated microbe. Immunization with live vaccines mimics natural infection and stimulates both Th1 and Th2 responses in the proper order to lead to the generation of lifelong immune memory. This is usually better than other immunization approaches due to a more natural presentation of the immunogen. However, live vaccines are associated with safety issues regarding reversion to a virulent form, incomplete attenuation and the susceptibility of immunocompromised individuals. Quality control, storage, and handling of a live vaccine can also be difficult since the infectivity of the vaccine must be standardized and maintained.

Inactivated vaccines consist of toxoid (inactivated toxin), inactivated viruses, microbial components, or specific proteins or peptides. These vaccines generally elicit Th2 responses and require booster shots but are completely safe for immunocompromised individuals and there is no chance of disease.

Protein and peptide vaccines offer the opportunity for use of a well defined immunogen which can be made by synthetic means and allows for GMC (good manufacturing conditions) production and distribution, important for approval by the Food and Drug Administration. Unlike a live vaccine, the vaccine manufacturer, rather than the patient's immune response, chooses the appropriate protein to be incorporated into the vaccine based on its ability to elicit protective immune responses. In most cases, the vaccine inventor must also facilitate the development of immune responses to the vaccine by stimulating the innate responses normally elicited by an infectious agent with an adjuvant.

Peptide vaccines allow selection of the appropriate immunogen to ensure the generation of a safe and appropriate response (Table 1). The peptide must include an epitope(s) which elicits a protective but not a suppressive immune response. Potential epitopes can be

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Table 1. Advantages of Peptide Vaccines

Defined immunogen	
	• Inclusion of protective epitopes
	• Exclusion of inhibitory/suppressive epitopes
	• Exclusion of autoimmune or allergy epitopes
Peptide	
	• Resembles pharmaceutical rather than biological
	• May be chemically synthesized under GMP conditions
	• Straightforward Biochemical Quality Control
Safety	
	• Non-infectious
	• Defined components to minimize side effects

Table 2. Approaches to Peptide Enhancement of Peptide Based Vaccines

L.E.A.P.S	Cel-Sci
PADRE	Epimmune
Ii-key peptide	Antigen Express

predicted by computer which use structural algorithms (22) but ultimately they must be tested for their ability to activate and be recognized by the immune response.

Adjuvants are used to enhance the response to an immunogen and some adjuvants can steer the immune response to a Th1 or Th2 response by activating different cytokine responses (18,23). This can be accomplished by providing a reservoir or depot for slow release of antigen, or by activating accessory cells to produce cytokines in order to initiate appropriate immune responses to the immunogen, oftentimes by binding to TLRs. Alum is the classical adjuvant used in human vaccines but this is not a very good adjuvant. For research purposes, Complete Freund's adjuvant (CFA) is the classical adjuvant but is too inflammatory for human use. CFA contains heat inactivated *Mycobacterium* sps bacteria, proteins, and lipid components in poorly metabolizable oils which entrap antigens and mycobacterium and very slowly allow them to disperse over the course of days or weeks. Newer adjuvants include liposomes, semi-synthetic lipid A (MPL or AGP) or other lipid molecules (MDP) molecules, block polymers, and co-administration of IL12 or other cytokines (GM-CSF, CD40-Ligand, Flt ligand). The proper matching of adjuvant with vaccine is important to potentiate the immunization.

Although the peptide epitopes that are recognized by T cells can be identified and even chemically synthesized, these peptides are generally too small to initiate an immune response and therefore cannot be used as immunogens. Several methods can be used for making an epitope into an immunogen. The classic method is to attach the peptide to a larger, carrier protein (e.g. keyhole limpet hemocyanin (KLH), Bovine serum albumin (BSA) or Heat Shock Proteins (HSP)). Multiple epitopes can be assembled into a larger complex by polymerization or by covalent attachment of many of the same or different epitope molecules to a branched backbone structure of lysine (24) or polyoxime backbone (25) as multiple antigen peptides (MAP) (26). Multimerization may also

allow for inclusion of overlapping or nested epitopes and epitopes which may be recognized by different MHC molecules. Another approach to enhancing the immunogenicity of an epitope is to incorporate its gene sequence into a viral or other gene and to express the fusion protein in bacteria, yeast or other cell type. As indicated by our preliminary studies and studies by others, attachment of an epitope to these protein carriers and administration of large amounts of immunogen usually limits the nature of the immune response to a Th2 response (12). Each of the carrier proteins (KLH, BSA or non human HSP and VLP) contain a large number of epitopes which can themselves elicit immune responses, perhaps even overshadowing the one desired. Some of these responses can be deleterious to the host, such as anti-seafood allergies associated with anti-KLH activity.

As an alternate to the large protein carriers, several relatively small 'helper' peptides have been identified which can be covalently linked to a T cell epitope to enhance its immunogenicity (Table 2). The resultant **heteroconjugates** are relatively small peptides (20-40 amino acids), small enough to be chemically synthesized under GMC conditions. Within the heteroconjugate, the 'helper' peptide can facilitate the binding, presentation and/or recognition of the epitope at the cell surface obviating the need for processing of the immunogen by antigen presenting cells. These peptides may also direct or define the subsequent immune responses to the epitope to allow optimization of the outcome. At the inception of this special series, there were three different heteroconjugate approaches to the development of peptide vaccines: the Ligand Epitope Antigen Presentation System (L.E.A.P.S.TM) developed by CEL-SCI Corp., Vienna VA.; the Ii-Key/hybrid peptide approach developed by Antigen Express of Worcester, MA; and the PADRE system developed by EPIMMUNE of San Diego, CA.

The Ligand Epitope Antigen Presentation System (L.E.A.P.S.TM) heteroconjugate approach to vaccine development provides a mechanism for converting epitopes or proteins into immunogens and electing the direction of the immune response to either a Th1 or Th2 type of response (27-31). Heteroconjugates are generated in which a peptide, even a minimal epitope (8 amino acids) is attached to an immune cell binding ligand (ICBL), a peptide which is known to interact with immune cell surface receptors. The two most effective ICBLs are peptides from MHC I and II molecules which promote interaction with CD8 and CD4 molecules. The entire L.E.A.P.S. heteroconjugate vaccine can be as small as 22 amino acids and depending upon the ICBL in the molecule, will direct the immune response to the epitope to either a Th1 or Th2 type of response.

Humphries *et al.* (32-35) developed the Ii-Key heteroconjugate approach by analyzing the natural mechanism for MHC II processing and expression on cell surfaces to identify a peptide which promotes epitope binding to MHC II molecules. A seven amino acid peptide portion of the invariant chain, the Ii-Key portion, preferentially binds to a site on MHC class II molecules

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which causes them to release the contents of the antigen-binding groove and allow substitution with the covalently linked epitope and stabilization of the complex. This promotes antigen presentation to CD4 T cells obviating the need for classical antigen processing prior to presentation by APCs.

The PADRE (Pan DR recognized epitope) system (36) utilizes a MHC II binding peptide in its heteroconjugate to promote binding and presentation of an epitope to CD4 T cells. The immunological peptide is from Tetanus toxin and was modified to limit its proteolysis and increase its recognition by many individuals with different MHC II type molecules. Attachment of an epitope to the PADRE core peptide minimizes MHC restriction for antigen presentation and promotes its presentation to the immune response.

The papers in this series will discuss the different approaches to promoting the immunogenicity of peptides by their incorporation into a heteroconjugate peptide vaccine. There will be a paper on the theory and background for each approach and at least one paper on the application of the approach to specific peptide vaccines.

REFERENCES

1. Takahashi, H.: Antigen presentation in vaccine development. *Comparative immunology microbiology and infectious diseases* 26, 309-328 (2003)
2. Gatti, E. & P. Pierre.: Understanding the cell biology of antigen presentation: the dendritic cell contribution. *Curr Opin Cell Biol* 15, 468-473 (2003)
3. Ackerman, A.L.: Cellular mechanisms governing cross-presentation of exogenous antigens. *Nature Immunology* 5,, 678-684 (2004)
4. Mosmann, T.R. & S. Sad: The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 17, 138-146 (1996)
5. Chtanova, T. & C.R. Mackay: T cell effector subsets: extending the Th1/Th2 paradigm. *Adv Immunol*, 78, 233-266 (2001)
6. Kemp, M., T.G.Theander, & A. Kharazmi: The contrasting roles of CD4+ T cells in intracellular infections in humans: leishmaniasis as an example. *Immunol Today* 17, 13-16 (1996)
7. Kamogawa, Y., L.A. Minasi, S.R. Carding, K. Bottomly, & R.A. Flavell: The relationship of IL-4- and IFN gamma-producing T cells studied by lineage ablation of IL-4-producing cells. *Cell* 75, 985-995 (1993)
8. Szabo, S.J., B.M. Sullivan, S.L. Peng, & L.H. Glimcher: Molecular mechanisms regulating Th1 immune responses. *Annu Rev Immunol* 21, 713-758 (2003)
9. Constant, S.L. & K. Bottomly.: Induction of Th1 and Th2 CD4+ T cell responses: the alternative approaches. *Annu Rev Immunol* 15, 297-322 (1997)
10. Kaya, Z., K.M. Dohmen, Y. Wang, J. Schlichting, M. Afanasyeva, F. Leuschner, & N.R. Rose: Cutting edge: a critical role for IL-10 in induction of nasal tolerance in experimental autoimmune myocarditis. *J Immunol* 168, 1552-1556 (2002)
11. Karlsson, H., P. Larsson, A.E. Wold, & A. Rudin: Pattern of cytokine responses to gram-positive and gram-negative commensal bacteria is profoundly changed when monocytes differentiate into dendritic cells. *Infect Immun* 72, 2671-2678 (2004)
12. Lopez, C.B., A. Fernandez-Sesma, J.L. Schulman, & T.M. Moran: Myeloid dendritic cells stimulate both Th1 and Th2 immune responses depending on the nature of the antigen. *J Interferon Cytokine Res* 21, 763-773 (2001)
13. Palucka, K. & J. Banchereau: How dendritic cells and microbes interact to elicit or subvert protective immune responses. *Curr Opin Immunol* 14, 420-431 (2002)
14. Granucci, F., S. Feau, I. Zanoni, N. Pavelka, C. Vizzardelli, G. Raimondi, & P. Ricciardi-Castagnoli: The immune response is initiated by dendritic cells via interaction with microorganisms and interleukin-2 production. *J Infect Dis* 187 Suppl 2, S346-350 (2003)
15. Shortman, K. & Y.J. Liu: Mouse and human dendritic cell subtypes. *Nat Rev Immunol* 2, 151-161 (2002)
16. John, B., D. Rajagopal, A. Pashine, S. Rath, A. George, & V. Bal: Role of IL-12-independent and IL-12-dependent pathways in regulating generation of the IFN-gamma component of T cell responses to Salmonella typhimurium. *J Immunol* 169, 2545-2552 (2002)
17. Steinkasserer, A: Dendritic Cells and Virus Infection. *Current topics in microbiology and immunology* 276 (2003)
18. Marciari, D.J: Vaccine adjuvants: role and mechanisms of action in vaccine immunogenicity. *Drug discovery today* 8, 934-943 (2003)
19. Kopp, E. & R. Medzhitov: Recognition of microbial infection by Toll-like receptors. *Current opinion in immunology* 15, 396-401 (2003)
20. Takeda, K., T. Kaisho, T. & S. Akira: Toll-like receptors. *Annu Rev Immunol* 21, 335-376 (2003)
21. Beutler, B: Innate immune responses to microbial poisons: discovery and function of the Toll-like receptors. *Annual review of pharmacology and toxicology* 43, 609-628 (2003)
22. Flower, D.R: Towards in silico prediction of immunogenic epitopes. *Trends Immunol* 24, 667-674 (2003)
23. Hunter, R.L: Overview of vaccine adjuvants: present and future. *Vaccine* 20 Suppl 3, S7-12 (2002)
24. Tam, J.P: Recent advances in multiple antigen peptides. *J Immunol Methods* 196, 17-32 (1996)
25. Rose, K., W. Zeng, L.E. Brown, & D.C. Jackson: A synthetic peptide-based polyoxime vaccine construct of high purity and activity. *Mol Immunol* 32, 1031-1037 (1995)
26. Sadler, K. & J.P. Tam: Peptide dendrimers: applications and synthesis. *J Biotechnol* 90, 195-229 (2002)
27. Zimmerman, D., K.F. Bergmann, K.S. Rosenthal, & D.A. Elliott: A new approach to T-cell activation: natural and synthetic conjugates capable of activating T-cells. *Vaccine Research* 5, 91-102 (1996)
28. Zimmerman, D., S. Morris, D. Rouse, K.F.W. Worthington, D.A. Elliott, D.A. & K.S. Rosenthal: Immunization with peptide heteroconjugates primes a T-helper cell type-1 associated antibody (IgG2a) response that recognizes the native epitope on the 38 kDa protein of Mycobacterium tuberculosis. *Vaccine Research* 5, 103-118 (1996)
29. Rosenthal, K.S., H. Mao, W.I. Horne, C. Wright, & D. Zimmerman: Immunization with a LEAPS heteroconjugate

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containing a CTL epitope and a peptide from beta-2-microglobulin elicits a protective and DTH response to herpes simplex virus type 1. *Vaccine* 17, 535-542 (1999)

30. Goel, N., Q. Rong, D. Zimmerman, & K.S. Rosenthal: A L.E.A.P.S. heteroconjugate vaccine containing a T cell epitope from HSV-1 glycoprotein D elicits Th1 responses and protection. *Vaccine* 21, 4410-4420 (2003)

31. Zimmerman, D H, J.P. Lloyd, D. Heisey, M.D. Winship, M Siwek, E. Talor, & P.S. Sarin: Induction of cross clade reactive specific antibodies in mice by conjugates of HGP-30 (peptide analog of HIV-1(SF2) p17) and peptide segments of human beta-2-microglobulin or MHC II beta chain. *Vaccine* 19, 4750-4759 (2001)

32. Gillogly, M.E., N.L. Kallinteris, M. Xu, J.V. Gulfo, R.E. Humphreys, & J.L. Murray: li-Key/HER-2/neu MHC class-II antigenic epitope vaccine peptide for breast cancer. *Cancer Immunol Immunother* 53, 490-496 (2004)

33. Humphreys, R.E., S. Adams, G. Koldzic, B. Nedelescu, B., E. von Hofe & M. Xu: Increasing the potency of MHC class II-presented epitopes by linkage to li-Key peptide. *Vaccine* 18, 2693-2697 (2000)

34. Gillogly, M. E., N.L. Kallinteris, M. Xu, J.V. Gulfo, R.E. Humphreys, & J.L. Murray: li-Key/MHC class II epitope hybrid peptide vaccines for HIV. *Vaccine* 21, 4128-4132 (2003)

35. Xu, M., J. Li, J.V. Gulfo, E. Von Hofe, & R.E. Humphreys: MHC class II allosteric site drugs: new immunotherapeutics for malignant, infectious and autoimmune diseases. *Scand J Immunol* 54, 39-44 (2001)

36. del Guercio, M F, J. Alexander, R.T. Kubo, T. Arrhenius, A. Maewal, E. Appella, S.L. Hoffman, T. Jones, D. Valmori, K. Sakaguchi, H.M. Grey, & A. Sette: Potent immunogenic short linear peptide constructs composed of B cell epitopes and Pan DR T helper epitopes (PADRE) for antibody responses *in vivo*. *Vaccine* 15, 441-448 (1997)

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