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REVIEW ARTICLE

Peptide and Glycopeptide Dendrimers. Part I

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Abstract: Recent progress in peptide and glycopeptide chemistry make the preparation of peptide and glycopeptide dendrimers of acceptable purity, with designed structural and immunochemical properties reliable. New methodologies using unprotected peptide building blocks have been developed to further increase possibilities of their design and improve their preparation and separation. Sophisticated design of peptide and glycopeptide dendrimers has led to their use as antigens and immunogens, for serodiagnosis and other biochemical uses including drug delivery. Dendrimers bearing peptide with predetermined secondary structures are useful tools in protein *de novo* design. This article covers synthesis and applications of multiple antigen peptides (MAPs), multiple antigen glycopeptides (MAGs), multiple antigen peptides based on sequential oligopeptide carriers (MAP-SOCs), glycodendrimers and template-assembled synthetic proteins (TASPs). Part I deals with the development of various structural forms of MAPs as well as their application as antigens, immunogens, and for immunodiagnostic and biochemical purposes.

Keywords: dendrimers; multiple antigen peptides (MAPs); template assembled synthetic proteins (TASPs); sequential oligopeptide carriers (SOCs); regioselectively addressable functionalized templates (RAFTs); multiple antigen glycopeptides (MAGs); glycodendrimers

Contents

Abbreviations: BSA, bovine serum albumin; CS, circumsporozoite; CTL, cytotoxic T lymphocyte; DCC, *N*,*N*′-dicyclohexylcarbodiimide; FCA, Freund's complete adjuvant; HBTU, *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluorophosphate; HIV, human immunodeficiency virus; HOBt, 1-hydroxybenzotriazole; KLH, keyhole limpet hemocyanin; MAG, multiple antigen glycopeptide; MAP, multiple antigen peptide; MHC, major histocompatibility complex; RAFT, regioselectively addressable functionalized templates; RIA, radiolabelled immunosorbent assay; sialosyl-Tn antigen, NeuNAcα2→6GalNAcα1→O-Ser/Thr; SOC, sequential oligopeptide carrier; T antigen, Galβ1→3GalNAcα1→O-Ser/Thr; TASP, template assembled synthetic protein; Tn antigen, GalNAcα1→O-Ser/Thr; TT, tetanus toxoid.

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INTRODUCTION

Dendrimers, also known as arborols, or cascade, cauliflower, or starburst polymers are attractive molecules due to their unique structures and physico-chemical properties [1–6]. Dendrimers are highly branched macromolecules formed by successive reactions of polyfunctional monomers around a core, characterized by a large number of terminal groups. The number of publications dealing with dendrimeric molecules, their use as potential candidates in the study of molecular recognition, self-assembly processes, development of new materials, etc. [7,8], emerging every year, indicates that dendrimers are valuable molecules in modern chemistry.

Dendrimeric macromolecules distinguish themselves from normal polymers in two ways. Firstly, they are constructed from AB_n (where n usually equals 2 or 3) monomers which produce hyperbranched structures. Secondly, they are synthesized in an iterative fashion. The combination of these two principles leads to the production of non-linear, highly branched polymeric structures which may differ significantly in their properties from their linear analogs [6].

Two main synthetic routes for the synthesis of dendrimers have appeared since the initial report on this class of molecules by Vögtle in 1978 [9], a divergent and a convergent strategy.

In the divergent strategy the dendrimers are built up from the initiation core out to the periphery. In each synthetic step the number of monomer units added as well as the number of coupling reactions performed doubles (AB_2) or triples (AB_3) compared with the previous synthetic step. Each new layer of monomer units attached to a growing molecule is called 'generation' and their number equals the number of repetitive synthetic steps performed during the synthesis and can be simply determined by counting the number of branching points when one proceeds from the core to the periphery. The selection of building units, i.e. initiation core and monomer unit, is fundamental for determining shape, size, and multiplicity of molecule and also influences the progress of synthesis.

On the contrary, in the convergent strategy the dendrimers are built up in an opposite fashion, i.e. from the periphery toward the central core. A key feature of this strategy is that the number of coupling reactions needed to add each new generation is constant throughout the synthesis making the product easier to separate. Dendrimers prepared by the convergent strategy are considered to be more homogenous than those prepared by the divergent strategy. This is especially true for dendrimers of higher generations, where number of coupling reactions is high and the incomplete coupling occurs.

The synthetic availability of dendrimers in a wide range of sizes (i.e. generations) combined with their unique structure and structural diversity in repeating units ranging from pure hydrocarbons to peptides and coordination compounds makes them versatile building blocks for a wide range of applications. To this rapidly growing class of molecules belong for example PANAM™ and poly (propylene imine) dendrimers [5], water soluble polyacryl ether dendrimers [10], dendrophanes [11], dendritic ruthenium complexes [12], crown ether-based dendrimers [13], peptide dendrimers [14] and glycodendrimers [15], various metal dendrimers [16–18], more complex nanometer-sized knedel-like structures [19] etc. For further details see [20,21].

Peptide and glycopeptide dendrimers are highly branched structures of non-natural origin that hold promise in various biochemical and biomedical applications, e.g. as synthetic vaccines, adjuvants, artificial protein-like structures, in mimicking the ion-channel structure, in the study of inter-cellular interactions, and as drug carriers. Their molecules consist of two parts: (1) a core or a template bearing several branching segments that give to the molecule an unique spatial architecture and dendrimeric character, and (2) multiple copies of biologically active structures, e.g. peptide antigens and saccharides, attached onto the core, that provide the molecule with designed biological properties, respectively.

The important points in the history of development of peptide dendrimers were experiments of Tam *et al.* [14], and Mutter *et al.* [22]. Tam firstly utilized branched oligolysine cores, developed by Denkewalter *et al.* [23], as synthetic carriers for the preparation of fully synthetic antigens, and termed these constructs as **Multiple Antigen Peptides (MAPs)** [14,24]. On a model compound, consisting of octavalent heptalysine branched core bearing eight copies of 14-amino acid residues long sequence, derived from the human T-cell antigen receptor β-chain constant region, he showed its ability to elicit antibodies capable of interacting not only with synthetic antigen, but also with intact native β-chain protein. The convenience of this approach in the vaccine development has been since

then many times demonstrated by successful design of compounds capable of eliciting both humoral and cellular immune responses. The recent progress in carbohydrate chemistry has enabled the preparation of MAPs with glycopeptide antigens, generally termed as **Multiple Antigen Glycopeptides (MAGs)**. These molecules seem to be prospective candidates in tumour vaccine development [25,26].

In the design of synthetic antigens, a novel class of carriers, called **Sequential Oligopeptide Carriers (SOCs)**, have been recently described. Molecules of SOCs are linear analogues of tripeptide Lys-Aib-Gly sequential motif which fold into defined helicoid secondary structure which directs and facilitates the arrangement of antigens into defined spatial conformation [27].

Glycodendrimers, described by Roy [15], are another approach utilized in the preparation of glycosylated dendrimers. The major characteristic is the use of thiolated saccharides which are bound to various types of dendrimeric cores. Glycodendrimers are prospective in the revealing of principles of carbohydrate–protein interactions that are, for example, involved in the intercellular regulatory processes or can serve as potent inhibitors of viral infection.

To overcome the well known folding problem in the protein *de novo* design, Mutter *et al.* suggested **Template-Assembled Synthetic Proteins (TASPs)** [28]. TASP molecules are built up on templates with β-structural motif, which induce the folding of attached amphiphilic peptide blocks to a complex protein-like arrangement. TASPs with various packing arrangements such as 4-helix bundle or 3-helix bundle mimicking ion-channel structure have been described. Recently, the use of TASPs as potent antigens was demonstrated showing a new area for their use. With the new requirements on the flexibility in TASP design, novel types of templates with selectively addressable lysine side-chains, called Regioselectively Addressable Functionalized Templates (RAFTs), have been recently developed [29].

The aim of this article is to give the reader a unifying view on different groups of peptide and glycopeptide dendrimers and similar compounds, i.e. MAPs, MAGs, MAP-SOCs, glycodendrimers, and TASPs. The scope of this article did not allow us to cover all the details in this area and we had to reduce and choose selectively articles cited herein.

MULTIPLE ANTIGEN PEPTIDE SYSTEM

Since the early 1970s it has been known that synthetic peptides can induce production of antibodies reacting both with synthetic peptide antigens and their cognate sequences in native proteins [30–32]. Such specific anti-peptide antibodies are useful in laboratory practice for confirming *de novo* proteins, in exploring biosynthetic pathways, in probing structural functions of proteins, in development of new fully synthetic vaccines etc.

Generally, the use of synthetic peptides in the vaccine design attracts considerable interest due to their evident advantages over the classical systems: (a) safety, no infectious materials are required, (b) synthetic availability, (c) chemical purity and homogeneity, and (d) better chemical stability. Although these factors make them prospective in vaccine design, currently only several peptide vaccines have been developed [33,34]. This may be in part explained by the findings that small peptides are poor immunogens. Furthermore, the requirement for T- and B-cell epitopes to be present in immunogen demands that multiple copies of different epitopes have to be presented to the immune system in a single covalent structure.

A conventional approach traditionally used in the preparation of immunogens is based on the conjugation of protein carriers such as bovine serum albumin (BSA), keyhole limpet hemocyanin (KLH), tetanus toxoid (TT) and others with synthetic peptides [35–40]. This approach possess several disadvantages. (a) Although these conjugates can produce high titres of antibodies toward the antigen, they also produce irrelevant anti-protein carrier antibodies that usually make up the major portion of all antibodies risen to the conjugate; this phenomenon has been known as the immunodominance of a carrier. (b) The process of conjugation can affect antigenic and immunogenic properties of peptide antigens [41–43]. (c) Exposure of an individual to the peptide–protein conjugate can reduce the antibody response to the synthetic peptide by carrier-induced epitope specific suppression [44–46]. (d) These conjugates are chemically non-defined. (e) The weight of attached peptide is small in relation to the weight of protein carrier, and (f) peptides are conjugated to the protein carrier without ensuring regular distribution in the molecule.

Co-injection of B- and T-cell epitope-containing peptides [47–50] as well as the tandem assembly of helper T-cell and B-cell peptide epitopes [51–53] to bypass the use of peptide carrier conjugates for the

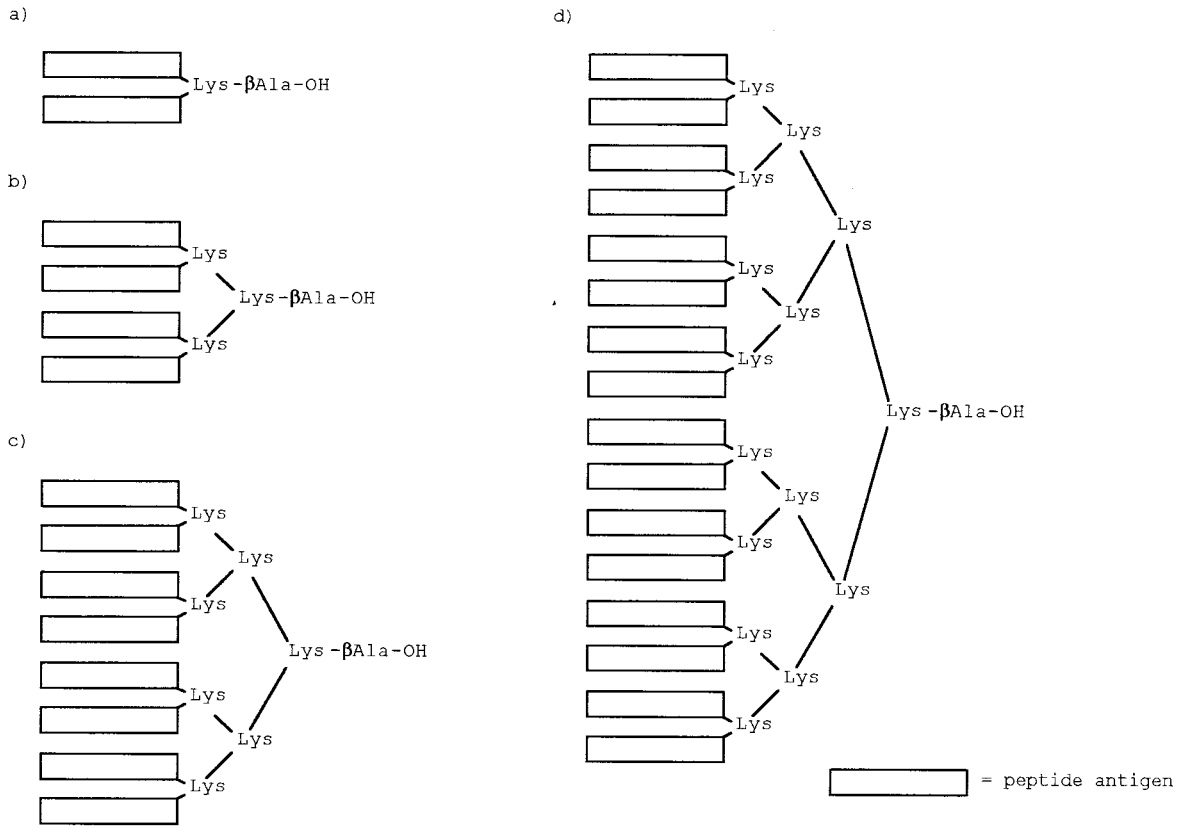

Figure 1 Schematic representation of (a) di-, (b) tetra-, (c) octa- and (d) hexadecavalent MAPs.

generation of specific antiserum have been also studied. Often low immunogenicity, in the case of co-injection, caused by the ineffectiveness of T-cell epitopes administered without prior covalent attachment to a particular B-cell epitope [54], and, in the case of tandem assembly, unresponsiveness due to the alternation in the relative position of the epitopes [55,56] or the presence of new undesirable antigen determinants in the place of linkage [57,58] make these approaches of limited use.

To avoid the above mentioned shortcomings, Tam *et al.* developed a totally synthetic **MAP** system [14,24] based on the use of a simple scaffolding of trifunctional amino acid lysine as a low-molecular synthetic carrier onto which multiple copies of peptide antigen are bound. The molecule of MAP consists of three structural features: (1) a simple amino acid such as glycine or β-alanine that stands for an internal standard, (2) an inner oligolysine core, and (3) multiple copies of synthetic peptide antigen. MAPs with two, four, eight or 16 copies of synthetic peptide antigens can be produced by utilizing oligolysine cores from one up to four sequential levels of lysine residues (see Figure 1). Generally used are tetra- and octavalent MAPs. Higher analogues are difficult to prepare and do not provide any improvement of immunological properties [59].

An effort to further elucidate the influence of core on the immunogenicity of MAPs led to the preparation of symmetrical cores with β-Ala-Lys dipeptide as a building unit, see Figure 2. No significant improvement of immunological properties has been detected, suggesting that asymmetrical cores are sufficient themselves and maybe more favourable for the presentation of antigens to the immune system. Moreover, the preparation of symmetrical cores was more complicated and additional steps including preparation and purification of dipeptide unit had to be added to the synthetic protocol [60]. Another modification is the preparation of cores with inserted linear non-natural amino acids, such as β-alanine [61] and γ-amino butyric acid [25]. Ranganathan *et al.* [62] recently described an interesting alternative to the oligolysine cores. This novel class of branched core is based on glutamic acid, with free carboxyl groups available for binding multiple copies of antigenic peptides. The disadvantage of this approach lies in the danger of racemization.

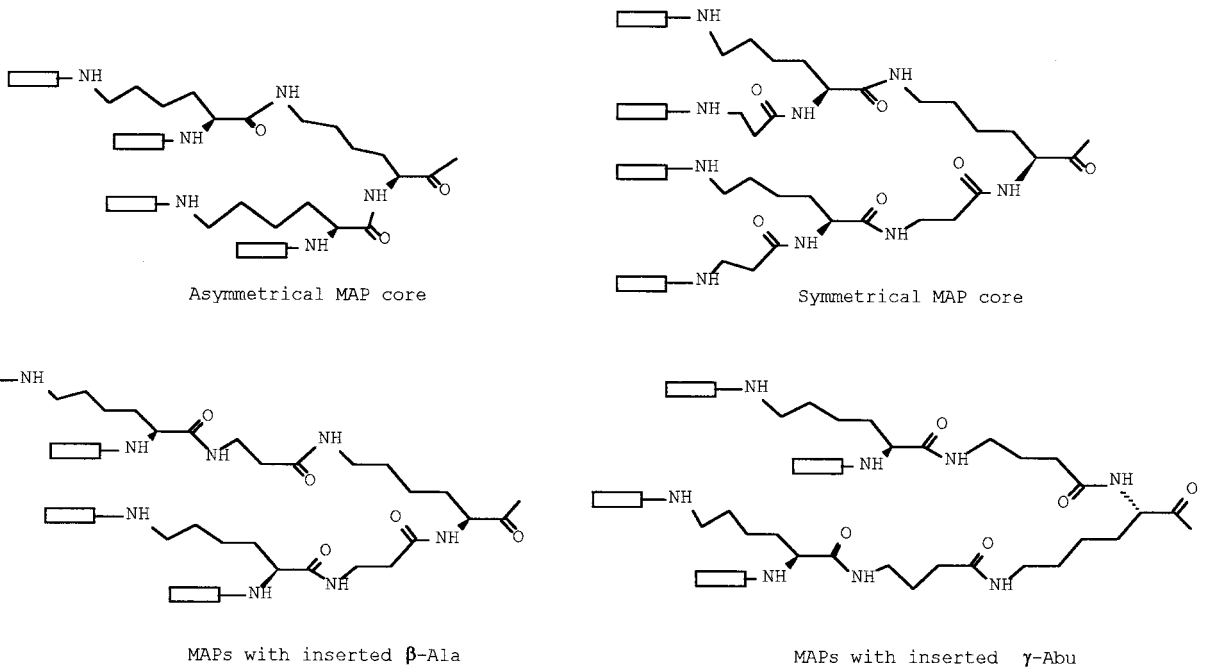

Figure 2 MAP cores with assymetrical and symmetrical design, and cores with inserted amino acids.

In the immunogen preparation MAPs offer several advantages over the peptide–protein conjugates. Briefly, (1) MAPs are synthetically available, (2) oligolysine cores are not immunogenic, (3) the ratio of antigen to carrier is defined and can be controlled by the design of the core, and (4) in MAPs, the core is small and the bulk is formed by a high density of regularly arranged peptide antigens; e.g. in the octavalent MAP the peptide antigen accounts for 92% of the total molecular weight, whereas in peptide–protein conjugates usually makes up less than 20%. Biological and physico-chemical properties of peptide–protein conjugates compared with MAPs, TASPs, and MAP-SOCs are given in Table 1.

Applications of MAPs

Popularity of MAPs for various biological and medicinal applications stems from the simplicity and unambiguity of their design, synthesis, and from their current commercial availability. Their favourable physico-chemical and biological properties are determining factors for their use in (1) antibody production and vaccine development [14,63], (2) immunoassays and serodiagnosis [59], (3) as inhibitors [64], (4) epitope mapping [65], and (5) for various biochemical studies [66,67].

MAPs in Antibody Production and Vaccine Development. MAPs are the subject of an increased interest for their use in antibody production and as prospective molecules for the preparation of the synthetic vaccines. Compared with peptide–protein conjugates, MAP constructs offer several indisputable advantages (see Table 1).

Briefly, strictly synthetic character ensures that unambiguous preparations will be studied. Branched character and predominance of the particular antigen in the molecule, that makes them immunologically focused and also confers to elimination of the immunodominance effect of a carrier, and the flexibility in B- and T-cell epitopes selection, are the main attributes that differentiate these two groups.

The first studies employing monoepitopic MAPs as antigenic structures revealed that such constructs were not always successful in eliciting humoral response even if they were, prior to their administration, emulsified in FCA (Freund's complete adjuvant) [68]. To enhance their immunogenicity, diepitopic MAPs bearing both B- and T-cell epitopes have been designed. B-cell epitopes provide the sequence and the shape against which the antibody is reactive. T-cell epitopes stimulate T-helper lymphocytes that influence the antibody

Table 1 Physico-Chemical and Biological Properties of Peptide–Protein Conjugates, MAPs, TASPs and MAP-SOCs

Properties	Peptide–protein conjugate	MAPs	TASPs	SOCs
Physico-chemical				
Structure	Branched, not defined	Branched, defined	Branched, defined	Branched, defined
Composition	Variable, difficult to control	Defined	Defined	Defined
Ratio of antigen to carrier	Variable and low	Defined and high	Defined and high	Defined and high
Stability	Unknown	Yes	Yes	Yes
Antigen amplification	Yes	Yes	Yes	Yes
Biological				
Need for conjugation	Yes	No	No	No
Ability to incorporate two or more epitopes	Questionable but possible, not defined	Yes, defined	Yes, defined	Yes, defined
Ability to incorporate built-in adjuvant	Questionable	Yes	Yes	Yes
Immunogenicity of the core	Yes	No	*	*
Source of Th epitopes	Yes	No, depends on the antigen peptide	*	*
Presence of not desired epitopes	High	Low	*	*

* Not described.

production in B-lymphocytes and induction of CTL, and thus T-cell mediated lysis. As T-cell epitopes, known universal or promiscuous T-helper epitopes from the same or extraneous source [69–72] are usually selected. MAPs with chimeric B-T or T-B constructs [63], B- and T-cell epitopes on particular arms [73], or dimers consisting of two monomeric MAPs, with bound B- or T-cell epitopes, attached to one another by a disulphide bridge [74] have been utilized to promote specific antibody response of antigen-specific B-cells [63,72,74,75] (see Figure 3). These studies revealed a real improvement of immunological properties in diepitopic MAPs. For example, in a study of synthetic vaccine model for hepatitis B [74] both monoepitopic and diepitopic MAPs, combining B-cell epitope (140–146 residue of the S protein) and T-cell epitope (12–26 residue of preS protein) were tested on mice and rabbits. Diepitopic MAPs were immunogenic in both mice and rabbits while monoepitopic MAPs with B-cell epitope elicited antibodies in rabbits only. In another study it was demonstrated that rabbits immunized with HIV-1 strain IIIB envelope protein rgp160 and boosted with diepitopic MAPs induced higher neutralizing titres and were superior in inducing both humoral and cellular reactivity than those boosted with rgp160 [76].

The arrangement, polarity and stochiometry of B- and T-cell epitopes also strongly affect the immunogenicity of MAPs [63,77,78]. In order to study this effect, Tam *et al.* prepared various monoepitopic and diepitopic MAPs in both tetra- and octavalent form, bearing B- (93–108 residue) and T-cell (265–276 residue) epitopes derived from the circumsporozoite (CS) protein of *Plasmodium berghei*, the rodent malaria parasite [63]. Diepitopic MAPs containing equimolar amounts of B- and T-cell epitopes elicited high antibody titres, while monoepitopic MAPs and BT monomer were not immunogenic in the A/J mice used in the experiment. Tetravalent MAPs with B epitope at the N-terminal part of the antigen, BT-(4), appeared to be the most efficient of all MAPs with chimeric antigens prepared. Furthermore, antibodies risen to BT-(4) protected from 80% against the intravenous challenge of the immunized mice with 2000 *P. berghei* sporozoites. Other tetra- and octavalent chimeric constructs offered only 50–60% protection, whereas monoepitopic MAPs as well as BT monomer showed no protectivity. The degree of protection correlated with the antibody titres obtained by the immunization [63]. Finally, MAPs were found to overcome the MHC class restriction in producing humoral response [79] and as potent immunogens capable of inducing long-lasting immunological memory [80].

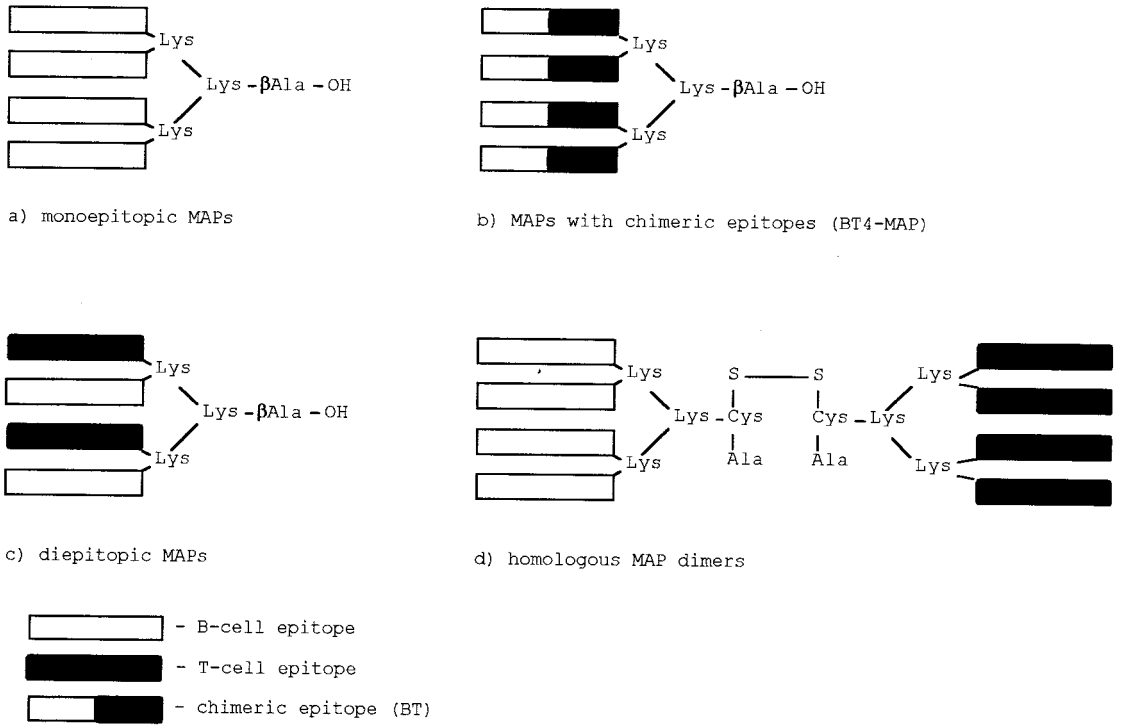

Figure 3 Schematic representation of (a) monoepitopic, (b) chimeric, (c) diepitopic, and (d) homologous MAP dimers.

Studies comparing the quality of antisera obtained by immunization with MAPs and peptide–protein conjugates show contradictory results [81]. In some studies antibodies elicited by MAPs cross-reacted with native protein, whereas those obtained by peptide–protein conjugates reacted poorly [82]. Moreover, several authors described opposite experiences. Their studies indicate poor cross-reactivity of anti-MAP antibodies to cognate sequences in native proteins, whereas peptide–protein conjugates elicited high titres of cross-reactive antibodies [83]. Whether this poor cross-reactivity is an intrinsic attribute of a given epitope sequence or is caused by inter-chain interactions in MAP format that influence the expression of the epitope to the immune system or selection of unsuitable and insufficiently immune antigens remains unclear.

The general trend is that sera obtained by immunization by peptides in MAP format are of higher titre and respond faster than those obtained by immunization with peptide–protein conjugate [84] (see Table 2).

Lipidated MAPs as a Novel Approach in Vaccine Design. In the vaccine design most of the MAP vaccine models have been directed toward the humoral response generating anti-peptide antibodies. MAPs without built-in adjuvant usually require Freund's complete adjuvant (FCA) [85,86] to elicit high titres of antibodies when administered to animal models. Its toxicity, pyrogenicity and ability to induce anterior uveitis in rabbits and arthritis in rats [87] make FCA unacceptable for use in human vaccines. The only adjuvants so far approved for humans are calcium and aluminium salts, which are known to be poor immunogens.

To eliminate the side effects of FCA and poor immunogenicity of alum salts, a strong effort has been made to identify new adjuvants favourable for the use in human vaccines. Muramyl dipeptide (MurNAc-L-Ala-D-iso-Gln, MDP) was identified as the minimal structure of FCA preserving its adjuvant activity [88–90] (see also reviews [91,92]). Similarly, the *N*-palmitoyl-S-[2,3-bis(palmitoyloxy)-(2RS)-propyl]-cysteine (tripalmitoyl-S-glyceryl-cysteine, Pam_3Cys, P3C) [93–95], which was derived from the immunologically active N-terminus of bacterial lipoprotein from the outer membrane of *Escherichia coli* and other Enterobacteriaceae was found as a potential candidate as adjuvant for vaccines [96].

Much attention has been paid to the use of P3C in the design of MAP vaccines [97]. P3C is a potent B-cell and macrophage activator [98,99], induces

Table 2 Recent Applications of MAPs in the Vaccine Design and Antibody Preparation (for Examples before 1995 see Review [136])

Antigen	Reference
CS protein	
*P. berghe*i	Verheul *et al.* [109]
P. falciparum	Ahlborg [73]
	Calvo-Calle *et al.* [137]
	Nardin *et al.* [138]
	Ahlborh *et al.* [139]
P. vivax	Herrera *et al.* [140]
P. yoelli	Wang *et al.* [141]
	Franke *et al.* [142]
B19, Parvovirus	Anderson *et al.* [143]
gp46, HTLV-1	Baba *et al.* [144]
Angiotensin II receptor AT2 type, mice	Železná *et al.* [145]
Spliceosome protein, human	James *et al.* [146]
Cyclin, human	Digweed *et al.* [147]
Phospholipase A, human	Sa *et al.* [148]
Kainate receptor, goldfish	Wo and Oswald [149]
IL-12, human; Amphiregulin, human; FALL-39, human	Ahlborg *et al.* [150]
V3, FIVA	Rigby *et al.* [151]
	Flynn *et al.* [152]
Pf332, *P. falciparum*	Ahlborg *et al.* [153]
	Ahlborg *et al.* [154]
NKTag, *Tetrahymena pyriformis*	Jaso-Friedmann *et al.* [155]
cRCP, chicken	Mahale *et al.* [156]
P126, *P. falciparum*	Gilardeau *et al.* [157]
Tn antigen, human cancer	Ježek *et al.* [25]
	Bay *et al.* [26]
	Ježek *et al.* [127]
	Velek *et al.* [129]
mPC-1, murine	Basak *et al.* [158]
Hemagglutinin, influenza virus	Rose *et al.* [159]
Prion protein (PrP), mouse	Yokoyama *et al.* [160]
ras V12, human carcinoma	Schott *et al.* [161]
VP1, JC virus	Aoki *et al.* [162]
CDR2 VH from anti-CD4 mAb, murine	Kanda *et al.* [163]
Poly(ADP-ribose) polymerase, higher eukaryots	Duriez *et al.* [164]
Protein M, Streptococci group A	Toth *et al.* [165]
Glucosyltransferase, *Streptococcus mutans*	Smith *et al.* [166]
gpS1, infectious bronchitis virus (IBV)	Jackwood *et al.* [167]
gp12, HIV-1	Singh *et al.* [168]

CTL *in vivo* [100], and can be used as a built-in adjuvant in MAP-P3C constructs [101]. The lipophilic character of MAP-P3C constructs enables further thousand-fold amplification of antigens by the Macromolecular Assemblage Approach by employing P3C as a lipidic membrane anchoring moiety in a lipidic matrix of liposomes or micelles [101].

The utility of P3C in the preparation of MAP vaccines was demonstrated by Defoort *et al.* [101]. Tetravalent MAPs with four copies of 24 amino acids long sequence derived from gp120 of HIV-1 (residue 308–331) with P3C at the C terminus (B1M-P3C) were prepared (see Figure 4) and administered both in free and liposomal form to rabbits and mice. In both animal models it showed humoral and T-cell response as measured by antibody production, cytolytic activity and IL-2 production [101,102]. The cytolytic response induced by MAPs only after one immunization was found to be superior to the response induced by a full cycle of immunization of B1M in FCA.

Furthermore, the ability of MAP-P3C constructs to elicit CTL response after a single injection, without the involvement of CD4 + T-lymphocytes [103], confirms previous findings that CD8 + cells are sufficient to control viral infection in the absence of CD4 + T-cells [104].

Another modification of MAPs toward targeting and delivery is the preparation of lipidated MAPs, lipoMAPs. The major advantages of the use of the simple monopalmitic acid–peptide conjugates are: (1) low costs of the fatty acid, (2) the coupling of lipid to peptide can be performed in the peptide synthesizer during the synthesis, and (3) standard peptide methodology can be used for purification.

Lipidation (palmitoylation) affects biodegradability, transportability through membranes, immunogenicity, etc. [105,106]. Site of acylation (palmitoylation) also influences the immunogenicity of peptide antigens [107]. S-palmitoylated peptides were found to be more immunogenic than *N*-palmitoylated ones and at least as immunogenic as KLH-peptide conjugates.

The influence of a linker, number and chirality of lipidated amino acids on the immunogenicity were studied. Tetravalent, symmetric lipoMAP with 18-amino acids long sequence (residue 312–329) derived from the V3 loop of gp120 of HIV-1 strain IIIB with a series of palmitoyl lysines (PL) or palmitoyl serines (PS) with alternating chirality of the D- and L-amino acids attached to the C-terminus by various linkers were prepared [108]. Hydrophilic linker, L-Ser-L-Ser, appears to be important since using the L-Ser-D-Ser linker which gives different orientation to the lipid did not produce the desired immunogenicity. Moreover, replacement of Lys(Palm) with Ser(Palm) also significantly reduces adjuvant effect of palmitoylation. In a series of

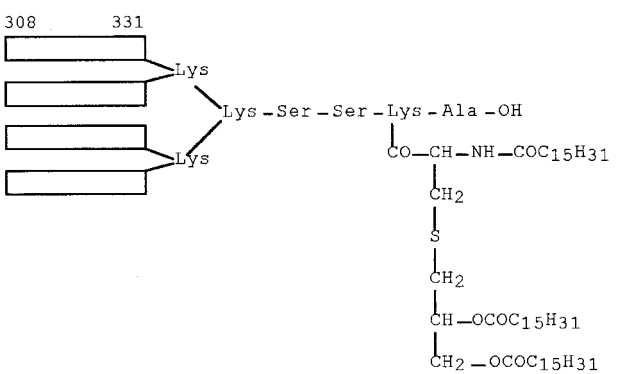

Figure 4 Tetravalent MAP with built-in adjuvant (B1M-P3C) [101].

derivatives from one up to four Lys(Palm) linked through the L-Ser-L-Ser linker, the lipoMAP with D-Lys(Palm)-L-Lys(Palm), B2SM-PL2, elicited the highest antibody responses. LipoMAPs were also versatile in inducing humoral response. Spleen cells of BALB/c mice immunized with B2SM-PL2 free and in liposomes showed strong cytolytic activity. These findings indicate that lipoMAPs may be a useful tool for the design of synthetic vaccines for humans [109,110].

Multiple Antigen Glycopeptides. The use of cancer vaccines to induce an anti-cancer immune response is an attractive idea for the treatment of cancer patients. This idea derives from the success of vaccinations in controlling viral infections. Since immune reaction is highly specific, vaccination is a preferable alternative to less specific treatments such as chemotherapy and radiation therapy.

Earlier studies on cancer utilized whole tumour cells or tumour cells extracts of uncertain composition. However, most of the studies were unsuccessful due to the poor understanding of the molecular nature of tumour antigens. Recent advances in immunology and in the identification of tumour-cell antigens have renewed the interest for the development of cancer vaccines. T (Galβ1 → 3GalNAcα1 → O-Ser/Thr), Tn (GalNAcα1 → O-Ser/Thr) and sialosyl-Tn (NeuNAcα2 → 6GalNAcα1 → O-Ser/Thr) structures have been recognized as tumour associated antigens [111,112] exclusively expressed on carcinoma associated mucins [111,113,114] (mucins are highly *O*-glycosylated, high molecular weight glycoproteins expressed on endodermal epithelial cells, particularly those showing glandular secretory activity). These antigens are usually expressed on normal cells in cryptic form. Incomplete glycosylation or neoglycosylation occurs in tumour cells and leads to the accumulation of these precursors or neostructures on the cell surface. Increased expression of Tn and sialosyl-Tn antigens has been correlated with tumour aggressiveness and poor prognosis in a number of epithelial tumours [113,115]. Tn antigen is expressed in over 70% of breast, lung, colon, and stomach carcinomas [111,113]. Tn antigens were found also on gp160 and gp120 of human immunodeficiency virus (HIV) and *in vitro* neutralization effect of anti-Tn antibodies was shown [116].

Several studies have shown some protection after immunization with these tumour antigens in experimental or clinical studies. In most of these studies tumour antigens linked to various protein carriers

were used [117–119]. As mentioned above, these conjugates are not unambiguous and produce irrelevant antibody responses. Still, the ideal vaccine should be totally synthetic, should not require any use of either carriers or extraneous adjuvants, and should protect after single administration and for a substantial period of time.

The synthetic preparation of tumour-associated antigens has been well described [120–124]. The chemistry of glycopeptides in general, and of *O*-glycopeptides especially, has been reviewed [125,126].

To circumvent the principal lack of use of protein conjugates we decided to use a fully synthetic vaccine model based on the MAP concept. We prepared MAGs [25,127] immobilized on undetachable biocompatible Tenta Gel S NH_2 [128] resin which acts as a synthetic hapten carrier for use in immunization. We synthesized several tetra- and octavalent MAGs both with and without inserted γ-Abu as spacers. Spacers were used to increase the mobility and accessibility of N-reactive ends as well as to study their influence on the coupling of bulky Tn antigens during SPPS. As a glycopeptide antigen we used *N*-acetylated Tn dimer with another γ-Abu spacer at the C-terminus (Ac-$(Tn)_2$-γ-Abu), to improve its accessibility to the immune system and thus immunogenicity.

$[Ac\text{-}(Tn)_2\text{-}\gamma\text{-}Abu]_4$-(Lys-X)$_2$-Lys-$\beta$-Ala-Tenta Gel (X = 0/$\gamma$-Abu), $[Ac(Tn)_2\text{-}\gamma\text{-}Abu]_8$-$(Lys\text{-}X)_4$-$(Lys\text{-}X)_2$-Lys-$\beta$-Ala-Tenta Gel (X = 0/$\gamma$-Abu) as well as corresponding negative standards, i.e. acetylated cores without antigens, were synthesized by Boc chemistry using standard DCC/HOBt or BOP coupling protocol [25]. Both tetravalent MAGs were readily synthesized, but during the synthesis of both octavalent MAGs we encountered difficulties during the coupling of Tn antigens. Repeated coupling steps as well as the use of aprotic solvent mixtures were necessary to achieve good coupling yields (for details see [25,127]).

To assess the antigenicity of our models we tested their specific recognition by mAb IgM anti-Tn (DAKO-HB-Tn1, DAKO, DK), anti-Tn/A (BRIC 66, IBGRL Research Products, UK) and anti-A (Birma-1, Gamma Biologicals). Both anti-Tn mAb recognized our models (DAKO-HB-Tn1 and BRIC 66) as determined by a rosetting test. Similar studies of Bay *et al.* [26] with tetravalent MAPs bearing chimeric epitopes $(BT)_4$-MAP, where the B-cell epitope represents one copy of Tn antigen and the T-cell epitope was the 103–115 sequence of the VP1 protein of poliovirus type 1, showed a good antibody recognition by mAb anti-Tn 83 D4 (IgM) and MLS 128 (IgG) which is in agreement with our own results.

More importantly, this concept seems to be promising for the vaccine model. An immunization study with octavalent MAG with inserted γ-Abu, $[Ac\text{-}(Tn)_2\text{-}\gamma\text{-}Abu]_8$-$(Lys\text{-}\gamma\text{-}Abu)_4$-$(Lys\text{-}\gamma\text{-}Abu)_2$-Lys-$\beta$-Ala-Tenta Gel, immobilized on Tenta Gel support, showed the ability of such a construct to elicit antibody response. BALB/c female mice (4–6 weeks old) were immunized with five doses, 300 μg each, at 3-week intervals. Afterwards, the sera and spleen cells were collected and the levels of anti-A as well as anti-Tn antibodies were assessed. Mice immunized with active compound showed a 100–1000 × increase in the titre of the agglutination reaction, whereas mice immunized with negative standard (without Ac-$(Tn)_2$-γ-Abu) did not show any significant increase of antibody level at all [127,129].

These studies show that our hypothesis is valid and that even small carbohydrate antigens alone can induce an antigen-specific immune response [130]. The selection of oligolysine carriers with or without γ-Abu spacers as well as the use of Tn dimers in the antigenic part are promising for the development of a fully synthetic anti-cancer vaccine. Specific recognition of our models makes them prospective molecules in affinity purification of Tn-related antibodies and also for immunodiagnosis. Currently, a detailed study with soluble analogues is underway.

Other Applications of MAPs. The multimeric format of MAPs makes them interesting also in other areas besides being immunogens in vaccine design (see Table 3). The utility of MAPs can be divided into several groups — immunoassays and serodiagnosis, inhibitors, epitope mapping, artificial proteins, intracellular delivery — for use in a variety of other biological applications including purification techniques.

Linear oligopeptides are known to have poor binding capacities to various plastic surfaces due to the lack of hydrophobic side-chains in their structures. The binding can also affect their antigenicity by employing particular epitopes in hydrophobic interactions with plastic surfaces. MAPs were found to possess better coating properties compared with linear analogues [131]. Only some of the multiple copies of a particular antigen in the molecule are employed in binding to a plastic surface, whereas others are exposed and thus available for effective interactions. This is especially advantageous for

Table 3 Applications of MAPs in Immunodiagnosis and Various Biochemical Uses

Application	Reference
Immunoassays and serodiagnosis	
Systemic lupus erythematosus	Caponi *et al.* [169]
HIV-1	Vogel *et al.* [170]
Hepatitis A virus	Firsova *et al.* [171]
EBV	Marchini *et al.* [172]
Inhibitors	
HIV-1 fusion and infection	Yahi *et al.* [173,174] Yahi *et al.* [175,176] Weeks *et al.* [177]
IL-6	Wallace *et al.* [178]
IL-2	Fassina *et al.* [179]
Fibronectin	Ingham *et al.* [180]
HeLa cells	Chillemi *et al.* [181]
Intracellular delivery	Flinn *et al.* [105] Sheldon *et al.* [182]
Molecular recognition	Wiegandt *et al.* [183]
Ab recognition by retro-, inverso peptides	Verdoliva *et al.* [184]
Purification methods	
Affinity purification of antibodies	Fassina *et al.* [133] Fassina *et al.* [185] Verdoliva *et al.* [186] Butz *et al.* [187]

solid-phase based immunoassays such as RIA and ELISA. The multimeric arrangement also improves avidity and coating efficiency and thus allows the detection of low affinity antibodies and identification of early stages of infections [132]. A multimeric nature is also favourable for the use of MAPs in affinity purification protocols [133]. Another promising application is their use in the design of inhibitors [134,135], where MAP format enables multiple-point contact and thus stronger binding compared with linear analogues.

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REFERENCES

1. J.M.J. Fréchet (1994). Functional polymers and dendrimers: reactivity, molecular architecture, and interfacial energy. *Science 263*, 1710–1715.
2. D.A. Tomalia and H.D. Durst (1993). Genealogically directed synthesis: starburst/cascade dendrimers and hyperbranched structures. *Top. Curr. Chem. 165*, 193–313.
3. H.-B. Mekelburger, W. Jaworek and F. Vögtle (1992). Dendrimers, arborols and cascade molecules: breakthrough into generations of new materials. *Angew. Chem. Int. Ed. Engl. 31*, 1571–1576.
4. N.N. Androin and D. Astruc (1995). Molecular trees — from syntheses towards applications. *Bull. Soc. Chim. Fr. 132*, 875–909.
5. D.A. Tomalia, A.M. Naylor and W.A. Goddard (1990). Starburst dendrimers: molecular-level control of size, shape, surface chemistry, topology, and flexibility from atoms to macroscopic matter. *Angew. Chem. Int. Ed. Engl. 29*, 138–175.
6. S.C. Stinson (1997). Delving into dendrimers. *Chem. Eng. News 75*, 28–30.

7. J.M. Lehn (1990). Perspectives in supramolecular chemistry – from molecular recognition towards molecular information processing and self-organization. *Angew. Chem. Int. Ed. Engl. 29*, 1304–1319.
8. J.M. Lehn (1994). Perspectives in supramolecular chemistry – from molecular recognition towards self-organisation. *Pure Appl. Chem. 66*, 1961–1966.
9. E. Buhleier, W. Wehner and F. Vögtle (1978). 'Cascade-' and 'Nonskid-Chain-like' syntheses of molecular cavity topologies. *Synthesis*, 155–158.
10. C.J. Hawker, K.L. Wooley and J.M.J. Fréchet (1993). Unimolecular micelles and globular amphiphiles: dendritic macromolecules as novel recyclable solubilization agents. *J. Chem. Soc., Perkin Trans. I*, 1287–1297.
11. S. Mattei, P. Seiler, F. Diederich and W. Gramlich (1995). Dendrophanes: water-soluble dendritic receptors. *Helv. Chim. Acta 78*, 1904–1912.
12. G.R. Newkome, R. Güther, C.N. Moorefield, F. Cardullo, L. Echegoyen, E. Pérez-Cordero and H. Luftmann (1995). Routes to dendritic networks: bis-dendimers by coupling of cascade macromolecules through metal centers. *Angew. Chem. Int. Ed. Engl. 34*, 2023–2026.
13. T. Nagasaki, O. Kimura, M. Ukon, S. Arimori, I. Hamachi and S. Shinkai (1994). Synthesis, metal-binding properties and polypeptide solubilization of 'crowned' arborols. *J. Chem. Soc., Perkin Trans. I*, 75–81.
14. J.P. Tam (1988). Synthetic peptide vaccine design: synthesis and properties of a high-density multiple antigenic peptide system. *Proc. Natl. Acad. Sci. USA 85*, 5409–5413.
15. R. Roy (1996). Glycodendrimers: a new class of biopolymers. *Polymer News 21*, 226–232.
16. G.R. Newkome, F. Cardullo, E.C. Constable, C.N. Moorefield and A.M.W. Cargill-Thompson (1993). Metallomicellanols: incorporation of ruthenium(II)-2,2′:6′,2″-terpyridine triads into cascade polymers. *J. Chem. Soc., Chem. Commun.*, 925–927.
17. E.C. Constable (1991). Helices, supramolecular chemistry and metal-directed self-assembly. *Angew. Chem. Int. Ed. Engl. 30*, 1450–1451.
18. J.W. Knapen, A.W. van der Made, J.C. de Wilde, P.W.N.M. van Leeuwen, P. Wijkens, D.M. Grove and G. van Koten (1994). Homogenous catalysts based on silane dendrimers functionalized with arylnickel(II) complexes. *Nature 372*, 659–663.
19. K.L. Wooley (1997). From dendrimers to knedel-like structures. *Chem. Eur. J. 3*, 1397–1399.
20. F. Zeng and S.C. Zimmerman (1997). Dendrimers in supramolecular chemistry: from molecular recognition to self-assembly. *Chem. Rev. 97*, 1681–1712.
21. G.R. Newkome, C.N. Moorefield and F. Vögtle (1996). *Dendritic Macromolecules: Concepts, Syntheses, Perspectives*, VCH, Weinheim, Germany.
22. M. Mutter, E. Altmann, K.-H. Altmann, R. Hersperger, P. Koziej, K. Nebel, G. Tuchscherer and S. Vuilleumier (1988). The construction of new proteins. Part III. Artificial folding units by assembly of amphiphilic secondary structures on a template. *Helv. Chim. Acta 71*, 835–847.
23. R.G. Denkewalter, J.F. Kolc and W.J. Lukasavage, US Pat. 4410688 (1983); *Chem. Abstr. 100*, (1984) 103 907 p.
24. D.N. Posnett, H. McGrath and J.P. Tam (1988). A novel method for producing anti-peptide antibodies. *J. Biol. Chem. 263*, 1719–1725.
25. J. Ježek, J. Velek, T. Trnka and M. Písačka. Solid phase synthesis of Tn antigens in both free and immobilized form, in: *Innovation and Perspectives in Solid Phase Synthesis and Combinatorial Libraries 1995*, R. Epton, Ed., p. 427–428, Mayflower Scientific Ltd., Birmingham, 1997.
26. S. Bay, R. Lo-man, E. Osinaga, H. Nakada, C. Leclerc and D. Cantacuzéne (1997). Preparation of a multiple antigen glycopeptide (MAG) carrying the Tn antigen. *J. Peptide Res. 49*, 620–625.
27. V. Tsikaris, C. Sakarellos, M.T. Cung, M. Marraud and M. Sakarellos-Daitsiotis (1996). Concept and design of a new class of sequential oligopeptide carriers (SOC) for covalent attachment of multiple antigenic peptides. *Biopolymers 38*, 291–293.
28. M. Mutter and S. Vuilleumier (1989). A chemical approach to protein design-template-assembled synthetic proteins (TASP). *Angew. Chem. Int. Ed.* Engl. *28*, 535–554.
29. P. Dumy, I.M. Eggleston, S. Cervigni, U. Sila, X. Sun and M. Mutter (1995). A convenient synthesis of cyclic peptides as regioselectively addressable functionalized templates (RAFT). *Tetrahedron Lett. 36*, 1255–1258.
30. R.A. Lerner (1982). Tapping the immunological repertoire to produce antibodies of predetermined specificity. *Nature 299*, 593–596.
31. J.G. Sutcliffe, T.M. Shinnick, N. Green and R.A. Lerner (1983). Antibodies that react with predetermined sites on proteins. *Science 219*, 660–666.
32. R. Arnon, E. Maron, M. Sela and C.B. Anfinsen (1971). Antibodies reactive with native lysozyme elicited by a completely synthetic antigen. *Proc. Natl. Acad. Sci. USA 68*, 1450–1455.
33. R.M. Hoksinson, R.D.G. Rigby, P.E. Mattner, V.L. Hyunh, M. D'Occhio, A. Neish, T.E. Trigg, B.A. Moss, M.J. Lindsey, G.D. Coleman and C.L. Schwartzkopf (1990). Vaxstrate: an anti-reproductive vaccine for cattle. *Aust. J. Biotechnol. 4*, 166–170.
34. M. Valero, L.R. Amador, C. Galindo, J. Figueroa, M.S. Bello, L.A. Murillo, A.L. Mora, G. Patarroyo, C.L. Rocha and M. Rojas (1993). Vaccination with SPf66, a chemically synthesised vaccine, against *Plasmodium falciparum* malaria in Colombia. *Lancet 341*, 705–710.
35. P.R. Hansen, H. Flyge, A. Holm, E. Lauritzen and B.D. Larsen (1996). Photochemical conjugation of peptides

to carrier proteins using 1,2,3-thiadiazole-4-carboxylic acid. *Int. J. Peptide Protein Res. 47*, 419–426.
36. G. Walter, K.H. Scheidtmann, A. Carbone, A.P. Laudano and R.F. Doolittle (1980). Antibodies specific for the carboxy- and amino-terminal regions of simian virus 40 large tumor antigen. *Proc. Natl. Acad. Sci. USA 77*, 5197–5120.
37. A.R. Neurath, S.B. Kent and N. Strick (1982). Specificity of antibodies elicited by a synthetic peptide having a sequence in common with a fragment of a virus protein, the hepatitis B surface antigen. *Proc. Natl. Acad. Sci. USA 79*, 7871–7875.
38. C.O. Jacob, R. Arnon and M.J. Sela (1985). Effect of carrier on the immunogenic capacity of synthetic cholera vaccine. *Mol. Immunol. 22*, 1333–1339.
39. G.H. Cohen, B. Dietzschold, M. Ponce de Leon, D. Long, E. Golub, A. Varrichio, L. Pereira and R.J. Eisenberg (1984). Localization and synthesis of an antigenic determinant of herpes simplex virus glycoprotein D that stimulates the production of neutralizing antibody. *J. Virol. 49*, 102–108.
40. E. Pfaff, M. Mussagy, H.O. Bohm, G.E. Schulz and H. Schaller (1982). Antibodies against a preselected peptide recognize and neutralize foot and mouth disease virus. *EMBO J. 1*, 869–874.
41. J.P. Briand, S. Muller and M.H. Van-Regenmortel (1985). Synthetic peptides as antigens: pitfalls of conjugation methods. *J. Immunol. Methods 78*, 56–69.
42. R. DiMarchi, G. Brooke, C. Gale, V. Cracknell, T. Doel and N. Mowat (1986). Protection of cattle against foot-and-mouth disease by a synthetic peptide. *Science 232*, 639–641.
43. H. MacArthur and G. Walter (1984). Monoclonal antibodies specific for the carboxy terminus of simian virus 40 large T antigen. *J. Virol. 52*, 483–491.
44. D. Di-John, S.S. Wasserman, J.R. Torres, M.J. Cortesia, J. Murillo, G.A. Losonsky, D.A. Herrington, D. Struchrer and M.M. Levine (1989). Effect of priming with carrier on response to conjugate vaccine. *Lancet 2*, 1415–1418.
45. M.P. Schutze, C. LeClerc, F. Jolivet, F. Audibert and L. Chedid (1985). Carrier-induced epitopic suppression, a major issue for future synthetic vaccines. *J. Immunol. 135*, 2319–2322.
46. A. Kumar, R. Arora, P. Kaur, V.S. Chauhan and P. Sharma (1992). 'Universal' T helper cell determinants enhance immunogenicity of a *Plasmodium falciparum* merozoite surface antigen peptide. *J. Immunol. 148*, 1499–1505.
47. D.M. Shaw, C.M. Stanley, C.D. Partidos and M.W. Steward (1993). Influence of the T-helper epitope on the titre and affinity of antibodies to B-cell epitopes after co-immunization. *Mol. Immunol. 30*, 961–968.
48. P. Sarobe, J.-J. Lasarte, J. Golvano, A. Gullon, M.-P. Civeira, J. Prieto and F. Borrás-Cuesta (1991). Induction of antibodies against a peptide hapten does not require covalent linkage between the hapten and a class II presentable T helper peptide. *Eur. J. Immunol. 21*, 1555–1558.
49. M. Bellone, P.I. Karachunski, N. Ostlie, S. Lei and B.M. Conti-Tronconi (1994). Preferential pairing of T and B cells for production of antibodies without covalent association of T and B epitopes. *Eur. J. Immunol. 24*, 799–804.
50. I. Prieto, S. Hervás-Stubbs, M. García-Granero, C. Berasain, J.I. Riezu-Boj, J.-J. Lasarte, P. Sarobe, J. Prieto and F. Borrás-Cuesta (1995). Simple strategy to induce antibodies of distinct specifity. Application to the mapping of gp120 and inhibition of HIV-1 infectivity. *Eur. J. Immunol. 25*, 877–883.
51. M.J. Francis, G.Z. Hastings, A.D. Syred, B. McGinn, F. Brown and D.J. Rowland (1987). Non-responsiveness to a foot-and-mouth disease virus peptide overcome by addition of foreign helper T-cell determinants. *Nature 330*, 168–170.
52. S. Sad, V.S. Chauhan, K. Arunan and R. Raghupathy (1993). Synthetic gonadotrophin-releasing hormone (GnRH) vaccines incorporating GnRH and synthetic T-helper epitopes. *Vaccine 11*, 1145–1150.
53. M. Srinivasan, S.Z. Domanico, P.T.P. Kaumaya and S.K. Pierce (1993). Peptides of 23 residues or greater are required to stimulate a high affinity class II-restricted T cell response. *Eur. J. Immunol. 23*, 1011–1016.
54. N.D. Zegers, C. van Holten, E. Classen and W.J.A. Boersma (1993). Peptide-induced memory (IgG) response, cross-reactive with native proteins, require covalent linkage of a specific B cell epitope with a T cell epitope. *Eur. J. Immunol. 23*, 630–634.
55. J.J. Golvano, J.J. Lasarte, P. Sarobe, A. Gullon, J. Prieto and F. Borass-Cuesta (1990). Polarity of immunogens: implications for vaccine design. *Eur. J. Immunol. 20*, 2363–2366.
56. C. Partidos, C. Stanley and M. Steward (1992). The effect of orientation of epitopes on the immunogenicity of chimeric synthetic peptides representing measles virus protein sequences. *Mol. Immunol. 29*, 651–658.
57. M.E. Levely, M.A. Mitchell and J.A. Nicholas (1990). Synthetic immunogens constructed from T-cell and B-cell stimulating peptides (T:B chimeras): preferential stimulation of unique T- and B-cell specificities is influenced by immunogen configuration. *Cell. Immunol. 125*, 65–78.
58. F. Ria, B.M.C. Chan, M.T. Scherer, J.A. Smith and M.L.Gefter (1990). Immunological activity of covalently linked T-cell epitopes. *Nature 343*, 381–383.
59. J.P. Tam and F. Zavala (1989). Multiple antigen peptide. A novel approach to increase detection sensitivity of synthetic peptides in solid-phase immunoassays. *J. Immunol. Methods 124*, 53–61.
60. W. Huang, B. Nardelli and J.P. Tam (1994). Lipophilic multiple antigen peptide system for peptide immunogen and synthetic vaccine. *Mol. Immunol. 31*, 1191–1199.

61. J. Bernillon and S.M. Wallach (1993). Design of a new multiple antigen peptide system using 9-fluorenylmethyloxycarbonyl (Fmoc) strategy. *Biotechnol. Tech. 7*, 603–608.
62. D. Ranganathan, S. Kurur, K.P. Madhusudanan, R. Roy and I.L. Karle (1998). Self-assembling bis-dendritic peptides: design, synthesis and characterization of oxalyl-linked bis-glutamyl peptides $[Glu_n(CO_2Me)_{n+1}–CO–]_2$; $n = 1, 3, 7$. *J. Peptide Res. 51*, 297–302.
63. J.P. Tam, P. Clavijo, Y.-A. Lu, V. Nussenzweig, R. Nussenzweig and F. Zavala (1990). Incorporation of T and B epitopes of the circumsporozoite protein in a chemically defined synthetic vaccine against malaria. *J. Exp. Med. 171*, 299–306.
64. G. Fassina and G. Cassani (1993). Peptide-based assay for the identification of endothelin-converting enzyme inhibitors. *Peptide Res. 6*, 73–78.
65. P. Simmonds, K.A. Rose, S. Graham, S.W. Chan, F. McOmish, B.C. Dow, E.A. Follett, P.L. Yap and H. Marsden (1993). Mapping of serotype-specific, immunodominant epitopes in the NS-4 region of hepatitis C virus (HCV): use of type-specific peptides to serologically differentiate infections with HCV types 1, 2, and 3. *J. Clin. Microbiol. 31*, 1493–1503.
66. G. Fassina (1992). Oriented immobilization of peptide ligands on solid supports. *J. Chromatogr. 591*, 99–106.
67. S. Butz, S. Rawer, W. Rapp and U. Birsner (1994). Immunization and affinity purification of antibodies using resin-immobilized lysine-branched synthetic peptides. *Peptide Res. 7*, 20–23.
68. J.P. Briand, C. Andre, N. Tuaillon, L. Herve, J. Neimark and S. Muller (1992). Multiple autoepitope presentation for specific detection of antibodies in primary biliary cirrhosis. *Hepatology 16*, 1395–1403.
69. F. Sinigaglia, M. Guttinger, S. Graham, S.W. Chan, F. McOrnish, B. Dow, E.A. Follette, P. Yap and H. Marsden (1993). Mapping of serotype-specific, immunodominant epitopes in the NS-4 region of hepatitis C virus (HCV): use of type-specific peptides to serologically differentiate infections with HCV types 1, 2, and 3. *J. Clin. Microbiol. 31*, 1493–1503.
70. P. Ho, D. Mutch, K. Winkel, A.J. Saul, G.I. Jones, T.J. Doran and C.M. Rzepczyk (1990). Identification of two promiscuous T cell epitopes from tetanus toxin. *Eur. J. Immunol 20*, 477–483.
71. V.S. Ivanov,L.N. Kulik, A.E. Gabrielian, L.D. Tchikin, A.T. Kozich and V.T. Ivanov (1994). Synthetic peptides in the determination of hepatitis A virus T-cell epitopes. *FEBS Lett. 345*, 159–161.
72. R.W. Tindle, G.J. Fernando, J.C. Sterling and I.H. Frazer (1991). A 'public' T-helper epitope of the E7 transforming protein of human papillomavirus 16 provides cognate help for several E7 B-cell epitopes from cervical cancer-associated human papillomavirus genotypes. *Proc. Natl. Acad. Sci. USA 88*, 5887–5891.
73. N. Ahlborg (1995). Synthesis of a diepitope multiple antigen peptide containing sequences from two malaria antigens using Fmoc chemistry. *J. Immunol. Methods 179*, 269–275.
74. J.P. Tam and Y.-A.Lu (1989). Vaccine engineering: enhancement of immunogenicity of synthetic peptide vaccines related to hepatitis in chemically defined models consisting of T- and B-cell epitopes. *Proc. Natl. Acad. Sci. USA 86*, 9084–9088.
75. B. Nardelli, J.P. Defoort, W. Huang and J.P. Tam (1992). Design of a complete synthetic peptide-based AIDS vaccine with a built-in adjuvant. *AIDS Res. Hum. Retroviruses 8*, 1405–1407.
76. M. Levi, U. Ruden, D. Birx, L. Loomis, R. Redfield, K. Lovgren, L. Akerblom, E. Sandstrom and B. Wahren (1993). Effects of adjuvants and multiple antigen peptides on humoral and cellular immune responses to gp160 of HIV-1. *J. Acquired Immune Defic. Syndr. 6*, 855–864.
77. J.M. Calvo-Calle, G.A. de Oliveira, P. Clavijo, M. Maracic, J.P. Tam, Y.-A. Lu, E.H. Nardin, R.S. Nussenzweig and A.H. Cochrane (1993). Immunogenicity of multiple antigen peptides containing B and non-repeat T cell epitopes of the circumsporozoite protein of *Plasmodium falciparum*. *J. Immunol. 150*, 1403–1413.
78. E.H. Nardin and R.S. Nussenzweig (1993). T cell responses to pre-erythrocytic stages of malaria: role in protection and vaccine development against pre-erythrocytic stages. *Annu. Rev. Immunol. 11*, 687–727.
79. A. Pessi, D. Valmori, P. Migliorini, C. Tougne, E. Bianchi, P.H. Lambert, G. Corradin and G. Del Giudice (1991). Lack of H-2 restriction of the *Plasmodium falciparum* (NANP) sequence as multiple antigen peptide. *Eur. J. Immunol. 21*, 2273–2276.
80. S.K. Chai, P. Clavijo, J.P. Tam and F. Zavala (1992). Immunogenic properties of multiple antigen peptide systems containing defined T and B epitopes. *J. Immunol. 149*, 2385–2390.
81. J-.P. Briand, C. Barin, M.H.V. Van Regenmortel and S. Muller (1992). Applications and limitations of the multiple antigen peptide (MAP) system in the production and evaluation of anti-peptide and anti-protein antibodies. *J. Immunol. Methods 156*, 255–265.
82. F. Troalen, A. Razafindratsita, A. Puisieux, T. Voeltzel, C. Bohuon, D. Bellet and J.M. Bidart (1990). Structural probing of human lutropin using antibodies raised against synthetic peptides constructed by classical and multiple antigen peptide system approaches. *Mol. Immunol. 27*, 363–368.
83. S.D. Mahale, J. Pereira, U. Natraj and K.S.N. Iyer (1996). Comparison of antibodies raised against the peptide 10–24 of chicken riboflavin carrier protein (cRCP) by classical and multiple antigen peptide (MAP) approaches. *J. Immunol. Methods 190*, 215–219.

84. G.W. McLean, A.M. Owsianka, J.H. Subak-Sharpe and H.S. Marsden (1991). Generation of anti-peptide and anti-protein sera. Effect of peptide presentation on immunogenicity. *J. Immunol. Methods 137*, 149–157.
85. J. Freund (1951). The effect of paraffin and mycobacteria on antibody formation and sensation. A review. *Am. J. Clin. Pathol. 21*, 645–656.
86. J. Freund, G.E. Thompson and M.M. Lipton (1955). Aspermatogenesis, anaphylaxis, and cutaneous sensitization induced in the guinea pig by homologous testicular extract. *J. Exp. Med. 101*, 591–603.
87. A. Adam. *Synthetic Adjuvants: Modern Concepts in Immunology Vol. 1*, C.A. Bona, Ed., p. 1–58, John Wiley & Sons, New York, 1984.
88. A. Adam, R. Ciorbaru, F. Ellouz, J.F. Petit and E. Lederer (1974). Adjuvant activity of monomeric bacterial cell wall peptidoglycans. *Biochem. Biophys. Res. Commun. 56*, 561–567.
89. F. Ellouz, A. Adam, R. Ciorbaru and E. Lederer (1974). Minimal structural requirements for adjuvant activity of bacterial peptidoglycan derivatives. *Biochem. Biophys. Res. Commun. 59*, 1317–1325.
90. S. Kotani, Y. Wanatabe, F. Kinoshita, T. Shimono, I. Morisaki, T. Shiba, S. Kusumoto, Y. Tarumi and K. Ikenaka (1975). Immunoadjuvant activities of synthetic *N*-acetyl-muramyl-peptides or -amino acids. *Biken J. 18*, 105–111.
91. A. Adam and E. Lederer (1984). Muramyl peptides: immunomodulators, sleep factors, and vitamins. *Med. Res. Rev. 4*, 111–152.
92. C. Leclerc and F.R. Vogel (1986). Synthetic Immunomodulators and synthetic vaccines. *Crit. Rev. Ther. Drug Carrier Syst. 2*, 353–406.
93. K.H. Wiesmüller, W.G. Bessler and G. Jung (1983). Synthesis of the mitogenic S-[2,3-bis-(palmitoyloxy)propyl]-*N*-palmitoyl pentapeptide from *Escherichia coli* lipopeptide. *Biol. Chem. Hoppe-Seyler's 364*, 593–606.
94. A. Reitermann, J. Metzgler, K.H. Wiesmüller, G. Jung and W.G. Bessler (1989). Lipopeptide derivatives of bacterial lipoprotein constitute potent immune adjuvants combined with or covalently coupled to antigen or hapten. *Biol. Chem. Hoppe-Seyler's 370*, 343–354.
95. J. Metzger, K.H. Wiesmüller, R. Schaude, W.G. Bessler and G. Jung (1991). Synthesis of novel immunologically active tripalmitoyl-S-glycerylcysteinyl lipopeptides as useful intermediates for immunogen preparations. *Int. J. Peptide Protein Res. 37*, 46–57.
96. V. Braun (1975). Covalent lipoprotein from the outer membrane of *Escherichia coli*. *Biochem. Biophys. Acta 415*, 335–377.
97. K.H. Wiesmüller, W.G. Bessler and G. Jung (1992). Solid phase peptide synthesis of lipopeptide vaccines eliciting epitope-specific B-, T-helper and T-killer cell response. *Int. J. Peptide Protein Res. 40*, 255–260.
98. F. Melchers, V. Braun, and C. Galanos (1975). The lipoprotein of the outer membrane of *Escherichia coli*: a B-lymphocyte mitogen. *J. Exp. Med. 142*, 473–482.
99. W.G. Bessler and B.P. Ottenbreit (1977). Studies on the mitogenic principle of the lipoprotein from the outer membrane of *Escherichia coli*. *Biochem. Biophys. Res. Commun. 76*, 239–246.
100. K. Deres, H. Schild, K.H. Wiesmüller, G. Jung and H.G. Rammensee (1989). *In vivo* priming of virus-specific cytotoxic T-lymphocytes with synthetic lipopeptide vaccine. *Nature 342*, 561–564.
101. J.-P. Defoort, B. Nardelli, W. Huang, D.D. Ho and J.P. Tam (1992). Macromolecular assemblage in the design of a synthetic AIDS vaccine. *Proc. Natl. Acad. Sci. USA 89*, 2879–2883.
102. J.-P. Defoort, B. Nardelli, W. Huang and J.P. Tam (1992). A rational design of synthetic peptide vaccine with built-in adjuvant. A modular approach for unambiquity. *Int. J. Peptide Protein Res. 40*, 214–221.
103. B. Nardelli and J.P. Tam (1993). Cellular immune responses induced by *in vivo* priming with a lipid-conjugated multimeric antigen peptide. *Immunology 79*, 355–361.
104. R.M.L. Buller, K.L. Holmes, A. Hugin, T.N. Frederickson and H.C. Morse III (1987). Induction of cytotoxic T-cell responses *in vivo* in the absence of CD4 helper cells. *Nature 328*, 77–79.
105. N. Flinn, S. Coppard, W.A. Gibbons, A. Shew, P. Arturson and I. Toth. Oral absorptions studies of lipidic conjugates of thyrotropin releasing hormone (TRH) and luteinizing hormone releasing hormone (LHRH), in: *Peptides: Chemistry, Structure and Biology*, P.T.P. Kaumaya and R.S. Hodges, Eds, p. 165–167, Mayflower Scientific Ltd., Kingswinford, 1996.
106. G. Zhong, I. Toth, R. Reid and R.C. Brunham (1993). Immunogenicity evaluation of a lipidic amino acid-based synthetic peptide vaccine for *Chlamydia trachomatis*. *J. Immunol. 151*, 3728–3736.
107. N.J.C.M. Beekman, W.M.M. Schaaper, G.I. Tesser, K. Dalsgaard, S. Kamstrup, J.M.P. Langeveld, R.S. Boshuizen and R.H. Meloen (1997). Synthetic peptide vaccines: palmitoylation of peptide antigens by a thioester bond increases immunogenicity. *J. Peptide Res. 50*, 357–364.
108. W. Huang, B. Nardelli and J.P. Tam (1994). Lipophilic multiple antigen peptide system for peptide immunogen and synthetic vaccine. *Mol. Immunol. 31*, 1191–1199.
109. A.F. Verheul, V. Udhayakumar, D.L. Jue, R.M. Wohlhueter and A.A. Lal (1995). Monopalmitic acid-peptide conjugates induce cytotoxic T cell responses against malarial epitopes: importance of spacer amino acids. *J. Immunol. Methods 182*, 219–226.
110. B. Nardelli, F.B. Haser and J.P. Tam (1994). Oral administration of an antigenic synthetic lipopeptide (MAP-P3C) evokes salivary antibodies and systemic humoral and cellular responses. *Vaccine 12*, 1335–1339.

111. G.F. Springer (1984). T and Tn, general carcinoma autoantigens. *Science 224*, 1198–1206.
112. S.I. Hakomori (1991). Possible functions of tumor-associated carbohydrate antigens. *Curr. Opin. Immunol. 3*, 646–653.
113. G.F. Springer and P.R. Desai. Pancarcinoma T and Tn epitopes: autoimmunogens and diagnostic markers that reveal incipient carcinomas and help establish prognosis, in: *Immunodiagnosis of Cancer*, 2nd Edition, R.B. Herberman and D.W. Mercer, Eds, p. 587–612, Marcel Dekker, New York, 1990.
114. J. Huang, J.C. Byrd, B. Siddiki, M. Yuan, E. Lau and Y.S. Kim (1992). Monoclonal antibodies against partially deglycosylated colon cancer mucin that recognize Tn antigen. *Dis. Markers 10*, 81–94.
115. G.F. Springer (1989). Tn epitope (*N*-acetyl-D-galactosamine-*O*-serine/threonine) density in primary breast carcinoma: a functional predictor of aggresiveness. *Mol. Immunol. 26*, 1–5.
116. J.E.S. Hansen, H. Clausen, S.L. Hu, J.O. Nielsen and S. Olofsson (1992). An O-linked carbohydrate neutralization epitope of HIV-1 gp 120 is expressed by HIV-1 env gene recombinant vaccinia virus. *Arch. Virol. 126*, 11–20.
117. A.K. Singhal, M. Fohn and S. Hakomori (1991). Induction of alpha-*N*-acetylgalactosamine-*O*-serine/threonine (Tn) antigen-mediated cellular immune response for active immunotherapy in mice. *Cancer Res. 51*, 1406–1411.
118. P.Y. Fung, M. Madej, R.R. Koganty and B.M. Longenecker (1990). Active specific inmmunotherapy of a murine mammary adenocarcinoma using a synthetic tumor-associated glycoconjugate. *Cancer. Res. 50*, 4308–4314.
119. F. Helling, S. Zhang, A. Shang, S. Adluri, M. Calves, R. Koganty, B.M. Longenecker, T.J. Yao, H.F. Oettgen and P.O. Livingston (1995). GM2-KLH conjugate vaccine: increased immunogenicity in melanoma patients after administration with immunological adjuvant QS-21. *Cancer. Res. 55*, 2783–2788.
120. M. Zheng, M. Gobbo, L. Biondi, F. Filira, S. Hakomori and R. Rocchi (1994). Synthetic immunochemistry of glycohexapeptide analogues characteristic of oncofetal fibronectin. Solid phase synthesis and antigenic activity. *Int. J. Peptide Protein Res. 43*, 230–238.
121. T. Toyokuni, B. Dean, S. Cai, D. Boivin, S. Hakomori and A.K. Singhal (1994). Synthetic vaccines: Synthesis of a dimeric Tn antigen-lipopeptide conjugate that elicits immune responses against Tn-expressing glycoproteins. *J. Am. Chem. Soc. 116*, 395–396.
122. M. Elofsson and L.A. Salvador (1997). Preparation of Tn and sialyl Tn building blocks used in Fmoc solid-phase synthesis of glycopeptide fragments from HIV gp120. *Tetrahedron 53*, 369–390.
123. B. Liebe and H. Kunz (1997). Solid-phase synthesis of a tumour-associated sialyl-Tn antigen glycopeptide with a partial sequence of the 'tandem repeat' of the MUC-1 mucin. *Angew. Chem. Int. Ed. Engl. 36*, 618–620.
124. J. Kihlberg and M. Elofsson (1997). Solid-phase synthesis of glycopeptides: immunological studies with T cell stimulating glycopeptides. *Curr. Med. Chem. 4*, 85–116.
125. H.G. Garg, K. von dem Bruch and H. Kunz (1994). Developments in the synthesis of glycopeptides containing glycosyl L-asparagine, L-serine, and L-threonine. *Adv. Carbohydr. Chem. Biochem. 50*, 277–310.
126. M. Meldal. Glycopeptide synthesis, in: *Neoglycoconjugates: Preparation and Applications*, Y.C. Lee and R.T. Lee, Eds, p. 145–198, Academic Press, San Diego, 1994.
127. J. Ježek, J. Velek, T. Trnka, M. Písačka and F. Mareček. Solid phase synthesis of glycopeptide dendrimers with Tn antigenic structure and their biological activities, in: *Peptides 1996*, R. Ramage and R. Epton, Eds, p. 503–504, Mayflower Scientific Ltd., London, 1998.
128. S. Butz, S. Rawer, W. Rapp and U. Birsner (1994). Immunization and affinity purification of antibodies using resin-immobilized lysine-branched synthetic peptides. *Peptide Res. 7*, 20–23.
129. J. Velek, J. Ježek, P. Vepřek, V. Velková, T. Trnka, J. Pecka and M. Písačka. Synthetic glycopeptide dendrimers with Tn antigenic structure: immunological study. Part II, in: *Innovation and Perspectives in Solid Phase Synthesis and Combinatoria1 Libraries 1997*, London, 1998 (in press).
130. T. Toyokuni and A.K. Singhal (1995). Synthetic carbohydrate vaccines based on tumour-associated antigens. *Chem. Soc. Rev. 24*, 231–242.
131. M. Marguerite, M. Bossus, C. Mazingue, I. Wolowczuk, H. Gras-Masse, A. Tartar, A. Capron and C. Auriault (1992). Analysis of antigenicity and immunogenicity of five different chemically defined constructs of a peptide. *Mol. Immunol. 29*, 793–800.
132. H.S. Marsden, A.M. Owsianka, S. Graham, G.W. McLean, C.A. Robertson and J.H. Subak-Sharpe (1992). Advantages of branched peptides in serodiagnosis. Detection of HIV-specific antibodies and the use of glycine spacers to increase sensitivity. *J. Immunol. Methods 147*, 65–72.
133. G. Fassina, A. Corti and G. Cassani (1992). Affinity enhancement of complementary peptide recognition. *Int. J. Peptide Protein Res. 30*, 549–556.
134. M. Nomizu, K. Yamamura, H.K. Kleinman and Y. Yamada (1993). Multimeric forms of Tyr-Ile-Gly-Ser-Arg (YIGSR) peptide enhance the inhibition of tumor growth and metastasis. *Cancer Res. 53*, 3459–3461.
135. P. Sinnis, P. Clavijo, D. Fenyo, B.T. Chait, C. Cerami and V. Nussenzweig (1994). Structural and functional properties of region II-plus of the malaria circumsporozoite protein. *J. Exp. Med. 180*, 297–306.

136. J.P. Tam. Synthesis and applications of branched peptides in immunological methods and vaccines, in: *Peptides: Synthesis, Structures, and Applications*, B.Gutte, Ed., p. 455–500, Academic Press, San Diego, 1995.
137. J.M. Calvo-Calle, J. Hammer, F. Sinigaglia, P. Clavijo, Z.R. Moya-Castro and E.H. Nardin (1997). Binding of malaria T cell epitopes to DR and DQ molecules *in vitro* correlates with immunogenicity *in vivo*: identification of a universal T cell epitope in the *Plasmodium falciparum* circumsporozoite protein. *J. Immunol. 159*, 1362–1373.
138. E.H. Nardin, J.M. Calvo-Calle, G.A. Oliveira, P. Clavijo, R. Nussenzweig, R. Simon, W. Zeng and K. Rose (1998). *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 16*, 590–600.
139. N. Ahlborg, E.H. Nardin, P. Perlmann, K. Berzins, R. Andersson (1998). Immunogenicity of chimeric multiple antigen peptides based on *Plasmodium falciparum* antigens: impact of epitope orientation. *Vaccine 16*, 38–44.
140. S. Herrera, C. De Plata, M. Gonzales, B.L. Perlaza, F. Bettens, G. Corradin and M. Arevalo-Herrera (1997). Antigenicity and immunogenicity of multiple antigen peptides containing *P. vivax* CS epitopes in Aotus monkeys. *Parasite Immunol. 19*, 161–170.
141. R. Wang, Y. Charoenvit, G. Corradin, R.L. Porrozzi, R. Hunter, G. Glenn, C.R. Alving, P. Church and S.K. Hoffman (1995). Induction of protective polyclonal antibodies by immunization with a *Plasmodium yoelii* circumsporozoite protein multiple antigen peptide vaccine. *J. Immunol. 154*, 2784–2793.
142. E.D. Franke, G. Corradin and S.L. Hoffman (1997). Induction of protective CTL responses against the *Plasmodium yoelii* circumsporozoite protein by immunization with peptides. *J. Immunol. 159*, 3424–3433.
143. S. Anderson, M. Momoeda, M. Kawase, S. Kajigaya and N.S. Young (1995). Peptides derived from the unique region of B19 parvovirus minor capsid protein elicit neutralizing antibodies in rabbits. *Virology 206*, 626–632.
144. E. Baba, M. Nakamura, K. Ohkuma, J. Kira, Y. Tanaka, S. Nakano and Y. Niho (1995). A peptide-based human T cell leukemia virus type I vaccine containing T and B cell epitopes that induces high titers of neutralizing antibodies. *J. Immunol. 154*, 399–412.
145. B. Železná, L. Veselský and J. Velek (1997). Antibody against angiotensin II receptor AT2 type as a tool for the study of receptor function. *Rev. Clin. Pharm. Pharmacokinet. Int. Ed. 11*, 85–87.
146. J.A. James, T. Gross, H.R. Scofield and J.B. Harley (1995). Immunoglobulin epitope spreading and autoimmune disease after peptide immunization: Sm B/B′-derived PPPGMRPP and PPPGIRGP induce spliceosome autoimmunity. *J. Exp. Med. 181*, 453–461.
147. M. Digweed, U. Gunthert, R. Schneider, H. Seyschab, R. Friedl and K. Sperling (1995). Irreversible repression of DNA synthesis in Fanconi anemia cells is alleviated by the product of a novel cyclin-related gene. *Mol. Cell. Biol. 15*, 305–314.
148. G. Sa, G. Murugesan, M. Jaye, Y. Ivashchenko and P.L. Fox (1995). Activation of cytosolic phospholipase A2 by basic fibroblast growth factor via a p42 mitogen-activated protein kinase-dependent phosphorylation pathway in endothelial cells. *J. Biol. Chem. 270*, 2360–2366.
149. Z.G. Wo and R.E. Oswald (1995). A topological analysis of goldfish kainate receptors predicts three transmembrane segments. *J. Biol. Chem. 270*, 2000–2009.
150. N. Ahlborg, S. Paulie and S. Braesch-Andersen (1997). Generation of antibodies to human IL-12 and amphiregulin by immunization of Balb/c mice with diepitope multiple antigen peptides. *J. Immunol. Methods 204*, 23–32.
151. M.A. Rigby, N. Mackay, G. Reid, R. Osborne, J.C. Neil and O. Jarrett (1996). Immunogenicity of a peptide from a major neutralising determinant of the feline immunodeficiency virus surface glycoprotein. *Vaccine 14*, 1095–1102.
152. J.N. Flynn, C.A. Cannon, G. Reid, M.A. Rigby, J.C. Neil and O. Jarrett (1995). Induction of feline immunodeficiency virus-specific cell-mediated and humoral immune responses following immunization with a multiple antigenic peptide from the envelope V3 domain. *Immunology 85*, 171–175.
153. N. Ahlborg, R. Andersson, P. Perlmann and K. Berzins (1996). Immune responses in congenec mice to multiple antigen peptides based on defined epitopes from the malaria antigen Pf332. *Immunology 88*, 630–635.
154. N. Ahlborg, J. Iqbal, M. Hansson, M. Uhlen, D. Mattei, P. Perlmann, S. Stahl and K. Berzins (1995). Immunogens containing sequences from antigen Pf332 induce *Plasmodium falciparum*-reactive antibodies which inhibit parasite growth but not cytoadherence. *Parasite Immunol. 17*, 341–352.
155. L. Jaso-Friedmann, J.H. Leary, Z. Weisman and D.L. Evans (1996). Activation of nonspecific cytotoxic cells with a multiple antigenic peptide: specifity and requirements for receptor crosslinkage. *Cell. Immunol. 170*, 195–201.
156. S.D. Mahale, J. Pereira, U. Natraj and K.S. Iyer (1996). Comparison of antibodies raised against the peptide 10–24 of chicken riboflavin carrier protein (cRCP) by classical and multiple antigen peptide (MAP) approaches. *J. Immunol. Methods 190*, 215–219.
157. M. Gilardeau Truffinet, M. Bossus, D. Camus, P. Delplace, C. Mazingue, E. Diesis, A. Tartar, S.

Moreau, H. Gras-Masse and D.M. Banic (1996). Induction of antibodies against the *Plasmodium falciparum* pl26 antigen in non-responder H-2b and partial-responder H-2d mice using synthetic peptides. *Peptide Res. 9*, 61–70.

158. A. Basak, A. Boudreault, A. Chen, M. Chretien, N.G. Seidah and C. Lazure (1995). Application of the multiple antigenic peptides (MAP) strategy to the production of prohormone convertases antibodies: synthesis, characterization and use of 8-branched immunogenic peptides. *J. Peptide Sci. 1*, 385–395.
159. K. Rose, W. Zeng, L.E. Brown and D.C. Jackson (1995). A synthetic peptide-based polyoxime vaccine construct of high purity and activity. *Mol. Immunol. 32*, 1031–1037.
160. T. Yokoyama, K. Kimura, Y. Tagawa and N. Yuasa (1995). Preparation and characterization of antibodies against mouse prion protein (PrP) peptides. *Clin. Diagn. Lab. Immunol. 2*, 172–176.
161. M.E. Schott, D.T. Well, J. Schlom and S.I. Abrams (1996). Comparison of linear and branched peptide forms (MAPs) in the introduction of T helper responses to point-mutated ras immunogens. *Cell. Immunol. 174*, 199–209.
162. N. Aoki, M. Mori, K. Kato, Y. Sakamoto, K. Noda, M. Tajima and H. Shimada (1996). Antibody against synthetic multiple antigen peptides (MAP) of JC virus capsid protein (VP1) without cross reaction to BK virus: a diagnostic tool for progressive multifocal leukoencephalopathy. *Neurosci. Lett. 205*, 111–114.
163. P. Kanda, D.A. Fritz, D.A. Gage and K.R. Shuler (1995). Dependence of murine antibody response to an anti-CDR2 VH peptide on immunogen formulation. *Mol. Immunol. 32*, 1319–1328.
164. P.J. Duriez, S. Desnoyers, J.C. Hoflack, G.M. Shah, B. Morelle, S. Bourassa, G.G. Poirier and B. Talbot (1997). Characterization of anti-peptide antibodies directed towards the automodification domain and apoptotic fragment of poly (ADP-ribose) polymerase. *Biochim. Biophys. Acta 1334*, 65–72.
165. I. Toth, N. Flinn, W.A. Gibbons, M. Good, W. Hayman and F. Brown. Immunological evaluation of the Lipid-Core-Peptide (LCP) adjuvant/carrier system, in: *Peptides, Chemistry, Structure and Biology*, P.T.P. Kaumaya and R.S. Hodges, Eds, p. 810–811, Mayflower Scientific Ltd., Kingswinford, 1996.
166. D.J. Smith, B. Shoushtari, R.L. Heschel, W.F. King and M.A. Taubman (1997). Immunogenicity and protective immunity induced by synthetic peptides associated with a catalytic subdomain of mutans group streptococcal glucosyltransferase. *Infect. Immun. 65*, 4424–4430.
167. M.W. Jackwood (1995). Production and immunogenicity of multiple antigenic peptide (MAP) constructs derived from the S1 glycoprotein of infectious bronchitis virus (IBV). *Adv. Exp. Med. Biol. 380*, 213–219.
168. M. Singh, J.P. McGee, X.M. Li, W. Koff, T. Zamb, C.Y. Wang, D.T. O'Hagan (1997). Biodegradable microparticles with an entrapped branched octameric peptide as a controlled-release HIV-1 vaccine. *J. Pharm. Sci. 86*,1229–1233.
169. L. Caponi, S. Perogano, V. Di Bartolo, P. Rovero, R. Revoltella and S. Bombardieri (1995). Autoantibodies directed against ribosomal P proteins: use of a multiple antigen peptide as the coating agent in ELISA. *J. Immunol. Methods 179*, 193–202.
170. T. Vogel, R. Kurth and S. Norley (1994). The majority of neutralizing Abs in HIV-1-infected patients recognize linear V3 loop sequences. Studies using HIV-1MN multiple antigenic peptides. *J. Immunol. 153*, 1895–1904.
171. T. Firsova, J.A. Pérez, I.V. Kruglov, F. Reig and I. Haro. Synthesis of a diepitope multiple antigen peptide containing sequences from VP1 and VP3 proteins of hepatitis A virus and its use in hepatitis diagnosis, in: *Book of Abstracts, 24th Symposium of the EPS*, Abstract No. 24, Edinburgh, Scotland, 1996.
172. B. Marchini, M.P. Dolcher, A. Sabbatini, G. Klein and P. Mogliorini (1994). Immune response to different sequences of the EBNA I molecule in Epstein–Barr virus-related disorders and in autoimmune diseases. *J. Autoimmun. 7*, 179–191.
173. N. Yahi, J. Sabatier, S. Baghdiguian, F. Gonzales-Scarano and J. Fantini (1995). Synthetic multimeric peptides derived from the principal neutralization domain (V3 loop) of human immunodeficiency virus type 1 (HIV-1) gp120 bind to galactosylceramide and block HIV-1 infection in a human CD4-negative mucosal epithelial cell line. *J. Virol. 69*, 320–325.
174. N. Yahi, J. Fantini, S. Baghdiguian, K. Mabrouk, C. Tamalet, H. Rochat, J. van Rietschoten and J.M. Sabatier (1995). SPC3, a synthetic peptide derived from the V3 domain of human immunodeficiency virus type 1 (HIV-1) gp120, inhibits HIV-1 entry into CD4 + and CD4- cells by two distinct mechanisms. *Proc. Natl. Acad. Sci. USA 92*, 4867–4871.
175. N. Yahi, J. Fantini, K. Mabrouk, C. Tamalet, P. DeMicco, J. van Rietschoten, H. Rochat and J.M. Sabatier (1994). Multibranched V3 peptides inhibit human immunodeficiency virus infection in human lymphocytes and macrophages. *J. Virol. 68*, 5714–5720.
176. N. Yahi, J.M. Sabatier, P. Nickel, K. Mabrouk, F. Gonzales-Scarano and J. Fantini (1994). Suramin inhibits binding of the V3 region of HIV-1 envelope glycoprotein gp120 to galactosylceramide, the receptor for HIV-1 gp120 on human colon epithelial cells. *J. Biol. Chem. 269*, 24349–24353.
177. B. Weeks, M. Nomizu, A. Otaka, C. Weston, A. Okusu, H. Tamamura, A. Matsumoto, N. Yamamoto and N. Fujii (1994). Lymphocytes and promonocytes attach to the synthetic [Tyr5,12, Lys7] polyphemusin II peptide. *Biochem. Biophys. Res. Commun. 202*, 470–475.

178. A. Wallace, S. Altamura, C. Toniatti, A. Vitelli, E. Bianchi, P. Delmastro, G. Ciliberto and A. Pessi (1994). A multimeric synthetic peptide combinatoria1 library. *Peptide Res*. *7*, 27–31.
179. G. Fassina, G. Cassani, P. Gnocchi, M.C. Fornasiero and A.M. Isetta (1995). Inhibition of interleukin-2/p55 receptor subunit interaction by complementary peptides. *Arch*. *Biochem*. *Biophys*. *318*, 37–45.
180. K.C. Ingham, S.A. Brew and M. Migliorini (1994). An unusual heparin-binding peptide from the carboxy-terminal hep-2 region of fibronectin. *Arch*. *Biochem*. *Biophys*. *314*, 242–246.
181. F. Chillemi, P. Francescato, R. Bossa, A. Fraccari, I. Galatulas (1997). Enhancement of cytotoxic activity by synthesis of peptide multimeric forms. *Anticancer Res*. *17*, 3609–3611.
182. K. Sheldon, D. Liu, J. Ferguson and J. Gariepy (1995). Loligomers: design of *de novo* peptide-based intracellular vehicle. *Proc*. *Natl*. *Acad*. *Sci*. *USA 92*, 2056–2060.
183. D.L. Wiegandt, W. Ma and A.F. Spatola. Homo-oligomeric and hetero-oligomeric multifunctional peptide analogs, in: *Peptides*, *Chemistry*, *Structure and Biology*, P.T.P.Kaumaya and R.S. Hodges, Eds, p. 555–557, Mayflower Scientific Ltd., Kingswinford, 1996.
184. A. Verdoliva, M. Ruvo, G. Cassani and G. Fassina. Antigenic cross-recognition of retro and inverso peptides, in: *Peptides in Immunology*, C.H. Schneider, Ed., p. 205–211, John Wiley & Sons, Chichester, 1996.
185. G. Fassina, A. Verdoliva, M.R. Odierna, M. Ruvo and J. Cassini (1996). Protein A mimetic peptide ligand for affinity purification of antibodies. *J*. *Mol*. *Recognit*. *9*, 564–569.
186. A. Verdoliva, G. Cassani and G. Fassina (1995). Affinity purification of polyclonal antibodies using immobilized multimeric peptides. *J*. *Chromatogr*. *B*, *Biomed*. *Appl*. *664*, 175–183.
187. S. Butz, S. Rawer, W. Rapp and U. Birsner (1994). Immunization and affinity purification of antibodies using resin-immobilized lysine-branched synthetic peptides. *Peptide Res*. *7*, 20–23.

