HUMAN INFLUENZA VIRUSES AND CD8⁺ T CELL RESPONSES

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Abstract

Influenza A viruses (IAVs) cause significant morbidity and mortality worldwide, despite new strain-specific vaccines being available annually. As IAV-specific CD8⁺ T cells promote viral control in the absence of neutralising antibodies, and can mediate cross-reactive immunity towards distinct IAVs to drive rapid recovery from both mild and severe influenza disease, there is great interest in developing a universal T cell vaccine. However, despite detailed studies in mouse models of influenza virus infection, there is still a paucity of data on human epitope-specific CD8⁺ T cell responses to IAVs. This review focuses on our current understanding of human CD8⁺ T cell immunity against distinct IAVs and discusses the possibility of achieving a CD8⁺ T cell mediated-vaccine that protects against multiple, distinct IAV strains across diverse human populations. We also review the importance of CD8⁺ T cell immunity in individuals highly susceptible to severe influenza infection, including those hospitalised with influenza, the elderly and Indigenous populations.

Highlights

- Pre-existing influenza-specific CD8⁺ T cells drive rapid recovery from both mild and severe influenza disease
- Established CD8⁺ T cell memory pools cross-react with distinct influenza strains, including avian viruses
- Universal CD8⁺ T cell vaccines may protect individuals highly susceptible to severe influenza infection
- CD8⁺ T cell vaccines need to be assessed for longevity of T cell memory, population coverage across different HLAs and ethnicities, vaccine-mediated immune escape and immunopathology

Human influenza viruses

Influenza viruses (IVs) belong to the Orthomyxoviridae family and are an enveloped virus, with a lipid bilayer encompassing an 8-stranded negative sense RNA genome that encodes 12 distinct proteins (1). They comprise of three distinct families: A, B and C (2). Influenza C viruses (ICV) generally causes a mild infection and thus is not typically considered a significant threat to population health. Conversely, Influenza A viruses (IAV) and Influenza B viruses (IBV) are responsible for seasonal epidemics (3), with IAV causing the majority of infections in the majority of IAV seasons. IAV is the only subtype associated with influenza pandemics (3). IAVs are categorized based on their surface Haemagglutinin (HA) and Neuraminidase (NA) glycoproteins, which are embedded within the lipid bilayer envelope and coat the entire surface of the virion. The combination of HA and NA glycoproteins expressed determines the subtype of a particular IAV and, according to the CDC, there are to date 18 distinct HA and 11 distinct NA glycoproteins (4).

Severity of IAV disease

IAVs cause respiratory disease and are readily transmitted between humans by the inhalation of virus-containing aerosols produced through coughing or sneezing (5). Furthermore, IAV is present in other animal hosts, in particular avian species, providing an additional reservoir for viral transmission. IAVs rapidly evolve through antigenic drift, whereby accumulating mutations in HA and NA glycoproteins result in evasion of pre-existing antibody responses (6). This, together with the high transmissibility of this virus, results in significant IAV-induced morbidity and mortality worldwide annually. Every year, seasonal IAV epidemics are responsible for 3-5 million cases of severe infection and up to 500,000 deaths worldwide, mostly in the young (<5 year-old (y/o)), elderly (>65 y/o), immunocompromised, pregnant and those with co-morbidities including obesity, smoking and respiratory disease (7). Furthermore, these epidemics carry a significant economic burden, estimated at around \$90 billion annually in the United States alone (8).

Pandemics can occur when a novel IAV strain or subtype, typically generated by antigenic shift, gains the capacity to transmit in humans. In this case, there is minimal to no pre-existing antibody-mediated immunity at a population level,

1 resulting in rapid spread of the virus and severe illness. This has been exemplified by
2 the 2009 “swine origin” H1N1 pandemic (pH1N1). Of the four pandemics recorded
3 during the last century (1918, 1975, 1968, and 2009), the most catastrophic was the
4 1918 “Spanish” pandemic, which resulted in >40 million fatalities (9-11). Antigenic
5 shift, which is often responsible for pandemic strains, typically involves the
6 reassortment of gene segments from two or more IAVs resulting in the formation of a
7 novel subtype with a distinct HA and NA combination. The ability of IAV strains to
8 infect different animals such as ducks, chickens and pigs plays a key role in the
9 emergence of new human strains via antigenic shift. Antigenic shift greatly
10 contributed to the generation of the 2009 “swine” H1N1 pandemic (pH1N1), which
11 comprised of genes from the North American swine lineage (HA, NA and NS),
12 Eurasian swine lineage (NP and NA), avian North American avian lineage (PA and
13 PB2) and human seasonal H3N2 lineage (PB1) (12) (6).

24 The outbreak of a new A/H7N9 strain in China in early 2013 (9) sparked fears
25 of a potential global IAV pandemic (13). This novel H7N9 avian-derived virus caused
26 >99% hospitalization and >30% mortality rates in infected individuals. Most H7N9-
27 infected patients were hospitalized with severe pneumonia and acute respiratory
28 distress syndrome, contributing to high rates of ICU admissions and mechanical
29 ventilation (14, 15). Furthermore, there is an ever-present threat of epidemics and
30 pandemics from other avian-derived viruses, such as H5N1 (16, 17), H5N6 (18),
31 H6N1 (19), H7N2 (20), H7N3 (21), H7N7 (22), H9N2 (23), H10N7 (24), H10N8
32 (25), which are highly virulent and capable of jumping hosts to human populations
33 (reviewed in (26)). Given that as few as 4-5 mutations can cause ferret-to-ferret
34 transmission of H7N9 (PB1-D76N, NP-I365V, NA-E73K and NA-I300V (27)) and
35 H5N1 (HA-Q222L, HA-G224S, HA-H103Y, HA-T156A and PB2-E627K (28)), the
36 capacity for direct human-to-human transmission may be acquired relatively easily
37 and rapidly in these highly virulent viruses, which would then present a significant
38 challenge to the human population around the globe.

39 **Adaptive immune control of IAV**

40 Current IAV vaccines illicit humoral immunity directed towards the surface HA and
41 NA glycoproteins and are highly effective in the control of IAV (29). However, due
42 to antigenic drift (6), these surface glycoproteins rapidly mutate, and thus humoral
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immunity established against one IAV strain is unlikely to protect against subsequent infections with distinct IAV strains (29). Conversely, CD8⁺ T cells typically recognise the more conserved internal proteins of IAV, and thus have the potential to be strain-cross protective (Figure 1). Whilst pre-existing CD8⁺ T cell immunity cannot prevent infection from occurring, IAV-specific CD8⁺ T cells are undeniably important in controlling and mediating recovery from influenza disease (30-32).

Murine studies on importance of CD8⁺ T cells in controlling IAV.

IAV infection of C57BL/6J (B6) mice provides a well-characterized model for dissecting CD8⁺ T cell immunity. This respiratory challenge system is characterized by an acute, transient, localized pneumonia, with virus clearance around day (d) 9 after infection (33, 34). Studies dating back to the 1970's have shown that, in this model, influenza-specific CD8⁺ T cells play an important part in promoting efficient elimination of the virus and subsequent host recovery via the production of pro-inflammatory cytokines and direct killing of virus-infected cells (35, 36). The importance of CD8⁺ T cells in influenza is further evidenced by delayed virus clearance in mice lacking CD8⁺ T cells (35, 37) and the generation of influenza virus escape mutants in the presence of CD8⁺ T cell-mediated immune pressure (38). Aside from reducing morbidity and mortality, CD8⁺ T cells also provide a level of protection following lethal IAV challenge (39-41) and heterosubtypic challenges (42-46).

CD8⁺ T cells and recovery from IAV in the human setting

While important, murine studies provide only a foundation to understanding the importance of CD8⁺ T cell responses in controlling IAV in humans. Although limited, studies in humans are so far consistent with murine data in highlighting the key role of CD8⁺ T cells in driving the recovery from IAV, especially when a new IAV strain emerges. In 1983, McMichael *et al.* (30) demonstrated that the magnitude of pre-existing CD8⁺ T cell populations, as assessed by ⁵¹Cr-mediated killing, inversely correlated with IAV viral titers and clinical symptoms in a cohort of 63 healthy volunteers infected with A/Munich/1/79. This provided the first direct evidence that CD8⁺ T cells are important in the control of IAV in humans. More recently, an elegant analysis of 342 healthy adults in household cohort study prior to and after

1 incidental pH1N1 infection (named as ‘an experiment of nature’) showed that the
2 frequency of pH1N1-specific pre-existing CD8⁺ T cells directed at conserved epitopes
3 inversely correlated with the clinical symptoms associated with IAV infection (31).
4 Furthermore, we have demonstrated that CD8⁺ T cell numbers correlate with recovery
5 from severe A/H7N9 disease (32). In this study, individuals who had early H7N9-
6 specific and IFN- γ -producing CD8⁺ T cell populations during influenza infection
7 recovered rapidly from severe infection, while patients with delayed or absent CD8⁺ T
8 cell response had increased morbidity and mortality following infection with the
9 novel avian H7N9 virus. As the latter study was performed during an outbreak of a
10 novel avian H7N9 influenza virus, the clinical symptoms could not be compared to
11 the baseline level of pre-existing CD8⁺ T cells, as in studies by McMichael *et al* and
12 Sridhar *et al*.
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23 Using *in vitro* approaches, others have highlighted the cross-reactive potential
24 of CD8⁺ T cells in the recognition of heterologous IAV strains. Boon *et al*. (47)
25 showed that seasonally-activated NP₄₁₈-specific CD8⁺ T cells could cross-react with
26 the serologically distinct pH1N1 virus, while Gras *et al*. (48) demonstrated CD8⁺ T
27 cell cross-reactivity towards related (but not distinct) NP₄₁₈ variants. Furthermore,
28 CD8⁺ T cells activated in response to infection with seasonal IAV strains can cross-
29 react with pH1N1 (49, 50) and highly pathogenic avian H5N1- (51, 52) and H7N9-
30 (53) derived variants. Thus, a CD8⁺ T cell based influenza vaccine could provide
31 considerable breadth of protection against distinct influenza strains.
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42 It is important to achieve a fine balance between protective CD8⁺ T cell
43 immunity and immunopathology in order to provide recovery from influenza disease
44 without severe damage to lung tissue (Figure 2). Our recent immune dissection of
45 early hypercytokemia/ immunopathology (54) as well as recovery from H7N9 (32) in
46 patients hospitalised at the Shanghai Public Health Clinical Centre shows that earlier
47 protective CD8⁺ T cell immunity (most likely recalled from cross-reactive memory
48 pools) was associated with less cytokine/chemokine-driven inflammatory disease. In
49 contrast, patients with minimal IAV-specific cellular immunity had increased
50 inflammation and viral titers (32, 54). Thus, there appears to be a need for early viral
51 control of influenza by CD8⁺ T cells (and other cellular responses) to prevent
52 exuberant inflammatory responses. However, potentially, CD8⁺ T cell responses
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cross-reactive between different influenza strains and subtypes might also induce enhanced immunopathology. Clearly, there is still much to learn about the contribution of human CD8⁺ T cells to influenza immunopathology.

Longevity of human CD8⁺ T cell memory pools

Cross-reactivity towards distinct IAV strains offers potential for universal immunity against influenza for as long as the established memory CD8⁺ T cell populations survive within an individual. Animal models have provided important insights into the persistence of CD8⁺ T cell memory. Influenza-specific CD8⁺ T cells can persist for the life-time of a laboratory mouse (2 years) when the animals are primed early (at 6 weeks) (55, 56). However, until recently, there has been debate about the longevity of human IAV-specific CD8⁺ T cell memory. Although persistence of influenza-specific CD8⁺ T cell memory in humans is understudied, an early study by McMichael *et al.* (57) using PBMCs obtained from individuals in 1977-1982 in a ⁵¹Cr cytotoxicity assay, showed that memory pools contract within a few years after influenza infection. It is, however, possible that the observed reduction in cytotoxicity could be related to diminishing killing capacity or cytolytic molecule expression (eg. granzyme A and/or B) in the long-term memory CD8⁺ T cells (58, 59), rather than reduction in number. Importantly, recent findings have shown that epitope-specific (PB1₅₉₁, M1₅₈, NP₂₆₅, NP₃₈₀ and NP₁₂₄) CD8⁺ T cells can be detected directly *ex vivo* by tetramer staining over a 13-year time-course (60). Similarly, following yellow-fever (61) and smallpox (62) vaccination, epitope-specific (NS4B₂₁₄ and, VV-CTL, respectively) CD8⁺ T cells can be detected by tetramers *ex vivo*, 10-50 years post infection (61, 62). Taken together, published evidence shows that influenza-specific memory CD8⁺ T cells may provide long-lasting protection from heterosubtypic IAV infection, thus supporting the rationale for developing an IAV-specific T cell-based vaccine.

The potential for an IAV-specific CD8⁺ T cell-mediated vaccine

The success of an IAV-specific T cell vaccine will depend greatly on selecting the

best approach to achieve long-term, heterosubtypic protection across diverse human populations. The live-attenuated influenza vaccine, Flumist (MedImmune, Gaithersburg, MD), contains specific mutations in PB1 (K391E, E581G and A661T), PB2 (N265S) and NP (D34G), preventing viral replication at temperatures present within the human respiratory tract (63). When given to children (aged 5-9), it induces limited T cell immunity (~0.3% increase in the IFN- γ ⁺CD8⁺ population), whilst none to minimal T cell responses are detected in adults (64). In contrast, the trivalent “split-virus” influenza vaccine (TIV) does not induce significant CD4⁺ and CD8⁺ T cell responses in children or adults (64).

Another approach for a CD8⁺ T cell-mediated vaccine is the use of viral vectors. Multiple studies from the same group have used a vaccine made from a modified Ankara vaccinia virus encoding one NP and M1 protein from IAV, named MVA-NP+M1 (65-68). Clinical trials involving 22 individuals showed that this vaccine was well tolerated in adults at low doses, and successfully reduced infection with IAV during a challenge experiment (67). Further characterization of the vaccine response revealed that epitope-specific CD8⁺ T cells were more highly activated and cytolytic compared to non-epitope-specific CD8⁺ T cells (68). However, this vaccine contains only one type of NP and M1 protein, and whilst these variants are highly conserved between circulating H1N1 and H3N2 viruses (>90%), they have little sequence identity with pH1N1 and H5N1 viruses (~8%), suggesting that the vaccine is unlikely to provide protection against variant IAVs that acquire mutations within these antigenic sites.

Peptide-based approaches are likewise being trailed for their induction of cellular immunity (69-71). Flu-v, manufactured by SEEK, contains four short (21-32aa) proteins with >70% conservation between all published IAV strains (69). Furthermore, these proteins, derived from IAV: M1 (M1₃₆₋₆₇), NP (NP₂₅₅₋₂₇₅), M2 (M2₃₂₋₅₅) and IBV NP (NP₃₀₆₋₃₂₆) contain >5 potential HLA-A*02:01-restricted epitopes, as predicted algorithmically. Flu-v was safe and well-tolerated in phase 1 clinical trials of 48 individuals, and IFN- γ -responses were detected in >80% of individuals immunised with an adjuvanted form of the vaccine following *in vitro* activation (70). Phase 1b clinical trials in 28 volunteers showed that Flu-v increases the IFN- γ ⁺CD8⁺ population ~8-fold and vaccination with Flu-v positively correlated

with a reduction in viral shedding and symptom scores following virus challenge (71). Whilst this study yields promising results, it is unlikely that these small increases in CD8⁺ T cell responses would protect against a range of IAVs. Furthermore, algorithmic approaches do not accurately identify immunogenic peptides (72) and indeed, few minimal epitopes within these short proteins (M1₃₆₋₆₇ (3 epitopes (73, 74)), NP₂₅₅₋₂₇₅ (1 epitope (75, 76)) M2₃₂₋₅₅ (1 epitope (77)) and NP₃₀₆₋₃₂₆ (0-epitopes)) have been published and are likely to only cover 16-57% of the population, depending on ethnicity and the associated HLA profile (78). The development of a universal IAV-specific CD8⁺ T cell-mediated vaccine requires further identification and characterisation of novel CD8⁺ T cell epitopes restricted by a range of HLA alleles.

Immunodominant CD8⁺ T cell epitopes in IAV

In order to provide protection across distinct ethnicities, a rationally designed vaccine would need to contain multiple viral peptides or peptide regions to elicit immunodominant CD8⁺ T cell responses restricted by a range of HLA alleles. To date, there have been 255 CD8⁺ T cell IAV-specific epitopes identified across 10 proteins using a range of epitope-identification techniques (72, 75) (reviewed in (78)). However, only a selected number of those influenza epitopes are immunogenic (53), while others, including the HLA-B*07:01-restricted NP₁₇₂₋₁₈₁ (53), HLA-A*24:02-restricted NP₃₉₋₄₇ (53) and HLA-B*44:03-restricted NP₃₃₈₋₃₄₆ (Grant EJ et al, unpublished), do not induce immunodominant CD8⁺ T cell responses. We have previously shown that the nucleoprotein (NP) of IAV is highly immunogenic (72, 75) and published epitopes within NP are 70-85% conserved (Quinones-Parra *et al.*, submitted). Five such NP peptides (HLA-A*03:01 NP₂₆₅₋₂₇₃, HLA-B*08:01 NP₂₂₅₋₂₃₃, B*18:01 NP₂₁₉₋₂₂₆, B*27:05 NP₃₈₃₋₃₉₁ and B*57:01 NP₁₉₉₋₂₀₇) (Table 1) elicited CD8⁺ T cell responses in all donors tested and were conserved between vaccine and Australian H1N1 and H3N2 isolates (53). These “universal” epitopes only provide ~16-57% coverage at the population level, depending on ethnicity. Thus, there is a need to identify novel influenza-specific immunodominant CD8⁺ T cell responses, especially in Indigenous populations, which may have distinct HLA and epitope profiles. Furthermore, it is important to understand the quality and thus the protective capacity of these CD8⁺ T cell responses. High quality CD8⁺ T cell characteristics

1 including high functional avidity (79-81), polyfunctionality (80) and diverse (79, 82-
2 85) and cross-reactive $\alpha\beta$ T cell receptor (TCR $\alpha\beta$) repertoires (86-88) are considered
3 superior for viral control (reviewed in (89)).
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6 Little is known about the importance of the TCR $\alpha\beta$ repertoire expressed by
7 IAV-specific CD8⁺ T cells for protection against infection. Until recently,
8 characterisation of the CD8⁺ TCR $\alpha\beta$ repertoire was limited to identifying TCR α or
9 TCR β gene usage by PCR (90) or V β staining (91). In the early 1990's, it was
10 established that the HLA-A*02:01-restricted M1₅₈-specific CD8⁺ TCR $\alpha\beta$ repertoire is
11 limited and displays a strong TRBV19 (V β 17 bias) (90-92). Advances in techniques
12 such as deep sequencing have allowed for clonal analysis epitope-specific CD8⁺ T
13 cell TCR α or TCR β repertoires. This has been utilized to confirm a strong TRBV19
14 bias for M1₅₈-specific T cells and identified new clonotypes (93), suggesting that the
15 A2⁺M1₅₈-specific TCR repertoire is not as restricted as previously thought. Using a
16 novel single-cell multiplex RT-PCR (94, 95), we are now able to identify, for the first
17 time, influenza-specific TCR $\alpha\beta$ paired clonotypes from single cells directly *ex vivo*
18 (Valkenburg SA *et al.*, submitted). Thus, we can now characterize the TCR $\alpha\beta$
19 repertoire for other influenza-specificities to understand the importance of TCR $\alpha\beta$
20 diversity and cross-reactivity in the recognition of distinct IAV strains (Grant EJ *et*
21 *al.*, unpublished). This will allow us to fully realize the protective potential of CD8⁺ T
22 cell responses in recovery from both mild and severe IAV disease.
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38 Taken together, there are a few challenges in the development of T cell-based
39 influenza vaccines, including persistence of T cell memory after influenza
40 immunization, population coverage across different HLAs and ethnicities, vaccine-
41 mediated immune escape and immunopathology. Firstly, the longevity of functional
42 influenza-specific memory CD8⁺ T cells in humans is still unclear. Most adults are
43 exposed to influenza virus infection (at least once) by the age of 15, elicit T cell
44 immunity and subsequently generate influenza-specific T cell memory. However,
45 despite this, some individuals fail to control influenza viruses following the
46 subsequent re-infection. A vaccine study of young children found that a threshold
47 level (of >100 SFU/10⁶ PBMCs) was required for effective T cell-mediated clinical
48 protection (96), thus the memory T cell numbers obviously matter. Therefore, the
49 varying levels of T cell immunity that exist in the wider population may result from
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different exposure histories, and thus contribute to the spectrum of disease severity. Early studies on cytotoxic T cells (determined by their killing capacity) in humans suggested that functional influenza-specific CD8⁺ T cell declines rapidly, after 2-3 years (57). However, recent study from Rimmelzwaan's group provides evidence that influenza-specific CTLs persist for at least 13 years (60). It is not clear, however, whether this resulted from repeated antigenic stimulations due to multiple influenza infections. The main purpose of a T cell-inducing vaccine may therefore be to maintain CD8⁺ T cells at levels needed to achieve clinical protection, especially in the elderly, which may require more frequent boosters. The presence of co-morbidities, age-related differences of innate responses in the young and immunological decline in the elderly (reviewed by (97)), which could also impair T cell recall responses. Importantly, an influenza T-cell based vaccine needs to address the issue HLA coverage in a diverse population and across different ethnicities.

Importantly, a T cell-based vaccine needs to address the issue HLA coverage in a diverse population and be appropriate for ethnic minorities with rare MHC alleles. This could be achieved by either utilization of peptide epitopes across HLA-super families (73), or the inclusion of full-length influenza-derived proteins in a form that enables endogenous antigen processing. Recently, a vector-based approach with a modified Vaccinia Virus Ankara (MVA) encoding the NP and M1 proteins (MVA-NP+M1) was used as a T cell-based influenza vaccine approach (65). MVA-NP+M1 has proven safe and well-tolerated, but at high dose induced adverse effects, including vomiting, malaise and rigors (65). A clinical trial of MVA-NP+M1 using an influenza challenge model (98), with 11 vaccinated subjects and 11 controls (all HIA-negative to the challenge strain), showed that vaccination reduced laboratory-confirmed influenza cases by 60%. The vaccine boosted T cell responses and increased expression of the cytolytic molecules granzyme A and perforin. However, to fully determine the efficacy of this vaccine, a larger scale vaccine trial is needed, especially as around half of the unvaccinated individuals did not develop the influenza disease after challenge. Surprisingly, the frequency of HLA-A*0201-restricted M1₅₈₋₆₆-specific CD8⁺ T cells did not increase in vaccinated HLA-A*0201⁺ participants, however these cells were more activated (as assessed by CD57 expression) and with high cytolytic potential (68). Importantly, when administered in combination with a TIV vaccine, MVA-NP+M1 did not prevent the production of antibodies (99).

Another approach is to use multiple epitope-based antigens, as trialled with multimeric-001 aiming to induce broadly-protective vaccines that trigger both humoral and cellular immunity by priming with five T-cell and four B-cell antigenic peptides from HA, NP and M1 (100). In a clinical trial involving 60 individuals, this vaccine (plus an adjuvant) was well tolerated at doses up to 500µg and increased Abs titres and ADCC activity. T cell responses to multimeric-001 were not as pronounced. This is obviously a promising result for those in greater need of an effective vaccine, yet the efficacy of Multimeric-001 in protecting against influenza viruses remains to be determined. Similarly, Flu-v, a synthetic peptide vaccine candidate composed M1, NP, PB1 and M2 internal conserved fragments, was safe, immunogenic and induced some CTL cross-reactivity against distinct influenza subtypes (101). The global population coverage across distinct HLAs and ethnicities is still unclear for those proposed multi-epitope vaccines.

Targeted IAV vaccinations for at-risk populations

Certain groups, including the young, elderly, immunocompromised, pregnant and Indigenous populations, are particularly susceptible to IAV infection. It is important to understand the mechanisms underlying this heightened susceptibility in order to determine whether IAV vaccines need to be targeted towards these specific populations.

The elderly

Studies in murine models have shown that aging has a profound impact on CD8⁺ T cell responses and thus the ability to control and clear viral infections (102). Naïve epitope-specific CD8⁺ T cell populations decline with age and have decreased ability to expand following infection with IAV (103). Furthermore, TCR repertoire diversity decreases with age (56, 93), which can negatively impact CD8⁺ T cell responses to IAV (104). These studies suggest that early priming of CD8⁺ T cells prior to aging (preferably in late childhood or early adulthood) is key for the establishment of long-lasting and protective immunity. Our analysis of CD8⁺ T cell immunity to the pH1N1 virus, showed that the new virus shared immunogenic peptides with the catastrophic 1918 H1N1 strain as well as viruses circulating prior to 1945 (105). Cross-reactive

CD8⁺ T cell (and antibody) immunity between the pH1N1 strain and the catastrophic Spanish H1N1 strain may have resulted in lower susceptibility to pH1N1 in individuals >65 years of age, who are usually more susceptible to seasonal IAVs (106, 107). In contrast, the majority of severe cases of pH1N1 infection occurred in young adults (the mean patient age was 24 years of age) (108, 109).

Although 18-70 year-old individuals show no difference in the frequency of influenza-specific CD8⁺ T cells, the lytic capacity of these cells in 68-70 year-old versus 18-20 year-old donors appears to be lower (110). Furthermore, the T cell proliferative response can be impaired in the elderly. It is still unknown whether this reduction in CD8⁺ T cell cytotoxicity and proliferation with age is mediated by intrinsic or extrinsic factors. Recent studies by Gil *et al.* (93) showed that the A2⁺M1₅₈-specific TCR repertoire was skewed with age, and analysis of prominent TRAV27, TRAV12 and TRBV19 genes revealed that older individuals display a more restricted TCR repertoire that appears to be more oligoclonal.

Overall, elderly individuals are more susceptible to IAV and the current vaccine displays ~30% efficacy in individuals >65 years of age. Thus, it is of great importance to further characterise and understand CD8⁺ T cell responses in this population to determine factors that could be targeted to induce optimal universal T cell immunity through vaccination.

Indigenous populations

Indigenous populations worldwide are particularly susceptible to IAV. For example, the 1918 “Spanish” influenza is estimated to have killed 10-20% of the Indigenous population in comparison to <1% of non-indigenous populations in Australia (111). Furthermore, Indigenous Australians were 12-times more likely to be hospitalised with 2009 pH1N1 infection (112), with 16% of those hospitalised and 9.7% of those admitted to ICU following IAV infection of indigenous heritage (113). Similar trends have been observed in New Zealand, Canada and the United States (114-116). Most strikingly, up to 100% of adult Indigenous Alaskans died during the 1918-1919 H1N1 pandemic (11). Indigenous populations display unique HLA profiles (53), and thus, it has been suggested that the resulting CD8⁺ T cell responses may be ineffective in driving recovery from severe IAV disease. Notably, high proportions (up to 70%) of Indigenous Australians and Alaskans are HLA-A*24:02-positive, an allele associated

with influenza-induced mortality (117) Therefore, an effective vaccine for these populations may contain a unique set of antigens, restricted by prevalent HLA alleles, to provide optimal CD8⁺ T cell-mediated protection against IAV.

Conclusions

Although there is great interest in developing a CD8⁺ T cell-mediated IAV vaccine, there remains much to be learned about human influenza-specific CD8⁺ T cell responses. Recent studies suggest an important role for human CD8⁺ T cells in driving recovery from IAVs, especially the newly-emerged viruses with pandemic potential. However, we need a more in depth understanding of the magnitude, quality and clonal TCR characteristics of influenza-specific CD8⁺ T cells in order to design vaccines that appropriately harness these cells to provide universal protection against influenza viruses. Critically, further studies are needed to identify and characterise novel immunogenic CD8⁺ T cell epitopes across a range of diverse HLA alleles, and to also understand their longevity and functionality in order to rationally design prime-and-boost strategies that optimally balance CD8⁺ T cell response magnitude and inflammation.

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Figure Legends

Figure 1. CD8⁺ T cells can provide protection against novel IAV strains. (A) IAV infection of a naïve individual will induce humoral and CD8⁺ T cell (cellular) immunity. Neutralizing antibodies predominately target the surface glycoproteins whilst CD8⁺ T cells will typically recognise the internal proteins. (B) Re-infection with the same IAV strain will re-activate immune memory. Antibodies will neutralise the virus, whilst CD8⁺ T cells will display cytotoxicity. (C) Upon infection with a

distinct IAV strain, antibodies will not bind, however CD8⁺ T cells that recognise the conserved internal antigens will still mediate immunity and thus provide protection against distinct IAV strains.

Figure 2. Balance between the magnitude of the CD8⁺ T cell immune response and immunopathology is key for optimal immune responses.

(A) Infection with IAV to which (i) there is a large immune memory pool results in recruitment of a large CD8⁺ T cell response and a moderate amount of immunopathology. (ii) Infection with an IAV to which there is a small CD8⁺ T cell memory pool elicits a moderate CD8⁺ T cell response with low levels of inflammation. (iii) Infection with a novel IAV to which there is no pre-existing memory will result in a high viral titre, a delayed CD8⁺ T cell response and a high and prolonged level of inflammation.

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Figure 1&2

Figure 1 Grant EJ *et al*

Figure 2 Grant EJ *et al*

Table 1

Table 1. Conserved CD8⁺ T cell epitopes across distinct influenza strains and subtypes.

Peptide	HLA	Sequence	Virus
M1 ₅₈₋₆₆	HLA-A*0201	GILGFVFTL	All human influenza virus subtypes
NP ₂₆₅₋₂₇₃	HLA-A*0301	ILRGSVAHK	All human influenza virus subtypes
NP ₃₈₃₋₃₉₁	HLA-B*2705	SRYWAITR	All human influenza virus subtypes when first emerged [#]
NP ₁₉₉₋₂₀₇	HLA-B*5701	RGINDRNFV	All human influenza virus subtypes
NP ₂₁₉₋₂₂₈	HLA-B*1801	YERMCNIL	All human influenza virus subtypes
NP ₂₂₅₋₂₃₃	HLA-B*0801	ILKGKFQTA	All human influenza virus subtypes

[#] Mutations can accumulate over time in H3N2 viruses

Highlights

- Pre-existing influenza-specific CD8⁺ T cells drive rapid recovery from both mild and severe influenza disease
- Established CD8⁺ T cell memory pools cross-react with distinct influenza strains, including avian viruses
- Universal CD8⁺ T cell vaccines may protect individuals highly susceptible to severe influenza infection
- CD8⁺ T cell vaccines need to be assessed for longevity of T cell memory, population coverage across different HLAs and ethnicities, vaccine-mediated immune escape and immunopathology