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The use of signal peptide domains as vaccine candidates

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Abbreviations: AE, adverse events; APC, antigen presenting cells; ADCC, antibody-dependent cell-mediated cytotoxicity; DC, dendritic cells; ER, endoplasmic reticulum; hGM-CSF, human granulocyte-macrophage colony-stimulating factor; LP, long peptide; MHC, major histocompatibility complex; MM, multiple myeloma; PBMC, peripheral blood mononuclear cells; SP, signal peptide; SPase, signal peptidase; SPP, signal peptide peptidase; TAP, transporter-associated with antigen processing; TAA, tumor associated antigen; VC, vaccine candidate.

Signal peptide (SP) domains have a common motif but also sequence specific features. This knowledge was mainly ignored by immunologists who considered SP as generic, short-lived, targeting sequences. Consequently, while SP-derived MHC class I, class II and HLA-E epitopes have been isolated, their use as antigen-specific vaccine candidates (VCs) was mostly neglected. Recently we demonstrated the rational of selecting entire SP domains as multi-epitope long peptide VCs based on their high T and B-cell epitope densities. This review summarizes preclinical and clinical results demonstrating the various advantages of human SP domain VCs derived from both bacterial and tumor antigens. Such vaccine design provides for a straightforward, yet unique immunotherapeutic means of generating robust, non-toxic, diversified, combined antigen-specific $CD4^+$/$CD8^+$ T/B-cell immunity, irrespective of patient HLA repertoire also in disease associated transporter-associated with antigen processing (TAP) deficiencies. Subsequent clinical trials will further assess the full potential of this approach.

Synthetic Peptides as Vaccines

Synthetic peptide vaccines, used for the induction of T-cell and antibody responses, are an established tool for the prevention and treatment of select malignancies. Among their advantages are (1) the induction of a highly specific response, with negligible short-term toxicity, (2) their chemical stability and absence of pathogens and other contaminating mammalian substances, and (3) their cost effective production, allowing for long-term maintenance use in therapeutic applications. However, to date, there is still no licensed peptide vaccine, suggesting that improvement in peptides' immunogenicity is highly needed. This review will present the properties of signal peptide (SP) domains that render them an immunogenic, antigen-specific, multi-epitope protein region with key immunological advantages as vaccine candidates (VCs).

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Antigen Processing and Presentation for T and B-cell Epitopes

Understanding the pathways of antigen processing and presentation is important for designing peptide vaccines. T-cells recognize major histocompatibility complex (MHC)-peptide complexes on the surface of host cells. MHC class I molecules bind 8–10-mer peptides, generated upon degradation of proteins of intracellular/cytosolic origin, while MHC class II molecules bind ~15-mer peptides of intracellular and extracellular origin.[1] To be presented to $CD8^+$ T-cells, peptides must enter the endoplasmic reticulum (ER) in a process which is largely mediated by the transporter associated with antigen processing (TAP) machinery located on the ER membrane.[2] In the ER lumen, peptides bind and stabilize MHC class I molecules, which are then transported to the cell membrane.[3] In parallel, $CD4^+$ T-cell activation is important for the induction of specific $CD8^+$ T-cell and humoral responses.[4,5] To be presented to B-cells[6] and $CD4^+$ T-cells, peptides enter the endosome, where they bind and stabilize MHC class II molecules. Alternatively, extracellular peptides can directly bind and stabilize MHC class II molecules situated on cell surfaces. For thymic-dependent humoral responses, B-cell epitopes, mostly originating from the extracellular domains of antigens,[7] bind to B-cell receptors in addition to MHC class II (**Fig. 1**).

Signal Peptide Domains: State of the Art

SP domains are short ~13–50 amino acid-long lipophilic targeting sequences, typically located at the N-terminus of proteins destined for secretion or for integration within cellular membranes.[8] SPs have a tripartite structure: a central hydrophobic h-region and flanking hydrophilic N- and C-terminal regions (**Fig. 2**). Once protein translation is completed, SPs incorporated in the ER membrane are generally removed from the mature protein by a dedicated signal peptidase (SPase) protease. However, they can still enter the ER lumen to bind and stabilize MHC class I molecules, either indirectly, via the TAP machinery, like most other degraded sequences,[9] or directly, using a novel TAP-

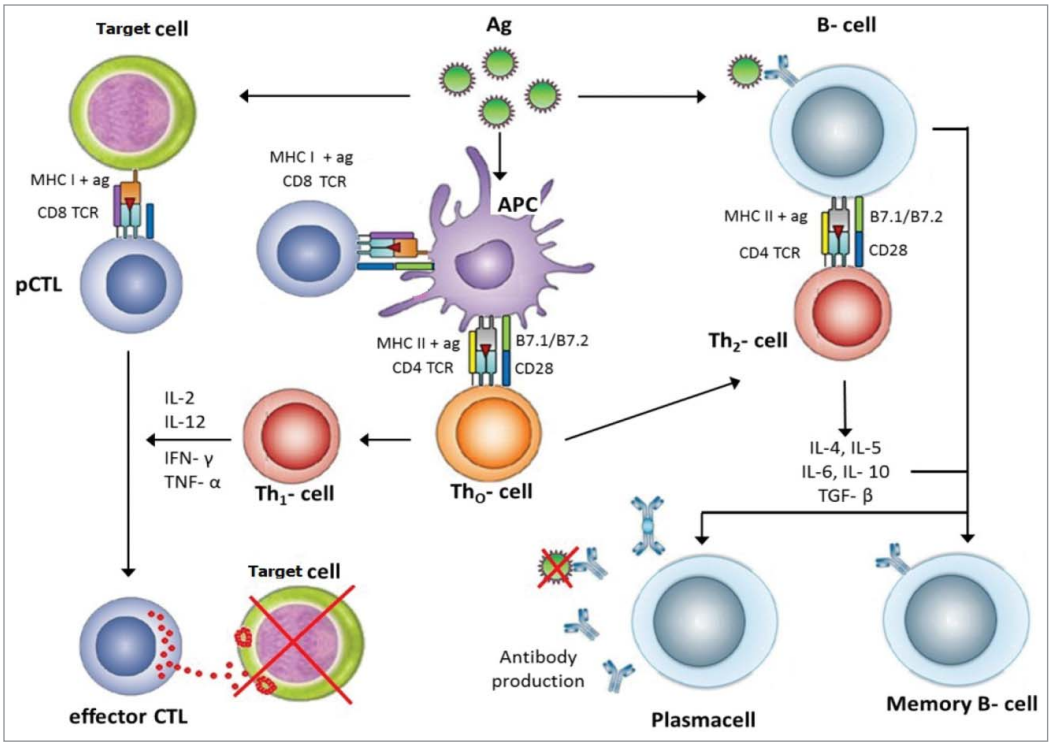

Figure 1. B-cell and T- cell immune responses.

independent pathway reliant on the protease activity of another ER-membrane-associated signal peptide peptidase (SPP) (**Fig. 2**).[8] This unique "ER-targeting property" of SP domains has been used to improve ER localization, and, consequently, the immunogenicity of non-SP-linked epitopes.[10,11] More recently, it has become evident, that apart from the consensus motif essential for the role of the SP as a targeting signal, SP domains exhibit high antigenic variability and specificity to the proteins they derived from,[8,12-14] enabling them to serve as VCs. Yet, while select MHC class I,[15-17] class II[6,18-20] and HLA-E[21,22] single epitopes have already been identified in different SP domains, and shown to specifically activate T-cells, and more rarely inhibit select NK-cells, the broader potential of using the entire SP domains as multi-epitope long peptides (LP) and their ability to induce robust antigen-specific T and B-cell response has not been explored.

The Advantages of SP Domains as Peptide-Based Vaccines

TAP restrictions

TAP demonstrates a significant initial barrier with respect to peptide binding; the affinity of peptides to TAP largely influences the probability of their presentation by MHC class I. However, as previously mentioned, this does not apply to select peptides, primarily SPs, which can access the ER in a TAP-independent manner. This novel TAP-independent presentation can better counteract immune escape mechanisms exploited by many pathogens and tumor cells, which lead to downregulation of MHC class I expression (**Figs. 2 and 3**). TAP-independent presentation can lead to an up to 2000-fold increase in the MHC class I presentation of SP epitopes.[23] Currently, most peptide vaccines targeting both tumor associated antigen (TAA) and microbial antigens, fail to specifically address TAP-independent presentation. Thus, while immunity is generated following vaccination with most vaccines, the expression of the same epitopes by MHC class I to $CD8^+$ T-cells might be reduced or fully eliminated on TAP-deficient target cells (see also **Table 1** for technology comparison for SP VCs).

Promiscuous MHC binding for T-cell immunity/total population coverage

Single MHC class I and II alleles

The extensive polymorphisms (a few hundred alleles) of both MHC class I and MHC class II molecules, each with a different binding restricted, is a major barrier for peptide-MHC binding and presentation. Thus, in silico prediction algorithms for MHC class I alleles[24] and more recently, for MHC class II alleles as well,[25] were developed and coupled with *reverse immunology* to assist in epitope mapping and isolation from appropriate targets. Yet, assuming that there are no preferred sequence with superior epitope densities, epitope selection and subsequent bio-validation are long and complicated processes, as they involve analysis of multiple epitopes derived from an entire protein sequence. To simplify this process, most research and consequently, most clinically evaluated peptide vaccines, have focused on abundant MHC class I alleles, mainly HLA-A2.1. While single MHC class I-restricted TAA and mycobacterium tuberculosis (MTb)[26] microbial antigen-derived peptides showed promising preclinical results, both in vitro and in vivo,[27-29] they have demonstrated limited clinical efficacy.[30] This outcome was suggested to reflect the limited polyclonal cytotoxic $CD8^+$ T-cell antigenic repertoire as well as the inadequate pan-MHC response,[31] mediated by MHC class II-restricted $CD4^+$ T-helper epitope(s). The absence of MHC class II epitopes was shown to induce immunological tolerance[32] to immunizing antigens, rather than long lasting $CD8^+$ T-cell activation-associated immunity. Likewise, clinical studies which utilized only

MHC class II-restriction epitopes, led to less optimal $CD8^+$ T-cell function.[6,19]

Combined MHC class I and II alleles

Improving immune responses to single MHC class I or MHC class II epitopes can be achieved via multi-epitope LPs featuring multiple MHC binding properties.[33] In this setting, antigen-specific $CD4^+$ T-cells can activate dendritic cells (DC), which, in turn, activate tumor-specific $CD8^+$ T-cells and cross-present specific epitopes.[34-38] There is increasing evidence that LP VCs combining mainly 1–2 MHC class I and class II epitopes, from select TAA-like HER-2/neu,[39] RAS,[40] and NY-ESO-1,[41] potentiate strong and long-term immunity.[33,39]More recently, an initial clinical study involving HPV-16-induced vulvar intraepithelial neoplasia treated with overlapping LPs derived from the E6 (9 LP) and E7 (4 LP) HPV-derived antigens, demonstrated a strong induction of immunity and encouraging anti-infective/anti-tumor efficacy.[42] The search for, and isolation of these peptides, however, remains a long, complicated process, resulting, so far, in very few candidate LPs[39-41] Moreover, despite the stronger immune response, with longer memory, none of these LP vaccines have sufficiently wide MHC coverage for both MHC class I and MHC class II, to enable universal use in the entire target population (HMC-wise). In addition, these LPs require dedicated adjuvants, such as incomplete Freund's adjuvant (see also **Table 1** for technology comparison).

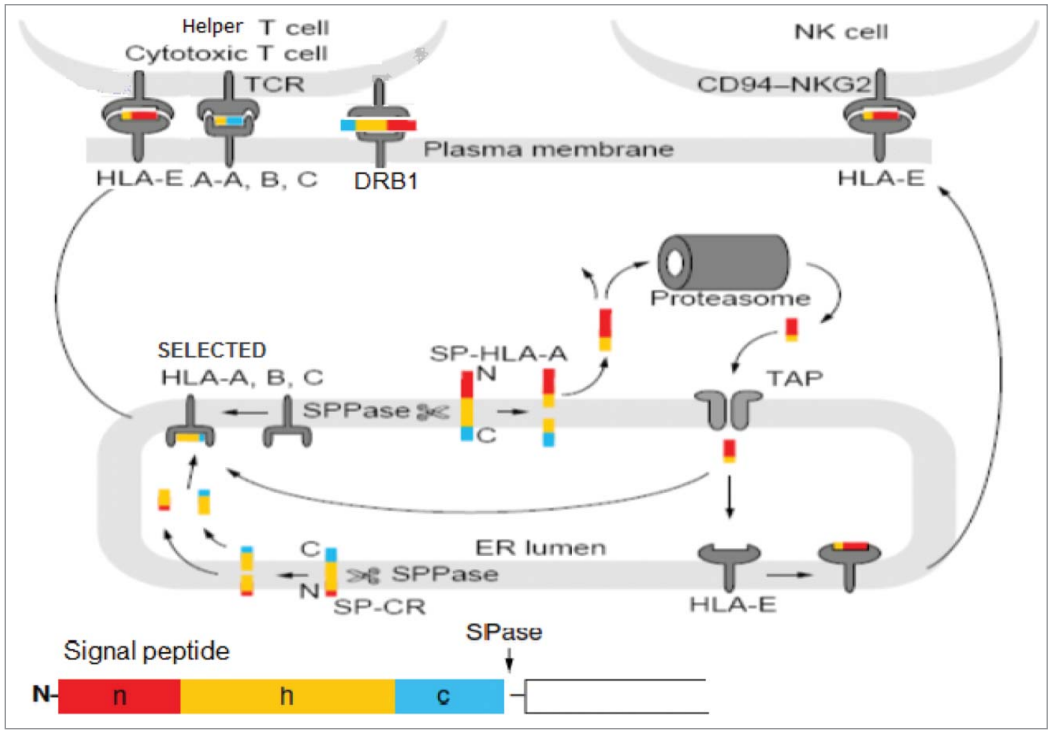

Figure 2. State of the art: The immunological properties of SP. Adapted from a scheme presented by Bruno Martoglio and Bernhard Dobberstein.[8] SPs feature a tripartite structure: a central hydrophobic h-region (yellow) and hydrophilic N- (red) and C-terminal (blue) flanking regions. SP fragments can be released directly into the ER lumen, by SPP activity (TAP-independent presentation), or into the cytosol, where they are proteolytically processed by the proteasome. The resulting fragments in the cytosol are transported by TAP-dependent machinery into the ER lumen. There, they bind and stabilize select MHC class I A, B, and C molecules and present them to cytotoxic $CD8^+$ T-cells. In parallel, N-region fragments of SP domain can selectively bind HLA-E molecules and present them to NK and cytotoxic $CD8^+$ T-cells. Longer SP fragments were found to activate $CD4^+$ T-cells, likely via binding to MHC class II molecules.

Combined MHC class I and II alleles in SP domains

A recent in silico analysis, corroborated by wet biological experimentation, was conducted in search of MHC class I epitope-rich regions in defined protein domains on the entire human and mouse genome. Results demonstrated that structurally defined SP domains have exceptionally high epitope densities, primarily for human MHC class I alleles, predominantly when analysis for restriction to TAP binding is not present.[43] Moreover, a similar in silico analysis performed on scrambled, human protein domains, showed significantly lower epitopes density (i.e., MHC binding) only for SP domains compared with the native none-scrambled sequences.[43] Furthermore, the high epitope density within SP domains, and not within any other human protein domain, stands in line with the significantly higher percentage of characterized SP epitopes in the IEDB[44] immune epitope database.[43] Taken together, these findings strongly suggest that while preferred MHC binding in general and SP domains in particular, relies partially on the domain hydrophobic nature, it is mainly dependent on SP antigen specificity (i.e., the protein they originate from). Surprisingly, while the sequence-specific feature of SP domains was a well-established fact in molecular cell biology,[8,13] this critical parameter, which can determine their suitability as VCs rather than just stimulatory molecules, was vastly neglected in immunology and vaccinology (see also previous paragraph: "Signal Peptide Domain: State of the Art"). Therefore, these results prove that when weighing antigen-specificity, SP domains should be considered an integral part of the proteins from which they are derived.

Following this rational, SP domains are multi-epitope LP VCs that overcome many of the aforementioned limitations. Firstly, as well-defined domains, SP isolation from known and novel antigens is a fast and straightforward process, and can be performed using established in silico programs (**Fig. 4**). Secondly, SP domains possess novel, promiscuous MHC class I and class II binding potential, leading to more robust combined

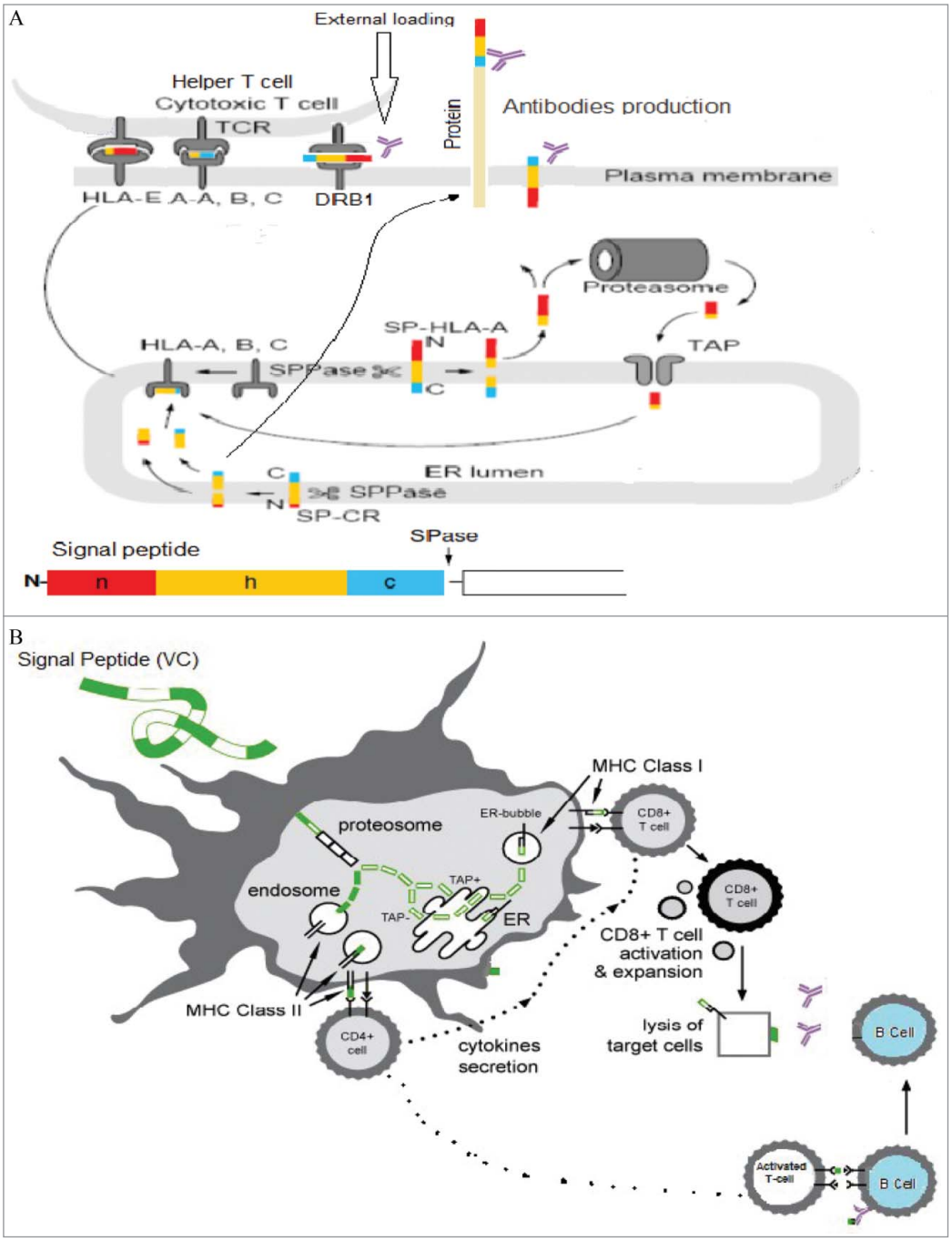

Figure 3. Beyond the state of the art: exploitation of entire SP domains as VCs. (**A**) Adapted from ref.[8] In select disorders (e.g., MUC1 TAA in cancer), the C-region, and, to some extent, the H-region SP fragments can reach the cell surface as independent molecules or as part of the entire antigen. This, in turn, can lead to the production of MUC1 SP-specific antibodies. (**B**) Entire multi-epitopes, LP, SP domains used as VCs can stabilize extracellular MHC class II on B-cells and APCs. In parallel, SP domains can rapidly enter these cells, where its internal overlapping epitopes (represented by green strip) can bind MHC class II in the endosome, to stimulate $CD4^+$ T-cell activation and B-cell receptors. The $CD4^+$ T helper and B-cell signals trigger antibody production. Other shorter overlapping SP fragments (represented by white strip with a green frame) enter the ER by TAP-dependent and TAP-independent pathways and are loaded as short 8-10-mers onto multiple MHC class I alleles. Different internal MHC class I and II epitopes are being selected in different human individuals based on their MHC repertoire. SP-specific $CD4^+$ T-cell activation, coupled with $CD8^+$ T-cell activation via the same antigen-specific SP domain, promotes more robust $CD8^+$ T-cell cytotoxic activity. Recently, anti-SP antibodies have been shown to mediate ADCC of target cells, seemingly by activation of innate immunity.

antigen-specific $CD4^+$ and $CD8^+$ T-cell immunity (**Fig. 3**). For initial screening, known and novel proteins which are overexpressed in selected disease are selected. Proteins with sub-cellular localization that do not require transport from the ER-Golgi (i.e., proteins with SP domains), as well as proteins with a function in basic homeostatic are excluded. Next, SP domain isolation and scoring is performed via SignalP or similar software. Their homology with other human sequence is evaluated, after which, MHC class I and II binding to selected alleles with good population coverage is analyzed and scored as previously published.[45,46] As SP domains preferably bind human MHC alleles, initial bio-validation of the selected SP domains and controlled antigen match peptides, is performed in-vitro on large sample pools of peripheral blood mononuclear cell (PBMC) obtained from both naïve and disease-associated donors. For an in-depth analysis, SP domain-specific T-cell lines are produced and analyzed for phenotype and function. Immunological, T/B cell properties and therapeutic potential are then assessed in vivo, in either syngeneic and/or HLA-transgenic mice.

Comprehensive in vitro and in vivo studies conducted on large (>50) sample pools of PBMCs, obtained from both cancer[45] and MTb-infected patients,[43,46] demonstrated significantly preferred proliferation across MHC, for the MUC1 TAA SP domain and 5 MTb-derived SP domains, (including the known Ag 85 and

Table 1. Comparative analysis of peptide-based vaccination approaches

Criteria	Single MHC epitope	Non-SP multi-epitope LP	SP multi-epitope LP
Isolation complexity[1]	2	2	3
Development complexity (synthesis and formulation)	3	2	1
Antigen specificity	3	3	3
Polyclonal $CD4^+$ plus $CD8^+$ T-cell induction[2]	1	2	3
Population applicability (MHC class I and II-wise)[3]	1	2	3
Induction of functional antibodies[4]	1	2	3
Modulation of non-classical HLA-E[5]	3	3	2
Adaptation for TAP- deficiency scenarios	1	1	3
Adjuvant/carrier requirements[6]	1	2	3

Scoring, defined from 1 to 3 where 1 represents poor; 2 moderate and 3 excellent performance. (1) VC isolation is easier for SP domains since it is focused on well-defined domains. (2, 3) Analysis is based on the ability of the peptides to bind multiple MHC alleles and consequently support polyclonal T-cell activation. (4) Analysis is based on the ability of the peptides to induce complement-dependent cytotoxicity and ADCC following vaccination. (5) A small portion of SP epitopes (both single and in LP) is more likely to bind non-classical HLA-E, and inhibit the killing activity of specific NK subsets. This inhibition is not VC-induced but can affect the total generated immunity. (6) The reduced need for adjuvant/carrier in SP multi-epitope LPs is related to their lipophilic sequence and improved MHC binding properties.

lipoprotein lpqH and novel Un char protein Rv0476/4941 and Un char protein Rv1334/1376 domains), when compared with antigen-matched epitope sequences. Furthermore, T-cell lines induced ex-vivo against these SP domains, using both naive and patient-derived PBMCs, demonstrated an effector memory profile ($CD45RO^+CD44^+CD62L^{high}$), coupled with robust antigen-specific IFN-γ production, as well as cytotoxic properties against MUC1-positive tumor[45] and MTb-infected cells.[46] Similarly, these SP domains induced a strong and specific cellular immune response[45-47] and anti-tumor activity[45] in both mice and in a first-in-human study.[48] More recently, an additional support for the potency of SP domains was published by Kerzerho et al., which demonstrated the existence of multiple $CD4^+$ T-cell epitopes[47] and functional anti-tumor $CD8^+$ T-cell cytotoxic properties[49] in the SP domain of Midkine, a heparin-binding growth factor and a TAA. In humans, 12 bi-weekly intradermal vaccinations of the formulated MUC1 SP vaccine ImMucin (100 μg dose), together with 250 μg human granulocyte-macrophage colony-stimulating factor (hGM-CSF), triggered robust $CD4^+/CD8^+$ T-cell-mediated immunity across HLA-barriers, in all 15 MUC1-positive asymptomatic multiple myeloma (MM) patients presenting minimal disease or relapse at the start of the study. The peak of T-cell-mediated immunity was achieved following 2–4 vaccinations. Patients exhibited robust IFN-γ production by both $CD4^+$ and $CD8^+$ T-cells, with mean baseline and peak postvaccination levels generated by 0.21% vs. 4.07% ($P < 1.4 \times 10^{-5}$, *t* test) and 0.21% vs. 11.76%, ($P < 0.0001$, *t* test) of T-cells, respectively. In

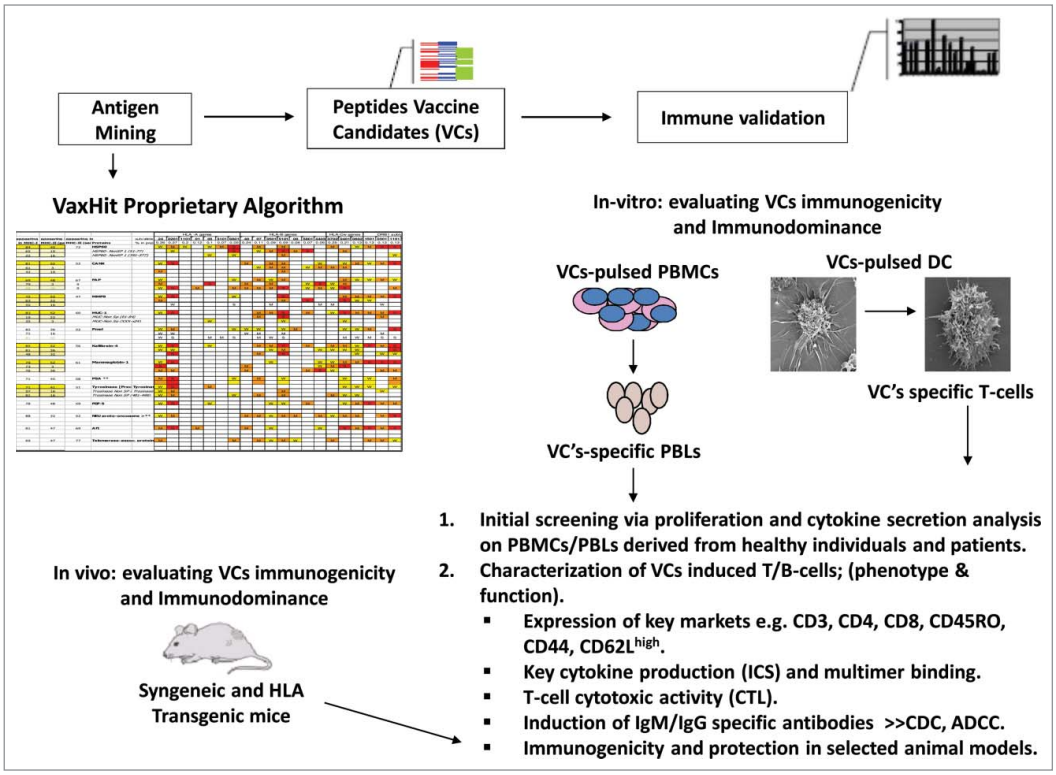

Figure 4. Isolation and development of SP domains. SP domains from selected proteins of interest are initially isolated and scored in silico. Next, the selected SP domains are analyzed for MHC class I and II binding. Biovalidation of selected SP domains is performed in-vitro on large sample pools of PBMCs and SP-induced T-cell lines for characterization of phenotype and function. Immunological and therapeutic potential is assessed in appropriate syngeneic and/or HLA-transgenic mice.

addition, a 35-fold increase in ImMucin-specific $CD4^+$ T-cell (range: 4–80-fold) and a 43.4-fold increase in $CD8^+$ T-cell (range: 18–80-fold) counts were observed post-vaccination. Because of the general motif shared by SP domains, it was important to ensure the specificity of the generated response. The T-cell response was confirmed to be ImMucin-specific, as demonstrated by a pronounced (>2%) population of MUC1 SP HLA-A2.1 multimer-positive $CD8^+$ T-cells and the absence of IFN-γ production in response to treatment with MUC1 and unrelated SP control peptides.[48] Despite the robust generated immunity, ImMucin was well tolerated, and no vaccine-related grade ≥3 adverse events (AE) were reported in over 160 vaccine (ImMucin plus hGM-CSF) administrations in patients. Common, primarily local vaccine administration-associated grade 1 or 2 AEs were observed, all of which self-resolved within 72 h (see also **Table 1** for technology comparison).

Generation of humoral immunity

A peptide-induced antibody response mostly requires B-cell epitopes derived from an extracellular domain of a target antigen, together with $CD4^+$ T-cell epitopes, mostly present on a different "helper sequence." Isolation and characterization of B-cell epitope(s) generally requires in silico prediction methodologies,[5,7] coupled with bio-validation efforts to assess the ability of the generated antibodies to recognize the target antigen in the context of the pathogen/tumor cells. Due to the different structure of B-cell vs. T-cell epitopes, a single antigen-specific peptide sequence is unlikely to be able to trigger both types of immunity.

Recent findings showed a significant elevation in the levels of autoantibodies against MUC1 SP in the bloodstream of MM patients,[50] when compared with those measured in healthy donors. Since soluble MUC1 SP was not detected in the sera of these patients, it was speculated that the naturally generated autoantibodies were primed by non-MHC-restricted, tumor MUC1-associated cell-bound SP. This hypothesis was confirmed using the MUC1 SP-specific polyclonal (R23IgG) and monoclonal (SPmAb-2.1 and SPmAb-6) antibodies,[51] which showed high specificity to MUC1 SP, without binding unrelated SPs. Cell-surface expression of MUC1 SP was detected on various MUC1-positive tumor cell-lines and primary tumors, but not on primary naïve blood and epithelial cells. While the mechanism by which MUC1 SP reaches the tumor cell surface has not been fully defined, published results with MUC1 SP suggested it to be mainly a consequence of tumor-associated SPP malfunction and to a lesser extent to SPase malfunction (**Fig. 3**).[51] In parallel, loading experiments with ImMucin's 9-mer epitopes MUC1-SP-S2 and MUC1-SP-S4 (which are SPmAb-2.1 and SPmAb-6 epitopes) on MUC1 negative, HLA-A2.1-transfected TAP-deficient RMA-S cells, revealed strong HLA-A2.1 stabilization but no MUC1 SP binding, thereby confirming that MUC1 SP surface expression most likely exists without MHC class I association (unpublished data). In the context of active vaccination, 10/15 MUC1 SP ImMucin-vaccinated MM cancer patients demonstrated a significant increase in anti-ImMucin IgG concentrations, with a mean 6.86-fold (range: 1.4–40) increase at the response peak, which was observed after receiving 6 or 7 immunizations. Importantly, the induced anti-ImMucin antibodies mixed with autologous PBMCs induced specific antibody-dependent cell-mediated cytotoxicity (ADCC) of autologous bone marrow-derived tumor cells, confirming the presence of the MUC1 SP domain on primary tumors, and the selective and prominent anti-tumor properties of the generated anti-MUC1 SP antibodies.[48]

The need for adjuvants and/or carriers

Immunity to peptides, specifically those derived from self-tumor antigens, usually require indirect presentation via DCs, as they are considered poor immunogens.[52] Likewise, injection of naked peptides without adjuvant has typically been shown to be minimally immunogenic; and co-administration with immunologic adjuvants is typically required to induce detectable T-cell or B-cell responses.[52] However, in vitro and in vivo results with MUC1's TAA[45,48] and MTb-derived[43,46] SP domains, somewhat deviated from the general observations. SP domains directly activated in vitro proliferation of PBMC samples isolated from naïve donors. Moreover, immunization of HLA-A2.1 transgenic mice and BALB/c syngeneic mice with the MUC1 SP domain or MTb SP domains, alone or in combination with murine GM-CSF, induced a strong antigen-specific T-cell response, and to some extent an antibody response as well, without involving any adjuvant.[48] In humans, strong cellular and moderate humoral responses were obtained in MM patients treated with 100 μg naked ImMucin vaccine plus 250 μg of hGM-CSF, but without a carrier or B-cell adjuvant,[48] (see sections on T-cell and antibody response). We attribute the strong immune response observed with SP domains to the following factors: first, SP domains contain hydrophilic/lipophilic sequences which are known to be more immunogenic, mainly with regard to T-cell induction (by enhancing MHC binding and cell penetration), which, in select cases, is comparable to that achieved when administering a vaccine with incomplete Freund's adjuvant[53] (and authors unpublished data). Second, as indicated, SP domains demonstrate promiscuous binding to MHC class I, mainly via TAP-independent presentation, and to MHC class II[47] and also present a high density of B-cell epitopes, which seemingly underlie the observed preferred dual T/B-cell immunogenicity.[8,54,55] Based on the limited available information on combining SP domains with different adjuvants in human studies, it is too early to predict if a given mixture will effectively reduce the amount of required antigen and/or the quality of the response. Moreover, it will be critical to assess if any of these modification will impact the final clinical outcome.

Other Points for Consideration When Selecting and Developing SP Domains as VCs

(1) It should be appreciated that when choosing SP domains as VCs, the vaccine antigenic repertoire is reduced to secreted and transmembranal proteins that contain SP domains.

(2) As briefly indicated earlier in this review, among the many features of SP-derived epitopes, is their preferred binding to non-classical HLA-E molecules on target cells (**Fig. 2**). HLA-E binding and stabilization, described so far on virus-infected targets,

by SP domains with a well-defined sequence, led to killing inhibition of CD94/NKG2-positive NK-cells.[56] It is important to emphasize that the specific inhibitory motif appears only on a select few SP domains. Moreover, the inhibition mechanism is not induced upon vaccination with SP LP VCs. Finally, the potential inhibition of CD94/NKG2-positive NK-cells is likely to work in parallel to NK-mediated ADCC activation, and to T/B-cell immune activation induced following vaccination with most SP domains.[48] Nevertheless, since HLA-E is also widely expressed on tumor cells, and this inhibitory process can affect the total generated immunity, it is wise to assess the presence and inhibitory properties of SP LP VC. Positive NK inhibition should be considered as part of the pro and cons in the initial development process of SP LP VCs.

(3) The hydrophobic properties of LP SP domains challenge the chemistry, manufacturing and control aspects of commercial SP domain development. Large-scale manufacturing of the active pharmaceutical ingredient and the clinical trial material, including its final configured formulation, requires a combination of superb peptide chemistry in addition to basic biology and immunology tools.

Concluding Remarks

While all SP domains share a common structure and motif, they have high antigen specific sequence variability that can enable, inhibit or activate innate (NK via ADCC), and adoptive (T-cell- and antibody-mediated) immunity. These unique features demonstrate that the activity of SP domains extends far beyond mere "targeting sequences." Moreover, SP-preferred binding to multiple human, rather than murine, MHC alleles, raises additional assumptions related to the co-evolution of pathogen-derived SP domains and the human immune system, which are beyond the scope of this review. However, until recently, the immunological properties of SP domains remain an unexplored niche. Work performed in recent years, showed that the use of human SP domains as VCs, presents an intuitive, yet unique immunotherapeutic approach, generating combined and diversified antigen-specific $CD4^+/CD8^+$ T-cell and B-cell immunity in a substantial number of subjects, irrespective of their HLA repertoire. In this manner, SP domain-based vaccines overcome the need for patient selection and treatment personalization. The induced immune response is safe, highly specific and effective, even with limited use of adjuvants, and results in in-vitro killing of appropriate targets, including transformed cancer cells and MTb-infected macrophages. Initial clinical experience using ImMucin, a MUC1 TAA entire SP domain-based therapeutic vaccine, further validated the preclinically observed robust and diversified T/B-cell immunological responses and also provided initial indications of its clinical efficacy. Nevertheless, further experiments will be required to better understand if the improved diversified immunity is translated to enhanced clinical outcomes. Moreover, additional research will be required to better understand the intracellular processes and mechanism of action of SP domains after ER localization, in order to broaden the commercial applications of these intriguing domains.

Disclosure of Potential Conflicts of Interest

R.K. is a senior scientist and L.C. is the founder and head of R&D and the CEO of Vaxil BioTherapeutics Ltd., which is developing vaccines and antibodies for cancer and tuberculosis using entire signal peptide domains.

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