

Multiple antigenic peptide (MAP): a synthetic peptide dendrimer for diagnostic, antiviral and vaccine strategies for emerging and re-emerging viral diseases

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Abstract The peptide dendrimer provides novel strategies for various biological applications. Assembling of peptide in macromolecular structure is expected to give rational models as drugs, their delivery and diagnostic reagents. Improved understanding of virus structure and their molecular interactions with ligands have paved the way for treatment and control of emerging and re-emerging viral diseases. This review presents a brief account of a synthetic peptide dendrimer used for diagnostic, therapeutic and prophylactic applications. The designs comprise of multiple antigenic peptides which are being used as alternate synthetic antigens for different viruses.

Keywords Synthetic peptide · Peptide dendrimers · Multiple antigenic peptides · Biosensor · Antiviral · Diagnostics

Introduction

Now a day's emerging and re-emerging viral infections are of global concern, especially the viral infections of zoonotic importance which not only affect the animal's health but also affect the health and livelihood of small and marginal farmers. These infections are responsible for

severe economic burden in the agriculture business and cripple the foreign and domestic trade of animals, their products and germplasms. The devastating consequence of such a disease was seen recently in an outbreak of avian influenza in Manipur in 2007 resulting in losses of 14 % of the total value of livestock output of the state [16]. Due to high impact of zoonotic diseases on social and health perspectives of human beings along with huge economic losses such outbreak necessitates special attention in terms of disease diagnosis and preventions. To create a competence for the disease prevention, treatment, and control, continuous efforts are required with the improvement in diagnostic, therapeutic and prophylactic strategies.

Recent developments in structural biology have paved the way for better understanding of biological behavior of the peptides and protein. Peptide science, the combination of 20 alphabets, can provide novel methodologies for the diagnostics, therapeutics and prophylactic measures. Further, the superior synthetic peptide designs can improve the practical applications of these methodologies. The peptides have wider biomedical applications in diverse areas like drug design, targeted drug delivery, chemotherapy, sero-diagnosis, oncology and vaccinology [1, 3, 4, 17, 28, 29, 40, 53, 65]. These approaches not only deal with infectious diseases but also found to have similar applications in metabolic and inherited disorders [36]. In this review, usage of superior synthetic peptides designs with special reference to multiple antigenic peptides (MAP) for viral diagnosis, vaccines and possible antiviral interventions are discussed. The different available peptide dendrimers can be broadly classified as covalent and non covalent dendrimers. MAP, polypeptide based dendrimers, amphiphilic dendrimers are covalent dendrimers whereas poly(propyleneimine) as mosaic in dendrimers with peptides such as of L-alanine, L-phenylalanine falls in category of non

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covalent dendrimers [8]. In addition to the peptide dendrimers other dendrimeric forms of biological importance reported are poly (aminodiamine) PAMAM, poly (propylene imine), polyester dendrimers and metallodendrimers [25, 45–47, 60, 61].

Synthetic peptide designs

Peptides are organized to super molecular designs such as dendrimers that increase bio-availability for desired action and are found to be more effective in disease diagnosis [53] as well as in vivo bio-imaging [36] in comparison with linear peptide chains. Peptide molecules can be conjugated in different ways to create a branched structure having dendrimeric form or other multimeric forms. The dendrimers can be covalent or non-covalent [46]. Among covalent dendrimers, MAP is known to be the most common form of peptide dendrimer as shown in Fig. 1 [53]. It comprises a core matrix forming the central component of branched architecture to give a cascade or a pennant type of arrangement [46]. In a MAP different amino acids have been used for core formation, but Lys is preferred the most because of its epsilon (ϵ) amino group which provides flexibility. In a cascade type of MAP, the core matrix contains two or three levels of geometrically branched lysine whereas pennant type of dendrimer contains a sequential linear lysinyl peptide. Their uses in magnetic resonance imaging (MRI), protein mimetic and vaccines directed towards bacterial, viral and parasitic pathogens show the dynamic applications of MAP and peptide dendrimers in the field of biomedical sciences [46]. The basic advantage of MAP is its flexibility to synthesize peptide in different structures which also help to increase loading capacity of antigen molecule. MAPs are actively used for immunodiagnosis of viral diseases like *Acquired Immuno Deficiency Syndrome* (AIDS), *Infectious Bursal Disease* (IBD) and *Hepatitis C* [2, 52, 54]. MAP format of peptide dendrimers for VP3 protein of HIV was found to be more sensitive when compared with linear peptide for serodiagnosis of AIDS [31]. The upcoming and promising applications of MAP and dendrimers are giving birth to

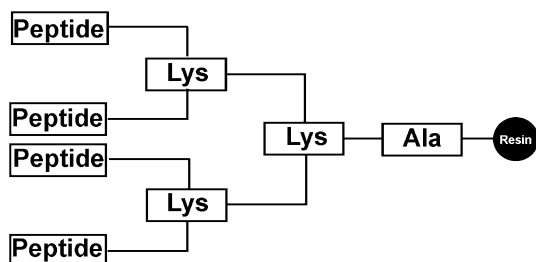


Fig. 1 Commonly used synthetic designs of multiple antigenic peptides

newer developments in peptide based disease diagnostics and therapeutics.

Chemistry of dendrimeric designs

Peptide dendrimers are branched architecture with higher molecular organization of peptides having stable structural configurations. They are commonly used for drug delivery, vaccine development and disease diagnosis and classified according to type of amino acid used, their chain arrangement and finally their three dimensional structures. Commonly used peptide dendrimers developed by Tam uses lysine core with 2–16 copies of similar or different peptide branches. This format of dendrimer grows on two reactive points N^α and N^ϵ of lysine making a multi-antigenic arm which is found to be favorable for induction of immune response [53]. Alternatively, use of different amino acids like proline [7], arginine [15], glutamic acid [50] and some of the unnatural amino acid such as ornithine was also documented and reviewed by Crespo et al. [8]. The arginine rich dendrimers were preferentially used for intracellular delivery of biomolecules such as nucleic acid [15]. Polyglutamic dendrimers were synthesized having π – π stacking or amide amide hydrogen bonding [50] and dendrimers with OAS (octa (3-aminopropyl) silsesquioxane) core are promising vectors for fabricating smart and targeting drug delivery systems. Polyproline dendrimers having structural plasticity were also evaluated as drug delivery models [7]. The peptide dendrimers were found to be efficient in non viral drug delivery, gene delivery and non invasive diagnostic methods [8].

Synthesis of peptides in dendrimers form is a tricky and cumbersome procedure involving high level organizations of peptide chains [8]. These super molecular peptide designs can be achieved by use of two synthesis strategies, namely convergent and divergent [53]. In divergent strategy, the synthesis occurs as a whole in a stepwise manner and synthesis diverges from core to outward as a single unit. Alternatively in convergent strategy, dendrons are synthesized separately and then assembled to form a complete dendrimer. The convergent strategies of dendrimer designs are commonly used now days for gene delivery and drug deliveries. Both strategies have their own merits and demerits. Divergent strategy is preferred for smaller products where synthesis of individual component is not feasible and avoided in cases where heavy branching is required. Whereas convergent strategies are used for super molecular assemblies, commonly with larger sized and intricate branching patterns; separate synthesis of each unit and their purification make this process difficult [53].

The peptide chains for dendrimer are usually synthesized by solid phase synthesis method [41] in which amino acids

are sequentially added one by one from C terminal to N terminal of peptide anchoring on solid resin beads. In this method amino acid derivatives used are either Fmoc or Boc protected at reactive N site as alpha position whereas side chains are suitably blocked by different protective groups so that they remain stable during synthesis procedure.

Diagnostic reagents

The diagnosis of infectious diseases plays an important role for better health management practices. Diagnostic reagents and methodologies should have high sensitivity and specificity. To achieve these goals various diagnostic tools have been suggested, synthetic peptide based diagnostics is one of them [19, 55]. These reagents have been used for the diagnosis of important viral diseases like AIDS, *Infectious Bronchitis* (IB), *Severe Acute Respiratory Syndrome* (SARS), and *Bluetongue* (BT) [2, 26, 56, 64]. Synthetic peptides as diagnostic reagents are more superior, specific and safe as compared to native antigen/inactivated virus [55]. The prediction and mapping of B cell and T cell epitopes are usually performed using various softwares like Immune Epitope Database (IEDB) analysis resources (<http://tools.immuneepitope.org>) and BCPREDS: B cell epitope prediction server (<http://ailab.cs.iastate.edu/bcpreds/>) and HHPred (<http://toolkit.tuebingen.mpg.de/hhpred>). Predictive analysis of antigenic epitopes are mainly based on different algorithms such as secondary structure prediction, hydrophilicity plot, flexibility, antigenicity index and surface probability [6, 14, 23, 27, 34]. These analyses together make it possible to identify the immunodominant epitopes having high reactivity with antibodies in serum. The secondary structure prediction tools generally determine the structure distribution on antigen sequence computing proclivity values of amino acid related to diverse conformations and configuration, in general beta and turn regions are chosen for B-epitope prediction. On the basis of predictive probabilities, Jameison and Wolf [27] developed a method which provides surface contour values called antigenic index for different segments of antigen. The suitability and flexibility of peptide based diagnosis were adapted in detection of antibodies of different viral pathogens of human and veterinary importance like *Human immunodeficiency virus* (HIV) [31], *Hepatitis C* [19], *Epstein–Barr virus* [62], *Infectious bursal disease virus* (IBDV) [55] respectively and also for the differential diagnosis of closely related agents like *Human T-lymphotropic virus* (HTLV-I) and HTLV-II [63], *Peste des petits ruminant virus* (PPRV) and *Rinderpest virus* (RP) [11].

In spite of their easy synthesis protocols and simple sequence characters, use of linear peptide suffers from

some of the practical difficulties such as poor coating efficiency and reactivity with antibody which ultimately affects the sensitivity of the test. Superior molecular designs such as MAPs, a form of peptide dendrimer improves the sensitivity of ELISA test when used as coating antigen. The multimeric nature of peptide dendrimers provides increased surface-binding in ELISA plate and thus improves the sensitivity with improved epitope projection. Tam and Zavala [58] reported superiority of MAP over linear peptides for binding on the plastic surface, and they found that MAP gave efficient surface coating even at as little as 0.11 µg/ml concentrations with specific reactivity to monoclonal and polyclonal antibodies on comparison to linear peptides. Multimeric forms of linear peptide show better reactivity as they can mimic native antigenic structure and therefore, have been used successfully in immunoassays for diagnosis of malaria [20], infectious bronchitis [26] and many more pathogens such as HIV, PPRV, BTV and IBDV [11, 31, 54, 56]. Peptide dendrimers used in serodiagnosis of naturally immunized or infected individuals have been tested for the presence of specific antibody [55]. The multimeric arrangement of dendrimeric peptide improves the detection of low affinity antibodies. In one of the study conducted in our laboratory MAP showed increased sensitivity in ELISA as compared to corresponding linear peptides as well as whole IBDV virus. A positive reaction (i.e., above the cut-off value) was obtained with the minimum amount of each MAP (5 ng/ml) in comparison to purified IBDV (500 ng/ml). This indicates that the amount of MAPs required was 100 times lesser than the purified virus antigen in ELISA which was due to the higher binding efficiency of MAPs as compared to whole virus [55]. MAP based enzyme immunoassays have been developed for the detection of *Simian Immunodeficiency Virus* (SIV) in non-human primates targeting V3 region of gp120 and immunodominant region of Env (gp41/36) gene using short peptide segments of 15 and 11 amino acids, respectively [44]. Routinely SIV was diagnosed by commercially available enzyme immune assay and western blotting however these commercial methods fail to diagnose all SIV infections [44]. The MAP based assay for SIV diagnosis provides an economical alternative to conventional western blotting. This MAP based assay gave 100 % sensitivity and specificity in detection of infection with different lineages of SIV, suggestive of its use in surveillance of the disease.

MAP can be designed with more than one epitopes of same or different proteins of virus targeting more than one immunodominant epitope. Such multiepitope MAP have higher diagnostic value and are termed as mixotopes. This chimeric MAP strategy was found to be successful in serodiagnosis of *Hepatitis C virus* (GBV-C) [19]. Avoiding complicated designs for more than two epitope in the same

MAP molecule, a cocktail of antigen/mixotope of different MAP molecules having individual epitope was successfully used in serodiagnosis of PPR. While using mixtures of different linear peptides failed to improve antibody detection in case of SARS virus and suggested inter-epitope interaction as a reason for lower activity with respective antigen [64] which is perhaps prevented in MAP format as documented in PPR [32]. The differential diagnosis of PPR virus and RP virus is complicated due to common epitope sharing in morbilliviruses that leads to cross-reactivity in serological assays and makes interpretations ambiguous. Synthetic peptides have been developed to address this problem. We reported an approach using MAPs and their mixotope (cocktail of different MAPs), for differential diagnosis of PPR and RP viruses [32, 33]. Presently RP has been eradicated globally and antigenic cross reactivity for PPR diagnosis is not an issue any longer, however such approach using MAP peptides mixotopes may be useful in devising sero-diagnosis of other immune cross reactive viral diseases of veterinary importance like West Nile Fever Virus with tick born encephalitis or dengue virus, and *blue tongue virus (BTV)* with *epizootic haemorrhagic disease virus (EHDV)*/*African horse sickness virus* [13]. For such immune cross reactive diseases whole viral/conventional antigens are not suitable antigen for specific diagnosis, and in case of highly antigenic variable infections the small specific segments of antigen used in dendrimeric design are better alternative.

Immune response to peptide

The hyper-immune sera raised against MAP or peptide dendrimers can also be used in serological diagnosis of infectious agents by detection of native antigen [54]. As MAP itself forms a stable macromolecular antigenic conformation, the pure antiserum can be raised against MAP without conjugation. In several instances, immune sera raised against MAPs were found to react with the cognate protein; whereas antisera raised by the corresponding linear peptide conjugated to a protein carrier may have interference from carrier protein antibodies [53]. Further, MAPs and their anti-peptide antibodies were produced for different epitope segments of VP2, VP3 and VP4 proteins in IBVD in our laboratory where anti-peptide antibodies demonstrated their ability to detect native viral antigen [54]. The MAPs for different peptide sequence of IBVD polypeptides were synthesized having peptide of 9–22 amino acid length and were used for induction of antibody response in rabbit to have specific serum for virus detection. On comparison with conventional agar gel precipitation test (AGPT) MAP antibody based test shown greater efficiency [54]. Results were suggestive of successful use

of MAP and anti-MAP antibodies as safe and non-infectious for antigen detection comparable to polyclonal and monoclonal antibodies [54]. Further, immune response of MAP was enhanced by supplementing universal T-helper epitope of human IL-1 β peptide in the region of 163–171 [11]. A 21 mer peptide segment from 454 to 472 amino acids in N protein of PPR virus was found highly immunogenic and induced antibody specific to PPRV [11].

Thus MAP offers a superior design to construct a synthetic peptide antigen of well defined structures that is safe to handle in sero-diagnosis and further be used to have specific serum for viral antigen detection. These reagents enable us to develop a strategy for clinical diagnosis of viral diseases, particularly emerging and re-emerging diseases. As such there is no better antigen than native viral antigen, which is a complete antigen providing both protective response as well as diagnostic reagent.

Peptides as biosensor and nanodiagnosics

Biosensors are believed to be more reliable sensitive and rapid diagnostic tools for confirmatory disease diagnosis, thus can play a role in disease surveillance and outbreak tackling programs. This technique has the potential to visualize antigen–antibody interactions in real time and possibly in a label-free mode. In real-time biospecific interaction analysis (BIA) measurements, the ligand of interest is bound directly to a coated layer by a defined covalent linkage which is freely accessible to three-dimensional antibody interactions. The antibody–antigen interactions are directly detected by measuring changes in the surface plasmon resonance (SPR) signal. This method differs from other surface interaction techniques like ELISA and immuno-chromatographic techniques, as a biospecific surface on SPR chip can easily be regenerated permitting a series of measurements to be performed repeatedly on the same surface in throughput manner. BIA eliminates the need for labeled reagents and provides a rapid one-step analytical procedure with better sensitivity. Assays can also be performed directly on crude plasma samples. Number of biosensors has been developed in the recent past which include a combination of metallic nanoparticles with the sensing probes like Nucleic acid, PNA or peptides and antibodies [19, 30]. The majority of biosensors use gold nanoparticles based detection system. Metallic nanoparticles like gold, silver, platinum and iron oxide are used because of their easy handling and provide easy differentiation between two samples. Use of peptides in biosensor technology is a regular practice; several modified versions of these biosensors are available now. Use of MAP in biosensor technology is also very well accepted in the field of bio imaging, cancer diagnostic and other disease diagnosis. MAP based biosensors have successfully been used for

the detection of Hepatitis A VP3 protein with reliable sensitivity and accuracy [18]. Efficiency of MAP biosensor to detect E2, NS4, and NS5 protein of GB virus-C (formerly known as *Hepatitis G virus*) infection from GBV-C infected patients showed its promising clinical application in viral disease diagnosis [19].

Use of biosensor based technologies has simplified disease diagnosis. Collateral studies exploiting Plasmon coupling effect for detection of analyte helps to eliminate elaborate diagnostic requirements [30]. The biomolecule such as peptides can be used in different formats in conjunction with nano-devices for disease diagnosis. The study conducted in our laboratory demonstrated the possibility of the use of the MAP directly coated with gold nanoparticles for detection of bluetongue antibodies. The identified immunodominant epitopes of VP7 protein of bluetongue virus were synthesized as MAP having Cysteine at C terminus and applied directly decorating the gold surface to detect of virus antibodies in serum samples. Nitrocellulose membrane was used to have spot-test wherein the presence of MAP specific antibodies resulted in apparent color change. In course, MAP antibodies bound to the gold surface with a pink colored solution and turned it into blue colored spot [56]. In addition to this, we made use of cysteine core MAP as well as linear peptide with C-terminal cysteine for direct coating on gold surface on surface plasmon resonance (SPR) biosensor platform as antigen with defined orientation and peptides giving reactivity with BTV antibodies (unpublished data). This approach does not involve conjugation chemistry generally required for antigen/antibody immobilization on SPR platform. We have also devised a MAP peptide mixotope based serodiagnostics for PPR virus as a visual test using MAP and immuno-gold in field diagnosis [32]. Such strategies using MAP antigen and their specific antibodies as diagnostic reagents can be helpful as blue print to address the timely diagnosis of emerging and reemerging diseases.

Peptide as antivirals

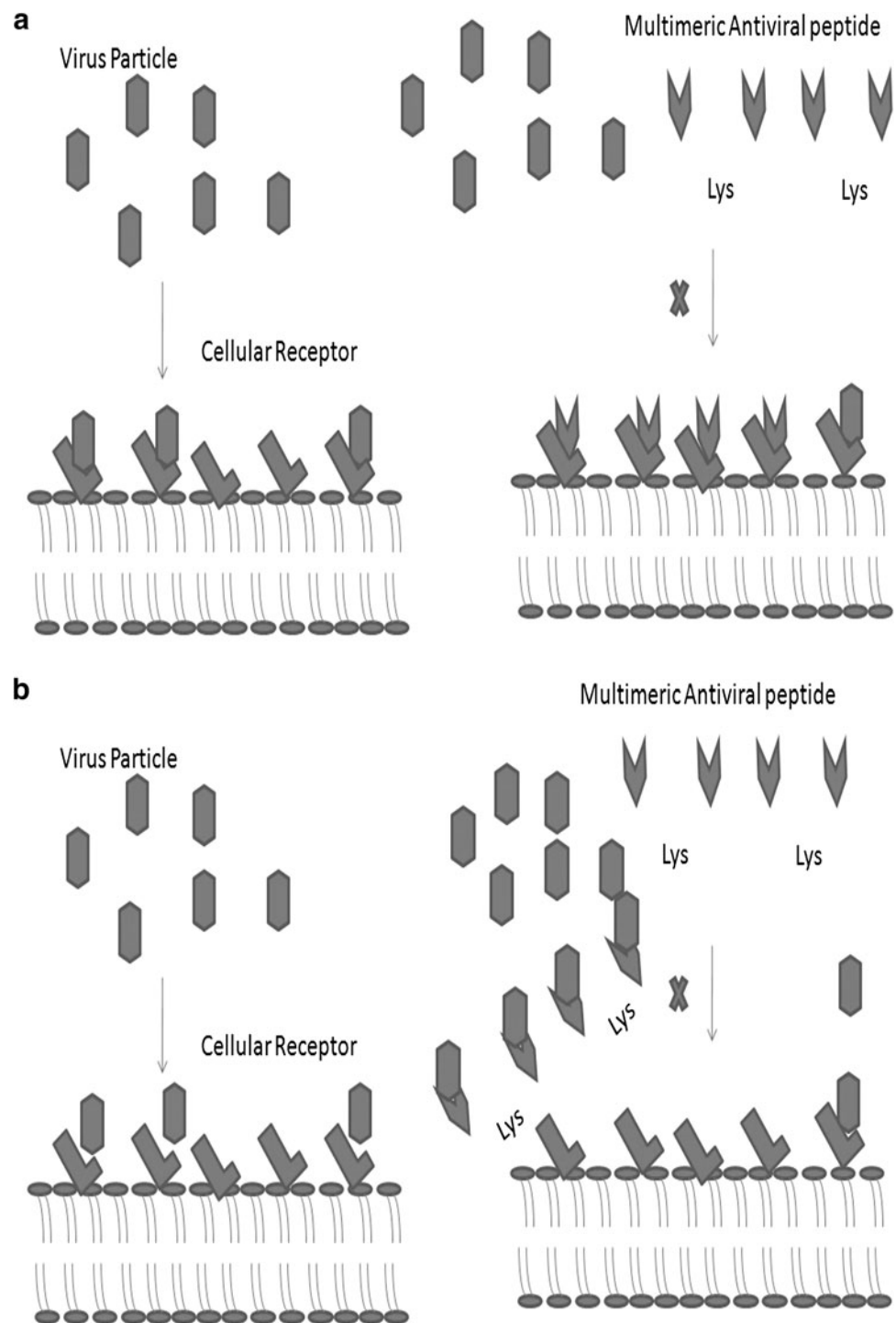
Peptide dendrimers have been used in various ways to tackle infective agents. It had been observed that several antimicrobial peptides when synthesized in dendrimeric forms show improved efficiency, well comparable to the commercially available antibiotics and antimicrobial molecules [28]. In case of viral pathogens, the interaction of viral pathogen with host cell is the principal event in pathogenesis and the blocking of viral entry by intervening with cellular receptor remains to be the first choice of viral therapy [28]. Being part of natural host defense system many of the peptides act as inherent antiviral molecule by limiting the spread of viral infection. Naturally occurring peptides such as defensin, tachyplesin, magnin and

lactoferrin show antiviral activity interfering with virus host interactions. Basic defensive mechanism used for antiviral designs can be aimed to restrict viral entry in the host cell and can be achieved with use of synthetic peptide mimetic of virus binding site or synthesizing virus binding site analogs for competitive inhibition of viral binding to target tissue (Fig. 2a, b), respectively. In both of the acts, inhibition occurs at the protein–protein interface which restricts virus primarily at cellular entry point. Identification of such peptide mimic is a pivotal step and phage display bio-panning technique provides a logical means to pin-point efficient antiviral peptides. However, identification of such antiviral targets is a difficult task. Use of such combinatorial phage display library lands in identification of many of potential antiviral peptide identification against many viruses like *Newcastle Disease Virus* (NDV) [48, 49], *Rabies virus* [51], and *Hepatitis A virus* [35]. Antiviral peptides are mostly used in two forms; linear/cyclic forms and most favored multimeric form. Linear and/or cyclic forms of peptide have been used in antiviral studies against NDV [49] and Rabies [51]. However, presence of multiple binding sites for a single viral particle practically needs a large number of neutralizing peptides and may limit their applications. This shortfall of antiviral peptides strategy can be bridged by devising multimeric designs. Hall et al. [21] reported improved antiviral efficiency of *anti-hanta-virus* peptides when used in multimeric forms and further anticipated that use of multivalent inhibitors may disrupt polyvalent protein–protein interactions. Recently, Donalizio et al. [12] reported successful use of dendrimeric heparin-sulfate binding peptide in inhibiting infectivity of genital types of *Human papillomavirus*. The study has shown that the tetra-branched compound SB105 and its derivative SB105-A10 was able to inhibit replication of genital type of *Human papillomavirus* in both 293TT and keratocarcinoma cells [12]. Lukanini et al. [37] showed antiviral activity of SB105-10A and SB105 against *Human cytomegalovirus* in both primary fibroblast and endothelial cells. Another study by the same group utilizes peptide to inhibits *Herpes Simplex Virus* type 1 and 2 replication in-vitro [37, 38]. All these studies show potential applications of superior peptide organizations for antiviral intervention and possible viral therapy. With time, progress is being made on understanding of virus pathogenesis and subsequent events happening in a cell for virus life cycle and this may provide a tool to intervene in these events using MAP peptide in a better way.

Peptide as vaccines

Vaccination is the most favored strategy in disease control programs. Generally heat killed or live attenuated vaccines

Fig. 2 Depicting the course of antiviral interventions blocking cellular entry of virus using multimeric peptide designs. **a** Antiviral MAP peptide blocks cellular entry of viruses by competitive binding with viral receptors on cell. **b** Antiviral intervention of MAP peptide by mimicking cell receptor binding site to prevent viral cell interactions competitively



are used for such regimes. However, these vaccines suffer from inherent drawbacks such as reversion of pathogenesis or limited protection. The subunit vaccines consisting of either whole recombinant proteins or synthetic peptides are coined as an alternate of classical vaccines. Similarly, immunodominant epitopes can be used to synthesize peptide based vaccines and give relative flexibility in terms of bulk peptide synthesis with proven chemical stability [53].

Many a time, linear peptide shows a poor immune response and reactivity, which limits their practical applications as a potential vaccine [53]. Superior synthetic designs such as MAP and peptide dendrimers can provide contemporary vaccine model for the important viral diseases of animals. However, challenge remains to put all required protective epitopes, B and T cell epitopes, in one single MAP molecule.

MAP can be used in two designs; homotropic, multimer of one epitope or heterotropic with combinations of different epitopes, the later synthesis is difficult. These designs proved to have high chemical stability and retain all immunological properties to act as surrogate antigen, required for induction of protection against the viral invasions. Most preferred MAPs are tetrameric and octameric constructs, however, larger constructs can also be used [53].

Mozdzanowska et al. [43] reported successful use of Me2 peptides MAP and Th determinant for immune-prophylactic applications in *Influenza type A* challenged mice. Use of Me2 determinant peptide showed high reactivity with mAb suggestive of its native antigenic nature with fixed viral Me2 protein. However, effective delivery of MAP for improved antigenic processing is essential which is the main driving force for developing hybrid MAP designs with liposomal entrapments. Haro et al. [22] studied these aspects of MAP based immunizations using tetrameric heterogeneous palmitoyl-derivative MAP for VP1 (11–25) and VP3 (102–121) gene of *hepatitis A virus* [22].

Use of lipopeptide dendrimers has also been studied for FMD virus [10]. Lipopeptide MAP as antigen eliminates the use of adjuvants for vaccination thus making it a better alternate to immunization. Use of MAP based mimotope to induce *Respiratory Syncytial Virus* (RSV) specific anti-mimotope antibody, and CTL responses suggest its possible application as vaccine for RSV [5]. Along with these initial studies, MAP based peptide vaccine designs are continuously evolving with improved mounting of immune response and better protection profile. Advancements in peptide chemistry in combination of nano-designs have drastically improved the performance of MAP based vaccines and widened its biomedical applications [57]. Recently MAP based vaccines were researched using combination of synthetic approaches to have size dependent nanomaterials that includes peptide self-assemble nanoparticles, lipophilic moieties, gold nanoparticle and non-peptide based dendrimeric molecules [57]. Few studies have also used cell penetrating peptides for induction of T cell mediated responses [4]. Such superior MAP designs not only improve the immune response induction but also help in safer delivery which is the biggest hurdle in case of DNA vaccines. Nanoparticle coated with MAP provides an easy cellular delivery and can be used as therapeutic vaccines.

Peptide dendrimers based vaccines containing T cell epitope with branches of multiple B cell epitope for increasing immune-reactivity and covering both CMI and HMI have been designed as the successful candidate vaccine as reported by Cubillos et al. [9]. The results of the study not only showed potential systemic immune response but also demonstrated mucosal immune response with IgA production, pointing at use of peptide dendrimer as a marker vaccine. The dendrimeric modulations having

combination of T cell and B cell epitopes were also used for vaccine development of *Classical swine fever* (CSF) virus wherein three different B cell epitope regions (covering regions of 694–712, 712–728 and 829–842 for constructs 1, 2 and 3, respectively) of E2 protein of CSF virus were selected with T cell epitope from NS3 protein covering 1,446–1,460 amino acid regions. Out of three constructs used, first construct was found to be more effective on assessment of ELISPOT assay having the highest value of $\text{INF}\gamma$ producing cell after CSFV challenge. Increase in $\text{INF}\gamma$ secreting cells production is believed to be characteristic of CSF infection [42, 59]. In recent time, multi epitope candidate vaccine against *Hepatitis C* has been developed [24] which includes non-structural protein having HLA-A2 epitope. The candidate vaccine contains epitopes from NS5A, NS4B and core protein of Hepatitis C virus. In this study, two approaches were used for MAP designs: the first one is VL-20 of 20 amino acid length having two CTL epitopes and the second one is MAP VL-44 of 44 amino acid design with Th epitope with two CTL epitopes. In VL-44 Lys was used as linker for epitope joining. This candidate vaccine elicits cellular immunogenicity with induction of $\text{INF}\gamma$ and IL2 responses [24]. In spite of several advantages MAP based vaccines products have not emerged in market as this type of vaccine cover the discontinuous epitopes and thus offers limited neutralization in many of cases. Another practical limitation is with delivery and biostability of peptide vaccine however these limitations can be overcome by using lipophilic domains in conjugation with peptides. As such there is no better antigen than native viral antigen, which is a complete antigen providing both protective response as well as diagnostic reagent. However, pathogenicity and cross reactivity are the issues associated with native antigen that have continuously derived the research in this area for development of safe and specific alternate antigen by developing subunit and synthetic peptide antigens. Subunit and synthetic peptide antigens (in both linear and MAP format), have applications as specific diagnostic reagents, but face the limitation to elicit protective immune response, which is possible only with native antigen. Many attempts have been made towards development of subunit synthetic peptide vaccine, but with limited success.

Such ameliorative peptide designs as discussed in review provide possible prophylactic, confirmative diagnostic strategies along with wholesome therapeutic alternative and thus can provide a combative ground for successful tackling of emerging and re-emerging viral diseases, the global concern of today. The collective applications of supramolecular designs of peptide may provide a viable rescue from various viral infections by targeting virus at any stage of its life cycle for better management of the emerging and re-emerging diseases.

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