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Advances in the study of HLA-restricted epitope vaccines

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Abbreviations: CMI, cell-mediated immunity; MHC, major histocompatibility complex; HLA, human leukocyte antigen; TCR, T-cell receptor; CTL, cytotoxic T-lymphocyte; TAA, tumor-associated antigen; DC, dendritic cells; APC, antigen-presenting cells; CEA, Carcinoembryonic antigen; NSCLC, non-small cell lung carcinoma; TLR, Toll-like receptor; HBV, hepatitis B virus; HPV, human papilloma virus; GM-CSF, granulocyte macrophage colony stimulating factor

Vaccination is a proven strategy for protection from disease. An ideal vaccine would include antigens that elicit a safe and effective protective immune response. HLA-restricted epitope vaccines, which include T-lymphocyte epitopes restricted by HLA alleles, represent a new and promising immunization approach. In recent years, research in HLA-restricted epitope vaccines for the treatment of tumors and for the prevention of viral, bacterial, and parasite-induced infectious diseases have achieved substantial progress. Approaches for the improvement of the immunogenicity of epitope vaccines include (1) improving the accuracy of the methods used for the prediction of epitopes, (2) making use of additional HLA-restricted $CD8^+$ T-cell epitopes, (3) the inclusion of specific $CD4^+$ T-cell epitopes, (4) adding B-cell epitopes to the vaccine construction, (5) finding more effective adjuvants and delivery systems, (6) using immunogenic carrier proteins, and (7) using multiple proteins as epitopes sources. In this manuscript, we review recent research into HLA-restricted epitope vaccines.

Introduction

Research for over 200 years has provided evidence of the benefits of vaccination. Edward Jenner provided the first scientific rationale for vaccination by demonstrating that individuals immunized with the cowpox virus were protected from the disease caused by the smallpox virus.[1] Historically, vaccines have consisted of live attenuated pathogens, whole inactivated organisms, purified antigens, or polysaccharides linked to a carrier protein or inactivated toxin. However, non-living vaccines have generally proven ineffective at inducing cell-mediated immunity (CMI), and some live attenuated vaccines can cause disease symptoms in immunosuppressed individuals. In addition, many traditional inactivated vaccines may contain components that can cause undesirable side effects and tolerability concerns.[2] Therefore, the induction of an effective and protective immune response with minimal adverse reactions is crucial to new vaccine development.

An "immunosense" vaccine is an epitope-based vaccine consisting of peptides derived from immunogenic proteins restricted by MHC supermotifs based on the theory of antigen processing and presentation. Epitopes are the antigenic determinant sections of antigens that are recognized by the immune system, specifically by antibodies, B cells, and T cells. In fact, these epitopes (antigenic determinants) rather than the entire antigens are recognized by immune cells. These can be specifically recognized by antibodies or by the antigen receptors of lymphocytes.[3] T-cell epitopes are presented on the surface of antigen-presenting cells (APCs), where they are bound to major histocompatibility complex (MHC) molecules. The T-cell epitopes presented by major histocompatibility complex class I (MHC I) molecules are $CD8^+$ T-cell epitopes, which are typically peptides 8–11 amino acids in length. In contrast, MHC class II molecules present longer peptides (13–17 amino acids in length), which are considered major $CD4^+$ T-cell epitopes.[4]

In humans, the MHC is also called the human leukocyte antigen (HLA). MHC class I molecules are encoded in humans by the HLA-A, -B, and -C genes, which present peptides from inside the cells. MHC class II molecules are encoded in humans by the HLA-DP, -DQ, and -DR genes, which present peptides from outside cells to T-lymphocytes. The HLA molecules recognize specific peptides that constitute the epitopes of pathogens. The molecular basis of the interactions between HLA molecules and antigenic peptides are anchor residues and consensus motifs.[5] In recent years, HLA transgenic mice have been used experimentally since the MHC molecules in these mice are the same as those involved in the human immune system.[6]

However, the diversity of HLA types in the human population is a potential obstacle for the study of epitope-based vaccines. HLA molecules exhibit high polymorphism; hundreds of different alleles exist. However, it has been demonstrated that a significant degree of overlap exists among the peptide-binding specificities of different HLA supertypes and that three peptides specificities corresponding to HLA-A02, A03, and B07 cover ~90% of the world's population.[7,8]

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The epitope-based approach offers some potential advantages over the more conventional whole-protein approaches. The focusing of the immune responses against highly conserved epitopes might be crucial for the prevention and treatment of infection with highly variable pathogens. Therefore, a protective "immunosense" vaccine would include antigenic epitopes that bind to the HLA supertype families that are present in a large proportion of the population, and would be able to elicit an effective and protective immune response.

Although research and development of HLA-restricted epitope vaccines to treat cancer and to prevent infectious diseases have made substantial progress, only one peptide-based renal cell cancer vaccine (IMA901,[9,10] which was developed by immatics biotechnologies GmbH) is known to have entered a Phase III clinical trial (www.immatics.com). There are many weaknesses and challenges associated with epitope vaccines. Thus, we should employ other useful strategies, such as the following: improving the accuracy of the prediction methods for epitopes, using more HLA-restricted $CD8^+$ T-cell epitopes, including specific $CD4^+$ T-cell and B-cell epitopes, identifying more effective adjuvants and delivery systems, using immunogenic carrier proteins, and using multiple proteins as epitope sources.

Methods Used for Epitope Identification

The first step in the design of an HLA-restricted epitope vaccine is the identification of high-affinity HLA epitopes. The methods used to identify epitopes are divided into two approaches: experimental and predictive.

Experimental approaches

The mapping of antigenic peptide sequences from proteins of relevant pathogens recognized by T and B cells is important for vaccine development. Experimental approaches depend on biochemical and immunological experiments, such as phage display libraries, overlapping peptides, enzyme-linked immunosorbent assay (ELISA), nuclear magnetic resonance (NMR), immunofluorescence, radioimmunoassay, immunoblotting, immunohistochemistry, and X-ray crystallography studies of the antibody/antigen structure.

The fusion phage display technology, which was first developed by Smith et al. in 1985,[11] is a strong tool for the study of the B-cell epitopes of proteins. Linear epitopes and conformational epitopes can be obtained from the protein antigens through the use of a random peptide library. The candidate gene fragment is first cloned into the phage coat protein gene region, and the exogenous polypeptide is expressed and displayed on the phage surface and maintained in a specific conformation. Immune sera or specific antibodies are used to screen the phage library, and positive binding phages are selected for sequence analysis. The amino acid sequences of the peptides displayed on the phage that are bound by the specific antibodies are then determined by sequencing the corresponding coding region in the pathogen DNAs. Tens of millions of short peptides can be easily surveyed for tight binding to an antibody, receptor or other binding protein using an "epitope library." In 1990, Scott et al. used a random phage peptide library to search for the localization of epitopes localization on antigens for the first time.[12] Since that time, related studies have been performed worldwide. This technology is widely used for epitope identification, and has greatly promoted the development of epitope vaccines. Beghetto et al. screened a phage-display library of T*oxoplasma gondii* cDNA fragments with sera from infected individuals and identified a panel of recombinant phage clones carrying B-cell epitopes encoding the *T. gondii* antigens SAG1, GRA1, GRA3, GRA7, GRA8, MIC3, and MIC5.[13]

In contrast, the T-cell epitope identification depends on functional assays, regardless of whether a T-cell function is detected. The following functional assays can be performed: MHC peptide, multimers[14] solid-phase MHC-peptide complexes,[15] intracytoplasmic cytokine staining,[16] ELISPOT (ELISA spot),[17] cytokine secretion and cell surface capture[18] and lymphoproliferation[19] Most of the assays reveal a functional T-cell response, such as the upregulation of activation markers, cytokine synthesis, proliferation, cytolytic, and helper function. Using different methods, immunodominant peptides have been identified on the proteins of various pathogens. However, because numerous peptide panels from antigenic proteins need to be screened, the research is time-consuming.

Predictive bioinformatics approaches

With the aid of software and databases, bioinformatics methods have become economical and effective tools for epitope prediction. The bioinformatics methods used for the prediction models can be divided into sequence-based methods and structure-based methods.

The sequence-based methods for T-cell epitope predictions are based on the linear amino acid sequence. The search for a motif with the combination of preferred amino acids at some of the peptide anchor binding positions is the most widely used method for the prediction of epitopes.[20] SYFPEIYHI was the first online database that was widely searched using the motif search approach.[21] This database comprises more than 7000 peptide sequences that are known to bind class I and class II molecules (http://www.syfpeithi.de/). EPIPREDICT and EPIMER are also motif-based tools that are used for the identification of MHC class II-binding epitopes from proteins and the prediction of HIV-related epitopes. In addition, many algorithms such as quantitative matrices (QM)-based techniques (EpiMatrix Meister,[22] Virtual matrix [VM],[23] and BIMAS[24]), and machine-learning techniques (Artificial Neural Networks [ANNs],[25–28] Hidden Markov Models [HMM],[29] and Support Vector Machine [SVM],[30,31]) have been developed over these years. However, a number of incorrectly identified false positives are predicted as non-binders through the comparison of the affinities predicted by the algorithm with that of the experimentally determined proteins.[32] The major limitation of these methods is their inability to discriminate between T-cell epitopes and non-epitope MHC binders.

The structure-based methods do not solely rely on binding data and sequence information. Instead, these methods use structural information based on three-dimensional structures of the protein and computational methods developed in the field of structural biology for the prediction.[20] Docking is a quick

and powerful technique for the investigation of intermolecular interactions. EpiDOCK is a structure-based server for the MHC-binding prediction of peptides using docking score-based QMs (DSQMs).[33] This method predicts the binding to 12 HLA-DR, six HLA-DQ and five HLA-DP proteins.[33] Threading algorithms, such as CEP, and DiscoTope, are used to discriminate the binding and non-binding peptides for particular MHC molecules without requiring previous data. In addition, DOT and PatchDock, among others, are based on the homology modeling method to predict epitopes.

A higher prediction accuracy can be achieved though the use of a combined approach such as nHLAPred (QM + ANN), CTLpred (QM + ANN + SVM), IEDB binding I (ANN + ARB + SMM), and IEDB binding II (Consensus Method + ARB + SMM + Sturniolo).[7] Bhasin et al. evaluated of the prediction of CTL epitopes using a method based on QM, ANN and SVM, and found that the performance was further enhanced by devising consensus and combined approaches based on SVM and ANN.[34] The combined prediction approach achieved a sensitivity of 79.4%, whereas the consensus approach obtained a sensitivity of 88.4%.[34]

Although many epitope prediction methods and approaches have been established and applied, epitope forecasting is not yet a complete and accurate reflection of the situation in vivo due to the complexity of the immune response mechanism. The bioinformatics-based prediction of immunogenic epitopes remains challenging. A higher degree of integration between these predictions and in vitro experiments should be considered to improve the accuracy of the predictions.

Epitope Vaccine Development

The development of vaccines based on the identification of immunogenic and protective HLA-restricted epitopes holds great promise for the treatment of cancer and the prevention and treatment of viral, bacterial, and parasitic diseases.

Epitope vaccines for the treatment of cancer

Due to the increasing incidence and mortality of cancer, the development of effective cancer vaccines against is important and urgent. Activated cytotoxic T lymphocytes (CTLs) can kill tumor cells directly or indirectly by secreting cytokines, such as IFN-γ and TNF-α. CTLs carry epitopes of tumor antigens associated with MHC-I molecules, and this association results in recognition of the MHC molecule and the bound tumor peptide. Thus, vaccines that contain CTL epitopes that recognize tumor antigens hold promise for the treatment of cancer.

Several peptides that bind to HLA molecules on tumor cells have been identified. A large number of tumor-associated antigens (TAAs) have been used for cancer vaccines[35] (**Table 1**).

The TAA MUC1 is overexpressed on various hematological and epithelial malignancies, and is therefore a suitable candidate for broadly applicable vaccine therapies. Brossart et al. identified two novel peptides with a high binding probability to the HLA-A2 molecule and showed that these peptides are shared among many tumors, including breast and pancreatic tumor cells and renal cell carcinoma cells.[36] Karl et al. evaluated the efficacy of immunizing mice transgenic (Tg) for human MUC1 with peptides derived from the amino acid sequence of the CT of MUC1 (MUC1 $CT_{3–27}$, MUC $CT_{18–49}$, and MUC $CT_{37–69}$).[37] Their data showed that survival can be significantly prolonged in vaccinated MUC1 Tg mice challenged with MUC1-expressing tumor cells without the induction of autoimmune responses.[37]

Carcinoembryonic antigen (CEA) is a TAA that can be overexpressed in individuals with various carcinomas.[38] Li et al. identified the long peptide $CEA_{625–667}$, and found that mice immunized with a plasmid encoding the CEA peptide induced strong antigen-specific T-cell proliferation; in particular, immunization with the plasmid encoding triple-repeated CEA peptides significantly elevated the levels of IFN-γ secreted by T cells.[39]

HER-2/*neu* is a member of the epidermal growth factor receptor family and is normally expressed during fetal development and overexpressed in 30% of breast cancers.[40,41] Fisk et al. identified an immunodominant peptide of the HER-2/*neu* proto-oncogene E75 (HER-2, 369–377: KIFGSLAFL) in ovarian tumor-specific CTL lines. The E75 was found to be efficient for the sensitization of T2 cells for lysis by all four CTL lines in an in-vitro assay.[42]

MAGE-A3 expresses both TCRs in human peripheral blood leukocytes (PBLs) and demonstrates antigen-specific reactivity against a range of melanoma and non-melanoma tumor cells. Chinnasamy et al. found that the TCR against $MAGE\text{-}A3_{112–120}$ has superior reactivity against tumor cells; thus, the MAGE-A3 TCR may be an ideal candidate for tumor immunotherapy.[43] These researchers immunized transgenic mice that expressed the human HLA-A*0201 molecule with two HLA-A*0201-restricted peptides of $MAGE\text{-}A3_{112–120}$ (KVAELVHFL) or $MAGE\text{-}A3_{271–279}$ (FLWGPRALV) to generate high-avidity TCRs against MAGE-A3.[43]

The immunogenic peptides from the MAGE-A3 epitope have received strong interest as a possible treatment for several types of cancer including melanoma,[44] non-small cell lung carcinoma (NSCLC),[45] head and neck squamous cell carcinoma,[46] hepatocellular carcinoma,[47] and multiple myeloma.[48] In addition, peptides derived from the T-1 protein,[49–51] TRP-2 protein,[52] and gp100 protein[53] also have been considered candidates for cancer treatments.

A number of HLA-restricted epitope vaccines against cancer have entered clinical trials. Hu et al. reported the first clinical trial of a melanoma antigen gene-1 (MAGE-1)-derived peptide vaccines in 1996.[54] These researchers immunized HLA-A*01-positive patients, whose melanoma cells expressed the MAGE-1 peptide, with a MAGE-1 gene-encoded nonapeptide (EADPTGHSY) pulsed with autologous APC-based vaccine, and found that this vaccine induced an autologous melanoma-reactive and peptide-specific cellular CTL response.[54] Takahashi et al. reported the first clinical findings in one patient diagnosed with pulmonary metastasis of colon cancer who was treated with an artificially synthesized MAGE-A4-helper/killer-hybrid epitope long peptide (H/K-HELP) cancer vaccine.[55] This hybrid peptide MAGE-A4-H/K-HELP was synthesized by conjugating $MAGE\text{-}A4_{278–299}$ helper epitope ($CD4^+$ T-cell epitope (ALAETSYVKV LEHVVRVNAR VR) with the

Table 1. HLA-restricted epitope vaccines against cancer

Protein	Epitope	Adjuvant	Patients/ Animals	Methods	Results	Ref.
MUC1	CT_{3-27}(CQCRRKNYGQ LDIFPARDTY HPMSEYPTYH) (#1) CT_{18-49}(HPMSEYPTYH THGRYVPPSS TDRSPYEKVS AG)(#2) CT_{37-69}(STDRSPYEKV SAGNGGSSLS YTNPAVAAAS ANL)(#3) MUC1 TR (PDTRPAPGST APPAHGVTSA)	GM-CSF	transgenic mice for human MUC1 (MUC1.Tg)	The mice was injected with a combination of 50 μg of peptide#1, 50 μg of peptide #2, and 50 μg of peptide #3 in a total volume of 100 μl	survival can be significantly prolonged in vaccinated MUC1.Tg mice challenged with MUC1-expressing tumor cells, without induction of autoimmune responses	37
CEA	$CEA_{625-667}$	aluminum hydroxide gel	Female BALB/c mice	Mice were immunized intramuscularly with 100μg of pcDNA3.0, pcDNA-CEA625–667, or pcDNA-triCEA625–667 in 1:1 (v/v) of 3% aluminum hydroxide gel, respectively	Induced strong antigen-specific T cell proliferation. Triple-repeated CEA peptides vaccine significantly elevated levels of IFN-γ secreted by T cells	39
MAGE	$MAGE\text{-}A3_{112-120}$ (KVAELVHFL) $MAGE\text{-}A3_{271-279}$ (FLWGPRALV)		HLA-A*0201 transgenic mice	Mice were immunized at the base of the tail with 100 mg of $MAGE\text{-}A3_{112-120}$ (KVAELVHFL) or $MAGE\text{-}A3_{271-279}$ (FLWGPRALV) plus 120 mg of hepatitis B virus core	generate high-avidity TCRs against MAGE-A3	43
	MAGE-1 peptide (EADPTGHSY)		HLA-A1 positive and melanoma cells expressed the MAGE-1 peptide, (EADPTGHSY) patients	Four monthly intradermal injections of increasing numbers of peptide-pulsed APCs (10^5, 5×10^5, 10^6, and 10^7cells) for the four consecutive injections	Induced autologous melanoma-reactive and peptide-specific cellular CTL response	54
	$MAGE\text{-}A4_{278-299}$ (ALAETSYVKV LEHVVRVNAR VR) $MAGE\text{-}A4_{143-154}$ (NYKRCFPVIF GK)	OK432 Montanide ISA-51	pulmonary metastatic colon cancer patients.	The patient was vaccinated with 1 or 10 mg MAGE-A4-H/K-HELP mixed with OK432 (0.02KE) and Montanide ISA-51 four times at 2-week intervals	Induced MAGE-A4-specific Th1 and T-cell 1 immune responses and the production of MAGE-A4-specific complement-fixing IgG antibodies. Significantly decreased Tumor growth and carcinoembryonic antigen tumor marker	55
gp100	gp100 (IMDQVPFSV, 209–217(210 M))	Interleukin-2 Montanide ISA-51	Stage III, IV cutaneous melanoma, expression of HLA-A0201, an absence of brain metastases	Patients were treated with gp100 peptide plus Montanide ISA-51 once every 3 weeks, followed by interleukin-2 intravenous bolus every 8 h	Compared with interleukin-2 alone, vaccine plus interleukin-2 significantly improved clinical response rate (16% vs. 6%) and progression-free survival (2.2 mo; 95% confidence interval [CI], 1.7 to 3.9 vs. 1.6 mo; 95% CI, 1.5 to 1.8)	53
HER-2/ *neu*	AE37 (Ac-LRMKGVGSPY VSRLLGICL-NH_2, Ii-Key hybrid of HER-2/*neu* peptide 776–790)	GM-CSF	Disease-free, node-negative breast cancer patients.	Each person received 100 μg, 500 μg or 1000 μg of AE37 peptide mixed with 250 μg, 125 μg, 30 μg or 0 μg GM-CSF inoculations for 6 mo.	The optimal biologic dose (OBD) of the novel AE37 hybrid vaccine: 500 μg of peptide with GM-CSF (30–125 μg) which significantly increased in proliferative responses at long-term follow-up.	57

Table 1. HLA-restricted epitope vaccines against cancer (continued)

Protein	Epitope	Adjuvant	Patients/ Animals	Methods	Results	Ref.
HER-2/ *neu*	E75 (KIFGSLAFL, HER-2/ *neu*; 369–377)	GM-CSF	Disease-free lymph node-positive (NP), lymph node-negative (NN) and HLA-A2/A3 breast cancer patients	Patients were vaccinated over 6 mo (3, 4, or 6 times) with different doses of E75 (100 μg, 500 μg, or 1000 μg) plus GM-CSF (250 μg or 125 μg)	**Phase I** The optimal biologic dose (OBD): 1000 μg E75 plus 250 μg GM-CSF monthly × 6. **Phase II** 24 mo landmark analysis disease free survival (DFS): Vaccinated group, 94.3%; control group, 86.8%	59
HER-2/ *neu*	GP2 (IISAVVGIL HER-2/ *neu*, 654–662)	GM-CSF	Disease-free, lymph node-negative and HLA-A2 breast cancer patients	Patients received 100 μg, 500 μg, or 1000 μg of GP2 peptide mixed with 250 μg of GM-CSF	Elicit an significant immune response	61

MAGE-A4$_{143–154}$ (NYKRCFPVIF GK) killer epitope though a glycine linker.. MAGE-A4-H/K-HELP induced MAGE-A4-specific Th1 and Tc1 immune responses and the production of MAGE-A4-specific complement-fixing IgG antibodies.[55] Tumor growth and the CEA tumor marker were significantly decreased in the final diagnosis.[55]

More excitingly, several clinical trials have evaluated immunogenic peptides from the HER2 protein (AE37 or E75 or GP2).[56] Holmes et al. conducted a Phase I trial to evaluate the AE37 peptide (Ac-LRMKGVGSPY VSRLLGICL-NH$_2$, Ii-Key hybrid of HER-2/*neu* peptide 776–790: GVGSPYVSRL LGICL) vaccine with or without granulocyte macrophage colony stimulating factor (GM-CSF) in disease-free, node-negative breast cancer patients.[57] This vaccine was found to be capable of eliciting HER-2/*neu* specific immune responses, even without the use of an adjuvant. Recently positive Phase II interim data on the AE37 cancer vaccine showed that disease-free survival in low-HER2-expressing patients was 89% in the treated group compared with 72% in the control group at a median follow-up period of 22 mo.[58] The Phase IIb trial plans to enroll 300 women and will provide additional valuable information on the efficacy and safety of AE37.[58] Mittendorf et al. conducted a Phase I/II clinical trial of the E75 peptide (KIFGSLAFL, HER-2/*neu*; 369–377). These researchers vaccinated disease-free lymph node-positive (NP), lymph node-negative (NN), and HLA-A2/A3 breast cancer patients, and the results suggested that the 24-mo landmark analysis disease-free survival (DFS) was 94%.[59] Moreover, the E75 and GP2 peptide vaccines derived from the HER2 protein can be used as adjuvant therapy to prevent disease recurrence.[59–61]

Immatics Biotechnologies is a clinical-stage biopharmaceutical company developing TAA peptide-based cancer vaccines that help to activate the body's self-defense mechanisms (www.immatics.com). Their most advanced product for the treatment of kidney cancer, IMA901, started a Phase III clinical trial in 2012.[9,10] Their second product for the treatment of colorectal cancer, IMA910, has completed a Phase II clinical trial. Their third product, IMA950 targeting brain cancer, recently entered Phase I clinical studies.[62]

Effective peptide vaccines against infectious disease agents

There is much experimental data demonstrating that epitope vaccines could be a new strategy for the development of effective vaccines for the prevention of some infectious diseases caused by viruses, bacteria, and parasites (**Table 2**).

Epitope vaccines against virus-infected cells

Recent years have seen advances in the study of HLA-restricted epitope vaccines against viral diseases. In the last few years, a wealth of HIV-related information has become available. Kloverpris et al. induced novel CD8$^+$ T-cell responses during chronic untreated HIV-1 infection though immunization with 10^7 monocyte-derived dendritic cells (MDDCs) subcutaneously pulsed with seven CD8$^+$ T-cell epitopes and three CD4$^+$ T-cell epitopes, and the immunization induced T-cell responses specific to one or more epitopes in all 12 individuals.[63] Jin et al. conducted a Phase I trial of a novel polypeptide vaccine of HIV T-helper epitopes (EP-1043) and a DNA vaccine of HIV CTL epitopes.[64] These researchers found that 68% (32/47) of the subjects had a positive CD4$^+$ T response after receiving the polypeptide vaccines, and the responding CD4$^+$ T cells exhibited a diverse poly-functional cytokine profile.[64]

An epitope-based vaccine against influenza virus might overcome the limitation of strain-specific restriction of the available vaccines. Tan et al. immunized HLA-transgenic mice expressing HLA class I A*0201, A*2402, and B*0702 and HLA class II DRB1*0301, and DRB1*0401 with 196 peptides from influenza H1N1, H3N2, H1N2, and H5N1 and avian influenza A strains.[65] These researchers found that 17 highly conserved H1N1 T-cell epitopes of the influenza virus PB1, PB2, and M1 proteins can induce a protective effect against live influenza virus challenge and may reduce the incidence of variant amino acids of the corresponding T-cell epitopes used in future influenza vaccines.[65] Ichihashi et al. subcutaneously vaccinated HLA-A24 transgenic mice with the three most immunogenic and highly conserved epitopes among three different influenza A virus subtypes (H1N1, H3N2, and H5N1), and the results showed that more than half of the mice survived a lethal influenza virus challenge during both effector and memory CTL phases.[66]

Table 2. Examples of HLA-restricted epitope vaccines in preventing infectious disease

Pathogen	Epitopes	Adjuvant	Patients/Animals	Immunization	Results	Ref.
HIV	Gag433 (FLGKIWPS), Gag150 (T2L, RLLNAWVKV), Vif23 (I9V, SLVKHHMYV), Env67 (V2I, NIWATHACV), Vif101 (M9L, GLADQLIHL), Vpu66 (A9V, ALVEMGHHV) and Pol606 (T9V, KLGKAGYVV)		18–50 y males, HLA-A*0201^{+}, HIV-1 seropositivity for 1 y or more, no clinical AIDS, never received antiretroviral therapy, and not received any other vaccine or immune-modulating medicine within 3 mo	Patients received 1 × 10^{7} monocyte-derived dendritic cells MDDCs subcutaneously (weeks 0, 2, 4 and 8), pulsed with seven CD8^{+} T-cell epitopes and three CD4^{+} T-cell epitopes	T-cell responses specific for one or more epitopes were induced in all 12 individuals	63
	EP1043 EP1090		Healthy adults	Patients received EP-1043 only (50 μg or 200 μg), DNA vaccine EP1090 only (4 mg), EP1043 plus EP1090 (200 μg + 4 mg in 4 single injections at 0, 1, 3 and 6 mo	Sixty-eight percent (32/47) of subjects had a positive CD4+ T response after two vaccinations	64
Influenza virus	196 influenza H1N1 peptides that contained residues of highly conserved proteome sequences of the human H1N1, H3N2, H1N2, H5N1, and avian influenza A strainss	TiterMax® Gold	HLA-A2(A*0201), A24(A*2402), B7 (B*0702), DR2 (DRB1*1501), DR3 (DRB1*0301), and DR4 (DRB1*0401) transgenic mice	Mice were injected subcutaneously at the base of tail with 100 μl of the immunization peptide pool in TiterMax® Gold adjuvant (1:1).	The most favorable sequences for a T cell epitope-based vaccine are the 17 H1N1 T cell epitopes of the PB1, PB2, and M1 proteins and can induce protective effect against live influenza challenge	65
	$PA_{130–138}$, $PB1_{430–438}$ $PB2_{549–557}$	CpG-ODN	HLA-A24 transgenic (A24Tg) mice	Mice were immunized with a mixture of peptide-liposome conjugates and CpG-ODN or poly(I:C) (10 μg/mouse) and re-immunized one and two weeks later	More than half of the mice survived lethal influenza virus challenge during both effector and memory CTL phases	66
HBV	HBx(52–60), HBx(92–100), HBx(115–123), HBx(140–148)		HLA-A*0201 transgenic (HLA-A2.1/^{Kb} Tg) mice C57BL/6$^{nu/nu}$ mice	HLA-A2.1/^{Kb} Tg mice received VLP- or epitope-pulsed DCs. C57BL/6$^{nu/nu}$ mice which were inoculated subcutaneously with 5 × 10^{6} SNU-398 tumor cells received 1 × 10^{8} stimulated splenocytes derived from immunized HLA-A2.1/Kb Tg mice	Induce high immunogenicity and significant antitumor effects	69
HPV	HPV-16 $E7_{12–20}$ (MLDLQPETT) HPV-16 $E7_{86–93}$ (TLGIVZPI)		HLA-A2 positive women with high-grade cervical or vulvar intraepithelial neoplasia who were positive for HPV 16	Patients received 50 mg of the HPV-16 E7 peptide in aqueous solution injected intradermally in a volume of 100 ml	12 of 18 patients cleared the virus from cervical scrapings by the fourth vaccine injection.	70

Some virus infections, i.e., with hepatitis B virus (HBV) and human papilloma virus (HPV), may eventually lead to the development of virus-induced tumors.[67,68] A virus-epitope-based vaccine may be used as a treatment for patients with cancer. Xiang et al. reported the formation of multi-epitope peptide-loaded virus-like particles (VLPs), which are composed of the HBV X protein (HBx)-derived epitopes $HBx_{52–60}$, $HBx_{92–100}$, and $HBx_{115–123}$, a novel subdominant CTL epitope $HBx_{140–148,}$ and the universal T helper epitope pan human leukocyte antigen DR-binding epitope (PADRE).[69] These researchers immunized HLA-A*0201

Table 2. Examples of HLA-restricted epitope vaccines in preventing infectious disease (continued)

Pathogen	Epitopes	Adjuvant	Patients/Animals	Immunization	Results	Ref.
Listeriolysin monocytogenes	listeriolysin O (LLO) 91–99 peptide, GYKDGNEYI. H2-K^d-restricted	DCs	BALB/c mice	2×10^5 LLO 91–99 peptide-pulsed DCs, intravenously. Two injections with a 1 week interval between them.	Strongly enhanced LLO 91–99-specific $CD8^+$ T cells exhibiting epitope-specific cytotoxic activity and IFN-γ production. Significantly improved protective immunity against *L. monocytogenes*	74
Trypanosoma cruzi	Ibosomal P2 protein (TcP2β). HLA-A*0201-restricted		HLA-A2.1 transgenic mice (HHD mice	pcDNA3-TcP2β or pcDNA3 alone (100 μg) injected into the tibialis anterior muscles Immunized 3 times at 15 d intervals.	Reduced parasitemia after challenge with a lethal *T. cruzi* dose.	77
Plasmodium falciparum	HLA-A2-restricted MAP-1,MAP-2 and MAP-3 multiple peptide.	Montanide ISA 51	C57BL/6 mice expressing the human HLA-A2 transgene BALB/c, A/J and outbred CD1 mice	20 μg of tetraepitope MAP in 100 μl PBS emulsified in 100 μl Montanide ISA 51 or PBS plus Montanide ISA 51 alone immunized groups of six mice each by three subcutaneous injections delivered at 4-week intervals.	All three MAPs could induce both antibody and cellular responses. MAP-2 vaccines could reduce the growth of blood stage parasites in erythrocyte cultures to various degrees.	78
Toxoplasma gondii	HLA-A02-restricted peptides HLA-A03-restricted peptides HLA-B07-restricted peptides	PARDE GLA-SE Pam_2Cys	HLA-A*0201 Kb transgenic mice HLA-A*1101 transgenic mice HLA-B*0702 transgenic mice	50 μg of each peptide was administered per mouse with PARDE,GLA-SE, orPam_2Cys. Mice were boosted once or twice in 2 week interval	Induced splenocytes to produce IFN-γ. Protected mice against challenge with high numbers of Type II parasites	75, 76, 82

Tg mice with VLP-pulsed dendritic cells, and found significantly high immunogenicity and antitumor effects.[69] Muderspach et al. conducted a Phase I trial to evaluate the peptide vaccines HPV16 $E7_{12–20}$ and $E7_{86–93}$.[70] These researchers immunized 18 HLA-A2-positive women with high-grade cervical or vulvar intraepithelial neoplasia who were positive for HPV16. The virological assays using cervical scraping showed that 12 of the 18 patients had cleared the virus by the fourth vaccine injection.[70]

A placebo-controlled randomized Phase II study was recently conducted in patients with HPV16-positive high-grade cervical squamous intraepithelial lesion (HSIL). Development of this type of treatment relies on the ability to motivate patients and in the reduction of the side effects.[71]

A multi-epitope chimeric DNA vaccine that expresses 25 glycoprotein epitopes from SEOV, HTNV and PUUV (designated as SHP chimeric gene) from hantavirus was constructed by Zhao et al.[72] The vaccination of BALB/c mice with the SHP multi-epitope chimeric DNA vaccine led to a marked dramatic augmentation of the humoral and cellular responses.[72]

Epitope vaccines against bacteria and parasites

Unlike many viral proteins, bacterial proteins are mainly exposed to B-cells and $CD4^+$ T-cells. However, bacterial type III secretion system (T3SS) effectors also have access to the host cytosol and may provoke CTL responses. Thus, we can assume that this group of proteins undergoes selection against the presentation of CTL epitopes, as observed in viral proteins.[73] Kono et al. used type-1 polarized DCs loaded with *Listeriolysin* O (LLO) 91–99, the H2-K^d-restricted epitope of *Listeriolysin monocytogenes*, and injected intravenously into BALB/c mice.[74] The results demonstrated that the vaccine strongly enhanced the LLO 91–99-specific $CD8^+$ T-cells exhibiting epitope-specific cytotoxic activity and IFN-γ production.[74]

$CD8^+$ T lymphocytes play a major role in protection against parasites, particularly intercellular protozoa such as *Trypanosoma cruzi*, *Plasmodium,* and *Toxoplasma*, through the secretion of IFN-γ, which activates macrophages to inhibit replication, kill the parasite, and induce lysis of infected cells. Thus, a $CD8^+$ T-cell epitope-based vaccine should contribute to the development of an effective vaccine against intercellular parasites. Cong et

al. evaluated protection against *Toxoplasma gondii* infection *by T. gondii*-specific HLA-A02, and -A03 restricted peptides.[75] These researchers immunized HLA-A*0201 and HLA-A*1101 transgenic mice with a number of CD8+ T-cell epitopes ($SAG2C_{38-46}$, $SAG2D_{180-189}$, $SAG2X_{44-52}$, $SAG2X_{351-359}$, $SAG3_{136-144}$, $SAG3_{375-383}$, SPA_{12-20}, SPA_{82-90}, $MIC1_{9-17}$, $MICA2P_{11-19}$, $SAG1_{224-232}$, $GRA6_{164-172}$, and $GRA7_{134-142}$), with PADRE and GLA-SE. The vaccine induced high levels of IFN-γ production and protected mice against challenge from type II parasites.[75,76] Garcia et al. characterized the T cell epitopes of the *T. cruzi* ribosomal P2 protein (TcP2β) that were recognized by HLA-A*0201-restricted CTLs in HLA-transgenic mice and humans,[77] and found that TcP2-P7 and $TSA\text{-}1_{514}$ peptide immunizations significantly reduced *T. cruzi* post-infection parasitemia.[77]

However, complex intracellular parasites, such as *Plasmodium* and *T. gondii*, present a plurality of antigenic epitopes. Thus, immunization with a compound polyvalent vaccine that stimulates immunity to a broad array of antigens is likely to be more effective than a single antigen. Therefore, a combination of epitopes from different stages is an optimal strategy to overcome the antigen complexity of the parasite. Mahajan et al. reported on three *P. falciparum* multiple antigen peptide (MAP) vaccines encoding several CD8+ and CD4+ T-cell epitopes and some B-cell epitopes. The antibody and cellular responses were determined in three inbred (C57BL/6, BALB/c, and A/J) strains, one congenic (HLA-A2 on the C57BL/6 background) strain, and one outbred strain (CD1) of mice.[78] All three MAP constructs were able to induce both antibody and cellular responses; furthermore, the MAP-2 vaccines reduced growth of blood-stage parasites in erythrocyte cultures to various degrees.[78]

Conclusions about epitope-based infectious disease vaccines

Promising progress has been made in the development of epitope-based vaccines against infectious diseases. However to date, very few of these are used in the clinic primarily due to. the low immunogenicity induced by the limited epitopes in the vaccine construction, relative to the immunogenicity of vaccines made by other approaches. It is therefore necessary to improve the immunogenicity of epitope vaccines.

How Can the Immunogenicity of Epitope Vaccines Be Improved?

A variety of strategies have been used to improve the immunogenicity of epitope-based vaccines. These include the construction of epitope vaccines with additional CD8+ T-cell epitopes accompanied by CD4+ T-cell epitopes and B-cell epitopes to induce a more complete immune response against the pathogen, the identification of more effective adjuvants or delivery system, the use of immunogenic carrier proteins, and the use of multiple proteins as the epitope sources.

CD4+ T-cell epitopes help CD8+ T-cell epitopes

Both cellular and humoral responses target a large number of antigens and epitopes of complex pathogens. HLA class II molecules are expressed by human professional APCs and display peptides derived from exogenous antigens to CD4+ T-cells[79] and HLA class II peptide ligands that are recognized by T-cells. These epitopes trigger the immune response.[80] Grover et al. identified a *T. gondii*-specific CD4+ T-cell epitope, AS15 AVEIHRPVPG TAPPS, through a CD4 T-cell hybridoma and found that immunization of mice with the corresponding peptides provided significant protection against subsequent parasite challenge and resulted in a lower parasite burden in the brain.[81]

Previous studies have illustrated a role for CD4+ responses in the development of CD8+ CTL responses, both in humans and in experimental animals. Vigorous CD4+ responses are important for developing a CTL response. We found that immunization of transgenic HLA-B*0702 mice with the CD8+ T-cell epitope GRA720–728 (LPQFATAAT) alone did not stimulate T cells to produce IFN-γ. The addition of PADRE (a synthetic non-natural pan HLA- DR binding epitope peptide) stimulated CD8+ T cells to secrete IFN-γ, indicating that PADRE is a universal effective CD4+ T-cell epitope that contributes to the peptide vaccine construct.[82] Oseroff et al. tested lipidated covalently linked HTL-CTL epitope constructs and showed that these are highly immunogenic for the induction of HBV- and HCV- specific CTL responses.[83]

Compared with the universal CD4+ T-cell epitope, specific epitopes may be more useful for the improvement of immunogenicity. Hughes et al. used a West Nile virus (WNV) CD4+ T-cell epitope to improve the immunogenicity of a dengue virus serotype 2 vaccine.[84] According to their data, 90% of mice were protected from lethal WNV challenge by dengue serotype 2, and 100% protection was achieved by dengue virus serotype 4.[84] The protection was temporally associated with a rapid influx of activated CD4+ T cells.[84] The CD4+ T cells from WNV-immunized mice could be stimulated by epitopes in the envelope protein transmembrane domain.[84] The incorporation of potent WNV epitopes into dengue virus serotype 2 DNA and VLP vaccines could significantly improve their immunogenicity.[84] The abovementioned studies demonstrated that both CD4+ and CD8+ epitopes should be included in an epitope vaccine constructs.

B-cell epitopes are necessary for the construction of an epitopes vaccine

B-cells play a required role in humoral immunity through the production of antibodies. Zhou et al. vaccinated specified-pathogen-free (SPF) piglets intramuscularly with two B-cell linear epitopes from the E2 glycoprotein of classical swine fever virus (CSFV): $rE2\text{-}a_{844-865}$ and $rE2\text{-}b_{693-716.}$ These researchers found that all of the rE2-ba-immunized pigs survived and exhibited no symptoms or signs of CSF after challenge infection.[85]

B-cells also can act work as APCs and are critical cellular adjuvants that facilitate optimal CD4+ T-cell activation and mediate other multiple roles in immune function.[86] Thus, it is necessary to include B-cell epitopes in HLA-restricted epitope vaccines. Wang et al. used multiple linear epitopes (B-cell, CTL, and Th epitopes) of Japanese encephalitis virus (JEV) expressed in recombinant MVA to obtain a multiple-epitope vaccine and found that this vaccine can induce adequate humoral and cellular immune responses as well as protection in challenged mice.[87]

Covalent or genetic linkage to a carrier protein with abundant helper epitopes

The fusion of the peptide antigen to a carrier protein is another approach that is often used to increase the immunogenicity of peptide vaccines. These carriers are foreign proteins that are likely to contain $CD4^+$ T-cell epitopes and can improve the CTL response that is induced by $CD8^+$ T-cell epitopes.[88] Many commonly used carrier proteins, such as calreticulin and invariant chain, also are likely to impact the subcellular localization of the antigen and hence may act via this mechanism.[88] For example, many vaccines have been explored to treat chronic HPV16 infections, and most of these vaccines consist of the fusion of E7 with a 'carrier-protein' that increases vaccine potency.[89] Kang et al. used a functionally linked Th1-polarizing chemokine (IFN-γ-inducible protein-10, IP-10) as a carrier-protein and found that it could enhance the HPV16 E7 DNA vaccine potency.[90]

Adjuvants and delivery systems play an important role in enhancing the immune response

Although peptide vaccines that contain $CD4^+$ and $CD8^+$ T-cell epitopes can prime CMI responses, low-molecular-weight synthetic peptide antigens are not highly immunogenic by themselves. These observations have led to investigations on co-administration of adjuvants with vaccine antigens to potentiate the weak immunogenicity of the synthetic epitopes.[91]

Adjuvanting antigens contribute to the success of vaccination. For example, 3-deacylated monophosphoryl lipid A (MPL), which is a detoxified derivative of the lipopolysaccharide from *Salmonella minnesota R595*, is a Toll-like receptor (TLR)-4 agonist and thus a potent activator of Th1 responses. It has been used as an adjuvant in licensed vaccines and in clinical trials for several infectious disease and cancer vaccines.[92] Other adjuvants in approved human vaccines include Alum, MF59™ (an oil-in-water emulsion), immunopotentiating reconstituted influenza virosomes (IRIV), and cholera toxin.[93] A novel AS03 (a tocopherol oil-in-water emulsion-based adjuvant system)-adjuvanted vaccine was used in humans during the 2009 influenza A/H1N1 pandemic, and was shown to be highly immunogenic in adults with a clinically acceptable safety profile.[94]

Vaccine adjuvants can be divided into two classes: immunostimulants and vehicles. Immunostimulants include cytokines, bacterial toxins, glycolipids, and TLR ligands (LPS, MPL QS21, and CpG DNA) and are used as adjuvants to enhance the immune responses.[95] Zonneveld-Huijssoon et al. treated rats with a heat shock protein 60 epitope (p1) and TLR9 ligand CpG and found that the nasal co-administration of p1/CpG amplified p1-specific T-cell proliferation and significantly augmented the arthritis-protective effect of p1.[96]

Particulate antigen delivery systems, e.g., lipid particles, nanoparticles, and microparticles, can act as vehicles that help present antigens to the immune system in a more optimal manner.[2,95] For example, poly (D,L-lactic-co-glycolic acid) nanoparticles (PLGA-NPs) in a cancer vaccine delivery system containing antigens and immunostimulatory molecules not only can actively target the antigens to DCs but also can provide immune activation and rescue impaired DCs from tumor induced immuosupression.[97] Partidos et al. injected CBA ($H\text{-}2^k$) mice intraperitoneally with the CTL epitope (T-NP6: LDRLVRLIG) from measles virus nucleoprotein encapsulated in PLGA (50:50) microparticles and elicited a higher T-cell response compared with results obtained with emulsion in incomplete Freund's adjuvant.[98]

Use of multiple proteins as sources of epitopes

The use of vaccines that include a mixture of multiple peptides derived from multiple proteins may have advantages compared with the single-protein vaccines. Multiple-epitope vaccines could increase the CTL induction,[99] and avoid the potential for antigenic escape which has been observed when all of the epitopes are derived from a single protein.

Recently, vaccines based on multiple peptides derived from multiple proteins have received increasing attentions. Suzuki et al. conducted Phase I trials in patients with advanced/recurrent NSCLC using a mixture of four peptides vaccines derived from four novel cancer antigens to evaluate their clinical response.[100] Vaccination achieved a median survival time of 398 d and a 1-y survival rate of 58%, while a cytotoxic chemotherapeutic drug only achieved a median survival time of about ~8 mo and a 1-y survival rate of ~30%.[100]

Conclusion

The study of HLA-restricted epitope vaccines has produced significant achievements and is receiving increasing attention. Many HLA-restricted epitope vaccines have exhibited potential benefits against pathogens, and some epitope vaccines have been evaluated clinically. However, we still face several key challenges in the development of epitope-based vaccines: (1) the immunogenicity of the epitope is weak by itself, and some natural epitopes are weakly bound to HLA molecules and are may cause immune tolerance; (2) the accuracy of epitope prediction methods is inconsistent; and (3) the diversity of HLA molecules in the human population limits the application of epitope-based vaccines. It is therefore important to (1) find and modify some epitopes to achieve higher affinity with the HLA molecules that are expressed by most of the population; (2) improve the methods of epitope identification, particularly the accuracy of the prediction methods; (3) construct epitope vaccines using additional $CD8^+$ T-cell epitopes, as well as $CD4^+$ T- and B-cell epitopes, in order to induce a more complete immune response against the pathogen;[101] (4) find more effective adjuvants or delivery systems to improve immunogenicity of epitope vaccine; (5) use immunogenic carrier proteins, and (6) use multiple proteins as epitope sources. We hypothesize that epitope vaccines are a promising vaccine approach and that, with further developments in bioinformatics, molecular biology and immunology, HLA-restricted epitope vaccines will make important breakthroughs and become effective vaccines to protect humans from various diseases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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