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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 vial 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 Unites 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,

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

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

Adaptive immune control of IAV

Current IAV vaccines illicit humoral immunity directed towards the surface HA and NA glycoproteins and are highly effective in the control of IAV (29). However, due to antigenic drift (6), these surface glycoproteins rapidly mutate, and thus humoral

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 ^{51}Cr-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

incidental pH1N1 infection (named as 'an experiment of nature') showed that the frequency of pH1N1-specific pre-existing $CD8^+$ T cells directed at conserved epitopes inversely correlated with the clinical symptoms associated with IAV infection (31). Furthermore, we have demonstrated that $CD8^+$ T cell numbers correlate with recovery from severe A/H7N9 disease (32). In this study, individuals who had early H7N9-specific and IFN-γ-producing $CD8^+$ T cell populations during influenza infection recovered rapidly from severe infection, while patients with delayed or absent $CD8^+$ T cell response had increased morbidity and mortality following infection with the novel avian H7N9 virus. As the latter study was performed during an outbreak of a novel avian H7N9 influenza virus, the clinical symptoms could not be compared to the baseline level of pre-existing $CD8^+$ T cells, as in studies by McMichael *et al* and Sridhar *et al*.

Using *in vitro* approaches, others have highlighted the cross-reactive potential of $CD8^+$ T cells in the recognition of heterologous IAV strains. Boon *et al.* (47) showed that seasonally-activated NP_{418}-specific $CD8^+$ T cells could cross-react with the serologically distinct pH1N1 virus, while Gras *et al.* (48) demonstrated $CD8^+$ T cell cross-reactivity towards related (but not distinct) NP_{418} variants. Furthermore, $CD8^+$ T cells activated in response to infection with seasonal IAV strains can cross-react with pH1N1 (49, 50) and highly pathogenic avian H5N1- (51, 52) and H7N9- (53) derived variants. Thus, a $CD8^+$ T cell based influenza vaccine could provide considerable breadth of protection against distinct influenza strains.

It is important to achieve a fine balance between protective $CD8^+$ T cell immunity and immunopathology in order to provide recovery from influenza disease without severe damage to lung tissue (Figure 2). Our recent immune dissection of early hypercytokemia/ immunopathology (54) as well as recovery from H7N9 (32) in patients hospitalised at the Shanghai Public Health Clinical Centre shows that earlier protective $CD8^+$ T cell immunity (most likely recalled from cross-reactive memory pools) was associated with less cytokine/chemokine-driven inflammatory disease. In contrast, patients with minimal IAV-specific cellular immunity had increased inflammation and viral titers (32, 54). Thus, there appears to be a need for early viral control of influenza by $CD8^+$ T cells (and other cellular responses) to prevent exuberant inflammatory responses. However, potentially, $CD8^+$ T cell responses

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 ^{51}Cr 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 ($PB1_{591}$, $M1_{58}$, NP_{265}, NP_{380} and NP_{124}) $CD8^+$ 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 ($NS4B_{214}$ 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 ($M1_{36-67}$), NP ($NP_{255-275}$), M2 ($M2_{32-55}$) and IBV NP ($NP_{306-326}$) contain >5 potential HLA-A*02:01-restricted epitopes, as predicted algorithmically. Flu-v was safe and well-tolerated in phase 1 clinical trails 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 ($M1_{36-67}$ (3 epitopes (73, 74)), $NP_{255-275}$ (1 epitope (75, 76)) $M2_{32-55}$ (1 epitope (77)) and $NP_{306-326}$ (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 $NP_{172-181}$ (53), HLA-A*24:02-restricted NP_{39-47} (53) and HLA-B*44:03-restricted $NP_{338-346}$ (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 $NP_{265-273}$, HLA-B*08:01 $NP_{225-233}$, B*18:01 $NP_{219-226}$, B*27:05 $NP_{383-391}$ and B*57:01 $NP_{199-207}$) (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

including high functional avidity (79-81), polyfunctionality (80) and diverse (79, 82-85) and cross-reactive αβ T cell receptor (TCRαβ) repertoires (86-88) are considered superior for viral control (reviewed in (89)).

Little is known about the importance of the TCRαβ repertoire expressed by IAV-specific $CD8^+$ T cells for protection against infection. Until recently, characterisation of the $CD8^+$ TCRαβ repertoire was limited to identifying TCRα or TCRβ gene usage by PCR (90) or Vβ staining (91). In the early 1990's, it was established that the HLA-A*02:01-restricted $M1_{58}$-specific $CD8^+$ TCRαβ repertoire is limited and displays a strong TRBV19 (Vβ17 bias) (90-92). Advances in techniques such as deep sequencing have allowed for clonal analysis epitope-specific $CD8^+$ T cell TCRα or TCRβ repertoires. This has been utilized to confirm a strong TRBV19 bias for $M1_{58}$-specific T cells and identified new clonotypes (93), suggesting that the $A2^+M1_{58}$-specific TCR repertoire is not as restricted as previously thought. Using a novel single-cell multiplex RT-PCR (94, 95), we are now able to identify, for the first time, influenza-specific TCRαβ paired clonotypes from single cells directly *ex vivo* (Valkenburg SA *et al.*, submitted). Thus, we can now characterize the TCRαβ repertoire for other influenza-specificities to understand the importance of TCRαβ diversity and cross-reactivity in the recognition of distinct IAV strains (Grant EJ *et al.*, unpublished). This will allow us to fully realize the protective potential of $CD8^+$ T cell responses in recovery from both mild and severe IAV disease.

Taken together, there are a few challenges in the development of T cell-based influenza vaccines, including persistence of T cell memory after influenza immunization, population coverage across different HLAs and ethnicities, vaccine-mediated immune escape and immunopathology. Firstly, the longevity of functional influenza-specific memory $CD8^+$ T cells in humans is still unclear. Most adults are exposed to influenza virus infection (at lease once) by the age of 15, elicit T cell immunity and subsequently generate influenza-specific T cell memory. However, despite this, some individuals fail to control influenza viruses following the subsequent re-infection. A vaccine study of young children found that a threshold level (of >100 SFU/10^6 PBMCs) was required for effective T cell-mediated clinical protection (96), thus the memory T cell numbers obviously matter. Therefore, the varying levels of T cell immunity that exist in the wider population may result from

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 $M1_{58-66}$-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 $A2^+M1_{58}$-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

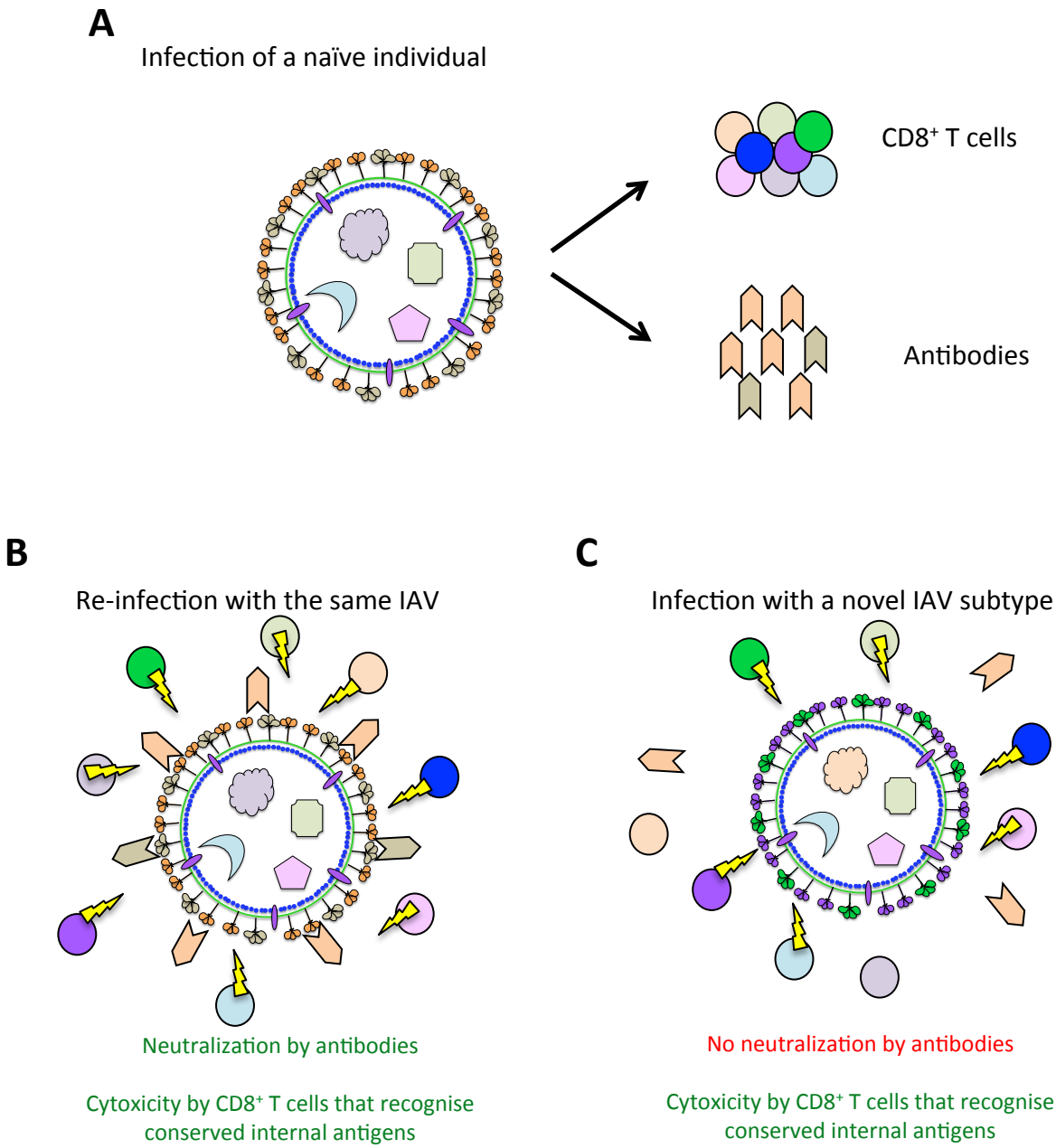

Figure 1 Grant EJ *et al*

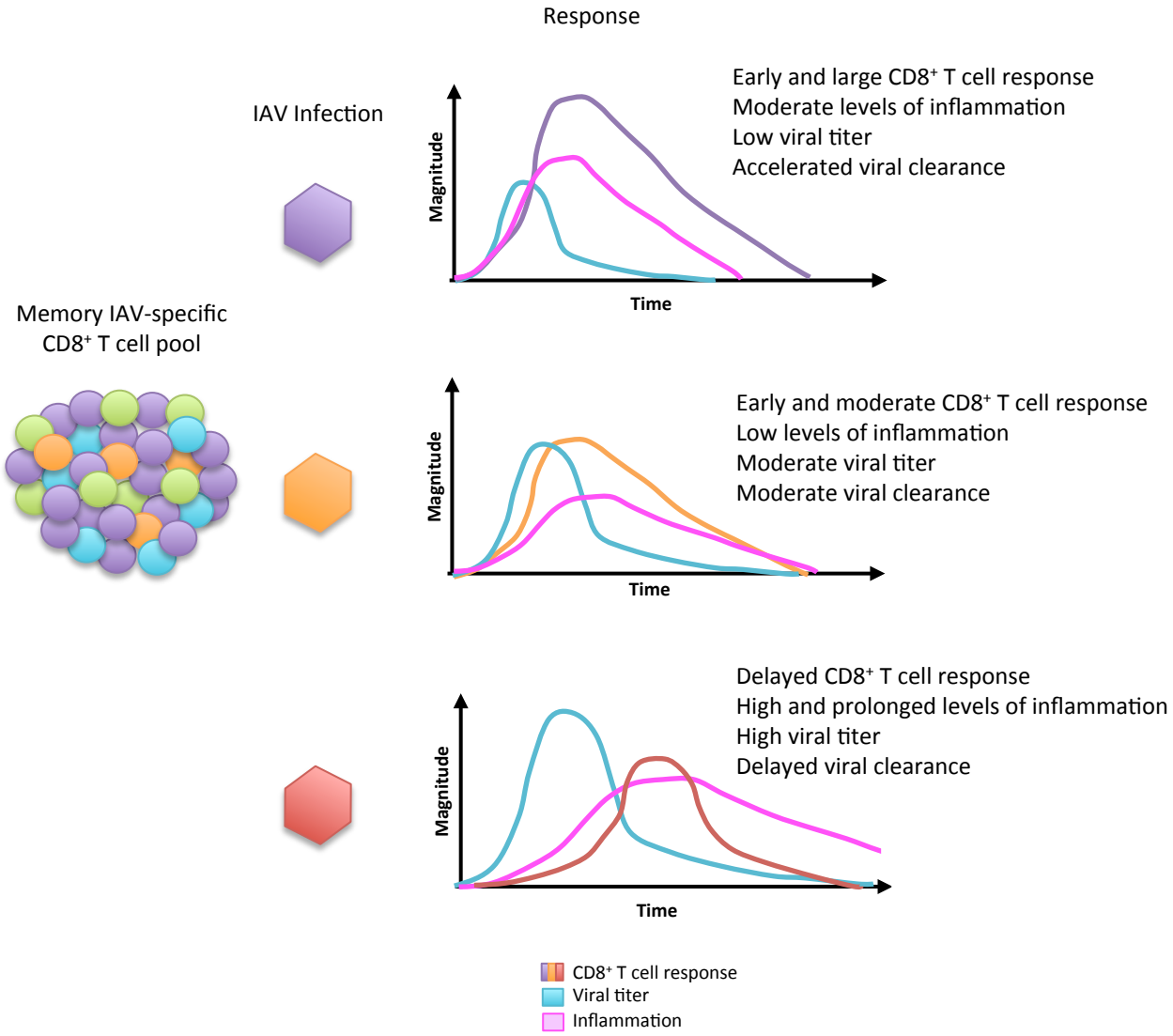

Figure 2 Grant EJ *et al*

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	RGINDRNFW	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