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Review article

Antigenic variability and conserved epitopes in influenza: Challenges and opportunities for universal vaccine design

Variabilidad antigénica de la influenza y epítopos conservados: Retos y oportunidades en el diseño de vacunas universales

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ABSTRACT

Influenza is a highly transmissible viral infection characterized by significant antigenic variability, leading to seasonal epidemics and occasional pandemics. The efficacy of current vaccines is constrained by the rapid evolution of the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA), necessitating annual reformulations. In contrast, conserved regions within internal viral proteins—such as nucleoprotein (NP), matrix proteins (M1, M2), and components of the polymerase complex (PB1, PB2, PA)—offer promising targets for the development of universal influenza vaccines. This review explores the antigenic mechanisms underlying immune evasion, with an emphasis on cross-reactive immune responses. It also summarizes recent advances in innovative vaccine platforms, including mRNA-based vaccines, polyepitope constructs, chimeric hemagglutinins, and nanoparticle formulations, which have demonstrated the ability to elicit broad and durable immunity in preclinical models. Despite significant progress, both scientific and logistical challenges remain. The findings discussed underscore the potential of targeting conserved epitopes and leveraging next-generation vaccine technologies to achieve universal protection against influenza and to enhance preparedness for future pandemics.

Keywords: Influenza; Virus; Antigenic variability; Conserved epitopes; Universal vaccine; Immune response.

RESUMEN

La influenza es una enfermedad viral de alta transmisibilidad y variabilidad antigénica, responsable de epidemias estacionales y pandemias. La eficacia de las vacunas actuales se ve limitada por la rápida evolución de las proteínas de superficie, la hemaglutinina (HA) y la neuraminidasa (NA), lo que exige actualizaciones anuales. Sin embargo, regiones conservadas en proteínas internas, como la nucleoproteína (NP), la matriz (M1, M2) y el complejo de polimerasa (PB1, PB2, PA), constituyen blancos prometedores para el desarrollo de vacunas universales. Esta revisión analiza los mecanismos antigénicos que favorecen la evasión inmune, así como la importancia de la reactividad cruzada. Se destacan los avances en plataformas vacunales innovadoras, como vacunas de ARNm, poli-epítopos, hemaglutininas quiméricas y nanopartículas, que han mostrado inducir inmunidad amplia y duradera en modelos preclínicos. A pesar de los progresos, persisten retos científicos y logísticos. Los hallazgos revisados subrayan el potencial de los epítopos conservados y de las nuevas plataformas para desarrollar una vacuna universal contra la influenza y enfrentar futuras pandemias.

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Palabras clave: influenza; virus; variabilidad antigénica; epítopos conservados; vacuna universal; respuesta inmune.

INTRODUCTION

Influenza is a highly contagious viral respiratory disease that can cause seasonal epidemics and large-scale pandemics. It is caused by influenza viruses, which are negative-sense, single-stranded RNA viruses in the family Orthomyxoviridae. Each year, millions of influenza infections occur worldwide. Annual epidemics are estimated to affect 5–15% of the global population, resulting in 3–5 million cases of severe illness and approximately 290000 to 650000 deaths.[1] In most instances, influenza presents as a self-limiting and mild illness; however, certain populations—including pregnant women, children under five years of age, older adults, and individuals with chronic medical conditions or immunosuppression—are at increased risk of developing severe disease and complications.[2]

Vaccination remains the most effective strategy currently available for the prevention and control of influenza. Current vaccines are designed to elicit neutralizing antibodies targeting the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). Licensed formulations include inactivated virus vaccines, live-attenuated virus vaccines, and recombinant HA-based vaccines, available in trivalent or quadrivalent formats. These formulations incorporate recent influenza A (H1N1 and H3N2) strains, in combination with one or both influenza B virus lineages (Yamagata and/or Victoria).[3] According to the Centers for Disease Control and Prevention (CDC), influenza vaccination during the 2018–2019 season in the United States prevented an estimated 3.1 million cases, 1.6 million medical visits, 43000 hospitalizations, and 2800 deaths.

Despite these public health achievements, several limitations remain: (i) reduced vaccine efficacy in older adults; (ii) strong dependence of vaccine performance on the antigenic match between vaccine strains and circulating viruses; and (iii) continuous antigenic drift and occasional antigenic shift, which diminish vaccine effectiveness over time. These factors necessitate annual updates to vaccine compositions by the World Health Organization (WHO). Implementing these updates requires substantial logistical coordination and financial investment from governments and healthcare systems to ensure timely distribution and broad population access to the most current formulations.[4]

Current research efforts increasingly focus on identifying universal influenza vaccine candidates that elicit broad, heterosubtypic immunity with strong, durable immunogenicity. Recent studies underscore the pivotal role of humoral immunity, particularly the induction of anti-HA neutralizing antibodies. Concurrently, growing attention is being directed toward pre-existing cellular immune responses targeting relatively conserved internal viral antigens, which may confer cross-protective immunity against a wide range of influenza strains.[5]

In this context, the objective of this review is threefold: (i) to examine the viral determinants underlying influenza antigenic variability and their implications for cross-immunity; (ii) to characterize key conserved epitopes with potential as targets for universal vaccine development; and (iii) to summarize recent advances in innovative vaccine platforms designed to overcome the limitations of conventional immunization strategies.

GENERAL CHARACTERISTICS AND PANDEMIC POTENTIAL OF INFLUENZA VIRUSES

Influenza viruses are enveloped, single-stranded, segmented, negative-sense RNA viruses belonging to the family Orthomyxoviridae. There are four types of influenza viruses: A, B, C, and D. Types A and B are primarily responsible for seasonal epidemics in humans; however, only type A viruses have been associated with pandemics. Type C viruses also infect humans but are typically associated with mild illness and have not been

linked to pandemics. Type D viruses have been identified exclusively in animals and are not known to infect humans.[6]

Influenza A viruses possess two highly variable surface glycoproteins—hemagglutinin (HA) and neuraminidase (NA)—which contain immunodominant epitopes and play critical roles in viral entry, release, and transmission. Based on the antigenic variability of these proteins and their phylogenetic relationships, influenza A viruses are classified into subtypes, clades, and subclades. To date, 18 HA subtypes (H1–H18) and 11 NA subtypes (N1–N11) have been identified. In total, more than 130 HA-NA subtype combinations have been documented, arising from the reassortment of gene segments between different influenza virus strains during co-infection of a host.[7]

In recent decades, pandemics caused by influenza A viruses have been significantly more prevalent than those caused by influenza B viruses. The most recent influenza A pandemic occurred in 2009 and was caused by the H1N1 subtype, which originated from a swine-derived virus and rapidly spread worldwide, resulting in substantial morbidity and mortality. This event underscored the capacity of influenza A viruses to cross species barriers and adapt to human hosts—a hallmark feature of their pandemic potential.[8]

Historically, influenza A viruses have caused several major pandemics in the 20th century, including the H1N1 pandemic in 1918, the H2N2 pandemic in 1957, and the H3N2 pandemic in 1968. The emergence of novel influenza A subtypes through genetic reassortment triggered these pandemics. This process enables the introduction of new combinations of viral gene segments into the human population.[9]

In contrast to influenza A viruses, influenza B viruses have not been associated with global pandemics. Although they contribute to seasonal epidemics, their pandemic potential is limited by low genetic diversity and a primary restriction to human hosts, with no significant animal reservoir to facilitate the emergence of novel strains.[10] Consequently, while influenza A viruses continue to pose a pandemic threat due to their capacity for interspecies transmission and adaptation, influenza B viruses remain confined to causing seasonal outbreaks. Ongoing surveillance and sustained pandemic preparedness efforts are essential to mitigate the impact of future influenza-related public health emergencies.

Viral proteins are essential in the spread of influenza spread

Influenza A viruses depend on several essential proteins to facilitate efficient replication and transmission. Among the most critical are the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). HA mediates viral entry by binding to host cell receptors and promoting the fusion of the viral envelope with the endosomal membrane. This interaction exhibits species specificity: avian influenza strains preferentially bind to α2,3-linked sialic acid glycans, whereas human-adapted strains show a preference for α2,6-linked sialic acid receptors.[11] In contrast, NA facilitates the release of progeny virions by cleaving sialic acids from host cell surface glycoproteins and from viral glycoproteins, thereby enabling efficient viral egress.[13]

In addition to HA and NA, matrix proteins such as matrix protein 1 (M1) and the M2 ion channel play pivotal roles in viral assembly and morphology. These proteins contribute to virion formation and membrane curvature—processes that are essential for the budding and release of infectious particles.[14] M1, in particular, forms a structural scaffold beneath the viral envelope, providing mechanical support and coordinating the assembly of viral components.[12]

The viral RNA polymerase complex, composed of the PB1, PB2, and PA subunits, is essential for the replication and transcription of the influenza genome. This complex orchestrates viral RNA synthesis through unique initiation mechanisms that ensure the accurate amplification of the virus's eight-segmented RNA genome.[13]

Additional non-structural proteins, including NS1, NS2, and the nuclear export protein (NEP), play critical roles in viral propagation and immune evasion. NS1 is a multifunctional virulence factor that antagonizes host immune responses through multiple mechanisms. It inhibits the nuclear export of host mRNAs by binding to the NXF1–NXT1 RNA export complex, thereby disrupting its interaction with nucleoporins and impairing mRNA translocation through the nuclear pore. This results in the nuclear retention of host transcripts and suppression of genes involved in antiviral defense. Moreover, NS1 inhibits type I interferon production and interferes with key antiviral effectors, including protein kinase R (PKR) and RNase L, thereby further dampening host immune responses.[14,15]

NS2, also known as nuclear export protein (NEP), is essential for the transport of viral ribonucleoprotein complexes (vRNPs) from the nucleus to the cytoplasm. By interacting with the cellular export receptor CRM1, NS2 mediates the translocation of vRNPs through the nuclear pore complex—a critical step in the influenza virus life cycle.[14]

DRIFT AND ANTIGENIC SHIFT

The influenza virus is highly dynamic and continuously evolves through two primary mechanisms: antigenic drift and antigenic shift. These evolutionary processes enable the virus to evade pre-existing population immunity and contribute to the emergence of novel variants with epidemic or pandemic potential.

Antigenic drift arises from the gradual accumulation of point mutations in the HA and NA genes, leading to alterations in key antigenic sites that enable the virus to evade host immune responses. These mutations disrupt neutralizing epitopes, thereby reducing the effectiveness of antibodies generated by prior infections or vaccination. This mechanism underlies the need for frequent updates to seasonal influenza vaccines.[16]

A clear example of antigenic drift was documented in Kenya, where A/H3N2 influenza viruses circulating between 2007 and 2013 exhibited substantial divergence from the WHO-recommended vaccine strains.[17] In 2012, 40% (2 out of 5) of the analyzed isolates displayed amino acid substitutions at positions I140R, R142G, K144N, N145S, and G186S within antigenic site A of the HA1 domain, relative to the A/Perth/16/2009 vaccine strain. These structural alterations significantly impaired antibody-mediated neutralization, resulting in an estimated vaccine effectiveness of −5%, indicating a complete lack of protection.[17] A similar pattern was observed in 2013, when Kenyan A/H3N2 strains acquired additional substitutions—T128A, R156H, and V186G—in the same antigenic region. These changes further diminished the immune response, with vaccine effectiveness dropping to just 8% against the 2010 reference strain A/Victoria/361/2011, which the WHO had recommended for that season. See Figure 1 and Table 1.[17]

In contrast, antigenic shift occurs through genomic reassortment between different influenza A subtypes, typically during co-infection of an intermediate host, such as pigs or aquatic birds. This mechanism can give rise to novel influenza strains with pandemic potential and has been responsible for several historical pandemics. A notable example is the emergence of the 2009 H1N1 pandemic virus, which resulted from a complex series of mutations and a quadruple reassortment involving swine, avian, and human influenza viruses.[18] Initially, a swine H1N2 virus emerged from a triple reassortment event that incorporated gene segments from a classical swine H1N1 virus, a North American avian H1N1 virus, and a human H3N2 virus. This reassortant virus continued to circulate within North American swine populations. Subsequently, it underwent further reassortment with an avian-like Eurasian H1N1 swine virus. The resulting strain—a novel swine-origin H1N1 virus—acquired the capacity for efficient human-to-human transmission and ultimately triggered the 2009 influenza pandemic. See Figure 2.[18]

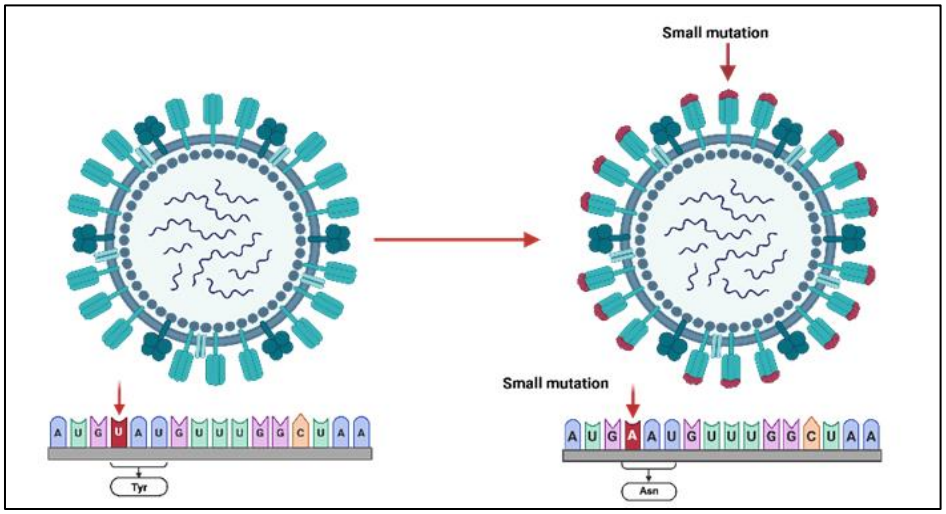

Figure 1. Antigenic drift occurs through the gradual accumulation of point mutations, resulting in minor antigenic changes in the surface glycoproteins of the influenza virus.

Table 1: Impact of antigenic drift on vaccine efficacy.

Year	Vaccine strain	Mutations in circulating strains	Vaccine efficacy %
2007	A/Wisconsin/67/2005	D122N; S138A; K140I	8
2008	A/Brisbane/10/2007	N144S	34
2010	A/Perth/16/2009	K144N	34
2012	A/Perth/16/2009	I140R; R142G; K144N; N145S; G186S	5
2013	A/Perth/16/2009	I140R; R142G; N145S	8

CONSERVED EPITOPES

Influenza A viruses exhibit substantial antigenic variability, particularly in their surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). Nevertheless, conserved regions have been identified not only within these surface proteins but also in internal viral components, including nucleoprotein (NP), matrix proteins (M1 and M2), and the polymerase complex (PB1, PB2, and PA), as summarized in Table 1. These conserved domains represent promising targets for the development of universal influenza vaccines and novel antiviral strategies.

Despite its high variability, HA contains several structurally and functionally conserved regions that are critical for viral attachment and membrane fusion. Among these, the receptor-binding site (RBS) facilitates interaction with host sialic acid receptors, while the hydrophobic groove within the stem domain contributes to structural stability and fusion capability.[19] Broadly neutralizing antibodies (bnAbs), such as FI6 and CR6261, have been shown to target these conserved regions and effectively inhibit viral entry.[20,21] Additional conserved epitopes have been identified in regions such as the HA monomer interface, the fusion peptide, and the vestigial esterase (VE) subdomain, all of which represent promising targets for cross-protective immune responses.[19]

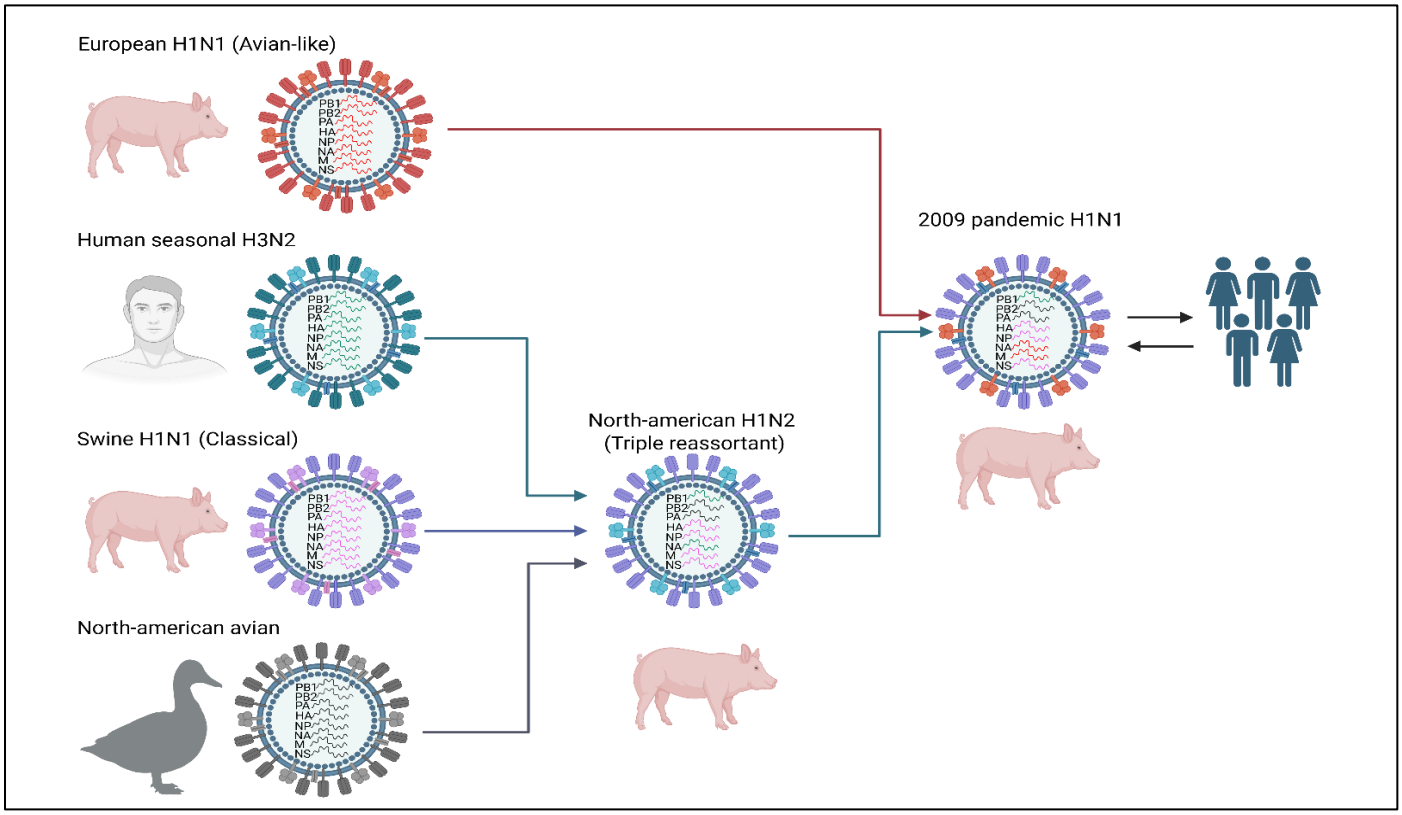

Figure 2. Antigenic shift. Schematic representation of the genetic origin of the 2009 pandemic H1N1 virus. The neuraminidase (NA) and matrix (M) gene segments were derived from the Eurasian "avian-like" H1N1 lineage, while the remaining six segments originated from the North American "triple reassortant" H1N2 virus, which incorporated gene segments from human, swine, and avian influenza viruses (adapted from Neumann et al.[18]). Created *with BioRender.com.*

The catalytic site of NA is another highly conserved domain and serves as the primary target of neuraminidase inhibitors such as oseltamivir and zanamivir.[22] Additionally, conserved epitopes located at the base of NA are recognized by bnAbs such as NC10 and CD6, which neutralize the virus by destabilizing the NA tetramer and obstructing access to the catalytic pocket.[23,24]

The internal proteins of influenza A are generally more genetically stable and play essential roles in viral replication and assembly. The nucleoprotein (NP) contains well-characterized immunodominant epitopes, such as $NP_{366–374}$ and $NP_{147–155}$, which elicit robust $CD8^+$ T cell responses and contribute to heterosubtypic immunity.[25,26] The M1 matrix protein harbors epitopes such as $M1_{58–66}$, which is presented by HLA-A*0201 and plays a key role in $CD8^+$ T cell activation.[27] The extracellular domain of M2 (M2e) is highly conserved and has been shown to induce protective antibody responses in preclinical studies.[28]

Table 1. Conserved epitopes identified in influenza viruses.

Protein	Preserved region	Function	Repetitive epitopes
Hemagglutinin	Receptor binding site (RBS) located in the head domain.	Adhesion to sialic acid receptors on the host cell	S139/1, C05, F045-092.[31–33]
	Hydrophobic groove located in the Stem Domain	Structural stabilization and viral fusion	FI6, CR6261, 27F3.[20,21,34]
	A hidden region in the monomer interface located between HA subunits	Transiently accessible, affects HA stability	FluA-20, S5V2-29.[19,35,36]

	The fusion peptide is located at the base of the stem.	Induces membrane fusion	CR8020, CR8043, 9H10.[37–39]
	Subdomain of vestigial esterase (VE) located at the head-stem interface.	Function poorly understood, possible structural regulation.	H3v-47, F005-126, H5M9.[40–42]
Neuraminidase	Globular domain of the protein, specifically in a hydrophilic cavity on the surface of the NA tetramer.	Residues involved in substrate binding.	R118, R292, R371, Y406.[43]
		Catalytic residues	E119, D151, E227, E277.[43]
		Stabilizing residues	R152, W178, S179.[43]
	Base of neuraminidase	Stability of the tetramer and/or exposure of the catalytic site.	NC10 y CD6.[23,24]
Nucleoprotein	Nucleoprotein (NP) C-terminal region	Interactions with viral polymerase (PB2, PB1, PA) and NP-NP oligomerization.	NP366-374.[25]
	Viral RNA binding domain (RBD, RNA Binding Domain)	ribonucleoprotein (RNP) complex formation.	NP147-155.[25]
M1	Central domain of the M1 protein in residues 58-66	Contributes to the structural stability of M1 and its interaction with other viral proteins.	M1 58−66 (GILGFVFTL) M1 180–197 (VLASTTAKAMEQ MAGSSE).[44,45]
M2	M2e (Extracellular domain of M2)	Target for antibodies, structural function	EVETPIRN (aa 6–13), SLLTEVET (aa 2–9).[46,47]
PB1	Inner region of PB1	Possibly involved in structural stability and interaction with other subunits of the polymerase complex.	PB130−38 (YSHGTGTGY).[48,49]
	Located within the catalytic domain of PB1.	Crucial for viral RNA transcription activity	PB1591−599 (VSDGGPNLY).[49,50]
	Located in the central region of the catalytic domain,	involved in viral RNA polymerization	TQIQTRRSF.[29]
	C-terminal end of PB1,	Stability and functionality of the polymerase complex, facilitating interaction with other subunits such as PB2 and PA	DTVNRTHQY.[29]
PA	Polymerase complex	Viral RNA processing	PA224-233 (SSLENFRAYV).[25]

The polymerase complex, composed of PB1, PB2, and PA, also contains multiple conserved T cell epitopes. In PB1, epitopes such as TQIQTRRSF, DTVNRTHQY, VSDGGPNLY, and YSHGTGTGY have been shown to stimulate $CD8^+$ T cell responses.[29] Similarly, PA contains the $PA_{224–233}$ epitope, which represents a key immunogenic determinant in cellular immunity.[30]

IMMUNE RESPONSE

Innate response

Influenza A virus initially infects epithelial cells of the respiratory tract by binding to sialic acid receptors, facilitating viral entry and subsequent intracellular replication.[51] Upon infection, viral RNA is recognized by pattern recognition receptors (PRRs), such as RIG-I in the cytoplasm and TLR7 in endosomes, which activate intracellular signaling pathways.[52] These pathways induce the production of type I interferons (IFN-α and IFN-β), which are essential for establishing an antiviral state, as well as proinflammatory cytokines (IL-1β, IL-6, TNF-α) that coordinate the early immune response.[53]

Alveolar macrophages, as the first line of defense in the lower respiratory tract, phagocytose viral particles and infected cells, thereby limiting viral spread.[54] In addition, they secrete chemokines such as CCL2 and CXCL10, which recruit monocytes, neutrophils, and dendritic cells to the site of infection.[55] Recruited monocytes can differentiate into macrophages or dendritic cells, amplifying the inflammatory response, while conventional dendritic cells migrate to the lymph nodes to activate T lymphocytes, marking the onset of the adaptive immune response.[56]

The NLRP3 inflammasome plays a central role in the inflammatory response to influenza. Its activation, triggered by viral RNA and damage-associated molecular patterns (DAMPs), promotes caspase-1 activation and the maturation of IL-1β and IL-18, thereby amplifying inflammation and immune cell recruitment.[57] Dysregulation of NLRP3 is associated with excessive inflammation and tissue damage in severe infections, positioning it as a potential therapeutic target. Preclinical models suggest that selective inhibition of NLRP3 can attenuate inflammation without compromising viral control, highlighting the importance of maintaining a balance between viral clearance and tissue protection to prevent severe complications.[58]

Plasmacytoid dendritic cells (pDCs) are distinguished by their capacity to produce large quantities of type I interferons in response to TLR7 activation, thereby enhancing the antiviral state in neighboring cells.[59] Natural killer (NK) cells, activated by the downregulation of MHC class I molecules on infected cells, eliminate these targets through the release of perforins and granzymes, and secrete IFN-γ to enhance macrophage activity.[60]

Neutrophils, attracted by chemokines such as IL-8, contribute to viral clearance through phagocytosis and the formation of neutrophil extracellular traps (NETs).[61] Although these structures are effective, their dysregulation can exacerbate tissue damage. The coordinated interaction among macrophages, dendritic cells, neutrophils, and NK cells ensures an efficient immune response, in which mediators such as IL-12 enhance NK cell activity and, consequently, macrophage function.[62]

Adaptive response

The adaptive immune response to influenza A virus in humans is a highly specialized system that integrates both cellular and humoral mechanisms to eliminate the pathogen and establish immunological memory. Activated following the initial phase of innate immunity, this response is characterized by specificity and the capacity to generate long-lasting protection. However, it is challenged by the virus's high mutation rate.[52,63]

Cellular mechanisms

The process begins when dendritic cells (DCs) in lung tissue capture viral antigens through pattern recognition receptors (PRRs), such as TLR7 and RIG-I, which detect viral RNA.[63,64] These cells then migrate to the mediastinal lymph nodes, where they present viral peptides associated with major histocompatibility complex (MHC) class I and II molecules to naïve T lymphocytes.[63,65] DCs serve as a critical bridge between innate and adaptive immunity by secreting cytokines such as IL-12, which promote the differentiation of T helper 1 (Th1) lymphocytes.[65]

Cytotoxic $CD8^+$ T lymphocytes recognize peptides presented by MHC class I molecules that are derived from internal viral proteins such as nucleoprotein (NP) and matrix protein 1 (M1), which are highly conserved across influenza strains.[63,65] Upon activation, these cells proliferate and differentiate into effectors capable of eliminating infected cells by releasing perforins and granzymes, which induce apoptosis.[63,64] Studies in murine models have demonstrated that $CD8^+$ T cell depletion increases viral load and mortality, underscoring the critical role of $CD8^+$ T cells in viral control.[63,65]

In parallel, $CD4^+$ T cells recognize peptides presented by MHC class II molecules and secrete cytokines such as IL-2 and IFN-γ, which are essential for amplifying the activity of $CD8^+$ T cells and NK cells.[63,65,66] Additionally, $CD4^+$ T cells facilitate B cell activation through CD40L–CD40 interactions, promoting affinity maturation and antibody isotype switching.[64,65] The cooperation between both T cell subsets ensures a balanced immune response: while $CD8^+$ T cells eliminate infected cells, $CD4^+$ T cells modulate inflammation and help prevent excessive tissue damage.[63,65]

Humoral mechanism

Humoral immunity, mediated by B lymphocytes, targets the surface glycoproteins of the influenza virus—hemagglutinin (HA) and neuraminidase (NA). Naïve B lymphocytes encounter viral antigens within the germinal centers of lymph nodes, where they receive $CD4^+$ T cell help to differentiate into antibody-producing plasmablasts.[64,65] Antibodies directed against the globular head domain of HA block its interaction with sialic acid receptors, thereby neutralizing viral entry. However, this domain is highly variable, necessitating frequent updates to seasonal vaccines.[64,67]

Recently, broadly neutralizing antibodies (bnAbs) targeting the conserved HA stem have been identified, providing heterosubtypic protection by inhibiting membrane fusion.[64,67] Among these, CR6261 and FI6v3 bind to hydrophobic epitopes on helix A of the HA2 subunit, preventing the conformational changes required for viral entry.[67] These antibodies utilize germline genes such as VH1-69 and VH3-30, offering valuable insights for the development of universal influenza vaccines.

Beyond neutralization, antibodies contribute to viral clearance through opsonization and antibody-dependent cellular cytotoxicity. Anti-NA antibodies inhibit enzymatic activity, thereby limiting virion release, while anti-M2 antibodies promote the elimination of infected cells via ADCC. Additionally, mucosal secretory IgA plays a critical role in early protection by preventing viral adhesion.[63,65]

CROSS-REACTIVITY AGAINST INTERNAL VIRAL PROTEINS

Internal antigens of the influenza virus, such as nucleoprotein (NP) and matrix protein 1 (M1), play a crucial role in cross-protection against diverse influenza A virus strains through T cell–mediated responses. These proteins are highly conserved among influenza A subtypes, enabling T cells specific to these antigens to reduce disease severity even in the context of antigenically divergent strains.

NP and M1 are efficiently presented by major histocompatibility complex (MHC) class I and II molecules, facilitating the expansion of antigen-specific $CD8^+$ and $CD4^+$ T cells that secrete interferon-gamma (IFN-γ) and tumor necrosis factor (TNF).[68] This efficient antigen presentation is critical for the development of cellular immune responses capable of recognizing and targeting multiple influenza virus variants.

Moreover, vaccines expressing NP and M1 have been shown to elicit robust $CD8^+$ T cell responses, including the generation of tissue-resident memory (TRM) T cells, which are essential for rapid and broad protection

against influenza reinfection.[69] These TRM cells, located in mucosal tissues, can mount immediate immune responses by recognizing conserved epitopes of NP and M1.

The ability of $CD8^+$ T cells to recognize conserved epitopes on these internal proteins has also been associated with significant cross-protection in animal models. In these models, immunization with vectors expressing NP and M1 conferred protection against lethal challenges with various influenza A subtypes.[70,71] This protection is attributed to the generation of memory T cell responses that recognize viral variants, supporting the potential of NP- and M1-based strategies for the development of universal influenza vaccines.

RECENT DEVELOPMENTS IN THE SEARCH FOR UNIVERSAL VACCINES

The development of a universal influenza virus vaccine has progressed significantly in recent years, driven by the need to overcome the limitations of current seasonal vaccines in the face of the virus's high antigenic variability. The most promising strategies focus on identifying and targeting conserved epitopes within key viral proteins, including hemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), matrix protein 1 (M1), and the proton channel M2. Table 2 provides a comparative summary of these strategies, the viral proteins involved, and the main findings from recent studies, offering an updated overview of progress toward a universal influenza vaccine.

One of the most innovative approaches has been the design of nucleoside-modified messenger RNA (mRNA) vaccines, which enable efficient expression of conserved viral antigens. For instance, a pentavalent mRNA-based vaccine demonstrated protection against influenza B even at very low doses, highlighting its high immunogenicity and efficacy in animal models.[72] Concurrently, strategies employing chimeric hemagglutinin (cHA) aim to redirect the immune response toward the conserved HA stem domain. These vaccines have elicited broadly reactive antibodies against group 1 hemagglutinins and provided cross-protection against heterologous strains of both influenza A and B.[73,74]

Another emerging strategy involves the use of headless HA viral particles in combination with NA, which promotes immune responses directed toward conserved epitopes and reduces the immunodominance of the HA head. This combination has been shown to induce protective hybrid immunity in preclinical models.[75]

In terms of cell-mediated immunity, the inclusion of conserved $CD8^+$ T cell epitopes in multi-epitope vaccine formulations has yielded promising results. For example, the rMVA-PE vaccine induced robust virus-specific T cell responses in humanized mice, while other recombinant formulations based on NP, M1, and HA achieved up to 67% protection against viral pneumonia caused by various influenza A strains.[45,49]

Complementing these strategies, the design of mosaic vaccines incorporating influenza B hemagglutinins has demonstrated cross-protection when administered in sequential vaccination regimens.[76] Additionally, multi-epitope mRNA vaccines combining HA, NA, NP, and M2 antigens have been shown to form stable, immunogenic complexes, supporting their potential as next-generation universal vaccine candidates.[77]

Table 2. Strategies for the development of universal influenza vaccines using conserved viral proteins and novel immunization technologies.

Estrategy	Viral proteins involved	Finding
Combination of viral particles.[75]	Headless hemagglutinin (HA), Neuraminidase (NA). A combination of wild-type (WT) influenza virus particles with viral particles engineered to exhibit a trimerized HA stalk.	Hybrid and protective responses against HA and NA head and stem proteins in both naïve and preimmune mice and ferrets.

Polyepitope-based vaccine (rMVA-PE vaccine).[49]	Artificial immunogen composed of twenty highly conserved influenza virus CD8+ T-cell epitopes with 99.50% HLA coverage of the global population.	Induced virus-specific CD8+ T cell responses in humanized mouse models.
Multi-epitope mRNA vaccine.[77]	Experimentally tested conserved T and B cell epitopes of hemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), and matrix proton channel-2 (M2).	Immune simulation and molecular docking with TLR2, TLR3, and TLR4 showed favorable immunogenicity and stable complex formation, with potential for protection against various influenza A and B subtypes.
Chimeric hemagglutinin (cHA)-based vaccine.[73]	Conserved hemagglutinin stem domain.	Induction of broad antibody responses, especially against group 1 hemagglutinins.
Vaccine based on conserved epitopes (rMVA-k1-k2 vaccine).[45]	Nucleoprotein (NP), Matrix 1 (M1), and Hemagglutinin (HA) of Influenza A and B.	67% or greater protection in mice against influenza viral pneumonia when infected with multiple strains of H1N1, H2N2, H3N2, and H5N1 subtypes
Nucleoside-modified mRNA vaccine encapsulated in lipid nanoparticles (mRNA-LNP).[72]	Pentavalent vaccine against influenza B directed to multiple antigens (B/Yamagata/16/1988 HA, B/Victoria/2/1987 HA, NA, NP, and M2 lineages).	Protection of mice against morbidity with very low doses (50 ng per antigen) after a single vaccination.
Mosaic" hemagglutinin approach.[76]	Type B hemagglutinins in mosaic format by replacing major antigenic regions with equivalent sequences derived from exotic hemagglutinins of influenza A viruses.	Sequential vaccination with "mosaic" hemagglutinin proteins provided cross-protection against homologous and heterologous strains of influenza B virus in the murine model.
Chimeric hemagglutinin (cHA)-based vaccine.[74]	Hemagglutinin stalk and conserved sites in the head domain.	Improving cHA antigens to elicit broad responses and protection against seasonal and pandemic influenza viruses.

CHALLENGES FOR THE DESIGN OF UNIVERSAL VACCINES

The development of a universal influenza virus vaccine faces several significant challenges, primarily due to the high genetic and antigenic variability of influenza A viruses and the need to elicit broad and durable immune responses. One of the main obstacles is the virus's capacity for antigenic drift and antigenic shift, which enables it to evade immunity induced by current vaccines that target hypervariable regions of surface proteins such as hemagglutinin (HA) and neuraminidase (NA).[16]

To address these limitations, universal vaccine strategies aim to redirect the immune response toward conserved regions of the virus, such as the HA stalk, which is less susceptible to antigenic variation. However, these conserved regions are often immunologically subdominant, making it difficult to induce a strong and sustained immune response.[78] Additionally, the genetic diversity of influenza A viruses—driven in part by animal reservoirs—adds further complexity to the design of a universal vaccine.[78]

Another major challenge is the difficulty of establishing immunological correlates of protection applicable across a wide range of influenza strains. This is essential for the development of vaccines that do not require precise antigenic matching with circulating viruses.[79] Moreover, the implementation of novel vaccine platforms, such as those based on nanoparticles or viral vectors, faces hurdles related to large-scale manufacturing and regulatory approval.[78]

CONCLUSIONS

Despite sustained efforts to control influenza through seasonal vaccination campaigns, the virus's high antigenic variability continues to undermine the efficacy of conventional vaccines. This ongoing challenge has driven the pursuit of universal vaccines capable of inducing broad, durable, and effective immunity against multiple influenza virus subtypes. Key advances in this field include the identification of conserved epitopes within internal viral proteins and structurally stable regions of HA and NA, as well as the incorporation of innovative technologies, including mRNA vaccines, viral vectors, and nanoparticles.

The evidence reviewed in this article highlights the complementary roles of humoral and cell-mediated immunity in achieving heterosubtypic protection. Moreover, T cell– and antibody-mediated cross-reactivity against conserved epitopes reinforces the potential of next-generation vaccine strategies. Nonetheless, significant scientific and logistical challenges remain, including overcoming the immunodominance of variable epitopes, defining broadly applicable immunological correlates of protection, and ensuring the scalability and regulatory approval of emerging technologies.

Taken together, recent advances not only represent meaningful progress toward developing a universal influenza vaccine but also provide a promising framework for the rational design of immunogens capable of addressing future influenza pandemics.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTION

RMM participated in the conceptualization, supervision, drafting, final revision, and approval of the manuscript.
JDD participated in the conceptualization, supervision, drafting, final revision, and approval of the manuscript.
XMS contributed to the study design, literature review, writing, drafting, translation, and approval of the manuscript.
ARS was responsible for the literature review, writing, topic analysis, translation, and approval of the manuscript.
YCL participated in the elaboration of the figures, literature review, and approval of the manuscript.
HSV contributed to the study design, literature review, manuscript drafting, topic analysis, and manuscript approval.
WMD participated in the conceptualization and contributed to the study design, literature review, writing, topic analysis, and manuscript approval.

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