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Influenza Virus-Derived CD8 T Cell Epitopes: Implications for the Development of Universal Influenza Vaccines

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ABSTRACT

The influenza virus poses a global health burden. Currently, an annual vaccine is used to reduce influenza virus-associated morbidity and mortality. Most influenza vaccines have been developed to elicit neutralizing Abs against influenza virus. These Abs primarily target immunodominant epitopes derived from hemagglutinin (HA) or neuraminidase (NA) of the influenza virus incorporated in vaccines. However, HA and NA are highly variable proteins that are prone to antigenic changes, which can reduce vaccine efficacy. Therefore, it is essential to develop universal vaccines that target immunodominant epitopes derived from conserved regions of the influenza virus, enabling cross-protection among different virus variants. The internal proteins of the influenza virus serve as ideal targets for universal vaccines. These internal proteins are presented by MHC class I molecules on Ag-presenting cells, such as dendritic cells, and recognized by CD8 T cells, which elicit CD8 T cell responses, reducing the likelihood of disease and influenza viral spread by inducing virus-infected cell apoptosis. In this review, we highlight the importance of CD8 T cell-mediated immunity against influenza viruses and that of viral epitopes for developing CD8 T cell-based influenza vaccines.

Keywords: Influenza A virus; Influenza vaccines; T cell-based vaccines; T cell epitopes; Cytotoxic T lymphocytes

INTRODUCTION

Globally, annual influenza epidemics cause approximately 3 to 5 million cases of severe disease and account for 290,000 to 650,000 deaths, putting enormous pressure on healthcare systems (1,2). Influenza is a contagious respiratory disease caused by the influenza virus (3). The incubation period for influenza ranges between 24 and 48 h, after which individuals infected with the influenza virus may present with acute symptoms, including headache, cough, runny nose, sore throat, muscle pain, chills, and fever. These acute symptoms may persist for 2–8

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Conflict of Interest
The authors declare no potential conflicts of interest.

Abbreviations
APC, Ag-presenting cell; alum, aluminum salts; CD40L, CD40 ligand; CPL, chemically enhanced peptide ligand; DC, dendritic cell; GDP, glycodendropeptide; gp96, glycoprotein 96; HA, hemagglutinin; M1, matrix protein 1; M2, matrix protein 2; M2e, M2 extracellular domain; NA, neuraminidase; NP, nucleoprotein; PA, polymerase acidic protein; PAQ11, Q11-based PA224–233 nanofibers; PB1, polymerase basic protein 1; Q11, Ac-QQKFQFQFEQQ-Am; TRM, tissue-resident memory T; vRNA, viral single-stranded RNA; vRNP, viral ribonucleoprotein; WIV, whole-inactivated influenza virus.

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days and correlate with high viral loads (4,5). In most cases, without medical treatment, fever and other influenza symptoms resolve within a week; however, in some cases, coughs can worsen and persist for longer than 2 weeks. Influenza can lead to serious illness or death, especially in high-risk groups such as children and the elderly (1,6).

Based on antigenic differences in nucleoproteins (NP) and matrix protein 1 (M1) of influenza virus, it is classified into 4 types (A, B, C, and D) (7). Among these, influenza A virus (IAV) and influenza B virus are the 2 leading causes of annual influenza epidemics virus (8). The influenza viral genome is segmented into eight RNA molecules, each folded into a rod-shaped, double-helical viral ribonucleoprotein (vRNP) complex. Each core vRNP complex consists of viral single-stranded RNA (vRNA), a heterotrimeric polymerase complex, containing polymerase acidic protein (PA), polymerase basic protein 1 (PB1), and PB2, and multiple copies of the viral-encoded NP. In addition to the proteins constituting the vRNP complex, influenza viral particle contains M1, a structural protein, and non-structural proteins. The host cell-derived envelope of IAV contains hemagglutinin (HA), neuraminidase (NA), and ion channel M2 (9,10).

Seasonal influenza vaccines are currently the primary means of providing cost-effective protection against influenza, reducing or counteracting influenza-associated mortality and morbidity. Vaccines mainly elicit Ab responses against the immunodominant hypervariable regions of HA and NA (11). These two viral glycoproteins are the key targets of neutralizing Abs induced by influenza virus infection or vaccination (3). However, the antigenic drift of influenza virus, in which the antigenic epitope is altered by point mutations, most frequently in genes encoding HA and NA during viral replication, allows viral escape, inhibiting natural infection and vaccination-induced herd immunity. Antigenic drift can also lead to the emergence of new influenza variants that can escape neutralization by Abs elicited by influenza vaccines (12). Thus, there is a need for the annual development of seasonal influenza vaccines that target emerging viral variants (13).

Therefore, to overcome the limitations of the current seasonal influenza vaccines, the development of a universal influenza vaccine has gained considerable interest. By targeting epitopes derived from conserved regions that are not affected by antigenic drift, a universal influenza vaccine should reduce symptomatic influenza cases more significantly than current vaccines. A universal vaccine should also elicit long-lasting, broadly cross-reactive and cross-protective immunity (10,14). Most universal influenza vaccines under development aim to reduce symptomatic influenza virus infection by inducing the production of neutralizing Abs against widely structurally conserved viral Ags, such as the M2 extracellular domain (M2e) or HA stalk domain (10,15). In contrast, other universal vaccines focus on inducing T cell responses against the conserved T cell epitopes derived from internal Ags, such as PA, PB1, PB2, NP, and M1 (10,16). In this review, we discuss the effector functions of influenza virus-derived Ag-specific CD8 T cells in influenza virus infection, the mechanisms of CD8 T cell-based immune responses against influenza virus infection, and influenza virus-derived CD8 T cell epitopes that can be targeted by universal vaccines.

IMPORTANCE OF CD8 T CELL-MEDIATED IMMUNE RESPONSES AGAINST INFLUENZA VIRUS

The effector functions of CD8 T cells in influenza virus infection

CD8 T cells are essential immune cells that specifically detect influenza virus-infected cells and eradicate the infected cells via immediate cytotoxic immune responses (**Fig. 1**) (17). However, naïve CD8 T cells cannot directly recognize influenza virus-derived Ags and require professional Ag-presenting cells (APCs), especially dendritic cells (DCs), which initially presents the viral Ag that is recognized by the CD8 T cell (18). DCs capture and phagocytize viral Ags derived from infected cells at the site of viral infection, and transport the Ags to the draining lymph nodes where naïve CD8 T cells are located (19). In the draining lymph nodes, DCs present viral 8–10 mer peptides bound to MHC class I (MHC-I) molecules to naïve CD8 T cells via cross-presentation (20,21). Consequently, naïve CD8 T cells are activated into viral peptide-specific CTLs, which are effector CD8 T cells that kill virus-infected cells (22). Activated viral peptide-specific CTLs are recruited to the site of viral infection (for example, into respiratory tissues) and contribute to virus clearance through diverse effector mechanisms to induce the killing of virus-infected cells (23). The effector mechanisms of viral peptide-specific CTLs include the ability to secrete a variety of cytotoxic molecules, such as perforin and granzymes, and activate apoptosis pathways, such as the FAS-FAS ligand or TNF-related apoptosis-inducing ligand pathways (18,24,25).

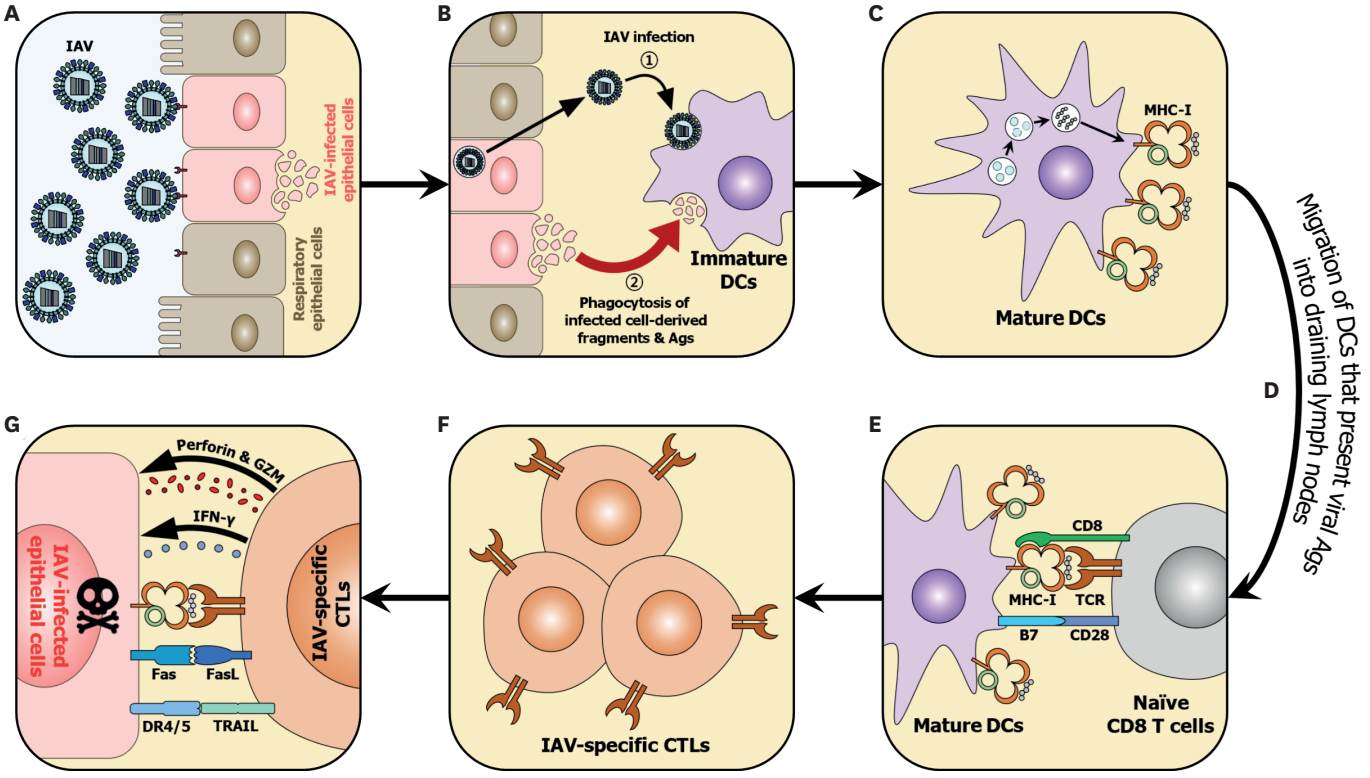

Figure 1. Influenza-specific CTL responses. (A) Infection of influenza virus is initiated via sialic acid receptor binding on respiratory epithelial cells. (B) Viral Ags derived from influenza virus-infected epithelial cells are captured by DCs. (C) Captured viral Ags are processed and cross-presented through MHC-I molecules of DCs. (D) Viral Ag-presented DCs migrate to the draining lymph node. (E) Naïve CD8 T cells contact viral Ags and receive co-stimulating signals via DCs. (F) The antigenic and co-stimulating signals induce the activation and expansion of influenza virus-specific CTLs. (G) Influenza virus-specific CTLs migrate to the site of viral infection, leading to the death of virus-infected cells, inhibiting viral spread.

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Why are CD8 T cells important in influenza virus infection?

Current seasonal influenza vaccines remain the most efficient way to reduce influenza-related morbidity and mortality. The vaccines act by inducing the production of neutralizing Abs against the virus, particularly against the receptor-binding site of HA or the enzymatic active site of NA. The current influenza vaccines are associated with several limitations, including short-term effectiveness, poor matches between the vaccine strains and the circulating strains of influenza virus resulting from substantial antigenic drift (26), and differences in HA glycosylation patterns following traditional egg-based virus production (27). In addition, owing to the antigenic drift of influenza, seasonal influenza vaccines need to be developed continuously. Therefore, the development of new vaccines is crucial (26).

A novel approach to influenza vaccine development focuses on the use of T cell- mediated immune responses, particularly CD8 T cell responses. Current influenza vaccines target highly variable HA and NA (nucleotide sequence homologies of 40%–70% between IAV subtypes), whereas the CD8 T cell-based influenza vaccine strategy aims to targets internal proteins with highly conserved nucleotide sequences (homologies of more than 90% between IAV subtypes) (28). Among the IAV proteins, NP, M1, and PB1 are potential targets for inducing CD8 T cell-mediated immune responses (29). Of these, NP has been extensively studied. NP possess key immunodominant CTL epitopes (30,31). M1 is one of the most abundant and highly conserved proteins of IAV (32). NP- and M1-specific CTL responses are key in combating IAV infections, comparable in importance to the CTL responses induced by the infection itself (29,33). This highlights the critical role of NP and M1 in stimulating immune defense mechanisms against the influenza virus. Thus, designing vaccines that effectively elicit CTL responses against NP and M1 will enhance immunity against the influenza virus and reduce the severity of infection (30,32). Therefore, understanding and leveraging the immunogenicity of NP and M1 as important targets for CTL responses is essential for the development of more effective influenza vaccines. PB1 elicits low cytolytic CTL activity *in vivo* and the PB1 epitope-MHC complex exhibits low stability, which can negatively impact CTL recognition and response (34); therefore, only a limited number of studies have focused on PB1. However, owing to high nucleotide conservation among all IAV strains, PB1 is still recognized as a potential target for the induction of CTL immune responses (29).

CD8 T cell-based targeting of conserved internal proteins may elicit cross-protective immune responses called heterosubtypic immune responses (35). These responses occur independently of humoral immune responses mediated by B cells and Abs (36). Cross-protective CTLs against the influenza virus do not mediate protection against direct viral infection of epithelial cells (37). Virus-specific CTLs exert effector functions by recognizing virus-derived peptides presented by MHC-I molecules on infected epithelial cells and lysing the infected cells before viral shedding, thereby preventing the spread of the influenza virus to neighboring cells (23,38). As a result, virus-specific CTLs limit the progression of diseases caused by influenza virus infection and alleviate symptoms by promoting viral clearance (39).

Unlike other memory CD8 T cell subsets that circulate in the blood or secondary lymphoid tissues, CD8 tissue-resident memory T (T_{RM}) cells co-expressing key identifiers of tissue residency, namely CD69 and CD103, reside in non-lymphoid mucosal tissues and act as front-line responders by coordinating local protective immune responses against local infections (40). Although CTL-mediated immune responses are essential for the initial clearance of influenza virus infection, the conversion of influenza virus-specific effector CD8 T cells

into CD8 T_{RM} cells is important for providing long-term protection against secondary IAV infection and eliciting cross-reactive immunity against other influenza virus strains (41). During the contraction phase following viral clearance, most influenza virus-specific CTLs are eliminated by apoptosis and only the surviving effector CD8 T cells are converted into CD8 T_{RM} cells (42). These cells are characterized by their proliferative potential and long-term viability (43). When secondary influenza virus infection occurs, CD8 T_{RM} cells are rapidly activated to clear the virus and prevent viral dissemination into neighboring cells (44).

CD4 Th cell activation is important for CTL activation and memory CD8 T cell development

CD4 Th cell activation is important for protective immunity by CTLs following viral challenge (**Fig. 2**) (45). CD4 Th cells are activated through Ag presentation by MHC-II molecules and co-stimulation by B7 molecules expressed on the surface of DCs, which increases the expression of CD40 ligand (CD40L) on CD4 Th cells (46). CD40L molecules on activated CD4 Th cells bind to CD40 molecules on DCs and trigger CTL activation by inducing contact between CTLs and Ag-presenting DCs. Activated CD4 Th cells secrete chemokines such as CCL3, CCL4, and CCL5 to guide naïve CD8 T cells toward the site of Ag presentation, increasing the number of encounters between CD8 T cells and APCs, thus facilitating the initiation and coordination of Ag-specific immune responses (47). Through direct interactions with CD40 molecules expressed on CTLs and the production of IL-2, CD4 Th cells also contribute to the survival, priming, and expansion of CTLs (42,48).

In acute influenza virus infection, primary antiviral CTLs may be independently activated, without the help of CD4 Th cells, because the virus induces sufficient DC maturation and a strong inflammatory response (47,49). However, CD4 Th cell assistance during the initial priming phase is critical for the generation and recall responses of long-lived memory CD8 T cells that can be converted to effector cells in response to a secondary viral challenge (42,49). After infection, CD4 Th cells rapidly migrate to the lung airways and secrete IFN-γ to mediate the release of chemokines from respiratory epithelial cells (47). This facilitates the recruitment of CD8 T cells to the infection site (48). The lung-

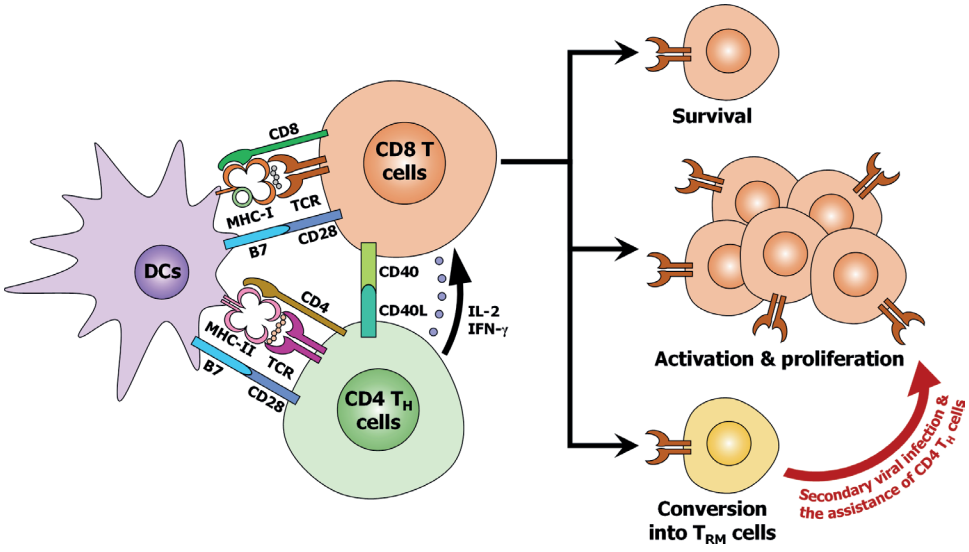

Figure 2. CD4 Th cells in influenza-specific CTL responses. CD4 Th cells assist the survival and expansion of CTLs. CD4 Th cells are essential for CD8 T_{RM} cell differentiation; CD8 T_{RM} cells elicit a rapid influenza virus-specific CTL responses to secondary influenza virus infection.

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recruited CD8 T cells are converted into CD8 T_{RM} cells via CD69 and CD103 upregulation by the local microenvironment of the lung tissue, which contributes to long-term immunity against influenza virus infection (47). CD8 T cells require CD4 Th cells for differentiation into memory CD8 T cells (50). The absence of memory CD8 T cell differentiation leads to defective recall responses that fail to elicit immediate immune responses upon secondary viral infection (51). Therefore, CD4 Th cells are essential for all influenza virus-specific CTL responses to induce robust secondary responses (49).

EPITOPES FOR CD8 T CELL-BASED INFLUENZA VACCINE

Murine CD8 T cell epitopes

Several IAV-derived murine peptides have been evaluated both *in vitro* and *in vivo* (**Table 1**). $NP_{366–374}$ (ASNENMETM), a naturally generated epitope in influenza A/Puerto Rico/8/34 virus (H1N1 PR8)-infected H-2^b-positive cells, is considered a potential influenza model epitope (52). Immunization of C57BL6/J (H-2^b) mice with $NP_{366–374}$ emulsified in incomplete Freund's adjuvant generates $NP_{366–374}$-specific CTLs, which can efficiently kill both $NP_{366–374}$-coated and virus-infected target cells *in vitro* (53). Vaccination using $NP_{366–374}$-pulsed DCs increases $NP_{366–374}$-specific CD8 T cells, the induction of a potent CTL response, and the production of IFN-γ

Table 1. IAV-derived murine CD8 T cell epitopes

Epitopes	Sequence	Formulation	Adjuvant	Description	References
$NP_{366-374}$	ASNENMETM (H-$2D^b$-restricted)	Emulsified	IFA	- Lysis of both $NP_{366-374}$-coated and virus-infected target cells by $NP_{366-374}$-specific CTLs	(53)
		DC vaccine	None	- Increases the number of $NP_{366-374}$-specific CD8 T cells in the lungs - Produces IFN-γ from $NP_{366-374}$-specific T cells - Produces TNF-α from ~30% of the restimulated $NP_{366-374}$-specific T cells - Induces potent CTL activity - Accelerates viral clearance	(54)
		Conjugated with OVA or BSA	Alum	- Virus-infected mice lose less weight and quickly regain starting weight - Generates $NP_{366-374}$-specific CD8 T cells in the lungs of virus-infected mice - Reduces viral titer - Provides the most rapid/protective memory response	(56)
		GDP	None	- Induces DC maturation, especially higher expression of CD83 - Increases CD8 T cell proliferation	(57)
$NP_{147-155}$	TYQRTRALV (H-$2K^d$-restricted)	Peptide	Heat shock protein gp96	- Provides partial protection against virus infection - Induces $NP_{147-155}$-specific CD8 T cell responses - Elicits a strong recall response after virus infection	(59)
		Peptide	CpG	- Elicits a modest $NP_{147-155}$-specific CTL response - Protects against lethal infection by the virus	(31)
		Peptide	Alum and CpG	- Elicits a stronger CTL response than CpG alone - Mice succumb to lethal dose influenza virus infection	(31)
$PA_{224-233}$	SSLENFRAYV (H-$2D^b$-restricted)	Dendritic cell vaccine	None	- Increases the number of $PA_{224-233}$-specific CD8 T cells in the lungs - Produces IFN-γ from $PA_{224-233}$-specific T cells - Produces TNF-α from ~75% of the restimulated $PA_{224-233}$-specific T cells - Induces potent CTL activity - Delays viral clearance	(54)
		Supramolecular peptide nanofiber	None	- Generates $PA_{224-233}$-specific CD8 T cell responses in lung-draining lymph nodes - Produces IFN-γ *ex vivo* - Lyses $PA_{224-233}$-pulsed target cells *in vivo* - Diminishes viral loads in infected mice - Increases the number of $PA_{224-233}$-specific CD8 T_{RM} cells in the lung	(63)
$PB1_{703-711}$	SSYRRPVGI (H-$2K^b$-restricted)	None	None	- Induces CTL activity in BAL cells isolated from virus-infected mice that can lyse target cells pulsed with $PB1_{703-711}$ - Stimulates an IFN-γ response by highly activated $PB1_{703-711}$-specific CD8 T cells in BAL	(66)
		None	None	- Induces $PB1_{703-711}$-specific CD8 T cells in the lung of virus-infected mice	(67)

IFA, incomplete Freund's adjuvant; OVA, ovalbumin.

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and TNF-α by $NP_{366–374}$-specific CTLs, resulting in accelerated viral clearance (54). Together with aluminum salts (alum), which is currently licensed as adjuvants for human IAV vaccines to boost Ab responses (55), immunization of mice with ovalbumin or bovine serum albumin-conjugated $NP_{366–374}$ elicits an immediate and protective memory immune response via the recruitment of $NP_{366–374}$-specific CD8 T cells and reduction of viral titers in the lungs (56). The Ag-specific immune activity of $NP_{366–374}$ was also investigated using glycodendropeptides (GDPs) (57). GDPs are mannose glycodendrons with multivalent peptide epitopes that enhance the immunogenicity of peptide epitopes by inducing DC internalization via interactions between the mannose of GDPs and mannose receptors expressed on the surface of immature DCs (58). Exposure to $NP_{366–374}$ outside of GDPs induces DC maturation, especially the expression of CD83 and the subsequent increase in the proliferation of $NP_{366–374}$-specific CD8 T cells (57).

Immunization using glycoprotein 96 (gp96) as an adjuvant with $NP_{147–155}$ (TYQRTRALV), a conserved NP epitope in H1N1 PR8, provides partial protection to mice against viral infection, resulting in a 50% survival rate (59). gp96 is a heat shock protein that functions as a cellular chaperone within the cells. External gp96 is internalized into DCs with Ags, and this process induces Ag-specific immune responses, including DC maturation, cross-presentation, and Ag-specific CTL activation (60). Immunization with $NP_{147–155}$ using gp96 as an adjuvant elicits CD8 T cell responses against $NP_{147–155}$ (61). $NP_{147–155}$-specific CD8 T cells, following H1N1 PR8 challenge, are generated from the vaccination-induced memory T cell pool, and recruited to the lungs from lung-draining lymph nodes, thereby allowing viral clearance (59). Vaccination with $NP_{147–155}$ has been investigated with CpG alone and with CpG + alum (as an adjuvant) (31). Immunization with $NP_{147–155}$ and CpG alone induces a modest $NP_{147–155}$-specific CTL response and protects mice against viral infection. Immunization with $NP_{147–155}$ and CpG + alum induces stronger $NP_{147–155}$-specific CTL responses than immunization with $NP_{147–155}$ and CpG alone (31).

Vaccination using DCs pulsed with $PA_{224–233}$ (SSLENFRAYV), a conserved PA epitope in H1N1 PR8, results in the expansion and migration of $PA_{224–233}$-specific T cells, the induction of a potent CTL response, and the production of IFN-γ and TNF-α from $PA_{224–233}$-specific CTLs (54). The Ag-specific immune response to $PA_{224–233}$ was examined using Ac-QQKFQFQFEQQ-Am (Q11)-based peptide nanofibers. Q11-based peptide nanofibers consist of a self-assembling Q11 domain at the C-terminus (62) and conserved $PA_{224–233}$ at the N-terminus (PAQ11) (63). Intranasally immunized Q11-based peptide nanofibers are taken up by DCs in lung-draining lymph nodes, leading to the activation of peptide-specific CD8 T cell responses (64). Intranasal vaccination with PAQ11 expands the $PA_{224–233}$-specific CD8 T cells. The $PA_{224–233}$-specific CD8 T cells induce IFN-γ production *ex vivo*, $PA_{224–233}$-pulsed target cell lysis *in vivo*, and viral titer reduction in virus-infected mice. Furthermore, intranasal delivery of PAQ11 markedly increases the recruitment of long-lasting $PA_{224–233}$-specific CD8 T_{RM} cells to the lungs, allowing for an immediate response to IAV infection (63).

Infection of mice with HKx31, a recombinant IAV of H1N1 PR8 that expresses the HA and NA of H3N2 (65), induces $PB1_{703–711}$ (SSYRRPVGI)-specific CD8 T cells. The $PB1_{703–711}$-specific CD8 T cells lyse $PB1_{703–711}$-pulsed target cells and produce IFN-γ upon $PB1_{703–711}$ re-stimulation (66). The IFN-γ-secreting $PB1_{703–711}$-specific CD8 T cells in H1N1 PR8-infected mice recruit approximately 10% of the total CD8 T cells into the lungs. $PB1_{703–711}$-specific CTLs exert cytotoxic effects on virus-infected targets, *in vitro* (67).

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Human CD8 T cell epitopes

IAV-derived human CD8 T cell-specific peptides have been identified (**Table 2**). $M1_{58–66}$ (GILGFVFTL), restricted by HLA-A*02:01 molecules, a human MHC-I molecule that is expressed at a high frequency in individuals worldwide (68), is highly conserved and is a well-known immunodominant epitope (69). After natural influenza virus infection, $M1_{58–66}$-specific CTLs are generated in most HLA-A*02:01-positive individuals (70,71). The $M1_{58–66}$-specific CTLs of HLA-A*02:01-positive individuals express CD107a, a known marker of activated CD8 T cells (72), and produce IFN-γ upon $M1_{58–66}$ re-stimulation (71). In addition, the frequency of $M1_{58–66}$-specific CD8 T cells in the blood of IAV-infected individuals is significantly higher than that in healthy donors, and $M1_{58–66}$-specific CD8 T_{RM} cells are observed in the lungs of IAV-infected donors (73). In animal experiments, immunization of $M1_{58–66}$ with whole-inactivated influenza virus (WIV), which is a vRNA-containing adjuvant that serves as a TLR7 agonist (74), induces increased levels of $M1_{58–66}$-specific CTLs compared to immunization with WIV only in HLA-A*02:01-transgenic mice. Co-administration of $M1_{58–66}$ and WIV at the same site is necessary to obtain the adjuvant effect of WIV on $M1_{58–66}$ (75).

$NP_{145–156}$ (DATYQRTRALVR), which can bind to HLA-A*68:01 molecules, is a highly conserved epitope except at amino acid position 146 (76). Amino acid position 146 in $NP_{145–156}$ is variable

Table 2. IAV-derived human CD8 T cell epitopes

Epitopes	Sequence	Description	References
$M1_{58-66}$	GILGFVFTL (HLA-A*02:01-restricted)	- Generated CTLs against $M1_{58-66}$ in the HLA-A*02:01-positive individuals - Increased frequency of CD107a-expressing CTLs in response to re-stimulation - Increased frequency of IFN-γ secretion of the total CD8 population in response to re-stimulation	(71)
		- Increased frequency of $M1_{58-66}$-specific CD8 T cells in the blood of IAV-infected individuals - $M1_{58-66}$-specific CD8 T_{RM} cells are observed in the lungs of IAV-infected individuals	(73)
		- Induced high levels of $M1_{58-66}$-specific CTLs - $M1_{58-66}$ with WIV significantly increased the induction of CTL responses	(75)
$NP_{145-156}$	DATYQRTRALVR (HLA-A*68:01-restricted)	- Three variants at position 146 of $NP_{145-156}$; alanine (89.1%), threonine (10.9%), or valine (<0.01%) - $NP_{145-156}$ has a high level of cross-reactivity between the variants - $NP_{145-156}$-specific CD8 memory T cells is induced in IAV-infected individuals	(77)
$NP_{265-273}$	ILRGSVAHK (HLA-A*03:01-restricted)	- IFN-γ and TNF-α production in $NP_{265-273}$-re-stimulated CD8 T cells	(81)
		- Observed memory phenotype of $NP_{265-273}$-specific CD8 T cells in the HLA-A*03:01-positive individuals - Observed naïve phenotype of $NP_{265-273}$-specific CD8 T cells at high proportions in some HLA-A*03:01-positive individuals	(82)
$NP_{338-346}$	FEDLRVLSF (HLA-B*37:01-restricted)	- IFN-γ and TNF-α production in $NP_{338-346}$-re-stimulated PBMCs derived from HLA-B*37:01-positive individuals - CD107a expression in $NP_{338-346}$-re-stimulated PBMCs derived from HLA-B*37:01-positive individuals - Elicited a high frequency of the memory phenotype among $NP_{338-346}$-specific CD8 T cells in HLA-B*37:01-positive individuals	(83)
$NP_{380-388}$	ELRSRYWAI (HLA-B*08:01-restricted)	- Induced potent CTL activity of $NP_{380-388}$-specific CD8 T cells derived from HLA-B*08:01-positive individuals	(84,85)
$NP_{383-391}$	SRYWAIRTR (HLA-B*27:05-restricted)	- Induced potent CTL activity of $NP_{383-391}$-specific CD8 T cells derived from HLA-B*27:05-positive individuals	(84,85)
		- IFN-γ and TNF-α production in $NP_{383-391}$-re-stimulated CD8 T cells	(81)
		- Increased production of IFN-γ and TNF via $NP_{383-391}$-specific responses compared to $M1_{58-66}$-specific in PBMCs co-expressing HLA-A*02:01/HLA-B*27:05	(86)
		- $NP_{383-391}$-specific CD8 T cell population has a higher proportion of the effector over the memory phenotype - CD107a expression in $NP_{383-391}$-specific CD8 T cells derived from HLA-A*27:05-positive individuals	
$PB1_{413-421}$	NMLSTVLGV (HLA-A*02:01-restricted)	- IFN-γ production upon stimulation of $PB1_{413-421}$-specific CD8 T cells derived from HLA-A*02:01-positive individuals	(73)
		- CD38 and Ki-67 expression in activated $PB1_{413-421}$-specific CD8 T cells - $PB1_{413-421}$-specific CD8 T_{RM} cells are observed in the lungs of IAV-infected individuals	
$PB1_{496-505}$	FYRYGFVANF (HLA-A*24:02-restricted)	- Induced CD8 T cell proliferation and IFN-γ and TNF production in HLA-A*24:02-positive individuals	(90)
		- IFN-γ production upon stimulation of $PB1_{496-505}$-specific CD8 T cells derived from HLA-A*24:02-positive individuals	(91,92)
$PB1_{498-505}$	RYGFVANF (HLA-A*24:02-restricted)	- Induced CD8 T cell proliferation and IFN-γ and TNF production in HLA-A*24:02-positive individuals	(90)
		- $PB1_{498-505}$-specific CD8 T_{RM} cells are observed in the lungs of IAV-infected individuals	
		- IFN-γ production upon stimulation of $PB1_{498-505}$-specific CD8 T cells derived from HLA-A*24:02-positive individuals	(92)

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and could be alanine (89.1%), threonine (10.9%), or valine (<0.01%). Re-stimulation of the $NP_{145–156}$-specific CD8 T cells expanded from HLA-A*68:01-positive PBMCs, using the three $NP_{145–156}$ variants, induces the production of IFN-γ, indicating cross-reactivity among all three variants, and the variation at position 146 does not affect $NP_{145–156}$-specific CD8 T cell responses (77). Additionally, owing to the high proportion of memory phenotypes among $NP_{145–156}$-specific specific T cells in some HLA-A*68:01-positive individuals, $NP_{145–156}$-specific memory T cells are induced by previous IAV infection (77).

HLA-A*03:01-restricted $NP_{265-273}$ (ILRGSVAHK) is an immunodominant epitope with 100% conservation among IAV strains circulating worldwide (78). HLA-A*03:01 molecules, which present $NP_{265-273}$ to CD8 T cells, are HLA alleles expressed at high frequencies in the global population (79,80). The $NP_{265-273}$-specific CD8 T cells expanded from HLA-A*03:01-positive PBMCs via $NP_{265-273}$ re-stimulation produce both IFN-γ and TNF-α (81). The $NP_{265-273}$-specific CD8 T cell populations expressing the memory phenotype are present in HLA-A*03:01-positive individuals. Furthermore, $NP_{265-273}$-specific CD8 T cell populations expressing the naïve phenotype are present at high frequencies in some HLA-A*03:01-positive individuals (82).

$NP_{338–346}$ (FEDLRVLSF) is identified as an immunodominant epitope in individuals expressing HLA-B*37:01 molecules (76). The re-stimulation of PBMCs derived from HLA-B*37:01-positive individuals using $NP_{338–346}$ elicits potent CD8 T cell responses through the increased production of IFN-γ and TNF-α, and the increased CD107a expression. Furthermore, the frequency of the memory phenotype is high among $NP_{338–346}$-specific CD8 T cells in healthy HLA-B*37:01-positive individuals (83).

$NP_{380–388}$ (ELRSRYWAI) and $NP_{383–391}$ (SRYWAIRTR), which have overlapping amino acid sequences, are conserved epitopes restricted by HLA-B*08:01 and HLA-B*27:05 molecules, respectively. $NP_{380–388}$- or $NP_{383–391}$-specific CD8 T cells expanded from the PBMCs of HLA-B*08:01- or HLA-B*27:05-positive individuals induce the killing of epitope-labeled target or IAV-infected cells (84,85). Moreover, the $NP_{383–391}$-specific CD8 T cells expanded from HLA-A*27:05-positive PBMCs via $NP_{383–391}$ re-stimulation produce both IFN-γ and TNF-α (81). Interestingly, in individuals that co-express HLA-A*02:01 and HLA-B*27:05, HLA-A*27:05-restricted $NP_{383–391}$ is a more immunodominant epitope than the HLA-A*02:01-restricted $M1_{58–66}$, which is considered as the highly immunodominant epitope (86). Although the frequencies of IFN-γ and TNF production upon $M1_{58–66}$ re-stimulation is high in HLA-A*27:05 non-expressing PBMCs, IFN-γ and TNF production by $NP_{383–391}$-specific responses in HLA-A*02:01/HLA-B*27:05 co-expressing PBMCs is even higher. Compared to $M1_{58–66}$-specific CD8 T cells, the proportion of the effector phenotype among $NP_{383–391}$-specific CD8 T cells is higher, whereas the proportions of the memory phenotype is reduced. $NP_{383–391}$-specific CD8 T cells also express CD107a in HLA-A*27:05-positive individuals (86).

$PB1_{413–421}$ (NMLSTVLGV), an HLA-A*02:01-restricted epitope that has a lower affinity for HLA-A*02:01 molecules compared to $M1_{58–66}$ (87), is an immunodominant and conserved (>99.9%) epitope (88). Stimulation of HLA-A*02:01-positive PBMCs using IAV-infected autologous PBMCs significantly increases the production of IFN-γ from $PB1_{413–421}$-specific CD8 T cells. The frequency of peripheral $PB1_{413–421}$-specific CD8 T cells in IAV-infected individuals is higher than that in healthy individuals (73). The expression of CD38 and Ki-67, which are related to the effector phenotype and proliferation during human viral infections (89), increases in $PB1_{413–421}$-specific CD8 T cells of IAV-infected individuals. Furthermore, $PB1_{413–421}$-specific CD8 T cells have been observed in the lungs of IAV-infected donors (73).

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$PB1_{496–505}$ (FYRYGFVANF) and $PB1_{498–505}$ (RYGFVANF), overlapping peptides and conserved epitopes restricted by HLA-A*24:02 molecules, are globally distributed (10%) (90). HLA-A*24:02-positive PBMCs treated with $PB1_{496–505}$ produce IFN-γ via $PB1_{496–505}$-specific responses. $PB1_{496–505}$ is immunogenic toward CTLs restricted by HLA-A*24:02 molecules (91,92). $PB1_{498–505}$ also elicits the production of IFN-γ from the $PB1_{496–505}$-specific CD8 T cells of HLA-A*24:02-positive PBMCs (92). Another study reported that both $PB1_{498–505}$ and $PB1_{496–505}$ induce the proliferation of CD8 T cells and the production of IFN-γ and TNF via $PB1_{498–505}$- and $PB1_{496–505}$-specific responses in HLA-A*24:02-positive individuals. In particular, $PB1_{498–505}$ contained within $PB1_{496–505}$ is considered an important epitope for HLA-A*24:02 binding. $PB1_{498–505}$-specific CD8 T cells are found in the lungs and secondary lymphoid organs, such as the spleen and tonsils, of HLA-A*24:02-positive individuals. During influenza virus infection, CD8 T_{RM} cells account for a large proportion of $PB1_{498–505}$-specific CD8 T cells within the human lung, and CD8 T cells with an effector memory phenotype are predominant in secondary lymphoid organs (90).

CONCLUSIONS

An influenza vaccination strategy based on virus-derived CD8 T cell epitopes, which aim to elicit influenza virus-specific CTLs and lung-resident CD8 T_{RM} cells, can effectively reduce the spread and health burden caused by the influenza virus (93). The CD8 T cell epitopes composed of short peptides (8–10 amino acids long) can be produced chemically on a large scale without cell culture or egg-based production, and are predictable using bioinformatics tools and computational algorithms, ensuring high specificity without causing infection or replication (9). However, the use of peptide vaccines is associated with several limitations such as low water solubility, low immunogenicity, and rapid degradation by peptidases in the blood and tissues (94). Therefore, vaccination using short peptide epitopes cannot sufficiently stimulate innate immunity, making it difficult to elicit satisfactory epitope-specific immune responses. To overcome these limitations and effectively induce viral epitope-specific immune responses, several studies have been conducted on delivery systems using various platforms (9,57,63,95-97), including combination with immune adjuvants (31,55,74,94). Vaccination with viral epitopes using various delivery systems can induce phagocytosis of epitopes by APCs, such as DCs, and the activation of DCs by increasing the production of co-stimulatory molecules and pro-inflammatory cytokines (98). Similarly, immune adjuvants activate innate immune processes, including the presentation of viral epitopes by DCs and increased expression of co-stimulatory molecules, thereby eliciting viral epitope-specific CTL responses (94).

In addition, recent studies have used chemically enhanced peptide ligands (CPLs), which replace one of the epitope amino acid sequences with a non-proteogenic amino acid that does not constitute a protein (87,99). When an epitope with a low binding affinity is modified with CPL, the binding affinity for HLA-I molecules is increased, with increased immunogenicity, allowing for the induction of viral epitope-specific immune responses (87).

Although this review only discusses immunodominant CD8 T cell epitopes for influenza viruses, several studies have reported numerous subdominant epitopes as well as immunodominant epitopes restricted to various HLA alleles (73,81,90,100). It is possible to develop influenza vaccines using various combinations of multiple CD8 T cell epitopes taking into account the diverse HLA haplotypes present in individuals. These vaccines will be more

effective than vaccines that use a single epitope, because it can induce individual CD8 T cell responses to combined epitopes.

Several studies have been performed to identify CD8 T cell epitopes for targeted CD8 T cell responses against the influenza virus. However, the development of efficacious vaccines that produce neutralizing Abs against influenza virus is ongoing. Here, we demonstrate the need to increase the relative importance of developing CD8 T cell epitope-based universal influenza vaccines.

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