Journal of Peptide Science
J. Peptide Sci. **11**: 707–712 (2005)
Published online 1 August 2005 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.700

JOURNAL OF PEPTIDE SCIENCE

Review

Peptides delivered by immunostimulating reconstituted influenza virosomes¶

NICOLE WESTERFELD and RINALDO ZURBRIGGEN*
Pevion Biotech Ltd., Rehhagstrasse 79, 3018 Bern, Switzerland

Received 26 April 2005; *Revised 9 June 2005*; *Accepted 10 June 2005*

Abstract: Vaccines have been well accepted and used effectively for more than 100 years. Traditional vaccines are generally composed of whole inactivated or attenuated microorganisms that have lost their disease-causing properties. These classical prophylactic live vaccines evoke protective immune responses, but have often been associated with an unfavorable safety profile, as observed, for example, for smallpox and polio myelitis vaccines [1,2]. First improvements were subunit vaccines that do not focus on attenuation of whole organisms but concentrate on particular proteins. These vaccines are able to generate protective immune responses (e.g. diphtheria, tetanus, pertussis) [3]. However, next generation vaccines should focus on specific antigens (e.g. proteins, peptides), since the requirements by regulatory authorities to crude biological material are becoming more stringent over time. An increasing number of such antigens capable of inducing protective humoral or cellular immune responses have been identified in the last few years. But most of these are weak immunogens. This reemphasizes the need for adjuvants to promote a potent immune response and also for delivery antigens to the immune system in an appropriate way (carrier capability). Here we review a new approach for prophylactic and therapeutic vaccines, which focuses on the induction of highly specific immune responses directed against antigen-derived peptides using a suitable carrier system.

Keywords: immunostimulating reconstituted influenza virosomes; conformation; vaccine; peptides; malaria; antibody; adjuvant

INTRODUCTION

A new generation of prophylactic and therapeutic vaccines is needed: vaccines capable of inducing a strong, specific immune response and possessing a more favorable safety profile than the classical preparations. These issues can be addressed by developing vaccines that are not derived from isolated biological material but which are produced synthetically and based on antigens (e.g. proteins, peptides or carbohydrates) capable of inducing humoral and cellular immune responses.

There are a number of advantages to using peptides rather than biologically derived material:

Although many vaccine candidates based on recombinant proteins were successfully tested preclinically, the production of large batches of clinical grade, recombinantly expressed protein is difficult and expensive. Peptides, however, are easy to manufacture and the upscaling has already been established. Moreover, peptides can be produced relatively cheaply in large amounts according to current good manufacturing practices. The immune response induced by a peptide antigen is highly specifically directed against a very defined region of the target protein. By contrast, whole proteins can induce a broad spectrum of immune responses, including antibodies that could also block binding of protective antibodies [4,5] and presentation of epitopes that might not be recognized very efficiently or elicit deleterious responses [6]. In addition, whole proteins may have some homologies to human proteins and may therefore not be immunogenic, or can induce an auto-immune response. Compared to attenuated viruses, there is no risk of backmutation into dangerous viruses. But we have to consider that peptide-based vaccines can be relatively susceptible to escape mutations of the pathogen due to their focused specificity, and it might be necessary to include several epitopes.

Nevertheless, no synthetic peptide-based vaccine is currently on the market. Polysaccharides, used as vaccine against *Haemophilus influenzae* type b (Hib) [7], are the only known synthetic vaccine for human use commercially available yet. The development of peptide-based vaccines is hampered by their poor immunogenicity and the lack of conformational similarities between small linear peptides and the corresponding sequence in the native protein, as many important neutralizing epitopes have a defined three-dimensional conformation [8]. In addition, unprotected linear peptides may be quickly degraded by proteases before they even reach their destination, namely, professional antigen-presenting cells. Furthermore, depending on the route

* Correspondence to: R. Zurbriggen, Pevion Biotech Ltd., Rehhagstrasse 79, 3018 Bern, Switzerland; e-mail: rinaldo.zurbriggen@pevion.com
¶ Selected paper presented at the 1st International Congress on Natural Peptides to Drugs, 30 November–3 December 2004, Zermatt, Switzerland.

of immunization, peptides can induce immunological tolerance rather than immunity [9]. As a consequence, suitable carriers or adjuvants are needed to improve the immunogenicity of peptides and prevent the induction of tolerance.

Peptide-based vaccines have already been tested in different animal models. Unfortunately, a good immune response could only be induced using strong adjuvants – adjuvants that are not allowed for human use.

Furthermore, it has been assumed that alum, one of the three adjuvants licensed for human use, is not a very suitable carrier for the delivery of peptides. Peptides often elicit weak antibody responses, and the aggregation process of the peptide antigen to the alum salts can destroy defined conformations, both aspects generally limiting the application as synthetic vaccine candidates for human use [10]. Alternative delivery systems include isolated virus coat proteins, which retain the ability to reassemble into virus-like particles. This has been described for hepatitis surface antigen and core and yeast transposon-derived virus-like particles (TyVLP) [11–13]. These particles stimulate both humoral and cellular immune responses but are not yet registered for human use.

DEVELOPMENT OF IMMUNOSTIMULATING RECONSTITUTED INFLUENZA VIROSOMES

The human immune system is able to generate a highly specific immune response against influenza viruses. After infection or immunization with a defined influenza virus, neutralizing antibodies protect us against a reinfection with the same influenza strain. Nevertheless, we are susceptible to other influenza strains, and our immune system adapts to the new virus variant, meaning that our immune system is able to recognize small modifications at the surface of the influenza virus. Influenza-like particles should therefore be an optimal antigen-carrier system for proteins and peptide antigens. This led to the development of immunostimulating reconstituted influenza virosomes (IRIVs).

In the meantime, IRIVs represent a very well characterized antigen carrier system. They display an improved tolerability compared to alum-adsorbed vaccines and have been shown to induce protective immune responses [14]. A very important advantage of influenza virosomes over other systems of reconstituted virus particles is the in-depth experience with two marketed products, the influenza vaccine Inflexal® and the Hepatitis A vaccine Epaxal®, of which more than 20 million doses have been given so far. To date, influenza virosomes are the only one of the three adjuvant systems approved by regulatory authorities, which has carrier capabilities.

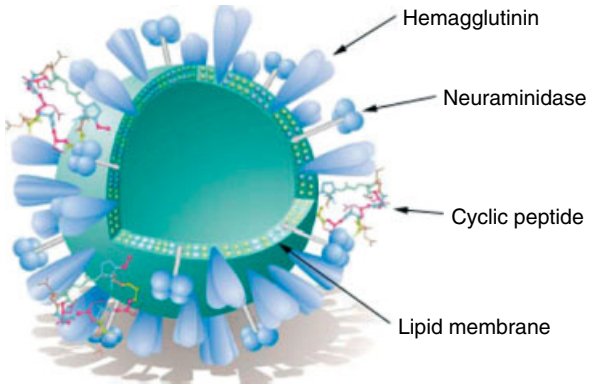

Figure 1 A schematic representing an IRIV carrying a cyclic peptide on the surface. (Reproduced with permission from [17] © 2003 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim).

CHARACTERISTICS OF INFLUENZA VIROSOMES

Immunostimulating reconstituted influenza virosomes are a liposomal carrier system characterized as spherical, unilaminar vesicles with a mean diameter of approximately 150 nm. They consist of 70% egg yolk phosphatidylcholine (EYPC), 20% phosphatidylethanolamin (PE) and 10% envelope phospholipids originating from H1N1 influenza virus (A/Singapore/6/86) (Figure 1). In contrast to liposomes, influenza hemagglutinin (HA) and neuraminidase (NA) are intercalated into the lipid bilayer, which give them their fusogenic activity and thereby play a key role in the mode of action of these virosomes [15]. Virosomes are prepared by the detergent removal as described in detail elsewhere [16].

Essentially, virosomes represent empty influenza virus envelopes, devoid of the nucleocapsid that contains the genetic material of the virus. Therefore they are not able to replicate, which is one aspect of their excellent safety profile. In addition, the influenza-derived HA confers stability to virosomal formulation and significantly contributes to the immunological properties of virosomes. Furthermore, due to the incorporation of hemagglutinin, virosomes retain the fusogenic activity of influenza particles, similar to the original influenza virus itself. It has been shown that a physical association between the virosome and the antigen of interest is a prerequisite to elicit the full adjuvant effect [18]. This association can be achieved by incorporation of the antigen molecule into the virosomal vesicle, adsorption to the surface, or integration into the lipid membrane via hydrophobic domains or lipid moieties.

Antigens or peptides attached to the surface of virosomes primarily elicit humoral immune responses, namely, antibodies as well as CD4+ T-cell help, while antigens and peptides inside the vesicle are capable of inducing CD8+ T-cell responses. As illustrated in

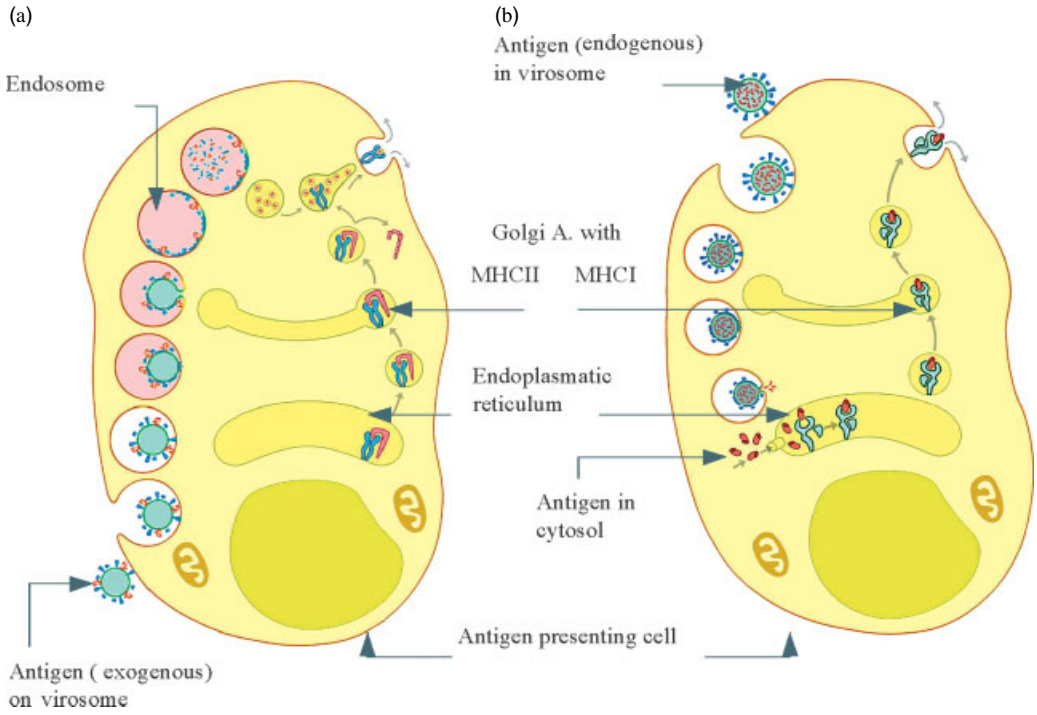

Figure 2 Virosome-based antigen processing in immune competent cells: (a) The processing of antigen protein or peptide localized on the virosome surface leading to MHC class II presentation. (b) The processing of antigen molecules delivered inside the virosome. The antigen is delivered to the cytoplasm triggering antigen-presentation on MHC class I.

Figure 2, in either case the influenza virosome attaches to the cell membrane of target cells (e.g. antigen-presenting cells) and enters it via the endosomal pathway. With decreasing pH in the endosome, the virus-derived hemagglutinin facilitates fusion of the virosomal membrane with the membrane of the endosome [19]. Consequently, antigen molecules on the surface of virosomes will remain in the endosome after fusion, and the pH shift in this compartment facilitates detachment and degradation of virosome-linked antigen molecules and the interaction of the released peptides with major histocompatibility complex (MHC) class II molecules localized in this compartment. The loaded MHC class II molecules are directed to the cell surface of the antigen-presenting cell to interact with CD4+ T-cells. Antigen molecules inside virosomes are delivered to the cytoplasm after fusion of the virosomes to the endosomal membrane. This leads to degradation of the antigen within the cytoplasm by the proteosome and subsequent transport of the antigen peptides into the endoplasmic reticulum (ER) and loading onto MHC class I. Peptide-loaded MHC class I molecules reach the cell surface of the antigen-presenting cell and interact with CD8+ T- cells to activate a cytotoxic T-cell response. The *in vitro* and *in vivo* activation of CD8+ T-cells by the immunization with peptides encapsulated in virosomes has been demonstrated in the context of Hepatitis C vaccine development by Hunziker *et al.* and Amacker *et al.* [20,21].

PEPTIDE VACCINE DEVELOPMENT USING IMMUNOSTIMULATING RECONSTITUTED INFLUENZA VIROSOMES

The development of classical vaccines has primarily concentrated on infectious diseases. With up to 300 million people currently infected, malaria continues to be one of the major burdens on public health in many tropical countries [22]. Spreading of drug resistance of the *Plasmodium* parasites as well as of the parasite-transmitting mosquitoes reemphasizes the need for an effective vaccine against the most severe form of the disease caused by *Plasmodium falciparum*. Many vaccine-target antigens are expressed at various stages of the parasite's life cycle and most of the mechanisms involved in protective immunity against malaria are incompletely understood. But there is good evidence that antibodies directed against surface antigens of the sporozoite stage, which represents the incoming parasite, and the blood stage can prevent initial infection of hepatocytes and reduce morbidity and mortality associated with the infection of erythrocytes, respectively [23,24].

Immunization of humans with x-irradiated sporozoites confers sterile immunity against the initial infection by *Plasmodium falciparum* with the surface antigen circum sporozoite protein (CSP) being the dominant antibody target [25]. Furthermore, antibodies directed against the central (NPNA) tandem repeat region of CSP confer protection against initial liver infection in

mice [23]. But first attempts to develop a peptide-based malaria vaccine were disappointing since a linear (NPNA) peptide linked to tetanus toxoid was poorly immunogenic in humans [26].

As a consequence, Moreno *et al.* further modified the (NPNA) repeat epitopes by preparing cyclic peptidomimetics [27]. These carry either one or two intact repeat units in a reverse-turn conformation and C(α)-backbone methylation of the proline to mimic the stable conformation of the (NPNA) repeats within CSP in contrast to the flexibility of linear peptides. The constructs were linked to PE via a succinate linker and incorporated into the membrane of influenza virosomes. The authors showed that formulating these cyclic peptides into virosomes improves the quality of the immune response compared to multiple antigen peptides (MAP) formulations carrying four of the same peptidomimetics formulated in alum: despite lower antibody titers against the mimetic, only mice immunized with NPNA repeats on the surface of virosomes developed antibodies that were able to recognize parasites as has been shown by indirect immune fluorescence analysis (IFA). In contrast to the virosomal formulations, the same peptides in MAP formulations adsorbed to alum seem to induce peptide conformations differing dramatically from the situation in the native protein, since these exclusively induce antibodies that did not recognize the parasite.

Circularization of 5 (NPNA) via a cross-linkage of the amino group at the β-position of proline at position 6 to a spatially adjacent side-chain carboxy group of glutamate also stabilizes the β-turn structure and induces parasite cross-reactive and even invasion-inhibitory antibodies when formulated in the membrane of influenza virosomes [17] (Figure 3).

Both attempts clearly demonstrate the importance of conformationally defined (NPNA) repeats to induce an immune response to recognize the parasite, which is a prerequisite to prevent the initial infection with *P. falciparum*.

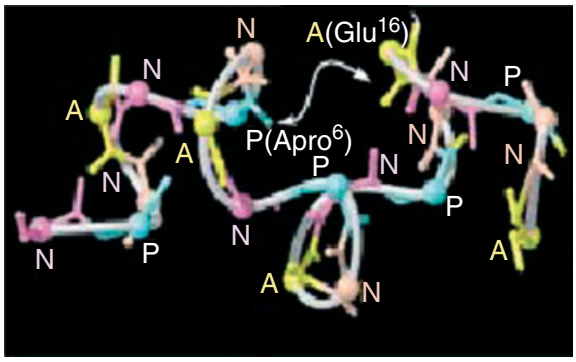

Figure 3 Theoretical structure of 5 (NPNA) units circularized via a cross link of the amino group at the β position of proline at position 6 to side-chain carboxy group of glutamate. (Reproduced with permission from [27] © 2003 Wiley–VCH Verlag GmbH & Co. KgaA, Weinheim).

The second strategy focused on induction of a protective immune response against the bloodstage of the parasite, where antibodies are known to inhibit the infection of erythrocytes. One of the leading vaccine candidates to be included into a malaria vaccine, the apical membrane antigen 1 (AMA-1), has been shown to be a target for protective antibodies [28,29]. The epitopes recognized by these antibodies against AMA1 are not characterized in detail, but they seem to be primarily directed against conformational epitopes as well [30].

The semiconserved loop I in domain III of AMA1 is one target for inhibitory human antibodies [31]. In contrast to the effect demonstrated for CSP-derived peptides, immunization of mice with linear and circular peptides mimicking the loop structure of AMA1 on the surface of virosomes was similarly efficient. The circularization of the 49-amino acid peptide did neither improve the quantity nor the quality of the antibody response. Both formulations were immunogenic and able to induce antibodies recognizing blood-stage parasites [32].

But fine analysis using monoclonal antibodies shows that, similar to CSP, conformation of the AMA1 epitopes still seems to be critical, as the majority of the monoclonal antibodies seem to bind to discontinuous epitopes including two monoclonal antibodies that inhibit parasite invasion of erythrocytes *in vitro*. Furthermore, shorter peptides could not induce antibodies capable of binding the parasite, and these peptides were not recognized by the hyperimmune sera of individuals living in endemic areas.

Both studies demonstrate the importance of the conformational properties of peptides with respect to developing malaria vaccines either dependent on circularization or on critical peptide length. Importantly, for both cases, the formulation with IRIV did not disturb the conformation in contrast to adsorption of peptide-formulations to alum as it has also been demonstrated for SPf66, one additional malaria vaccine candidate [33].

Furthermore, influenza-virosome formulations carrying synthetic malaria peptides have demonstrated to be well tolerated, safe and highly immunogenic in a first ongoing clinical phase I trial (personal communication, Degen, L.). This further confirms the preclinical data and is in sharp contrast to the use of linear peptides adsorbed on alum, which are poorly immunogenic when applied to humans [26]. The study will be continued with a third immunization to evaluate further immunological aspects of the vaccination.

One very important issue concerning influenza virosomes is preexisting immunity to the carrier: nearly every human being has experienced influenza infections. The influence of preexisting influenza-neutralizing mechanisms has been addressed by comparing the potency of peptide-bearing virosomes

in mice with or without pre-immunization against influenza. In fact, the immune response against the carrier further improves the immunogenicity of the additional peptide antigen, maybe by supporting the alternative uptake of virosomes by antigen-presenting cells due to binding of opsonizing antibodies to the virosomal particles [14].

FURTHER APPLICATIONS FOR IMMUNOSTIMULATING RECONSTITUTED INFLUENZA VIROSOMES

Apart from vaccine development, influenza virosomes can also be used as carriers for pharmaceutically active substances that are encapsulated inside the vesicle. Furthermore, these virosomes can be engineered to specifically target certain cell types by integration of ligands or antibodies into the virosome surface. This has been proven by Ernst Waelti *et al.* who used IRIV to specifically introduce drugs directly into target cells of mice [34]. They show that it is possible to improve drug delivery into tumor cells by the use of surface modified influenza virosomes.

CONCLUSION

The main goal for most efforts on vaccine development is to protect humans from infectious diseases. A lot has been done on the development of subunit vaccines focusing on pathogen-derived proteins or peptides. But so far there are only three adjuvant systems licensed for human use: Alum, MF59 and IRIVs. Most subunit vaccines licensed for human use are based on adsorption to alum, and for several vaccines this has been demonstrated to support the induction of a protective immune response. However, its use as an adjuvant to carry peptide vaccine candidates has been disappointing so far: low immunogenicity and conformational changes of the peptide antigen were consequences of the combination with alum. Immunogenicity and conformational integrity can be improved by the use of influenza virosomes as carriers. Furthermore, virosomes display a higher tolerability compared to alum with less side effects after injection, proven by two marketed products. By defining the localization of the peptide, influenza virosomes can be actively targeted to induce either humoral or cellular immune responses, depending on the bias needed for protection. Pre-existing immunity to influenza even further enhances the targeted immune responses. Taking these together, reconstituted influenza virosomes as carriers further improve the use of peptides as human vaccine candidates by solving major obstacles observed in combination with alum.

REFERENCES

1. Bray M. Pathogenesis and potential antiviral therapy of complications of smallpox vaccination. *Antiviral Res.* 2003; **58**: 101–114.
2. Pearce JM. Salk and Sabin: poliomyelitis immunisation. *J. Neurol. Neurosurg. Psychiatr.* 2004; **75**: 1552.
3. Godfroid F, Denoel P, de Grave D, Schuerman L, Poolman J. Diphtheria-tetanus-pertussis (DTP) combination vaccines and evaluation of pertussis immune responses. *Int. J. Med. Microbiol.* 2004; **294**: 269–276.
4. Blackman MJ, Scott FT, Shai S, Holder AA. Antibodies inhibit the protease-mediated processing of a malaria merozoite surface protein. *J. Exp. Med.* 1994; **180**: 389–393.
5. Guevara Patino JA, Holder AA, McBride JS, Blackman MJ. Antibodies that inhibit malaria merozoite surface protein-1 processing and erythrocyte invasion are blocked by naturally acquired human antibodies. *J. Exp. Med.* 1997; **186**: 1689–1699.
6. Berzofsky J. Development of artificial vaccines against HIV using defined peptides. *FASEB J.* 1991; **5**: 2412–2418.
7. Verez-Bencomo V, Fernandez-Santana V, Hardy E, Toledo ME, Rodriguez MC, Heynngnezz L, Rodriguez A, Baly A, Herrera L, Izquierdo M, Villar A, Valdes Y, Cosme K, Deler ML, Montane M, Garcia E, Ramos A, Aguilar A, Medina E, Torano G, Sosa I, Hernandez I, Martinez R, Muzachio A, Carmenates A, Costa L, Cardoso F, Campa C, Diaz M, Roy R. A synthetic conjugate polysaccharide vaccine against Haemophilus influenzae type b. *Science* 2004; **305**: 522–525.
8. Ling IT, Ogun SA, Holder AA. Immunization against malaria with a recombinant protein. *Parasite Immunol.* 1994; **16**: 63–67.
9. Aichele P, Brduscha-Riem K, Zinkernagel RM, Hengartner H, Pircher H. T cell priming versus T cell tolerance induced by synthetic peptides. *J. Exp. Med.* 1995; **182**: 261–266.
10. Schild H, Rammensee HR. *Peptid Based Vaccines*. Walter de Gruyter: Berlin, 1996.
11. Wunderlich G, Moura IC, del Portillo HA. Genetic immunization of BALB/c mice with a plasmid bearing the gene coding for a hybrid merozoite surface protein 1-hepatitis B virus surface protein fusion protects mice against lethal Plasmodium chabaudi chabaudi PC1 infection. *Infect. Immun.* 2000; **68**: 5839–5845.
12. Walther M, Dunachie S, Keating S, Vuola JM, Berthoud T, Schmidt A, Maier C, Andrews L, Andersen RF, Gilbert S, Poulton I, Webster D, Dubovsky F, Tierney E, Sarpotdar P, Correa S, Huntcooke A, Butcher G, Williams J, Sinden RE, Thornton GB, Hill AV. Safety, immunogenicity and efficacy of a pre-erythrocytic malaria candidate vaccine, ICC-1132 formulated in Seppic ISA 720. *Vaccine* 2005; **23**: 857–864.
13. Gilbert SC, Hill AV. Protein particle vaccines against malaria. *Parasitol. Today* 1997; **13**: 302–306.
14. Zurbriggen R, Gluck R. Immunogenicity of IRIV- versus alum-adjuvanted diphtheria and tetanus toxoid vaccines in influenza primed mice. *Vaccine* 1999; **17**: 1301–1305.
15. Tsurudome M, Gluck R, Graf R, Falchetto R, Schaller U, Brunner J. Lipid interactions of the hemagglutinin HA2 NH2-terminal segment during influenza virus-induced membrane fusion. *J. Biol. Chem.* 1992; **267**: 20 225–20 232.
16. Ollivon M, Lesieur S, Grabielle-Madelmont C, Paternostre M. Vesicle reconstitution from lipid-detergent mixed micelles. *Biochim. Biophys. Acta* 2000; **1508**: 34–50.
17. Pfeiffer B, Peduzzi E, Moehle K, Zurbriggen R, Gluck R, Pluschke G, Robinson JA. A virosome-mimotope approach to synthetic vaccine design and optimization: synthesis, conformation, and immune recognition of a potential malaria-vaccine candidate. *Angew. Chem. Int. Ed. Engl.* 2003; **42**: 2368–2371.
18. Zurbriggen R, Novak-Hofer I, Seelig A, Gluck R. IRIV-adjuvanted hepatitis A vaccine: in vivo absorption and biophysical characterization. *Prog. Lipid Res.* 2000; **39**: 3–18.
19. Durrer P, Galli C, Hoenke S, Corti C, Gluck R, Vorherr T, Brunner J. H+-induced membrane insertion of influenza virus

hemagglutinin involves the HA2 amino-terminal fusion peptide but not the coiled coil region. *J. Biol. Chem.* 1996; **271**: 13 417–13 421.
20. Hunziker IP, Grabscheid B, Zurbriggen R, Gluck R, Pichler WJ, Cerny A. In vitro studies of core peptide-bearing immunopotentiating reconstituted influenza virosomes as a non-live prototype vaccine against hepatitis C virus. *Int. Immunol.* 2002; **14**: 615–626.
21. 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.
22. World Health Organization, World malaria situation in 1994. *Wkly. Epidemiol. Rec.* 1997; **69**: 309–314.
23. Nussenzweig V, Nussenzweig RS. Rationale for the development of an engineered sporozoite malaria vaccine. *Adv. Immunol.* 1989; **45**: 283–334.
24. Cohen S, McGregor IA, Carrington SC. Gamma globulin and acquired immunity to malaria. *Nature* 1961; **192**: 733–737.
25. Herrington D, Davis J, Nardin E, Beier M, Cortese J, Eddy H, Losonsky G, Hollingdale M, Sztein M, Levine M, Nussenzweig RS, Clyde D, Edelmann R. Successful immunization of humans with irradiated malaria sporozoites: humoral and cellular responses of the protected individuals. *Am. J. Trop. Med. Hyg.* 1991; **45**: 539–547.
26. Herrington DA, Clyde DF, Losonsky G, Cortesia M, Murphy JR, Davis J, Baqar S, Felix AM, Heimer EP, Gillessen D, Nardin E, Nussenzweig RS, Nussenzweig V, Hollingdale MR, Levine MM. Safety and immunogenicity in man of a synthetic peptide malaria vaccine against *Plasmodium falciparum* sporozoites. *Nature* 1987; **328**: 257–259.
27. Moreno R, Jiang L, Moehle K, Zurbriggen R, Gluck R, Robinson JA, Pluschke G. Exploiting conformationally constrained peptidomimetics and an efficient human-compatible delivery system in synthetic vaccine design. *Chembiochem* 2001; **2**: 838–843.
28. Hodder AN, Crewther PE, Anders RF. Specificity of the protective antibody response to apical membrane antigen 1. *Infect. Immun.* 2001; **69**: 3286–3294.
29. Kocken CH, Narum DL, Massougbodji A, Ayivi B, Dubbeld MA, van der Wel A, Conway DJ, Sanni A, Thomas AW. Molecular characterisation of Plasmodium reichenowi apical membrane antigen-1 (AMA-1), comparison with P. falciparum AMA-1, and antibody-mediated inhibition of red cell invasion. *Mol. Biochem. Parasitol.* 2000; **109**: 147–156.
30. Anders RF, Crewther PE, Edwards S, Margetts M, Matthew ML, Pollock B, Pye D. Immunisation with recombinant AMA-1 protects mice against infection with Plasmodium chabaudi. *Vaccine* 1998; **16**: 240–247.
31. Nair M, Hinds MG, Coley AM, Hodder AN, Foley M, Anders RF, Norton RS. Structure of domain III of the blood-stage malaria vaccine candidate, Plasmodium falciparum apical membrane antigen 1 (AMA1). *J. Mol. Biol.* 2002; **322**: 741–753.
32. Mueller MS, Renard A, Boato F, Vogel D, Naegeli M, Zurbriggen R, Robinson JA, Pluschke G. Induction of parasite growth-inhibitory antibodies by a virosomal formulation of a peptidomimetic of loop I from domain III of Plasmodium falciparum apical membrane antigen 1. *Infect. Immun.* 2003; **71**: 4749–4758.
33. Poltl-Frank F, Zurbriggen R, Helg A, Stuart F, Robinson J, Gluck R,Pluschke G. Use of reconstituted influenza virus virosomes as an immunopotentiating delivery system for a peptide-based vaccine. *Clin. Exp. Immunol.* 1999; **117**: 496–503.
34. Waelti E, Wegmann N, Schwaninger R, Wetterwald A, Wingenfeld C, Rothen-Rutishauser B, Gimmi CD. Targeting her-2/neu with antirat Neu virosomes for cancer therapy. *Cancer Res.* 2002; **62**: 437–444.

