Journal of Peptide Science
J. Pept. Sci. 2007; **13**: 776–786
Published online 13 September 2007 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.896

JOURNAL OF PEPTIDE SCIENCE

Review

Viral peptide immunogens: current challenges and opportunities

ALI AZIZI[a,b,c] and FRANCISCO DIAZ-MITOMA[a,b,c]*

[a] Variation Biotechnologies Inc., 22 de Varennes, Suite 210, Gatineau, QC J8T 8R1, Canada
[b] Vaccine Research Centre, Research Institute, Children's Hospital of Eastern Ontario, 401 Smyth, Ottawa, ON, K1H 8L1, Canada
[c] Department of Pathology and Laboratory Medicine, University of Ottawa, Ottawa, ON, K1H 8M2, Canada

Received 16 May 2007; Revised 28 May 2007; Accepted 2 June 2007

Abstract: Synthetic peptide vaccines have potential to control viral infections. Successful experimental models using this approach include the protection of mice against the lethal Sendai virus infection by MHC class I binding CTL peptide epitope. The main benefit of vaccination with peptide epitopes is the ability to minimize the amount and complexity of a well-defined antigen. An appropriate peptide immunogen would also decrease the chance of stimulating a response against self-antigens, thereby providing a safer vaccine by avoiding autoimmunity. In general, the peptide vaccine strategy needs to dissect the specificity of antigen processing, the presence of B-and T-cell epitopes and the MHC restriction of the T-cell responses. This article briefly reviews the implications in the design of peptide vaccines and discusses the various approaches that are applied to improve their immunogenicity. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: vaccine; immune response; viral infection; peptide epitopes

INTRODUCTION

Despite the success rate of human vaccines (inactivated or attenuated) to control viral infections such as smallpox, polio, measles and hepatitis B, their effectiveness is limited against hypervariable viruses. The antigens contained in inoculated human vaccines are frequently processed by endosomal proteases and not cytosolic proteasomes. Therefore, antigens are presented via MHC class II and not MHC class I, resulting in a lack of cytotoxic CD8 + T cell immune responses [1–4]. The expression of MHC class II molecules are limited to APCs, while MHC class I molecules are presented on the surface of all nucleated cells. As a result, CD8 + T cells are able to eradicate a variety of infected cells [5–9]. Attenuated viruses such as the Varicella–Zoster OKA strain or the attenuated measles virus are likely to induce immune responses through the MHC class I pathway [10–12]. MHC class I-peptide binding with their capability in the induction of a repertoire specific immune responses initiated a new era in vaccine design. The idea of peptide epitopes was conceived from the scrutiny of hundreds of overlapping synthetic peptides. This analysis revealed that only a small number of regions in a protein are immunogenic and capable of provoking humoral and cellular immune responses. B cells recognize epitopes exposed on the surface of antigens, while T cells distinguish specific amino acid sequences that are first recognized by MHC class I and II molecules on the surface of APCs [13–15]. Over the past few years, the specific T- and B-cell epitopes have been characterized in tumor and viral antigens, which has advanced the design and testing of peptide vaccines in animal models. However, only a limited number of these vaccine candidates moved to human clinical trials. Although peptide vaccines are considered generally safe, they suffer from low immunogenicity. This article briefly reviews the current trends and challenges in the design of peptide epitopes and discusses the various approaches that are applied to improve peptide immunogenicity.

PITFALLS IN DEVELOPMENT OF PEPTIDE IMMUNOGENS

Synthesis of peptides for use in vaccines requires an understanding of T- and B-cell immunodominant epitopes in the protein structure and their interaction with MHC or HLA complexes [16–19]. Previous experiments indicate that only a small number of predicted peptides are able to bind to MHC motifs with a high affinity but even these epitopes do not necessarily induce protective immunity [20–22]. On the other hand, induction of the

Abbreviations: DCs: dendritic cells; APCs: antigen presenting cells;CTL: cytotoxic T lymphocyte; Th: T helper; TCR: T-cell receptor; MHC: major histocompatibility complex; HLA: human leukocyte antigen; HCV: hepatitis C; CM: central memory; EM: effector memory; TLR: toll-like receptor; ER: endoplasmic reticulum; Nabs: neutralizing antibodies.

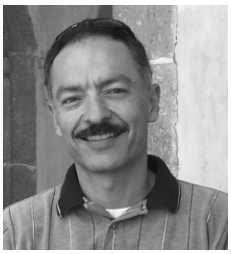
*Correspondence to: Francisco Diaz-Mitoma, Vaccine Research Centre, Research Institute, Children's Hospital of Eastern Ontario, 401 Smyth, Ottawa, ON, K1H 8L1, Canada; e-mail: diaz@cheo.on.ca

BIOGRAPHIES

Ali Azizi – Biography

Ali Azizi received his Ph.D. in Immunology from the University of Ottawa. After a postdoctoral fellowship at the National Research Council of Canada, he advanced his career by working as a Research Scientist in the Variation Biotechnologies Inc. Recently, he was appointed as a Research Investigator and an Adjunct Professor at the Children's Hospital of Eastern Ontario and the Department of Pathology and Laboratory Medicine at the University of Ottawa, respectively. Ali has been focusing his research on the induction of protective immunity against hypervariable viruses such as HIV-1, hepatitis C and influenza.

Francisco Diaz-Mitoma – Biography



Francisco Diaz-Mitoma has served as an infectious disease and clinical virology consultant for the Ottawa Hospital and the Children's Hospital of Eastern Ontario and is a member of the American Society for Microbiology; Faculty of Graduate Studies and Research, University of Ottawa; and a Fellow of the Royal College of Physicians and Surgeons of Canada. He holds numerous externally funded, peer-reviewed grants, industry grants and contracts. His main areas of research include antivirals and diagnosis of viral infections, pathogenesis of Hepatitis C and HIV and the development of vaccines for these viral infections. His research work has been published in more than 100 peer-reviewed international journals. Francisco is also a founder of the Herridge Health Research Center which conducts clinical trials for the pharmaceutical industry in collaboration with major North American academic centers. Repeat clients have included Merck, GSK and Chiron.

protective immune response depends on more essential factors that can ultimately affect the immunogenicity of peptides. For example, T-cell immune responses may not be generated due to immunoregulatory phenomena, deficiency in transportation of peptides to the ER, thymic deletion or peripheral tolerance [23,24]. In the past few years, several algorithms to epitope mapping have been developed; however, the epitopes of many viral antigens are still unknown. The immunodominance hierarchies that exist in human antiviral responses by peptides are more immunodemocratic and less predictable compared to mouse models. However, most viral epitopes have been described on the basis of mouse studies. For instance, while many mouse B-cell epitopes are known, there is only one known human B-cell epitope for influenza A [25]. It has been shown that less than 1% of predicted peptides are able to bind with high affinity to a given MHC class I, form a stable complex and activate naïve CD8 T cells [26,27]. The representation of MHC diversity and TCR variability are also obdurate tasks in peptide vaccine design. HLA have a very polymorphic structure even within the same animal species and therefore the level of T-cell responses to peptides could be highly variable between individuals [28–30]. More than 100 MHC variants have been identified in humans, and therefore selecting peptides with MHC-binding specificities is a complicated issue in the designing of peptide vaccines [31,32]. Computational algorithms such as EpiMatrix, ClusiMer (EpiVax) and Epicover predict the MHC-binding potential of peptides to the number of various HLA molecules; but these approaches need improvement [33–35].

Heterogeneity among viruses such as HIV-1 and HCV circulating throughout the world poses a significant challenge to vaccine development [36–38]. For instance, HIV-1 has eight subtypes, with a high degree of diversity within each subtype [39]. In addition, recombinant hybrid subtypes have emerged recently in regions where HIV has high endemicity. Thus, multiple variants of HIV-1 are usually encountered even within the HIV viruses infecting an individual. The epitope sequence differences between viral strains pose an interesting challenge for vaccine development. This dilemma may be partially solved by utilizing multiple epitopes corresponding to diverse HLA types in each population [40,41]. This strategy may be desirable to thwart viral variants existing at the population level. The other major drawback in the design of peptide vaccines is the swift degradation by extracellular proteases present on the surface of DCs. The endo- and exo-peptidases incise peptide epitopes, resulting in a decreased presentation of the correct epitopes to MHC molecules [42–44]. To overcome the proteolytic effects of proteases, peptides may be encapsulated into chimeric unilamellar vesicules such as liposomes, virus-like particles, virosomes or particles made of biomaterials. These hollow structures may be fusogenic and deliver encapsulated peptide immunogens to APCs without any degradation. In an interesting study by Amacker *et al.*, a strong anti-HCV CTL response was detected in HLA-A2.1 mice that received the HCV-core peptide incorporated into influenza virosomes compared to mice immunized with peptide alone (Figure 1) [45].

DOI: 10.1002/psc

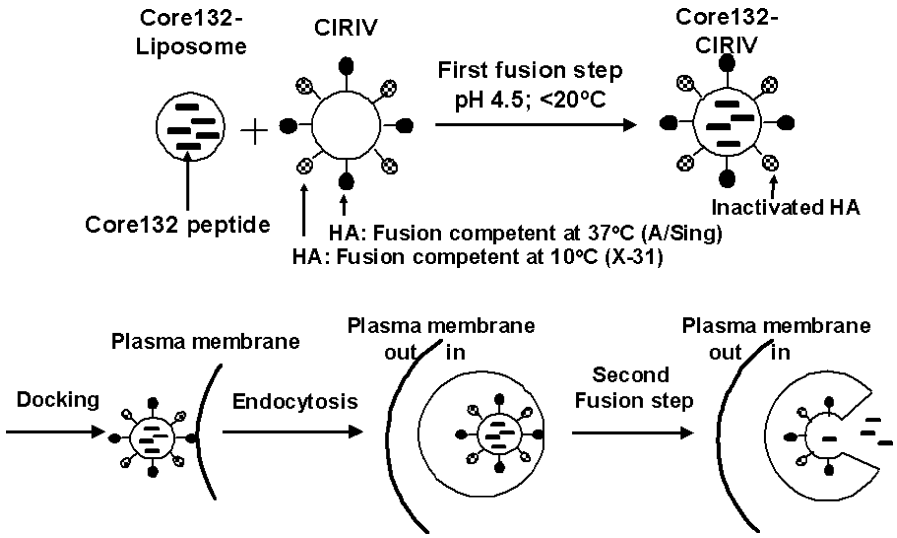


Figure 1 Schematic representation of the preparation of chimeric virosomes containing the Core132 peptide with the two fusion steps. In a first fusion step, chimeric virosomes with HA from the A/Sing and the X-31 strains were fused with homogenized liposomes of a diameter of 200 nm containing the Core132 peptide inside the particle. Fusion takes place at a pH ~4.5 and at a temperature < 20 °C and is mediated by the HA derived from X-31. The resulting, neutralized fusion products were used for vaccination of mice. After receptor-mediated endocytosis, a second fusion step triggered by the low pH within endosomes and mediated by A/Sing HA takes place, releasing the Core132 peptide into the cytosol [45].

There are also some manufacturing and chemistry issues that need to be addressed before a peptide vaccine formulation is taken to clinical testing. Although peptide vaccines may be more stable than other vaccine approaches, the existence of cysteine residues at the end of peptide chains could cause dimerization in the presence of oxygen, changes in the peptide conformation and a decrease in the stability in the peptides [46]. Peptide aggregation or insolubility at physiologic pH range may also represent difficulties in peptide vaccine formulation.

DECISIVE APPRAISALS IN CELLULAR IMMUNITY

The induction of long-lived specific cellular and humoral immune responses is a critical aspect in the development of an effective vaccine [47–50]. The role of neutralizing antibodies (Nabs) in control of hypervariable viruses like HIV or HCV is not deniable, but to date, only a limited number of antibodies with neutralization capabilities have been identified [51–54]. Predicaments in the induction of Nabs against diverse virus variants have encouraged scientists to focus on cell-mediated immune responses [55,56]. CD8 + T cells are frequently referred to as cytotoxic T lymphocytes (CTLs), which recognize and destroy infected cells by different mechanisms, including perforin-mediated killing as well as secreting antiviral cytokines. CD4 + T cells are referred to as the helper cells (Th) and secrete cytokines, which provide support for the generation and preservation of CD8 + T cells and B cells. T cells recognize epitopes derived from viral proteins that are presented by the MHC antigens. CD4 + T cells recognize endosome-derived antigens on MHC class II molecules, and CD8 + T cells recognize peptides in association with MHC class I, which usually present antigens derived from the cytosolic compartment [57–59].

Central memory (CM) and effector memory (EM) T cells are recognized as two main populations of memory T cells. In particular, CM T cells express CD28, CD95, CCR7 and L-selectin which home in the lymph nodes, whereas EM T cells do not express CD28 or CCR7 and home in on the peripheral tissues [60–63]. The generation of memory T cells is not clear; however, the type and potency of antigens may have influences in the quantity and differentiation of memory T cells [64].

The antigenic variation, lack of immune correlates of protection and scarcity of animal models has thwarted vaccine development in hypervariable viruses [65–67]. In an impressive study, Jones *et al.* showed the presence of a high frequency of CD8 + T cells in HIV-resistant prostitutes in Nairobi [68]. These results concluded that CD8 + T cell function is inversely correlated with HIV-1 viral load and may be associated with protection from this disease [69–72]. The question arises whether a peptide-based vaccine is able to provoke broad cellular immunity against hypervariable viral variants. Several studies have been performed on conserved epitopes of HIV-1 but only a limited breadth of reactivity was raised against HIV-1 variants [73–76]. It may be that not all CD8 + T cells are identical in terms of their ability to eliminate virus-infected cells. Essential factors such as avidity between TCR-MHC and peptide, frequency of effective CTL activity and vigor constraints on the epitope region play crucial

DOI: 10.1002/psc

roles in cellular immunity [77,78]. One of the most important, yet least studied, areas in peptide-based vaccines are the hypervariable regions of viruses such as HIV-1. These regions, unlike the conserved domains, contain immunogenic regions encompassing decisive B- and T-cell epitopes, which are under constant selective pressure. Although the highly variable HIV regions allow the virus to escape from the immune response, targeting the immunodominant multiple epitopes in the hypervariable regions may prove to be more effective in the induction of immunity with greater depth compared to the vaccines targeting conserved regions [79–81]. While some amino acid positions within an epitope are quite variable, limits exist in their variation. For example, five or fewer amino acids comprise the majority of amino acids present at any given variable position, and there is little evidence for the presence of all 20 amino acids at any single position within an epitope [82].

MODIFICATION OF PEPTIDE EPITOPES TO ENHANCE IMMUNOGENICITY

Over the past decade, tremendous progress has been made in the understanding of T-cell immunodominant epitopes and their interaction with MHC molecules [83–86]. However, many questions pertaining to bear on the feasibility of designing an effective peptide immunogen still remain unclear, including characterization of appropriate epitopes in eliciting a broad, specific immune response. To increase the immunogenicity of peptide epitopes, a number of parameters could be considered. Previous experiences show that the existence of spacer sequences between individual epitopes might enhance immune response on the specific epitopes. Velders *et al.* showed that addition of the AAY spacer sequence between human papillomavirus (HPV) epitopes was critical in the induction of protective immunity [87]. Some programs such as EpiSort have been developed that are able to optimize spacer sequences between two epitopes [88]. Previous studies showed that the presence of costimulatory molecules and APC signals mediated via CD28 is vital for T-cell activation [89–91]. The choice of cytokines and chemokines to peptide immunogens could activate innate immunity and increase costimulatory molecules on the surface of T cells [92–94].

It has been shown that adjuvants play an important role in designing an effective peptide vaccine candidate. Adjuvants increase the breadth and depth of the immune response of weakly immunogenic peptides [95–100]. Many different types of adjuvants such as lipidation of peptides, MPL, cholera toxin and Freund's incomplete have been used in peptide vaccine studies [101–103]. The oil-based adjuvants such as Montanide and TiterMax have recently been studied in phase I and II human clinical trials and showed an increase in the half-life of peptide immunogens at the site of immunization [104–106]. However, one of the struggles is the paucity of adjuvants for human use. So far, the only FDA-approved adjuvants are alum and monophosphoryl lipid A (MPL). However, alum is not able to activate APCs such as DCs and consequently induce a low amount of IL-12 [107,108]. Furthermore, alum may aggregate with a range of peptide immunogens, which could change epitope conformation [109–111]. MPL is a TLR-4 agonist and has been used in several vaccine studies, but its efficacy in combination with viral peptide immunogens has not been studied in clinical trials [112,113]. Encapsulation of the peptide immunogens by polymer microspheres is another approach to increase the immunogenicity of peptides. With this approach, antigens are released slowly and antigen encapsulation may promote phagocytosis. The slow release of antigen by microspheres averts the need for a vaccination boost [114–116].

One interesting strategy to promote cell-mediated immune response is by targeting the epitope immunogens to the proteasome of APCs. Ubiquitination of proteins lead target proteins to the proteasomes and therefore augment the proteolytic degradation of the epitopes inside the host cells [117,118]. Likewise, targeting the epitopes with the same strategy may boost cellular immunity. Previous studies showed that ligation of lysosome-associated membrane proteins (LAMPs) to epitopes can pilot them to lysosomes and increase presentation of MHC II molecules [119].

Most MHC class I molecules on the surface of APCs express inconsistent antigens and only a limited number of these molecules are accessible [120–123]. Therefore, binding between short peptide epitopes and MHC molecules on the surface of APCs happens infrequently. These peptides occasionally bind to MHC molecules on the surface of nonprofessional antigens, which leads to tolerance and immune response down regulation [124,125]. Vice versa, longer peptide immunogens enter DCs, and get internalized into phagosomes and transferred into the cytosol where they pursue the classical endogenous MHC class I pathway [126]. Alternatively, immunogenicity of short peptides could be increased by chemical conjugation to a carrier protein such as the keyhole limpet haemocyanin (KLH) [127]. Furthermore, previous experiences point out that small peptides (9–11mer) are more sensitive to enzymes compared to long peptides. Thus, small peptides may be truncated non-naturally and form cryptic epitopes, resulting in immunodominant irregular epitopes that are not recognized by specific T cells [128–130]. Progress in the manufacture of longer synthetic peptides may increase the immunogenicity of peptide vaccines. For instance, Lopez *et al.* demonstrated an increase in the frequency of specific CTLs by a 102mer

DOI: 10.1002/psc

malaria polypeptide representing the *C*-terminal region of the circumsporozoite (CS) protein of plasmodium [131].

One option to enhance immune response and induce protection by peptide immunogens against viral infections is the fusion between Th and CTL or B-cell epitopes. In fact, the appearance of Th/CTL or Th/B peptide epitopes on a single APC is more competent than two epitopes on diverse APCs, which may happen with injection of multiple peptides [132]. This could be due to upregulation of CD40L on the surface of Th cells, which subsequently augment the production of IL-12 by APCs [133,134]. This phenomenon skews Th cells toward a Th1 bias, which leads to stimulation of CTL responses. Linkage between Th and CTL epitopes and extension in the length of the peptide may be another vindication in the context of protective immunity.

Using the lipidated form of peptide immunogens is another scheme in the improvement of immunogenicity. The presences of lipidated peptide variants serve to elicit both humoral and cellular immune responses [135,136]. Langhans *et al.* showed that HCV lipidated peptides are more immunogenic than nonlipidated peptides and can initiate specific HCV humoral immune responses from HCV-naïve blood donors. [137] Jackson *et al.* has shown that the lipid moiety present on the peptides prolongs the duration of antigen presentation, enhances cytosolic uptake of peptide immunogens, activates innate immunity due to TLR2 recognition and differentiates nonactivated B cells into immunoglobulin-secreting plasma cells [138]. They found that a synthetic peptide vaccine composed of a Th epitope, target epitope (CTL or B-cell epitope) plus a lipid moiety (Pam2Cys) could increase adaptive immune responses compared to other peptide vaccines (Figure 2).

These studies indicated that the lipid moiety in peptide epitopes is a crucial issue in the design of peptide vaccines. In an interesting study, Espuelas *et al.* studied the characterization of different lipopeptide analogs incorporated into liposomes on the maturation

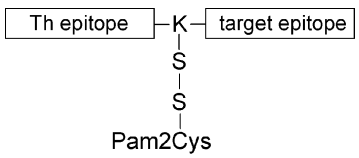
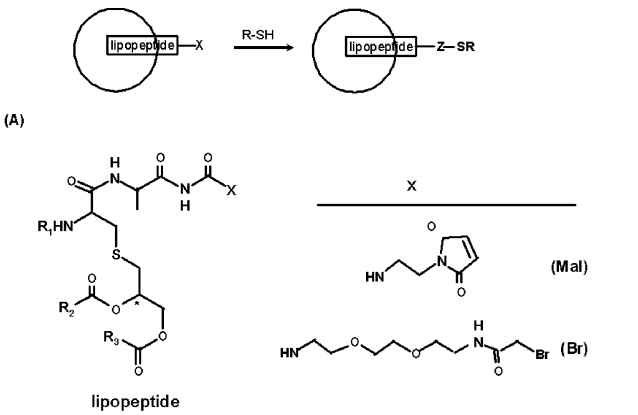


Figure 2 Schematic representation of the epitope-based vaccine candidates examined during this study. Each vaccine contains a Th epitope and a target epitope that is either a CTL-inducing epitope or an antibody-inducing epitope. In all cases, the Th epitope occupies the *N*-terminal position and is separated from the target epitope by a single lysine (K) residue. Where the lipid is attached, this was done through the ϵ-amino group of the lysine residue such that the self-adjuvant lipid, linked through two serine residues (S), forms a branch between the Th and target epitopes [138].

of human DCs (Figure 3). They found that slight modifications in the peptide moiety of lipopeptides have an immense impact on upregulation of cell-surface markers such as CD80, CD83, CD86 and HLA-DR on the surface of human DCs [139]. The presentation of peptide immunogens to DCs and the processing of epitopes via the endosomal compartment play a crucial role in the activation of antiviral immunity [140,141].

The dose and route of peptide immunogens play an important role in the maturity of the immune response. A few studies compared different routes of administration but dosage, volume and nature of immunogen, and choice of adjuvants varied in each study. In one study, Johansen *et al.* showed that intralymphatic administration of a peptide epitope from lymphocytic choriomeningitis virus augments the frequency of CD8 + T cells compared to subcutaneous and intradermal vaccination [142]. A number of studies also have been performed on peptide dosages but drawing a clear conclusion from these studies is complicated. A few studies show that high peptide concentration could change the direction of immune



Compound	Abbreviation	R_1	R_2	R_3	X
1	Pam_3CAG	$C_{15}H_{31}CO$	$C_{15}H_{31}$	$C_{15}H_{31}$	OH
2	Pam_3CAG - Mal	$C_{15}H_{31}CO$	$C_{15}H_{31}$	$C_{15}H_{31}$	Mal
3	Pam_2CAG-Mal	H	$C_{15}H_{31}$	$C_{15}H_{31}$	Mal
4	Ol_3CAG-Mal	Z-$C_{17}H_{33}$.CO	Z-$C_{17}H_{33}$	Z-$C_{17}H_{33}$	Mal
5	Ol_3CAG-Br	Z-$C_{17}H_{33}$.CO	Z-$C_{17}H_{33}$	Z-$C_{17}H_{33}$	Br

(B)

Figure 3 (A) Liposomal formulation of functionalized di- or triacylated lipopeptides. Preformed liposomes (SUV) composed of PC/PG/Chol (75/20/50 molar ratio) containing lipopeptides (5 mol%) functionalized with thiol-reactive groups (X: maleimide or bromoacetyl) (**2**–**5**) were reacted with 2-mercaptoethanol or coupled to HA 307-319-C peptide (R-SH). These constructs were then tested for their capacity to stimulate DC maturation. (B) Structure of the synthetic lipopeptides used in this work. Pam: palmitoyl chain; Ol: oleoyl chain (contains a *Z*-unsaturation at position 9, 10). The nonfunctionalized lipopeptide Pam_3CAG (compound **1**) is terminated by a carboxylic group (X = OH); compound **3** was synthesized with the *R*-configuration at position 2 of the glycerol chain (marked with a star), the triacylated lipopeptides were racemates [139].

DOI: 10.1002/psc

response into T-cell tolerance. It appeared that the administration of peptides with higher concentrations was less effective than lower doses [143,144].

CONCLUDING REMARKS

In creating the next generation of vaccines against hypervariable viruses, we must learn from our past experiences, rather than ignore them. Viruses including influenza, HIV-1 and HCV, have the ability to mutate and avoid the specific immunity directed against them. Genetic variation of these viruses result from transcription errors, rearrangement or recombination which may negate the efficacy of existing vaccines [145–147]. One of the problems facing traditional vaccines is the lack of a broad cell-mediated immune response against variable pathogens [148–151]. Humoral immunity may prevent infection; however, induction of cell-mediated immune responses with a large repertoire of immune specificities has emerged as an essential characteristic for the clearance or control of viral infections such as HCV and HIV [152–155]. The risk of reversion to the wild-type phenotype is another risk factor with attenuated viral vaccines [156,157]. A number of approaches have been developed as an alternative for traditional vaccines. One of the promising technologies in the induction of broad and potent antiviral CD8 + T cell responses is based on the binding between synthetic peptide epitopes and MHC molecules. The peptide immunogens also offer several advantages such as simple antigenic composition, low cost, control of production at scale, absence of risk of reversion to the wild-type form and better stability compared to other vaccine technologies [64,158,160–162]. Advances in the design of synthetic peptide can be applied to increase the breadth and magnitude of immune responses, including increased peptide length, incorporation into microspheres or vesicles, inclusion of more potent adjuvants in peptide vaccine formulation, ubiquitination and fusion between immunodominant epitopes and lipidated moieties. Over the past decade, peptide immunogens have been directed against various viral infections to evaluate relevant specific immune responses. Although some studies demonstrate a strong immunogenicity with both breadth (humoral and cellular immunity) and depth against hypervariable viruses, most of these studies have been directed in animal models and only a limited number of them have moved to human clinical trials. The results in animal models may not be always predictive of human clinical utility. Future studies may elucidate whether synthetic peptide vaccines are able to protect against infectious diseases that have a major public health impact.

REFERENCES

1. Men Y, Audran R, Thomasin C, Eberl G, Demotz S, Merkle HP, Gander B, Corradin G. MHC class I- and class II-restricted processing and presentation of microencapsulated antigens. *Vaccine* 1999; **17**: 1047–1056.
2. Goletz TJ, Klimpel KR, Arora N, Leppla SH, Keith JM, Berzofsky JA. Targeting HIV proteins to the major histocompatibility complex class I processing pathway with a novel gp120–anthrax toxin fusion protein. *Proc. Natl. Acad. Sci. U. S. A.* 1997; **94**: 12059–12064.
3. Maecker HT, Maino VC. T cell immunity to HIV: defining parameters of protection. *Curr. HIV. Res.* 2003; **1**: 249–259.
4. Boles JW, Pitt ML, LeClaire RD, Gibbs PH, Torres E, Dyas B, Ulrich RG, Bavari S. Generation of protective immunity by inactivated recombinant staphylococcal enterotoxin B vaccine in nonhuman primates and identification of correlates of immunity. *Clin. Immunol.* 2003; **108**: 51–59.
5. Celia H, Wilson–Kubalek E, Milligan RA, Teyton L. Structure and function of a membrane–bound murine MHC class I molecule. *Proc. Natl. Acad. Sci. U. S. A.* 1999; **96**: 5634–5639.
6. Fruh K, Ahn K, Peterson PA. Inhibition of MHC class I antigen presentation by viral proteins. *J. Mol. Med.* 1997; **75**: 18–27.
7. Ljunggren HG, Thorpe CJ. Principles of MHC class I–mediated antigen presentation and T cell selection. *Histol. Histopathol.* 1996; **11**: 267–274.
8. Jones RA, Scott CS, Child JA. Expression of MHC class I and class I–like gene products on the cell membrane of mature and immature T cells. *Leuk. Res.* 1988; **12**: 799–804.
9. Daar AS, Fuggle SV, Fabre JW, Ting A, Morris PJ. The detailed distribution of HLA–A, B, C antigens in normal human organs. *Transplantation* 1984; **38**: 287–292.
10. Habermehl P, Lignitz A, Knuf M, Schmitt HJ, Slaoui M, Zepp F. Cellular immune response of a varicella vaccine following simultaneous DTaP and VZV vaccination. *Vaccine* 1999; **17**: 669–674.
11. Arvin AM. Immune responses to varicella–zoster virus. *Infect. Dis. Clin. North Am.* 1996; **10**: 529–570.
12. Ovsyannikova IG, Dhiman N, Jacobson RM, Vierkant RA, Poland GA. Frequency of measles virus–specific CD4+ and CD8+ T cells in subjects seronegative or highly seropositive for measles vaccine. *Clin. Diagn. Lab Immunol.* 2003; **10**: 411–416.
13. Jung MC, Diepolder HM, Pape GR. T cell recognition of hepatitis B and C viral antigens. *Eur. J. Clin. Invest.* 1994; **24**: 641–650.
14. Takahashi H. Antigen presentation in vaccine development. *Comp Immunol. Microbiol. Infect. Dis.* 2003; **26**: 309–328.
15. Lu NQ. A speculative view of immune recognition. *Immunol. Invest.* 1994; **23**: 53–71.
16. Van Kaer L, Ashton–Rickardt PG, Eichelberger M, Gaczynska M, Nagashima K, Rock KL, Goldberg AL, Doherty PC, Tonegawa S. Altered peptidase and viral–specific T cell response in LMP2 mutant mice. *Immunity* 1994; **1**: 533–541.
17. Toes RE, Nussbaum AK, Degermann S, Schirle M, Emmerich NP, Kraft M, Laplace C, Zwinderman A, Dick TP, Muller J, Schonfisch B, Schmid C, Fehling HJ, Stevanovic S, Rammensee HG, Schild H. Discrete cleavage motifs of constitutive and immunoproteasomes revealed by quantitative analysis of cleavage products. *J. Exp. Med.* 2001; **194**: 1–12.
18. York IA, Goldberg AL, Mo XY, Rock KL. Proteolysis and class I major histocompatibility complex antigen presentation. *Immunol. Rev.* 1999; **172**: 49–66.
19. Mo XY, Cascio P, Lemerise K, Goldberg AL, Rock K. Distinct proteolytic processes generate the C and N termini of MHC class I–binding peptides. *J. Immunol.* 1999; **163**: 5851–5859.
20. Deng H, Fosdick L, Sercarz E. The involvement of antigen processing in determinant selection by class II MHC and its relationship to immunodominance. *APMIS* 1993; **101**: 655–662.

21. Yewdell JW, Bennink JR. Immunodominance in major histocompatibility complex class I–restricted T lymphocyte responses. *Annu. Rev. Immunol.* 1999; **17**: 51–88.
22. Schaeffer EB, Sette A, Johnson DL, Bekoff MC, Smith JA, Grey HM, Buus S. Relative contribution of "determinant selection" and "holes in the T–cell repertoire" to T–cell responses. *Proc. Natl. Acad. Sci. U.S.A.* 1989; **86**: 4649–4653.
23. Chen W, Norbury CC, Cho Y, Yewdell JW, Bennink JR. Immunoproteasomes shape immunodominance hierarchies of antiviral CD8(+) T cells at the levels of T cell repertoire and presentation of viral antigens. *J. Exp. Med.* 2001; **193**: 1319–1326.
24. Lauvau G, Kakimi K, Niedermann G, Ostankovitch M, Yotnda P, Firat H, Chisari FV, van Endert PM. Human transporters associated with antigen processing (TAPs) select epitope precursor peptides for processing in the endoplasmic reticulum and presentation to T cells. *J. Exp. Med.* 1999; **190**: 1227–1240.
25. Bui HH, Peters B, Assarsson E, Mbawuike I, Sette A. Ab and T cell epitopes of influenza A virus, knowledge and opportunities. *Proc. Natl. Acad. Sci. U. S. A.* 2007; **104**: 246–251.
26. Parker KC, Shields M, DiBrino M, Brooks A, Coligan JE. Peptide binding to MHC class I molecules: implications for antigenic peptide prediction. *Immunol. Res.* 1995; **14**: 34–57.
27. Parker KC, Bednarek MA, Coligan JE. Scheme for ranking potential HLA–A2 binding peptides based on independent binding of individual peptide side–chains. *J. Immunol.* 1994; **152**: 163–175.
28. Zhan X, Martin LN, Slobod KS, Coleclough C, Lockey TD, Brown SA, Stambas J, Bonsignori M, Sealy RE, Blanchard JL, Hurwitz JL. Multi–envelope HIV–1 vaccine devoid of SIV components controls disease in macaques challenged with heterologous pathogenic SHIV. *Vaccine* 2005; **23**: 5306–5320.
29. Le Gal FA, Ayyoub M, Dutoit V, Widmer V, Jager E, Cerottini JC, Dietrich PY, Valmori D. Distinct structural TCR repertoires in naturally occurring versus vaccine–induced CD8+ T–cell responses to the tumor–specific antigen NY–ESO–1. *J. Immunother.* 2005; **28**: 252–257.
30. Vider–Shalit T, Raffaeli S, Louzoun Y. Virus–epitope vaccine design: informatic matching the HLA–I polymorphism to the virus genome. *Mol. Immunol.* 2007; **44**: 1253–1261.
31. Lau M, Terasaki PI, Park MS. International cell exchange, 1994. *Clin. Transpl.* 1994; 467–488.
32. van Endert PM. Designing peptide vaccines for cellular cross–presentation. *Biologicals* 2001; **29**: 285–288.
33. Martin W, Sbai H, De Groot AS. Bioinformatics tools for identifying class I–restricted epitopes. *Methods* 2003; **29**: 289–298.
34. Culshaw S, Larosa K, Tolani H, Han X, Eastcott JW, Smith DJ, Taubman MA. Immunogenic and protective potential of mutans streptococcal glucosyltransferase peptide constructs selected by major histocompatibility complex class II allele binding. *Infect. Immun.* 2007; **75**: 915–923.
35. De Groot AS, Bosma A, Chinai N, Frost J, Jesdale BM, Gonzalez MA, Martin W, Saint–Aubin C. From genome to vaccine: in silico predictions, ex vivo verification. *Vaccine* 2001; **19**: 4385–4395.
36. Gaschen B, Taylor J, Yusim K, Foley B, Gao F, Lang D, Novitsky V, Haynes B, Hahn BH, Bhattacharya T, Korber B. Diversity considerations in HIV–1 vaccine selection. *Science* 2002; **296**: 2354–2360.
37. Azizi A, Anderson DE, Ghorbani M, Gee K, Diaz–Mitoma F. Immunogenicity of a polyvalent HIV–1 candidate vaccine based on fourteen wild type gp120 proteins in golden hamsters. *BMC. Immunol.* 2006; **7**: 25.
38. Naas T, Ghorbani M, Alvarez–Maya I, Lapner M, Kothary R, De Repentigny Y, Gomes S, Babiuk L, Giulivi A, Soare C, Azizi A, Diaz–Mitoma F. Characterization of liver histopathology in a transgenic mouse model expressing genotype 1a hepatitis C virus core and envelope proteins 1 and 2. *J. Gen. Virol.* 2005; **86**: 2185–2196.
39. Gao F, Robertson DL, Carruthers CD, Li Y, Bailes E, Kostrikis LG, Salminen MO, Bibollet–Ruche F, Peeters M, Ho DD, Shaw GM, Sharp PM, Hahn BH. An isolate of human immunodeficiency virus type 1 originally classified as subtype I represents a complex mosaic comprising three different group M subtypes (A, G, and I). *J. Virol.* 1998; **72**: 10234–10241.
40. Arnon R, Ben Yedidia T. Old and new vaccine approaches. *Int. Immunopharmacol.* 2003; **3**: 1195–1204.
41. Kawada M, Igarashi H, Takeda A, Tsukamoto T, Yamamoto H, Dohki S, Takiguchi M, Matano T. Involvement of multiple epitope–specific cytotoxic T–lymphocyte responses in vaccine–based control of simian immunodeficiency virus replication in rhesus macaques. *J. Virol.* 2006; **80**: 1949–1958.
42. Falo LD, Colarusso LJ, Benacerraf B, Rock KL Jr. Serum proteases alter the antigenicity of peptides presented by class I major histocompatibility complex molecules. *Proc. Natl. Acad. Sci. U. S. A.* 1992; **89**: 8347–8350.
43. Amoscato AA, Prenovitz DA, Lotze MT. Rapid extracellular degradation of synthetic class I peptides by human dendritic cells. *J. Immunol.* 1998; **161**: 4023–4032.
44. Larsen SL, Pedersen LO, Buus S, Stryhn A. T cell responses affected by aminopeptidase N (CD13)–mediated trimming of major histocompatibility complex class II–bound peptides. *J. Exp. Med.* 1996; **184**: 183–189.
45. Amacker M, Engler O, Kammer AR, Vadrucci S, Oberholzer D, Cerny A, Zurbriggen R. Peptide–loaded chimeric influenza virosomes for efficient in vivo induction of cytotoxic T cells. *Int. Immunol.* 2005; **17**: 695–704.
46. Zeng W, Gauci S, Ghosh S, Walker J, Jackson DC. Characterisation of the antibody response to a totally synthetic immunocontraceptive peptide vaccine based on LHRH. *Vaccine* 2005; **23**: 4427–4435.
47. Del Giudice G. Vaccination strategies. An overview. *Vaccine* 2003; **21**(Suppl 2): S83–S88.
48. Esser MT, Marchese RD, Kierstead LS, Tussey LG, Wang F, Chirmule N, Washabaugh MW. Memory T cells and vaccines. *Vaccine* 2003; **21**: 419–430.
49. Yang LT, Peng H, Zhu ZL, Li G, Huang ZT, Zhao ZX, Koup RA, Bailer RT, Wu CY. Long–lived effector/central memory T–cell responses to severe acute respiratory syndrome coronavirus (SARS–CoV) S antigen in recovered SARS patients. *Clin. Immunol.* 2006; **120**: 171–178.
50. Jangpatarapongsa K, Sirichaisinthop J, Sattabongkot J, Cui L, Montgomery SM, Looareesuwan S, Troye–Blomberg M, Udomsangpetch R. Memory T cells protect against Plasmodium vivax infection. *Microbes Infect.* 2006; **8**: 680–686.
51. Srivastava IK, Ulmer JB, Barnett SW. Neutralizing antibody responses to HIV: role in protective immunity and challenges for vaccine design. *Expert Rev. Vaccines* 2004; **3**: S33–S52.
52. Li M, Gao F, Mascola JR, Stamatatos L, Polonis VR, Koutsoukos M, Voss G, Goepfert P, Gilbert P, Greene KM, Bilska M, Kothe DL, Salazar–Gonzalez JF, Wei X, Decker JM, Hahn BH, Montefiori DC. Human Immunodeficiency Virus Type 1 env Clones from Acute and Early Subtype B Infections for Standardized Assessments of Vaccine–Elicited Neutralizing Antibodies. *J. Virol.* 2005; **79**: 10108–10125.
53. Sarmati L, d'Ettorre G, Nicastri E, Ercoli L, Uccella I, Massetti P, Parisi SG, Vullo V, Andreoni M. Neutralizing antibodies against autologous human immunodeficiency virus Type 1 isolates in patients with increasing CD4 cell counts despite incomplete virus suppression during antiretroviral treatment. *Clin. Diagn. Lab. Immunol.* 2001; **8**: 822–824.
54. Locher CP, Grant RM, Collisson EA, Reyes–Teran G, Elbeik T, Kahn JO, Levy JA. Antibody and cellular immune responses in breakthrough infection subjects after HIV type 1 glycoprotein 120 vaccination. *AIDS Res. Hum. Retroviruses* 1999; **15**: 1685–1689.

55. Duerr A, Wasserheit JN, Corey L. HIV vaccines: new frontiers in vaccine development. *Clin. Infect. Dis.* 2006; **43**: 500–511.
56. Luckay A, Sidhu MK, Kjeken R, Megati S, Chong SY, Roopchand V, Garcia–Hand D, Abdullah R, Braun R, Montefiori DC, Rosati M, Felber BK, Pavlakis GN, Mathiesen I, Israel ZR, Eldridge JH, Egan MA. Effect of plasmid DNA vaccine design and in vivo electroporation on the resulting vaccine–specific immune responses in rhesus macaques. *J. Virol.* 2007; **81**: 5257–5269.
57. Oliveira–Ferreira J, Daniel–Ribeiro C. Protective CD8+ T cell responses against the pre–erythrocytic stages of malaria parasites: an overview. *Mem. Inst. Oswaldo Cruz* 2001; **96**: 221–227.
58. Blattman JN, Antia R, Sourdive DJ, Wang X, Kaech SM, Murali–Krishna K, Altman JD, Ahmed R. Estimating the precursor frequency of naive antigen–specific CD8 T cells. *J. Exp. Med.* 2002; **195**: 657–664.
59. Bousso P, Wahn V, Douagi I, Horneff G, Pannetier C, Le Deist F, Zepp F, Niehues T, Kourilsky P, Fischer A, de Saint BG. Diversity, functionality, and stability of the T cell repertoire derived in vivo from a single human T cell precursor. *Proc. Natl. Acad. Sci. U. S. A.* 2000; **97**: 274–278.
60. Acierno PM, Schmitz JE, Gorgone DA, Sun Y, Santra S, Seaman MS, Newberg MH, Mascola JR, Nabel GJ, Panicali D, Letvin NL. Preservation of functional virus–specific memory CD8+ T lymphocytes in vaccinated, simian human immunodeficiency virus–infected rhesus monkeys. *J. Immunol.* 2006; **176**: 5338–5345.
61. Haegele KF, Stueckle CA, Malin JP, Sindern E. Increase of CD8+ T–effector memory cells in peripheral blood of patients with relapsing–remitting multiple sclerosis compared to healthy controls. *J. Neuroimmunol.* 2007; **183**: 168–174.
62. Kamath A, Woodworth JS, Behar SM. Antigen–specific CD8+ T cells and the development of central memory during Mycobacterium tuberculosis infection. *J. Immunol.* 2006; **177**: 6361–6369.
63. Stock AT, Jones CM, Heath WR, Carbone FR. Cutting edge: central memory T cells do not show accelerated proliferation or tissue infiltration in response to localized herpes simplex virus–1 infection. *J. Immunol.* 2006; **177**: 1411–1415.
64. Letsch A, Keilholz U, Kern F, Asemissen AM, Thiel E, Scheibenbogen C. Specific central memory T cells in the bone marrow of patients immunized against tyrosinase peptides. *J. Immunother.* 2006; **29**: 201–207.
65. Levy JA. HIV pathogenesis: knowledge gained after two decades of research. *Adv. Dent. Res.* 2006; **19**: 10–16.
66. Gallo RC. The end or the beginning of the drive to an HIV–preventive vaccine: a view from over 20 years. *The Lancet* 2005; **366**: 1894–1898.
67. Girard MP, Osmanov SK, Kieny MP. A review of vaccine research and development: the human immunodeficiency virus (HIV). *Vaccine* 2006; **24**: 4062–4081.
68. Rowland–Jones SL, Dong T, Fowke KR, Kimani J, Krausa P, Newell H, Blanchard T, Ariyoshi K, Oyugi J, Ngugi E, Bwayo J, MacDonald KS, McMichael AJ, Plummer FA. Cytotoxic T cell responses to multiple conserved HIV epitopes in HIV–resistant prostitutes in Nairobi. *J. Clin. Invest* 1998; **102**: 1758–1765.
69. Jin X, Gao X, Ramanathan M, Deschenes GR, Nelson GW, O'Brien SJ, Goedert JJ, Ho DD, O'Brien TR, Carrington M Jr. Human immunodeficiency virus type 1 (HIV–1)–specific CD8+–T–cell responses for groups of HIV–1–infected individuals with different HLA–B*35 genotypes. *J. Virol.* 2002; **76**: 12603–12610.
70. Monceaux V, Viollet L, Petit F, Ho Tsong FR, Cumont MC, Zaunders J, Hurtrel B, Estaquier J. CD8+ T cell dynamics during primary simian immunodeficiency virus infection in macaques: relationship of effector cell differentiation with the extent of viral replication. *J. Immunol.* 2005; **174**: 6898–6908.
71. Kan–Mitchell J, Bisikirska B, Wong–Staal F, Schaubert KL, Bajcz M, Bereta M. The HIV–1 HLA–A2–SLYNTVATL is a help–independent CTL epitope. *J. Immunol.* 2004; **172**: 5249–5261.
72. Jennes W, Sawadogo S, Koblavi–Deme S, Vuylsteke B, Maurice C, Roels TH, Chorba T, Nkengasong JN, Kestens L. Positive association between beta–chemokine–producing T cells and HIV type 1 viral load in HIV–infected subjects in Abidjan, Cote d'Ivoire. *AIDS Res. Hum. Retroviruses* 2002; **18**: 171–177.
73. Nyambi PN, Mbah HA, Burda S, Williams C, Gorny MK, Nadas A, Zolla–Pazner S. Conserved and exposed epitopes on intact, native, primary human immunodeficiency virus type 1 virions of group M. *J. Virol.* 2000; **74**: 7096–7107.
74. Braibant M, Brunet S, Costagliola D, Rouzioux C, Agut H, Katinger H, Autran B, Barin F. Antibodies to conserved epitopes of the HIV–1 envelope in sera from long–term non–progressors: prevalence and association with neutralizing activity. *AIDS* 2006; **20**: 1923–1930.
75. Kothe DL, Li Y, Decker JM, Bibollet–Ruche F, Zammit KP, Salazar MG, Chen Y, Weng Z, Weaver EA, Gao F, Haynes BF, Shaw GM, Korber BT, Hahn BH. Ancestral and consensus envelope immunogens for HIV–1 subtype C. *Virology* 2006; **352**: 438–449.
76. Gao F, Weaver EA, Lu Z, Li Y, Liao HX, Ma B, Alam SM, Scearce RM, Sutherland LL, Yu JS, Decker JM, Shaw GM, Montefiori DC, Korber BT, Hahn BH, Haynes BF. Antigenicity and immunogenicity of a synthetic human immunodeficiency virus type 1 group m consensus envelope glycoprotein. *J. Virol.* 2005; **79**: 1154–1163.
77. Yang OO, Sarkis PT, Ali A, Harlow JD, Brander C, Kalams SA, Walker BD. Determinant of HIV–1 mutational escape from cytotoxic T lymphocytes. *J. Exp. Med.* 2003; **197**: 1365–1375.
78. Friedrich TC, Watkins DI. The role of the SIV model in AIDS vaccine research. *IAVI. Rep.* 2005; **9**: 6–1, 8.
79. Carlos MP, Anderson DE, Gardner MB, Torres JV. Immunogenicity of a vaccine preparation representing the variable regions of the HIV type 1 envelope glycoprotein. *AIDS Res. Hum. Retroviruses* 2000; **16**: 153–161.
80. Nabel GJ. Immunology. Close to the edge: neutralizing the HIV–1 envelope. *Science* 2005; **308**: 1878–1879.
81. Gomez CE, Abaitua F, Rodriguez D, Esteban M. Efficient CD8+ T cell response to the HIV–env V3 loop epitope from multiple virus isolates by a DNA prime/vaccinia virus boost (rWR and rMVA strains) immunization regime and enhancement by the cytokine IFN–gamma. *Virus Res.* 2004; **105**: 11–22.
82. Anderson DE, Carlos MP, Nguyen L, Torres JV. Overcoming original (antigenic) sin. *Clin. Immunol.* 2001; **101**: 152–157.
83. Sijts AJ, Ossendorp F, Mengede EA, van den Elsen PJ, Melief CJ. Immunodominant mink cell focus–inducing murine leukemia virus (MuLV)–encoded CTL epitope, identified by its MHC class I–binding motif, explains MuLV–type specificity of MCF–directed cytotoxic T lymphocytes. *J. Immunol.* 1994; **152**: 106–116.
84. Li H, Natarajan K, Malchiodi EL, Margulies DH, Mariuzza RA. Three–dimensional structure of H–2Dd complexed with an immunodominant peptide from human immunodeficiency virus envelope glycoprotein 120. *J. Mol. Biol.* 1998; **283**: 179–191.
85. Pahl–Seibert MF, Juelch M, Podlech J, Thomas D, Deegen P, Reddehase MJ, Holtappels R. Highly protective in vivo function of cytomegalovirus IE1 epitope–specific memory CD8 T cells purified by T–cell receptor–based cell sorting. *J. Virol.* 2005; **79**: 5400–5413.
86. Cheuk E, D'Souza C, Hu N, Liu Y, Lang H, Chamberlain JW. Human MHC class I transgenic mice deficient for H2 class I expression facilitate identification and characterization of new HLA class I–restricted viral T cell epitopes. *J. Immunol.* 2002; **169**: 5571–5580.
87. Velders MP, Weijzen S, Eiben GL, Elmishad AG, Kloetzel PM, Higgins T, Ciccarelli RB, Evans M, Man S, Smith L, Kast WM. Defined flanking spacers and enhanced proteolysis is essential for eradication of established tumors by an epitope string DNA vaccine. *J. Immunol.* 2001; **166**: 5366–5373.

88. Livingston BD, Newman M, Crimi C, McKinney D, Chesnut R, Sette A. Optimization of epitope processing enhances immunogenicity of multiepitope DNA vaccines. *Vaccine* 2001; **19**: 4652–4660.
89. Nierkens S, Aalbers M, Bol M, Bleumink R, van Kooten P, Boon L, Pieters R. Differential requirement for CD28/CTLA–4–CD80 /CD86 interactions in drug–induced type 1 and type 2 immune responses to trinitrophenyl–ovalbumin. *J. Immunol.* 2005; **175**: 3707–3714.
90. Kuchroo VK, Das MP, Brown JA, Ranger AM, Zamvil SS, Sobel RA, Weiner HL, Nabavi N, Glimcher LH. B7–1 and B7–2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: application to autoimmune disease therapy. *Cell* 1995; **80**: 707–718.
91. Bour–Jordan H, Blueston JA. CD28 function: a balance of costimulatory and regulatory signals. *J. Clin. Immunol.* 2002; **22**: 1–7.
92. Hodge JW, Sabzevari H, Yafal AG, Gritz L, Lorenz MG, Schlom J. A triad of costimulatory molecules synergize to amplify T–cell activation. *Cancer Res.* 1999; **59**: 5800–5807.
93. Higgins SC, Jarnicki AG, Lavelle EC, Mills KH. TLR4 mediates vaccine–induced protective cellular immunity to Bordetella pertussis: role of IL–17–producing T cells. *J. Immunol.* 2006; **177**: 7980–7989.
94. Zuckermann FA, Martin S, Husmann RJ, Brandt J. Use of interleukin 12 to enhance the cellular immune response of swine to an inactivated herpesvirus vaccine. *Adv. Vet. Med.* 1999; **41**: 447–461.
95. Mapletoft JW, Oumouna M, Townsend HG, Gomis S, Babiuk LA, van Drunen Littel–van den Hurk S. Formulation with CpG oligodeoxynucleotides increases cellular immunity and protection induced by vaccination of calves with formalin–inactivated bovine respiratory syncytial virus. *Virology* 2006; **353**: 316–323.
96. Asahi–Ozaki Y, Itamura S, Ichinohe T, Strong P, Tamura S, Takahashi H, Sawa H, Moriyama M, Tashiro M, Sata T, Kurata T, Hasegawa H. Intranasal administration of adjuvant–combined recombinant influenza virus HA vaccine protects mice from the lethal H5N1 virus infection. *Microbes. Infect.* 2006; **8**: 2706–2714.
97. Carrington AC, Secombes CJ. A review of CpGs and their relevance to aquaculture. *Vet. Immunol. Immunopathol.* 2006; **112**: 87–101.
98. Gluck R, Burri KG, Metcalfe I. Adjuvant and antigen delivery properties of virosomes. *Curr. Drug Deliv.* 2005; **2**: 395–400.
99. Pascual DM, Morales RD, Gil ED, Munoz LM, Lopez JE, Casanueva OL. Adjuvants: present regulatory challenges. *Vaccine* 2006; **24**(2): S2–S9.
100. Dzierzbicka K, Kolodziejczyk AM. [Adjuvants–essential components of new generation vaccines]. *Postepy Biochem.* 2006; **52**: 204–211.
101. Beignon AS, Brown F, Eftekhari P, Kramer E, Briand JP, Muller S, Partidos CD. A peptide vaccine administered transcutaneously together with cholera toxin elicits potent neutralising anti–FMDV antibody responses. *Vet. Immunol. Immunopathol.* 2005; **104**: 273–280.
102. Egan MA, Chong SY, Hagen M, Megati S, Schadeck EB, Piacente P, Ma BJ, Montefiori DC, Haynes BF, Israel ZR, Eldridge JH, Staats HF. A comparative evaluation of nasal and parenteral vaccine adjuvants to elicit systemic and mucosal HIV–1 peptide–specific humoral immune responses in cynomolgus macaques. *Vaccine* 2004; **22**: 3774–3788.
103. Fiorentini S, Marini E, Bozzo L, Trainini L, Saadoune L, Avolio M, Pontillo A, Bonfanti C, Sarmientos P, Caruso A. Preclinical studies on immunogenicity of the HIV–1 p17–based synthetic peptide AT20–KLH. *Biopolymers* 2004; **76**: 334–343.
104. Head E, Barrett EG, Murphy MP, Das P, Nistor M, Sarsoza F, Glabe CC, Kayed R, Milton S, Vasilevko V, Milgram NW, Agadjanyan MG, Cribbs DH, Cotman CW. Immunization with fibrillar Abeta(1–42) in young and aged canines: Antibody generation and characteristics, and effects on CSF and brain Abeta. *Vaccine* 2006; **24**: 2824–2834.
105. Audran R, Cachat M, Lurati F, Soe S, Leroy O, Corradin G, Druilhe P, Spertini F. Phase I malaria vaccine trial with a long synthetic peptide derived from the merozoite surface protein 3 antigen. *Infect. Immun.* 2005; **73**: 8017–8026.
106. Morita S, Oka Y, Tsuboi A, Kawakami M, Maruno M, Izumoto S, Osaki T, Taguchi T, Ueda T, Myoui A, Nishida S, Shirakata T, Ohno S, Oji Y, Aozasa K, Hatazawa J, Udaka K, Yoshikawa H, Yoshimine T, Noguchi S, Kawase I, Nakatsuka S, Sugiyama H, Sakamoto J. A phase I/II trial of a WT1 (Wilms' tumor gene) peptide vaccine in patients with solid malignancy: safety assessment based on the phase I data. *Jpn. J. Clin. Oncol.* 2006; **36**: 231–236.
107. Petrovsky N. Novel human polysaccharide adjuvants with dual Th1 and Th2 potentiating activity. *Vaccine* 2006; **24**(Suppl 2): S2–S9.
108. Stills HF Jr. Adjuvants and antibody production: dispelling the myths associated with Freund's complete and other adjuvants. *ILAR. J.* 2005; **46**: 280–293.
109. Rosenthal KS, Zimmerman DH. Vaccines: all things considered. *Clin. Vaccine Immunol.* 2006; **13**: 821–829.
110. Kirkley JE, Goldstein AL, Naylor PH. Adjuvant properties of montanide CSA 720 with a recombinant HIV P17 gag protein and synthetic peptide antigens. *Scand. J. Immunol.* 1996; **43**: 431–438.
111. Westerfeld N, Zurbriggen R. Peptides delivered by immunostimulating reconstituted influenza virosomes. *J. Pept. Sci.* 2005; **11**: 707–712.
112. Baldridge JR, McGowan P, Evans JT, Cluff C, Mossman S, Johnson D, Persing D. Taking a Toll on human disease: Toll–like receptor 4 agonists as vaccine adjuvants and monotherapeutic agents. *Expert. Opin. Biol. Ther.* 2004; **4**: 1129–1138.
113. Bevaart L, Jansen MJ, van Vugt MJ, Verbeek JS, van de Winkel JG, Leusen JH. The high–affinity IgG receptor, FcgammaRI, plays a central role in antibody therapy of experimental melanoma. *Cancer Res.* 2006; **66**: 1261–1264.
114. Mata E, Carcaboso AM, Hernandez RM, Igartua M, Corradin G, Pedraz JL. Adjuvant activity of polymer microparticles and Montanide ISA 720 on immune responses to Plasmodium falciparum MSP2 long synthetic peptides in mice. *Vaccine* 2007; **25**: 877–885.
115. Rajkannan R, Dhanaraju MD, Gopinath D, Selvaraj D, Jayakumar R. Development of hepatitis B oral vaccine using B–cell epitope loaded PLG microparticles. *Vaccine* 2006; **24**: 5149–5157.
116. Jaganathan KS, Vyas SP. Strong systemic and mucosal immune responses to surface–modified PLGA microspheres containing recombinant hepatitis B antigen administered intranasally. *Vaccine* 2006; **24**: 4201–4211.
117. Lord MJ, Jolliffe NA, Marsden CJ, Pateman CS, Smith DC, Spooner RA, Watson PD, Roberts LM. Ricin. Mechanisms of cytotoxicity. *Toxicol. Rev.* 2003; **22**: 53–64.
118. Tobery TW, Siliciano RF. Targeting of HIV–1 antigens for rapid intracellular degradation enhances cytotoxic T lymphocyte (CTL) recognition and the induction of de novo CTL responses in vivo after immunization. *J. Exp. Med.* 1997; **185**: 909–920.
119. Rodriguez F, Whitton JL. Enhancing DNA immunization. *Virology* 2000; **268**: 233–238.
120. Kurts C, Heath WR, Carbone FR, Kosaka H, Miller JF. Cross–presentation of self antigens to CD8+ T cells: the balance between tolerance and autoimmunity. *Novartis Found. Symp.* 1998; **215**: 172–181.
121. Humrich J, Jenne L. Viral vectors for dendritic cell–based immunotherapy. *Curr. Top. Microbiol. Immunol.* 2003; **276**: 241–259.
122. Stefanova I, Dorfman JR, Germain RN. Self–recognition promotes the foreign antigen sensitivity of naive T lymphocytes. *Nature* 2002; **420**: 429–434.

DOI: 10.1002/psc

123. Speiser DE, Miranda R, Zakarian A, Bachmann MF, McKall-Faienza K, Odermatt B, Hanahan D, Zinkernagel RM, Ohashi PS. Self antigens expressed by solid tumors Do not efficiently stimulate naive or activated T cells: implications for immunotherapy. *J. Exp. Med.* 1997; **186**: 645–653.
124. Jonuleit H, Schmitt E, Steinbrink K, Enk AH. Dendritic cells as a tool to induce anergic and regulatory T cells. *Trends Immunol.* 2001; **22**: 394–400.
125. Taams LS, Vukmanovic–Stejic M, Smith J, Dunne PJ, Fletcher JM, Plunkett FJ, Ebeling SB, Lombardi G, Rustin MH, Bijlsma JW, Lafeber FP, Salmon M, Akbar AN. Antigen–specific T cell suppression by human CD4 + CD25+ regulatory T cells. *Eur. J. Immunol.* 2002; **32**: 1621–1630.
126. Nickoloff BJ, Turka LA. Immunological functions of non–professional antigen–presenting cells: new insights from studies of T–cell interactions with keratinocytes. *Immunol. Today* 1994; **15**: 464–469.
127. Fan J, Liang X, Horton MS, Perry HC, Citron MP, Heidecker GJ, Fu TM, Joyce J, Przysiecki CT, Keller PM, Garsky VM, Ionescu R, Rippeon Y, Shi L, Chastain MA, Condra JH, Davies ME, Liao J, Emini EA, Shiver JW. Preclinical study of influenza virus A M2 peptide conjugate vaccines in mice, ferrets, and rhesus monkeys. *Vaccine* 2004; **22**: 2993–3003.
128. Tine JA, Firat H, Payne A, Russo G, Davis SW, Tartaglia J, Lemonnier FA, Demoyen PL, Moingeon P. Enhanced multiepitope–based vaccines elicit CD8+ cytotoxic T cells against both immunodominant and cryptic epitopes. *Vaccine* 2005; **23**: 1085–1091.
129. Ho O, Green WR. Alternative translational products and cryptic T cell epitopes: expecting the unexpected. *J. Immunol.* 2006; **177**: 8283–8289.
130. Cardinaud S, Moris A, Fevrier M, Rohrlich PS, Weiss L, Langlade–Demoyen P, Lemonnier FA, Schwartz O, Habel A. Identification of cryptic MHC I–restricted epitopes encoded by HIV–1 alternative reading frames. *J. Exp. Med.* 2004; **199**: 1053–1063.
131. Lopez JA, Weilenman C, Audran R, Roggero MA, Bonelo A, Tiercy JM, Spertini F, Corradin G. A synthetic malaria vaccine elicits a potent CD8(+) and CD4(+) T lymphocyte immune response in humans. Implications for vaccination strategies. *Eur. J. Immunol.* 2001; **31**: 1989–1998.
132. Fong L, Hou Y, Rivas A, Benike C, Yuen A, Fisher GA, Davis MM, Engleman EG. Altered peptide ligand vaccination with Flt3 ligand expanded dendritic cells for tumor immunotherapy. *Proc. Natl. Acad. Sci. U. S. A.* 2001; **98**: 8809–8814.
133. van der Burg SH, Bijker MS, Welters MJ, Offringa R, Melief CJ. Improved peptide vaccine strategies, creating synthetic artificial infections to maximize immune efficacy. *Adv. Drug Deliv. Rev.* 2006; **58**: 916–930.
134. Slansky JE, Rattis FM, Boyd LF, Fahmy T, Jaffee EM, Schneck JP, Margulies DH, Pardoll DM. Enhanced antigen–specific antitumor immunity with altered peptide ligands that stabilize the MHC–peptide–TCR complex. *Immunity* 2000; **13**: 529–538.
135. Hertz CJ, Kiertscher SM, Godowski PJ, Bouis DA, Norgard MV, Roth MD, Modlin RL. Microbial lipopeptides stimulate dendritic cell maturation via Toll–like receptor 2. *J. Immunol.* 2001; **166**: 2444–2450.
136. Duesberg U, von dem BA, Kirschning C, Miyake K, Sauerbruch T, Spengler U. Cell activation by synthetic lipopeptides of the hepatitis C virus (HCV)–core protein is mediated by toll like receptors (TLRs) 2 and 4. *Immunol. Lett.* 2002; **84**: 89–95.
137. Langhans B, Schweitzer S, Nischalke HD, Braunschweiger I, Sauerbruch T, Spengler U. Hepatitis C virus–derived lipopeptides differentially induce epitope–specific immune responses in vitro. *J. Infect. Dis.* 2004; **189**: 248–253.
138. Jackson DC, Lau YF, Le T, Suhrbier A, Deliyannis G, Cheers C, Smith C, Zeng W, Brown LE. A totally synthetic vaccine of generic structure that targets Toll–like receptor 2 on dendritic cells and promotes antibody or cytotoxic T cell responses. *Proc. Natl. Acad. Sci. U. S. A.* 2004; **101**: 15440–15445.
139. Espuelas S, Roth A, Thumann C, Frisch B, Schuber F. Effect of synthetic lipopeptides formulated in liposomes on the maturation of human dendritic cells. *Mol. Immunol.* 2005; **42**: 721–729.
140. Kang TH, Lee JH, Bae HC, Noh KH, Kim JH, Song CK, Shin BC, Hung CF, Wu TC, Park JS, Kim TW. Enhancement of dendritic cell–based vaccine potency by targeting antigen to endosomal/lysosomal compartments. *Immunol. Lett.* 2006; **106**: 126–134.
141. Moris A, Nobile C, Buseyne F, Porrot F, Abastado JP, Schwartz O. DC–SIGN promotes exogenous MHC–I–restricted HIV–1 antigen presentation. *Blood* 2004; **103**: 2648–2654.
142. Johansen P, Haffner AC, Koch F, Zepter K, Erdmann I, Maloy K, Simard JJ, Storni T, Senti G, Bot A, Wuthrich B, Kundig TM. Direct intralymphatic injection of peptide vaccines enhances immunogenicity. *Eur. J. Immunol.* 2005; **35**: 568–574.
143. Vitiello A, Ishioka G, Grey HM, Rose R, Farness P, LaFond R, Yuan L, Chisari FV, Furze J, Bartholomeuz R. Development of a lipopeptide–based therapeutic vaccine to treat chronic HBV infection. I. Induction of a primary cytotoxic T lymphocyte response in humans. *J. Clin. Invest.* 1995; **95**: 341–349.
144. Pinilla–Ibarz J, Cathcart K, Korontsvit T, Soignet S, Bocchia M, Caggiano J, Lai L, Jimenez J, Kolitz J, Scheinberg DA. Vaccination of patients with chronic myelogenous leukemia with bcr–abl oncogene breakpoint fusion peptides generates specific immune responses. *Blood* 2000; **95**: 1781–1787.
145. Fiers W, De Filette M, Birkett A, Neirynck S, Min JW. A "universal" human influenza A vaccine. *Virus Res.* 2004; **103**: 173–176.
146. Roberts JD, Bebenek K, Kunkel TA. The accuracy of reverse transcriptase from HIV–1. *Science* 1988; **242**: 1171–1173.
147. Ji JP, Loeb LA. Fidelity of HIV–1 reverse transcriptase copying RNA in vitro. *Biochemistry* 1992; **31**: 954–958.
148. Carruth LM, Greten TF, Murray CE, Castro MG, Crone SN, Pavlat W, Schneck JP, Siliciano RF. An algorithm for evaluating human cytotoxic T lymphocyte responses to candidate AIDS vaccines. *AIDS Res. Hum. Retroviruses* 1999; **15**: 1021–1034.
149. Niedrig M, Gregersen JP, Fultz PN, Broker M, Mehdi S, Hilfenhaus J. Immune response of chimpanzees after immunization with the inactivated whole immunodeficiency virus (HIV–1), three different adjuvants and challenge. *Vaccine* 1993; **11**: 67–74.
150. Nabel GJ. HIV vaccine strategies. *Vaccine* 2002; **20**: 1945–1947.
151. Yasutomi Y, Palker TJ, Gardner MB, Haynes BF, Letvin NL. Synthetic peptide in mineral oil adjuvant elicits simian immunodeficiency virus–specific CD8+ cytotoxic T lymphocytes in rhesus monkeys. *J. Immunol.* 1993; **151**: 5096–5105.
152. Letvin NL, Mascola JR, Sun Y, Gorgone DA, Buzby AP, Xu L, Yang ZY, Chakrabarti B, Rao SS, Schmitz JE, Montefiori DC, Barker BR, Bookstein FL, Nabel GJ. Preserved CD4+ central memory T cells and survival in vaccinated SIV–challenged monkeys. *Science* 2006; **312**: 1530–1533.
153. Cristillo AD, Wang S, Caskey MS, Unangst T, Hocker L, He L, Hudacik L, Whitney S, Keen T, Chou TH, Shen S, Joshi S, Kalyanaraman VS, Nair B, Markham P, Lu S, Pal R. Preclinical evaluation of cellular immune responses elicited by a polyvalent DNA prime/protein boost HIV–1 vaccine. *Virology* 2006; **346**: 151–168.
154. Azizi A, Ghorbani M, Soare C, Mojibian M, Diaz–Mitoma F. Synergistic effect of combined HIV/HCV immunogens: a combined HIV–1/HCV candidate vaccine induces a higher level of CD8+ T cell–immune responses in HLA–A2.1 mice. *Curr. HIV. Res.* 2007; **5**: 211–219.
155. Azizi A, Ghorbani M, Kryworuchko M, Aucoin S, Diaz–Mitoma F. Potency of cell–mediated immune responses to different combined HIV–1 immunogens in a humanized murine model. *Hum. Vaccin.* 2005; **1**: 170–176.

156. Cann AJ, Stanway G, Hughes PJ, Minor PD, Evans DM, Schild GC, Almond JW. Reversion to neurovirulence of the live–attenuated Sabin type 3 oral poliovirus vaccine. *Nucleic Acids Res.* 1984; **12**: 7787–7792.
157. Voltan R, Robert–Guroff M. Live recombinant vectors for AIDS vaccine development. *Curr. Mol. Med.* 2003; **3**: 273–284.
158. Bermudez A, Reyes C, Guzman F, Vanegas M, Rosas J, Amador R, Rodriguez R, Patarroyo MA, Patarroyo ME. Synthetic vaccine update: applying lessons learned from recent SPf66 malarial vaccine physicochemical, structural and immunological characterization. *Vaccine* 2007.
159. Calvo–Calle JM, Oliveira GA, Nardin EH. Human CD4+ T cells induced by synthetic peptide malaria vaccine are comparable to cells elicited by attenuated Plasmodium falciparum sporozoites. *J. Immunol.* 2005; **175**: 7575–7585.
160. Jiang S, Song R, Popov S, Mirshahidi S, Ruprecht RM. Overlapping synthetic peptides as vaccines. *Vaccine* 2006; **24**: 6356–6365.
161. Purcell AW, Zeng W, Mifsud NA, Ely LK, Macdonald WA, Jackson DC. Dissecting the role of peptides in the immune response: theory, practice and the application to vaccine design. *J. Pept. Sci.* 2003; **9**: 255–281.

DOI: 10.1002/psc