Technological Advances in Antigen Delivery and Synthetic Peptide Vaccine Developmental Strategies

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Abstract: Significant advances have been made in the field of peptide chemistry, especially in the design of synthetic peptide immunogens which has led to new concepts and strategies for human vaccine development. This article reviews some of these technological advances and approaches to vaccine design including the multiple antigenic peptide system, the lipid polylysine core peptide system, the polymerization of peptides into multivalent immunogens and self adjuvanting synthetic peptide-based immunogens.

INTRODUCTION

Vaccines are one of the most cost-effective public health interventions. This is illustrated by the worldwide eradication of smallpox by vaccination. In addition to infectious diseases, autoimmune disorders and some cancers may also be amenable to prophylactic and therapeutic treatment by vaccines. There is therefore a formidable incentive for scientists in designing new and improved vaccines, and for public health organizations in delivering vaccines to the community.

There are a number of problems associated with vaccine design and development, such as instability and the availability of suitable carriers and adjuvants for delivery to the immune system. Some of these problems have been overcome with current technological advances, and will be discussed. Furthermore, significant advances in the areas of biotechnology, biochemistry and peptide synthesis have led to the availability of large quantities of pure, potent and highly specific peptides, which can be used to generate the appropriate immune responses necessary to combat many human diseases. The ability to produce peptide antigens, however, surpasses that of vaccine adjuvant technology particularly with respect to the identification of human-compatible mucosal adjuvants. Vaccine adjuvants pose various problems including toxicity, hypersensitivity and short-term side effects. Attack by enzymes, instability in the gut, short retention times and the barriers to absorption and transport also represent some of the challenges confronting the oral delivery of vaccines.

Prior to a discussion of new advances in vaccine design and delivery, it is important to give a brief overview of the immune system with respect to the initiation of an immune response. The reader is referred to the following reviews for more detailed information [1, 2]. In this review a peptide, epitope or determinant is the usually short sequence of amino acids that is recognized by an antibody, T-cell receptor (TCR) or major histocompatibility complex (MHC)-class I or class II; an antigen is the epitope-containing structure recognized by these elements of the immune system and an immunogen is the structure capable of eliciting an immune response. The terms antigen and immunogen are often used interchangeably, as are peptide and epitope.

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INDUCTION OF AN IMMUNE RESPONSE AND THE ROLE OF T- AND B-CELLS

(Fig. **1**) illustrates the basic recognition events leading to the production of antibody in response to antigen. The first step in the generation of an antibody response is the uptake of antigen by antigen presenting cells (APC) and B-cells. These antigens then undergo proteolysis producing peptide fragments containing epitopes, some of which are bound by class II molecules of the MHC and are then transported to the APC and B-cell surface. Helper T-cells that possess TCRs capable of interacting with the epitope/class II MHC complexes can then bind to the APC, an event which is accompanied by additional cell-cell interactions through co-stimulatory molecules. The activated T-cell is now able to recognize those B-cells that display the same epitope/class II MHC complexes on their surfaces which have been acquired as a result of internalization of the same antigen through specific B-cell surface immunoglobulin (Ig) receptors. It is this interaction between T-cells and B-cells that results in triggering of the B-cell to differentiate into a plasma cell capable of secreting antibody of the same specificity as that of the original Ig receptor. Cytokines are also produced by each cell type which can influence the type of immune response elicited. To elicit an antibody response, the immunogen therefore must contain a helper T-cell epitope, which is a short linear sequence recognized by the TCR, as well as a B-cell epitope which is usually made up of a three

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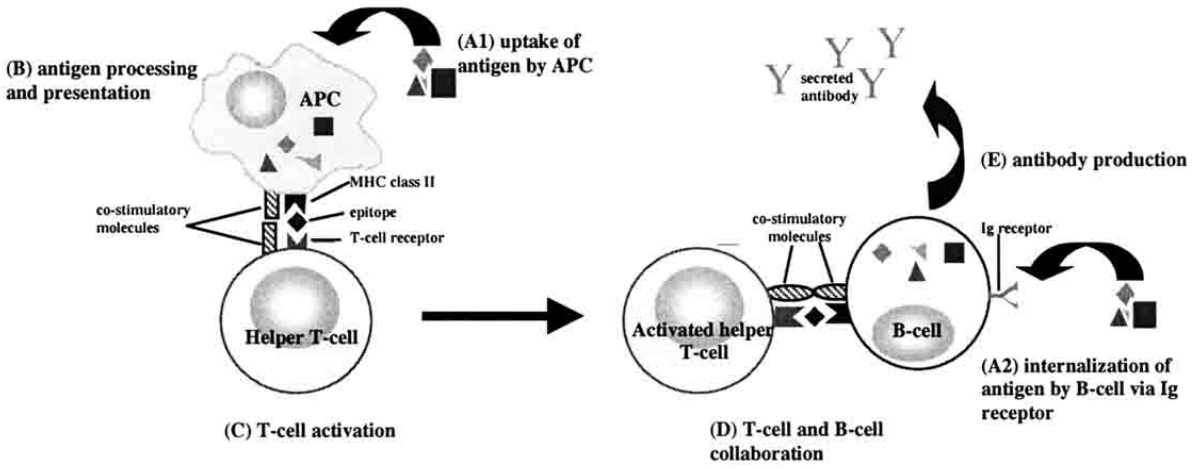

Fig. (1). The generation of an antibody response involves a number of steps. (A) The uptake of antigen by antigen presenting cells (APC) and the internalization of antigen by B-cells following recognition of a B-cell epitope by a specific immunoglobulin (Ig) receptor. (B) These antigens then undergo antigen processing to produce peptides that contain helper T-cell epitopes, some of which are bound by class II molecules of the major histocompatibility complex (MHC) and are then transported to the APC and B-cell surface. (C) Helper T-cells that possess T-cell receptors specific for the epitope/class II complexes bind to the APC which leads to T-cell activation following co-stimulation. (D) The activated T-cell recognizes B-cells that display the same epitope/class II complexes on their surfaces. (E) The interaction between T-cells and B-cells results in triggering of the B-cell to differentiate into a plasma cell capable of secreting antibody of the same specificity as that of the original Ig receptor.

dimensional conformational structure that is recognized by the Ig receptor.

In the case of viral infections and in many cases of cancer, the immune system responds by inducing cytotoxic T-cells (CTL) which cause the virus-infected or cancer cell to undergo apoptosis which leads to cell death. Like helper T-cells, CTL are first activated by interaction with APC bearing a specific epitope presented on the cell surface, in this case in association with MHC class I molecules [3]. The APC also receives signals from helper T-cells to upregulate the expression of co-stimulatory molecules. Once differentiated, the effector CTL can lyse cells bearing the same epitope/class I MHC complex.

VACCINATION AND THE IMMUNE SYSTEM

The goal of vaccination is to educate the immune system to recognize a non-virulent form of an infectious agent or a small portion of the pathogen such that when the immune system encounters the intact pathogen, a strong immune response is rapidly elicited to prevent illness. The use of defined antigenic determinants which play an important role in protective immunity is often preferable to the whole organism or protein antigen for several reasons, one of which is to eliminate the possibility of the induction of autoimmunity due to immunological cross-reactivity between foreign and host self proteins [4, 5]. As vaccine candidates, antigens or parts of antigens may be represented in several forms which include recombinant proteins, purified proteins or synthetic peptides [reviewed in 6]. Immunization with these immunogens can induce humoral and/or cellular immune responses specific for the epitope within the native antigen. This is essential where antibody is important for immunity but also in cases where CTL are required for the elimination of virus-infected or cancer cells.

The design of vaccines is often complicated by the polymorphism of MHC proteins [7], the essential presentation elements of the immune system, and also by the variability of pathogenic proteins. These two factors make it difficult to cover all host MHC types as well as multiple pathogenic serotypes in a single vaccine. An example of the problem of target antigen variability is evident in the design of a group A streptococcal (GAS) vaccine based on the M protein. The M protein is an α-helical coiled-coil bacterial surface protein and is the major virulence factor in GAS infection which allows the bacteria to escape destruction by phagocytosis in the absence of serotype-specific opsonic antibodies [8-10]. Vaccine strategies have therefore focused on the M protein in the induction of protective immunity to GAS. However, serotype-specific determinants of the M protein are located in the highly variable amino terminus with over 80 known GAS serotypes [8, 10]. Attempts have been made to solve the problem of variant target antigens by defining conserved epitopes within pathogenic proteins. In the case of the GAS M protein, there is >98% identity in the carboxyl terminus C-repeat region [8] and researchers have therefore focused on this region in developing GAS vaccines [11, 12]. GAS candidate vaccines based solely on the C-region have proved extremely promising in the mouse system in both protection from systemic GAS infection (unpublished observations) and in the reduction of nasopharyngeal GAS colonization [11, 12]. These approaches, however, do not address the issue of MHC diversity.

SYNTHETIC PEPTIDES AS IMMUNOGENS

Synthetic peptides representing individual B-cell epitopes are generally short peptide sequences which are poorly immunogenic either because conformational integrity, that is the correct three dimensional structure, is lacking in the B-cell epitope or because they do not contain an appropriate helper T-cell epitope required for antibody production. In addition, peptides may be rapidly degraded before recognition by the immune system. The immunogenicity of peptide vaccine candidates can be enhanced with an adjuvant that is designed to facilitate effective uptake and presentation of antigenic determinants by APCs and augment peptide-specific immune responses. An example of an adjuvant which has been used in experimental animal models is complete Freund's adjuvant (CFA) [13] which is, however, too reactogenic for human use. Current adjuvants which are approved for use in humans include the calcium and aluminium salts (phosphate or hydroxide) but in general these have little effect in adjuvanting peptides in the absence of appropriate T-cell help. Recent advances in peptide vaccine technology, however, have in some cases obviated the need for additional adjuvant by including non toxic, self adjuvanting moieties, and will be discussed later in this review.

THE NEED FOR CONFORMATIONAL INTEGRITY OF B-CELL EPITOPES

Peptide sequences do not usually contain sufficient structural information to fold correctly and consequently a major problem in vaccine design is to induce the correct conformational shape into synthetic peptide epitopes to induce antibody of the correct specificity. There are, however, approaches to mimicking conformational integrity of peptides that have proved successful. An example of a conformational-dependent epitope is found in the M protein of GAS [14]; the M protein is a coiled-coil α-helical surface protein of the bacterium and requires α-helical conformation in order to be recognized by antibody [8]. The minimal B- and T-cell epitopes within a GAS M protein conserved region peptide sequence (p145) that is recognized by antibodies in the sera of most adults living in areas of high GAS exposure were mapped using a series of overlapping chimeric peptides, and two reactive chimeric peptides were identified. These peptides, called J8 and J14, were each devoid of a potentially deleterious T-cell autoepitope but contained a protective B-cell epitope [15]. Synthetic peptides representing the p145-specific sequences alone did not possess the correct helical structure for B-cell epitope integrity but were immunogenic in mice when flanked by non-M protein yeast-derived GCN4 sequences, yielding chimeric peptides that possessed a propensity for folding into an α-helix [14, 15]. The resulting antibodies were opsonic and protective against GAS infection (unpublished observations). This highlights that B-cell epitope mapping of antigenic determinants within a protein is a prerequisite for the design of synthetic peptide-based vaccines, but also, that some knowledge of the three dimensional structure of the native antigen may be required.

SOURCE OF HELPER T-CELL EPITOPES

Carrier Proteins

Small synthetic peptides usually do not contain an appropriate helper T-cell epitope required to induce an antibody response and are therefore not effective vaccines by themselves. Such peptides, however, can be rendered immunogenic by conjugation to a carrier molecule which contains helper T-cell epitopes. Although carrier proteins are traditionally used as a source of helper T-cell epitopes, there are significant disadvantages to their use. For example, the use of carrier proteins can result in the induction of antibodies to the carrier protein itself and divert the response away from the peptide epitope of interest [16]. Antibody of irrelevant specificity may also be induced unless the immunogen is constructed with the individual epitopes in the correct order and orientation; this has been demonstrated in studies which assessed the antibody production of specific peptides conjugated to either the N- or C-terminus of the carrier protein, keyhole limpet hemocyanin [17]. Chemical coupling of peptide to a carrier protein can also lead to modification of the antigenic determinant(s) of interest [18]. Furthermore, it is difficult to control the coupling reaction between the peptide and the carrier leading to heterogeneous conjugates.

Synthetic Helper T-Cell Epitopes

The assembly of vaccines containing synthetic T-cell epitopes would solve some of the problems associated with the use of carrier proteins as a source of helper T-cell epitopes. Immune responses to peptide-based immunogens have been achieved in experimental models using a number of different strategies including non-specific polymerization of peptides [19] and assembly of linear tandem (T-cell epitope)-(B-cell epitope) constructs. For example, a synthetic peptide vaccine based on luteinizing hormone releasing hormone (LHRH), which plays a central role in the reproductive process, and a synthetic T-cell epitope was immunogenic in mice eliciting the production of antibodies and abolishing fertility [20]. Secondly, incorporation of a helper T-cell epitope from ovalbumin or sperm whale myoglobin led to the induction of a response to a B-cell epitope from foot-and-mouth disease virus [21]. It should be noted that there is also the potential for antibody generation against epitopes formed at the junction of the T-cell and B-cell fragments [22]. The use of synthetic peptides representing T-cell epitopes, however, may alleviate the problem of carrier protein-induced epitope suppression [23]. Furthermore, branched immunogens containing B- and T-cell epitopes have been shown to elicit higher titres of antibody than the corresponding tandem linear arrangement of epitopes [24]. The use of simple synthetic B- and T-cell epitope constructs, however, do not address MHC polymorphism and target antigen variability. The use of universal T-cell epitopes that bind to different MHC alleles represents a feasible approach to overcome such problems [25].

EPITOPE IDENTIFICATION USING SYNTHETIC PEPTIDE COMBINATORIAL LIBRARIES

An understanding of human B-cell and T-cell recognition phenomena is fundamental for the determination of immune response mechanisms and their role in the prevention and cause of human disease. In recent years, an understanding of B-cell and T-cell specificity has been advanced significantly by the development of mixture-based synthetic combinatorial libraries made up of millions of peptides with all possible sequence permutations of amino acids for a peptide of a certain length (for example, 64 million for a hexapeptide composed of the 20 amino acids) [26-28].

Although evidence indicates that the majority of antibodies recognize conformational regions of protein antigens, it has been shown that antibodies can cross-react with linear parts of the protein. Furthermore, although antibodies usually recognize shapes and not amino acid sequences *per se*, peptide libraries can be used effectively to define antibody specificity with a peptide not necessarily related to the native sequence. The use of synthetic peptide combinatorial libraries (SCL) is a technological advance which has the potential to identify novel epitopes (both B- and T-cell) and peptide vaccine candidates that may be important in inducing protective immunity to infection. A positional scanning SCL (PS-SCL) hexapeptide library contains 20 mixtures for each of X00000, 0X0000, 00X000, 000X00, 0000X0, and 00000X in which X in each of positions 1 to 6 is fixed [29]. For example, in X00000, position 1 is fixed and represented by any one of the 20 amino acids. The remaining amino acid residues are random. In total there are 20 x 6= 120 peptide mixtures with each PS-SCL containing 64 million peptides (20^6). For each PS-SCL the same peptides will be represented. By screening each peptide mixture for each of the fixed positions, the amino acids that are recognized can be identified and using an iterative process, a hexapeptide can be established in a single assay that gives maximum recognition [29].

SCL have previously been used for epitope mapping in the identification of antigenic peptide determinants recognized by monoclonal antibodies, the identification of receptor ligands, antimicrobial compounds and enzyme inhibitors [reviewed in 29] and the study of T-cell antigen recognition [27, 28].

THE MULTIPLE ANTIGENIC PEPTIDE SYSTEM

The multiple antigenic peptide (MAP) system [30] allows the assembly of multiple, but usually identical, peptide sequences attached to a core of branching lysine residues (Fig. **2**) to yield a multivalent construct. The MAP comprises a core matrix formed from a number of lysine residues. This core matrix presents terminal primary amine groups, each of which acts as a growth point for a synthetic peptide. Alternatively, a synthetic peptide can be attached to these amine groups by chemoselective ligation. Significantly higher antibody titres have been obtained by coupling immunogenic peptides to a polylysine core to form a MAP when compared to carrier protein-conjugated peptides [30] although adjuvant was still required. Despite the success of this technique in experimental models, a limitation of the method is the difficulty in assuring purity of the product. The synthetic process involves the construction of structures with high molecular weight. Because of the simultaneous synthesis of all epitopes, there is a risk that steric constraints and aggregation will reduce the fidelity of the sequences. A further disadvantage of the MAPS is that the number of different epitopes that can be introduced is limited.

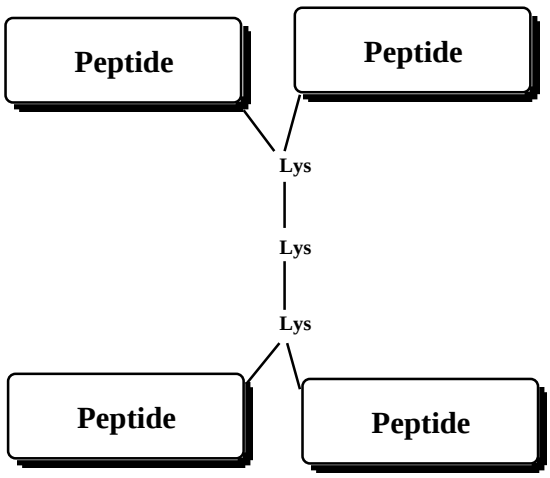

Fig. (2). Schematic representation of a tetravalent MAP formed by the addition of a lysine residue to each of the two primary amine groups of a central lysine residue, an arrangement that provides four primary amine groups from which separate peptides can be covalently coupled.

LIPOSOMES AND LIPOAMINO ACIDS

The delivery of vaccines or therapeutic drugs to the desired site of action in the body can be enhanced using liposomes, where the vaccine or drug is incorporated either by dispersing it in the intra-vesicular space or by anchoring it to the liposome wall [31]. A disadvantage of liposomes, however, is their limited bioavailability. Liposomes as a delivery system can be modified to increase their stability *in vivo* [32] and to enhance their immunogenicity and potential use as adjuvants for oral immunization [33]. Recently, a novel method has used biotin-coated liposomes to improve their delivery and retention in lymph nodes [34].

The synthesis of lipoamino acids has been described for increasing peptide immunogenicity [35]. Furthermore, lipoamino acid conjugates have the potential for oral delivery of peptides and drugs [36]. Another approach for the development of the lipoamino acid system is to exploit the particulate-forming properties of these amphiphiles, to develop particulate antigens. The amphipathic structure of these moieties gives rise to characteristic aggregation behaviour. NMR spectroscopy has demonstrated that lipoamino acids coupled to highly hydrophilic compounds (lactic, glycolic and gluconic acids) formed micelles in aqueous environments, and inverted micelles in the presence of organic solvents [37]. Preliminary experiments with a limited number of lipoamino acid constructs have

demonstrated their ability to form vesicles alone or in the presence of cholesterol [38]. The vesicle forming property of the system could be utilized for oral vaccine delivery.

There are a number of potential advantages to a combined vaccine-adjuvant particulate carrier system. For example, labile antigens can be protected within the particles from the degradative conditions of the gastrointestinal tract. In addition, oral particulate antigens generally provide a more effective stimulus for the induction of an immune response than do orally ingested soluble antigens [39]. The demonstrated accumulation of particles in the lymphoid tissues of the gastrointestinal tract [40] allows passive targeting of the antigens to the immunocompetent tissues of the gastrointestinal tract for the subsequent induction of a disseminated mucosal immune response [41]. Lipoamino acid vesicles, in contrast to liposomes, are prepared without phospholipid and thus may be less affected by the disruptive effects of pancreatic lipase - a major limitation in the use of liposomes for oral administration. The problem of leakage from liposomes and other types of particulate carrier are also avoided because the antigen does not have to be physically entrapped within a leaky bilayer but can be covalently bound to the vesicles. Finally, many modifications to the lipoamino acids are possible, allowing the preparation of vesicles with optimal stability, targeting and adjuvant properties for each potential antigen candidate.

LIPID POLYLYSINE CORE PEPTIDE SYSTEM

An advanced system was designed to enhance synthetic peptide immunogenicity by using a lipidic anchor moiety (lipoamino acid) together with the polylysine system, to form a lipid polylysine core peptide (LCP) [42] with potential adjuvant and carrier properties. In the LCP system, the lipopamino acids are incorporated as a lipidic anchor moiety at the C-terminal of a polylysine peptide system (Fig. **3**). LCPs can be readily synthesized in a single reaction vessel, by step-wise solid phase methods, without isolating any of the intermediates. Properties such as molecular weight, charge, lipophilicity, targeting moieties, and radiolabels can readily be varied in the LCP system. LCP-based vaccine candidates from the variable domains of *Chlamydia trachomatis* outer membrane protein were shown to significantly enhance peptide immunogenicity when compared to peptide monomers given alone in adjuvant [43].

CHEMICAL LIGATION

A solution to the problem of achieving homogeneity when MAPs are assembled is to synthesize and purify core and peptide epitopes separately and then chemically ligate the products. Cyclic, linear and branching templates have all been reported for the construction of synthetic peptide-based immunogens [44]. The ligation process can therefore be specifically directed to ensure that the correct final structure is achieved. Particular approaches have included the use of oxime chemistry [45] in which the specific reaction of aldehyde groups with aminooxy groups to form an oxime is used.

The use of ligation chemistries allows flexibility in the attachment of epitopes through different points of their sequence thereby determining orientation and overall structure of the molecule, which can influence the immune response that is elicited. Despite the fact that high purity products can be made using chemical ligation, a limitation of the technology is that only relatively small numbers of epitopes can be incorporated into a single molecule.

FREE RADICAL INDUCED POLYMERIZATION OF ACRYLOYL PEPTIDES INTO MULTIVALENT IMMUNOGENS

A method was recently developed for the assembly of large numbers of identical or different pure epitopes into polyvalent artificial proteins using free radical initiated polymerization [19]. Similar to the polymerization of acrylamide to form polyacrylamide, peptides acylated with the acryloyl group are polymerized to form peptide side chains that are pendant from an inert alkane backbone. In this way, large synthetic structures can be assembled while avoiding errors inherent in long sequential syntheses. Each peptide sequence is first assembled using conventional solid phase synthesis but before deprotection of the side chains

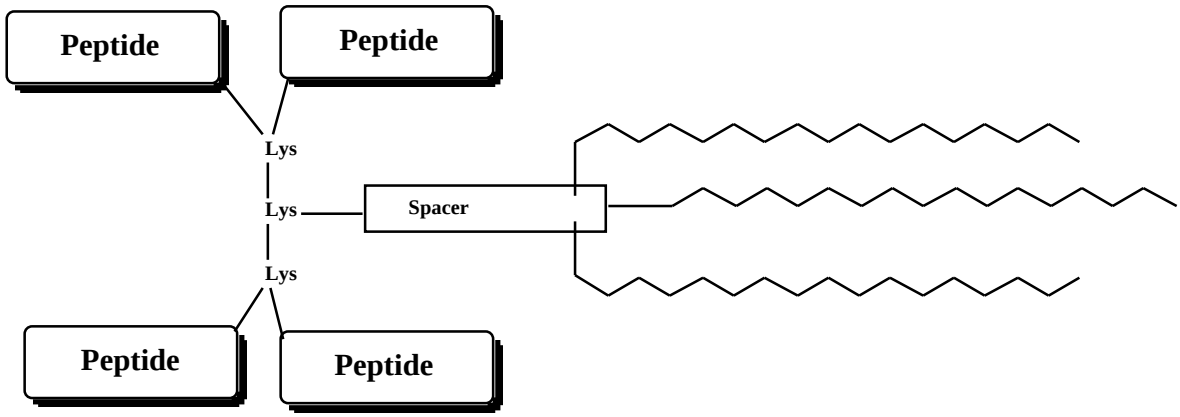

Fig. (3). Schematic representation of a tetravalent LCP system, where the MAP system is extended by a 3 alkyl chain containing lipophilic anchor via covalent chemical linkages.

and release from the solid support, the terminal amino group is acylated using acryloyl chloride (Fig. **4**). Following cleavage from the solid phase support, individual acryloyl peptides can be purified and then polymerized to yield polymers with peptides attached to the alkane backbone. In practice, the size of peptide epitopes prevents polymerization by steric hindrance and so acrylamide or a similar "spacer" is added to separate the pendant epitopes along the length of the backbone. An advantage of this approach, as with other chemical ligation strategies mentioned above, is the ability to purify individual defined epitopes prior to polymerization.

The immunological properties of polymeric epitopes produced by this type of free radical induced polymerization have been reported showing that their immunogenicity is superior to that of monomeric peptides [19]. More recently this approach has been used to co-polymerize peptide epitopes of GAS in a novel strategy to develop a new and improved GAS vaccine with broader strain coverage [46]. Multiple M protein peptides from both the amino terminal and conserved regions of the M protein were combined into a single immunogen, in the design of a multi-epitope "heteropolymer" vaccine [46]. The GAS vaccine was designed specifically to target GAS strains found in the Australian Aboriginal population, and contains the non-host cross-reactive C-region peptide, J14, in addition to seven opsonic amino terminal serotypic M protein peptide sequences found to be common in GAS-endemic communities of the Northern Territory. The rationale for the study was that GAS strains not represented on the heteropolymer by their amino terminal serotypic determinants would potentially be targeted by antibodies to the C-region. In addition, sera from Australian Aboriginals reacted to a number of these peptides in an age-related manner [46] indicating that immunity to these peptides may be important in protection from GAS infection and that protective immunity may potentially be mimicked by vaccination using these peptides. Complete protection from GAS infection was observed in mice immunized with heteropolymer and challenged with two different GAS strains [46]. While the data are encouraging, some limitations have been encountered with the use of this "heteropolymer" technology in vaccine delivery. The incorporation of peptides onto the alkane backbone is random and hence there is variation between heteropolymer syntheses which affects the immunogenicity of the individual peptides (unpublished observations). There is also the potential for steric hindrance with the addition of multiple peptides. In this regard, new approaches are currently being sought in the design of a multi-epitope synthetic GAS vaccine.

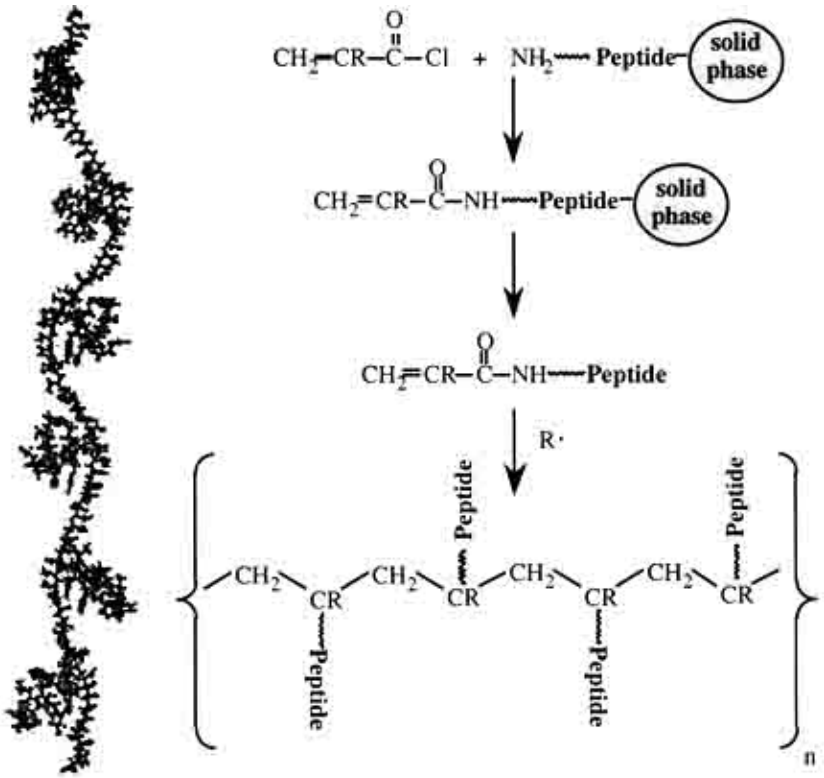

Fig. (4). Schematic of the reaction sequence used to acryloylate and subsequently polymerize individual purified peptide epitopes into high molecular weight polymers. The strategy lends itself to the co-polymerization of the same or different peptides as well as the inclusion of other materials into the backbone. The molecular model on the left hand side represents a part of a copolymer composed of a single type of peptide epitope and an acrylamide backbone. In this model, a ratio of acrylamide:peptide of 10:1 is represented and six epitopes pendant from the backbone are shown.

Using a different approach in the design of multivalent immunogens, Dale *et al.* [47, 48] have developed recombinant tetravalent and octavalent constructs containing epitopes from the amino terminal regions of different M protein serotypes. In each case, the vaccines were shown to be immunogenic in rabbits. Furthermore, the antisera produced were shown to be opsonic against the GAS strains represented in the recombinant constructs and there was no cross-reactivity with human tissues, indicating the potential use of these vaccines in inducing broadly protective immune responses. Theoretically, however, these vaccines would only provide protection against the GAS strains represented in the constructs, and are likely to be faced with the problems of steric hindrance and correct epitope folding that are associated with the addition of multiple peptide epitopes.

SELF ADJUVANTING SYNTHETIC PEPTIDE-BASED IMMUNOGENS

The incorporation of lipids into synthetic peptides has been shown to be capable of adjuvanting otherwise poorly immunogenic peptides and can elicit antibody and cellular responses [49, 50]. In lipopeptide constructs, a lipidic moiety with known adjuvanticity is chemically coupled to the peptide to generate a fully synthetic, totally characterized and potentially safe vaccine [51].

The most effective experimental adjuvants have an immunostimulatory component such as killed bacteria or a biologically active bacterial cell wall component such as bacterial lipopeptide, the lipid A portion of bacterial lipopolysaccharide or muramyl dipeptide from bacterial peptidoglycan. These components are known to be potent activators of macrophages [52, 53]. Efficient synthetic adjuvants based on these materials show promise for use with synthetic peptide vaccines.

Proteosomes are based on the outer membrane proteins of bacteria such as meningococci to which peptides can be incorporated forming vesicular structures which facilitate optimal antigen orientation for recognition by the immune system [reviewed in 54]. Proteosomes serve as both a carrier and adjuvant, and have been used to enhance the immunogenicity of a variety of vaccine antigens including proteins, peptides and lipopolysaccharides [54] particularly at the mucosal level [55, 56]. In addition, they are suitable for human use [57, 58].

In 1983, Wiesmuller, Bessler and Jung [59] described a synthetic analog of the N-terminal moiety of bacterial lipoprotein from *E.coli* (tripalmitoyl-S-glyceryl cysteine), termed Pam_3Cys (Fig. **5**). When this compound was covalently coupled to a peptide epitope, tetrameric forms of a MAP or polyoxime constructs, the resulting lipopeptide constructs were found to be potent immunogens with self adjuvanting properties enabling humoral and cellular responses irrespective of the route of administration [60-63].

Recently Pam_2Cys, an analogue of Pam_3Cys containing a free amino group has been synthesized [64] and shown to be the lipid moiety of MALP-2, a macrophage activating lipopeptide isolated from mycoplasmas lacking cell walls [52, 53]. It has been reported that Pam_2Cys is a more potent stimulator of splenocytes [64] and macrophages [52] than Pam_3Cys. Successful delivery of Pam_3Cys-containing MAP immunogens by the oral route has also been reported [61] and offers potential for vaccination not only against gastrointestinal diseases but also against pathogens which infect other mucosal sites of entry.

TRIS COUPLED LIPIDS IN VACCINE TECHNOLOGY

T-cells are essential components of the immune system and are responsible for immune surveillance and recognition of foreign pathogenic antigens like bacteria and viruses. Loss of "self-tolerance" and subsequent recognition of "self" antigens by T-cells leads to a large spectrum of autoimmune diseases including diabetes, psoriasis, multiple sclerosis and rheumatoid arthritis. Recently, it has been shown that this T-cell mediated response can be inhibited in a number of animal models of inflammation using small peptides from the TCR transmembrane region [65]. The mode of action is not known but is thought to involve the inhibition of assembly between the α chain of the TCR and the δ subunit of the CD3 complex which is closely associated with the TCR on the T-cell surface. The sequence of one of the peptides (a nonapeptide) represents part of the transmembrane region of the TCR α chain and contains four hydrophobic amino acids flanked by two positively charged

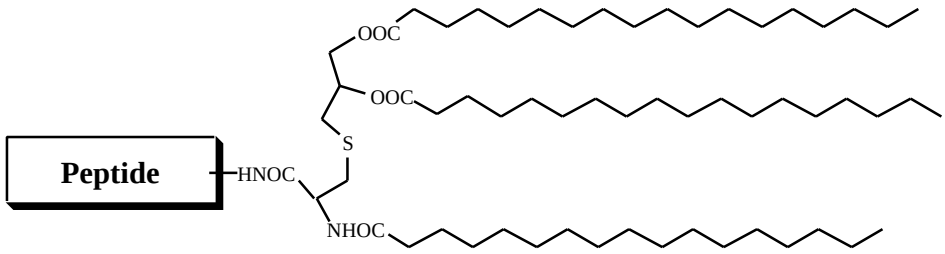

Fig. (5). Schematic representation of the Pam_3Cys/peptide system, where the antigenic peptide is covalently coupled to tripalmitoyl-S-[2,3-bis(palmitoyl)propyl]cysteine.

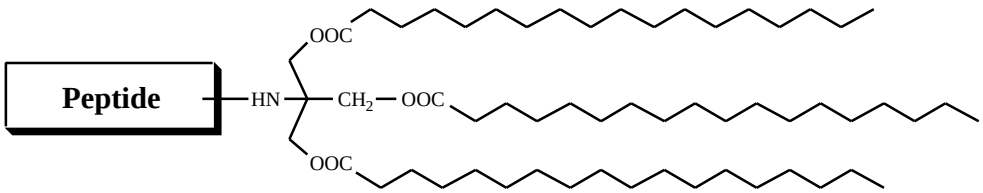

Fig. (6). Schematic representation of lipophilic adjuvant carriers utilizing tris, where the antigenic peptide is coupled to the amine function of the tris. The hydroxyl functions of the tris are acylated by three palmitoyl moieties providing the lipophilic anchor.

ones, arginine and lysine. When the C-terminal of the peptide was reacted with glycine-Tris-palmitates *via* an active ester to give the corresponding lipopeptides (Fig. **6**), inhibition of IL-2 production, a measure of T-cell function following antigen stimulation, was considerably enhanced. More dramatic was the protective effect against the induction of arthritis in an animal model (adjuvant-induced arthritis). Tris lipidation of peptides also has the potential for the delivery of DNA and drugs into cells [66].

SUMMARY

The advent of technological advances in antigen delivery has led to new strategies for the design and development of vaccines. This is especially highlighted by the progress in the design of synthetic peptide-based vaccines which have the potential to overcome a number of obstacles associated with current vaccine delivery technologies, especially pertaining to the issues of MHC and antigen diversity, immunogenicity and adjuvanticity. With the new technological advances, it can be envisaged that synthetic peptide-based vaccines will prove useful in the future.

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REFERENCES

[1] Parker D. T cell-dependent B cell activation. *Annu. Rev. Immunol.,* **1993**, *11*, 331-360.

[2] Allison JP. Structure, function, and serology of the T-cell antigen receptor complex. *Ann. Rev. Immunol.,* **1987**, *5*, 503-540.

[3] Rotzschke O.; Falk K.; Deres K.; Schild H.; Norda M.; Metzger J.; Jung G.; Rammensee H-G. Isolation and analysis of naturally processed viral peptides as recognized by cytotoxic T cells. *Nature,* **1990**, *348*, 252-254.

[4] Karlsen AE.; Dyrberg T. Molecular mimicry between non-self, modified self and self in autoimmunity. *Semin. Immunol.,* **1998**, *10*, 25-34.

[5] Froude J.; Gibofsky A.; Buskirk DR.; Khanna A.; Zabriskie JB. Cross-reactivity between streptococcus and human tissue: A model of molecular mimicry and autoimmunity. *Current Topics in Microbiology and Immunology,* **1989**, *145*, 5-26.

[6] Hess J.; Schaible U.; Raupach B.; Kaufmann SH. Exploiting the immune system: toward new vaccines against intracellular bacteria. *Advances in Immunology,* **2000**, *75*, 1-88 .

[7] Marsh SG. Nomenclature for factors of the HLA system.; update October 2000. *Hum. Immunol.,* **2000**, *61*, 1404-1405.

[8] Fischetti V. Streptococcal M protein. *Scientific American,* **1991**, *264*, 32-39.

[9] Robinson JH.; Kehoe MA. Group A streptococcal M protein: virulence factors and protective antigens. *Immunology Today*, **1992**, *13*, 362-367.

[10] Fischetti VA. Streptococcal M protein: Molecular design and biological behavior. *Clinical Microbiology Reviews*, **1989**, *2*, 285-314.

[11] Bessen D.; Fischetti VA. Synthetic peptide vaccine against mucosal colonization by group A streptococci. I. Protection against a heterologous M serotype with shared C repeat region epitopes. *J. Immunol.,* **1990**, *145*, 1251-1256.

[12] Bessen D.; Fischetti VA. Influence of intranasal immunization with synthetic peptides corresponding to conserved epitopes of M protein on mucosal colonization by group A streptococci. *Infect. Immunity,* **1988**, *56*, 2666-2672.

[13] Pruksakorn S.; Galbraith A.; Houghten RA.; Good MF. Conserved T and B cell epitopes on the M protein of group A streptococci: Induction of bactericidal antibodies. *J. Immunol.,* **1992**, *149*, 2729-2735.

[14] Relf WA.; Cooper J.; Brandt ER.; Hayman WA.; Anders RF.; Pruksakorn S.; Currie B.; Saul A.; Good MF. Mapping a conserved conformational epitope from the M protein of group A streptococci. *Peptide Research,* **1996**, *9*, 12-20.

[15] Hayman WA.; Brandt ER.; Relf WA.; Cooper J.; Saul A.; Good MF. Mapping the minimal murine T cell and B cell epitopes within a peptide vaccine candidate from the conserved region of the M protein of group A streptococcus. *Int. Immunol.,* **1997**, *9*, 1723-1733.

[16] Schutze M-P.; Leclerc C.; Jolivet M.; Audibert F.; Chedid L. Carrier-induced epitopic suppression, a major issue for future synthetic vaccines. *J. Immunol.*, **1985**, *135*, 2319-2322.

[17] Dyrberg T.; Oldstone MB. Peptides as antigens. Importance of orientation. *J. Exp. Med.*, **1986**, *164*, 1344-1349.

[18] Briand.; JP.; Muller S.; Van Regonmortel MHV. Synthetic peptides as antigens: pitfalls of conjugation methods. *J. Immunol. Meth.*, **1985**, *78*, 59.

[19] Jackson DC.; O'Brien-Simpson NM.; Ede NJ.; Brown LE. Free radical induced polymerization of synthetic peptides into polymeric immunogens. *Vaccine*, **1997**, *15*, 1697-1705.

[20] Ghosh S.; Jackson DC. Antigenic and immunogenic properties of totally synthetic peptide-based anti-fertility vaccines. *Int. Immunol.*, **1999**, *11*, 1103-1110.

[21] Francis MJ.; Hastings GZ.; Syred AD.; McGinn B.; Brown F.; Rowlands DJ. Non-responsiveness to a foot-and-mouth disease virus peptide overcome by addition of foreign helper T-cell determinants. *Nature*, **1987**, *300*, 168-170.

[22] Sharma P.; Kumar A.; Batni S.; Chauhan VS. Co-dominant and reciprocal T-helper cell activity of epitopic sequences and formation of junctional B-cell determinants in synthetic T:B chimeric immunogens. *Vaccine*, **1993**, *11*, 1321-1326.

[23] Etlinger HM.; Knorr R. Model using a peptide with carrier function for vaccination against different pathogens. *Vaccine*, **1991**, *9*, 512-514.

[24] Fitzmaurice CJ.; Brown LE.; McInerney TL.; Jackson DC. The assembly and immunological properties of non-linear synthetic immunogens containing T-cell and B-cell determinants. *Vaccine*, **1996**, *14*, 553-560.

[25] Panina-Bordignon P.; Tan A.; Termijtelen A.; Demotz S.; Corradin G.; Lanzavecchia A. Universally immunogenic T cell epitopes: promiscuous binding to human MHC class II and promiscuous recognition by T cells. *Eur. J. Immunol.*, **1989**, *19*, 2237-2242.

[26] Pinilla C.; Martin R.; Gran B.; Appel JR.; Boggiano C.; Wilson DB.; Houghten RA. Exploring immunological specificity using synthetic peptide combinatorial libraries. *Current Opinion in Immunology*, **1999**, *11*, 193-202.

[27] Hemmer B.; Vergelli M.; Pinilla C.; Houghten R.; Martin R. Probing degeneracy in T-cell recognition using peptide combinatorial libraries. *Immunol. Today*, **1998**, *19*, 163-168.

[28] Hemmer B.; Pinilla C.; Appel J.; Pascal J.; Houghten R.; Martin R. The use of soluble synthetic peptide combinatorial libraries to determine antigen recognition of T cells. *J. Pep. Res.*, **1998**, *52*, 338-345.

[29] Eichler J.; Houghten R.A. Generation and utilization of synthetic combinatorial libraries. *Molecular Medicine Today*, **1995**, 174-180.

[30] Tam J. P. Synthetic peptide vaccine design: synthesis and properties of a high-density multiple antigenic peptide system. *Proc. Natl. Acad. Sci. USA*, **1988**, *85*, 5409-5413.

[31] Kim CK.; Jeong EJ.; Kim MH. Comparison of in vivo fate and immunogenicity of hepatitis B surface antigen incorporated in cationic and neutral liposomes. *J. Microencapsul.*, **2000**, *17*, 297-306.

[32] Mahnke IR.; Rivera M.; Hong K.; Isdell F.; Steinman RM.; Pope M.; Stamatatos L. Presentation of proteins encapsulated in sterically stabilized liposomes by dendritic cells initiates CD8(+) T-cell responses in vivo. *Blood*, **2000**, *15*, 3505-3513.

[33] Venkatesan N.; Vyas SP. Polysaccharide coated liposomes for oral immunization-development and characterization. *Int. J. Pharm.*, **2000**, *10*, 169-177.

[34] Phillips WT.; Klipper R.; Goins B. Novel method of greatly enhanced delivery of liposomes to lymph nodes. *J. Pharmacol. Exp. Ther.*, **2000**, *295*, 309-313.

[35] Flinn N.; Danton M.; Hillery AM.; Wood IP.; Gibbons WA.; Toth I. A lipidic alpha-amino acid based synthetic adjuvant peptide complex for increasing immunogenicity of vaccines. *Biochem. Soc. Trans.*, **1994**, *22*, 1055-1058.

[36] Toth I. A novel chemical approach to drug delivery: lipid amino acid conjugates. *J. Drug Target*, **1994**, *2*, 217-239.

[37] Toth I.; Anderson GJ.; Hussain R.; Wood IP.; del Olmo Fernandez E.; Ward P.; Gibbons WA. Lipidic Peptides X. Synthesis.; structural and physico-chemical evaluation of lipidic amino acid conjugates with hydrophilic compounds. *Tetrahedron*, **1992**, *48*, 923-930.

[38] Drouillat B.; Hillery AM.; Dekany G.; Falconer R.; Wright K.; Toth I. Novel liposaccharide conjugates for drug and peptide delivery. *J. Pharm. Sci.*, **1998**, *87*, 25-30.

[39] Wilding IR.; Davis SS.; O'Hagan DT. Targeting of drugs and vaccines to the gut. *Pharmacol. Ther.*, **1994**, *62*, 97-124.

[40] Florence AT.; Hillery AM.; Hussain N.; Jani PU. Nanoparticles as carriers for oral peptide absorption: studies on particle uptake and fate. *J. Control. Rel.*, **1995**, *36*, 39-46.

[41] McGhee JR.; Mestecky J.; Detzbaugh MT.; Eldridge JH.; Hirasawa M.; Kiyono H. The mucosal immune system: from fundamental concepts to vaccine development. *Vaccine*, **1992**, *10*, 75-88.

[42] Toth I.; Danton M.; Flinn N.; Gibbons WA. A combined adjuvant and carrier system for enhancing synthetic peptides immunogenicity utilising lipidic amino acids. *Tetrahedron Lett.*, **1993**, *34*, 3925-3924.

[43] Zhong G.; Toth I.; Reid R.; Brunham RC. Immunogenicity evaluation of a lipidic amino acid-based synthetic peptide vaccine for *Chlamydia trachomatis*. *J. Immunol.*, **1993**, *151*, 3728-3736.

[44] Mutter M.; Tuchscherer GG.; Miller C.; et. al. Template-assembled synthetic proteins with four-helix-bundle topology. *J. Am. Chem. Soc.*, **1992**, *114*, 1463-1470.

[45] Nardin EH.; Calvo-Calle JM.; Oliveira GA.; Clavijo P.; Nussenzweig R.; Simon R.; Zeng W.; Rose K. *Plasmodium falciparum* polyoximes: highly immunogenic synthetic vaccines constructed by chemoselective ligation of repeat B-cell epitopes and a universal T-cell epitope of CS protein. *Vaccine*, **1998**, *16*, 590-600.

[46] Brandt ER.; Sriprakash KS.; Hobb RI.; Hayman WA.; Zeng W.; Batzloff MR.; Jackson DC.; Good MF. Novel multi-epitope strategy for a group A streptococcal vaccine designed for the Australian Aboriginal population. *Nature Medicine*, **2000**, *6*, 455-459.

[47] Dale JB.; Chiang EY.; Lederer W. Recombinant tetravalent group A streptococcal M protein vaccine. *J. Immunol.*, **1993**, *151*, 2188-2194.

[48] Dale JB.; Simmons M.; Chiang EC.; Chiang EY. Recombinant.; octavalent group A streptococcal M protein vaccine. *Vaccine*, **1996**, *14*, 944-948.

[49] Martinon F.; Gras-Masse H.; Boutillon C.; Chirat F.; Deprez B.; Guillet JG.; Gomard E.; Tartar A.; Levy JP. Immunization of mice with lipopeptides bypasses the prerequisite for adjuvant. Immune response of BALB/c mice to human immunodeficiency virus envelope glycoprotein. *J. Immunol.*, **1992**, *149*, 3416-3422.

[50] Sauzet JP.; Deprez B.; Martinon F.; Guillet JG.; Gras-Masse H.; Gomard E. Long-lasting anti-viral cytotoxic T lymphocytes induced in vivo with chimeric-multirestricted lipopeptides. *Vaccine*, **1995**, *13*, 1339-1345.

[51] BenMohamed L.; Gras-Masse H.; Tartar A.; Daubersies P.; Brahimi K.; Bossus M.; Thomas A.; Druilhe P. Lipopeptide immunization without adjuvant induces potent and long-lasting B, T helper, and cytotoxic T lymphocyte responses against a malaria liver stage antigen in mice and chimpanzees. *Eur. J. Immunol.*, **1997**, *27*, 1242-1253.

[52] Muhlradt PF.; Kiess M.; Meyer H.; Sussmuth R.; Jung G. Structure and specific activity of macrophage-stimulating lipopeptides from *Mycoplasma hyorhinis*. *Infect. Immunity*, **1998**, *66*, 4804-4810.

[53] Sacht G.; Marten A.; Deiters U.; Sussmuth R.; Jung G.; Wingender E.; Muhlradt PF. Activation of nuclear factor-kappaB in macrophages by mycoplasmal lipopeptides. *Eur. J. Immunol.*, **1998**, *28*, 4207-4212.

[54] Lowell GH. New Generation Vaccines, Second Edition, Revised and Expanded. Proteosomes for improved nasal, oral, or injectable vaccines. **1997**, Chapter *15*, 193-206.

[55] Levi R.; Aboud-Pirak E.; Leclerc C.; Lowell GH.; Arnon R. Intranasal immunization of mice against influenza with synthetic peptides anchored to proteosomes. *Vaccine*, **1995**, *14*, 1353-1359.

[56] Mallett CP.; Hale TL.; Kaminski RW.; Larsen T.; Orr N.; Cohen D.; Lowell GH. Intranasal or intragastric immunization with proteosome-*Shigella* lipopolysaccharide vaccines protects against lethal pneumonia in a murine model of *Shigella* infection. *Infect. Immunity*, **1995**, *63*, 2382-2386.

[57] Haneberg B.; Dalseg R.; Wedege E.; Hoiby EA.; Haugen IL.; Oftung F.; Andersen SR.; Naess LM.; Aase A.; Michaelsen TE.; Holst J. Intranasal administration of a meningococcal outer membrane vesicle vaccine induces persistent local mucosal antibodies and serum antibodies with strong bactericidal activity in humans. *Infect. Immunity*, **1998**, *66*, 1334-1341.

[58] Oftung F.; Naess LM.; Wetzler LM.; Korsvold GE.; Aase A.; Hoiby EA.; Dalseg R.; Holst J.; Michaelsen TE.; Haneberg B. Antigen-specific T-cell responses in humans after intranasal immunization with a meningococcal serogroup B outer membrane vesicle vaccine. *Infect. Immunity*, **1999**, *67*, 921-927.

[59] Wiesmuller KH.; Bessler W.; Jung G. Synthesis of the mitogenic S-[2,3-bis(palmitoyloxy)propyl]-N-palmitoyl-pentapeptide from *Escherichia coli* lipoprotein. *Hoppe Seylers Z. Physiol. Chem.*, **1983**, *364*, 593-606.

[60] Deres K.; Schild H.; Wiesmuller KH.; Jung G.; Rammensee HG. *In vivo* priming of virus-specific cytotoxic T lymphocytes with synthetic lipopeptide vaccine. *Nature*, **1989**, *342*, 561-564.

[61] Nardelli B.; Haser PB.; Tam JP. Oral administration of an antigenic synthetic lipopeptide (MAP-P3C) evokes salivary antibodies and systemic humoral and cellular responses. *Vaccine*, **1994**, *12*, 1335-1339.

[62] Mora AL.; Tam JP. Controlled lipidation and encapsulation of peptide as a useful approach to mucosal immunizations. *J. Immunol.*, **1998**, *161*, 3616-3623.

[63] Zeng W.; Jackson DC.; Murray J.; Rose K.; Brown LE. Totally synthetic lipid-containing polyoxime peptide constructs are potent immunogens. *Vaccine*, **2000**, *18*, 1031-1039.

[64] Metzger JW.; Wiesmuller KH.; Jung G. Synthesis of Nα-Fmoc protected derivatives of S-(2,3-dihydroxypropyl)-cysteine and their application in peptide synthesis. *Int. J. Pept. Protein Res.*, **1991**, *38*, 545-554.

[65] Manolios M.; Collier S.; Taylor J.; Pollard J.; Harrison LC.; Bender V. T-cell antigen receptor transmembrane peptides modulate T-cell function and T-cell mediated disease. *Nature Med.*, **1997**, *3*, 84-88.

[66] Lockett T.; Reilly W.; Manthey M.; Wells X.; Cameron F.; Moghaddam M.; Johnston J.; Smith K.; Francis C.; Yang Q.; Whittaker R. Tris lipidation: a chemically flexible technology for modifying the delivery of drugs and genes. *Clin. Exp. Pharmacol. Physiol.*, **2000**, *27*, 563-567.