Published in final edited form as:
Hum Vaccin. 2010 February 19; 6(2): .

Time for T? Thoughts about the 2009 novel H1N1 influenza outbreak and the role of T cell epitopes in the next generation of influenza vaccines

Anne S. De Groot, M.D.[1,2,3], **Elizabeth McClaine**[1], **Lenny Moise**[1,2], and **William Martin**[1]

[1]EpiVax, Inc. [2]Institute for Immunology and informatics, University of Rhode Island [3]Pediatric Infectious Disease, Brown University Medical School, Providence Rhode Island

Keywords

H1N1; Influenza; T cell; B cell; cognate; help; epitope; immunoinformatics

A new strain of H1N1 influenza struck Central and North America in early March, 2009 and quickly spread across the globe. Early clinical reports suggested that the novel H1N1 influenza would be more virulent among younger individuals, since almost half of the first wave of H1N1 hospitalizations involved persons under the age of 18 years and greater than 35% of cases were identified among individuals age 19–49 [i]. Adding to the sense of urgency, public health officials were alarmed by official reports that antibodies resulting from vaccination with the inactivated trivalent influenza vaccines against seasonal flu (TIV) did not cross react with the new isolate [ii,iii]. And yet, it was also observed that older people infected by the novel H1N1 were not experiencing the severity of symptoms typically observed with infection by typical seasonal influenza strains. Oddly, despite the lack of B cell epitope conservation in novel H1N1, older individuals were relatively protected (based on general population incidence rates as well as hospitalization rates) [1]. This suggested that there might be cross-protective immunity due to previously circulating influenza virus and the novel H1N1. By all accounts, antibody levels were low or non-existent. Was it possible that – in the absence of cross-protective antibodies - T cells were contributing enough of a protective immune response to protect some individuals against novel H1N1?

T cell skeptics

Of course, the idea that T cells might protect against influenza well enough to reduce the burden of disease at a global level is heresy to those who consider antibodies to be the only fully protective arm of the immune system [iv]. And so it was not surprising that the concept of cross-protective immunity by T cells – and their role in the current pandemic - has been hotly debated in vaccine circles. At one vaccine conference, held in Singapore, officials from several of the largest influenza vaccine manufacturing companies openly derided individuals who raised the possibility that T cells might be responsible for the unusual epidemiology of novel H1N1, and went further, suggesting that T cells could actually

Corresponding Author: Anne S. De Groot M.D. AnnieD@EpiVax.com 146 Clifford Street Providence, Rhode Island, 02903.

Conflict of interest: Two of the contributing authors, Anne S. De Groot, and Bill Martin, are majority shareholder at EpiVax, Inc., a privately owned vaccine design company located in Providence, RI. Dr. De Groot is also a faculty member at Brown University and University of Rhode Island.

contribute to pathology, citing as evidence unpublished data from Canada that seasonal flu vaccine "might make H1N1 worse". Exacerbation following vaccination would have course been associated with a nightmare scenario, and millions upon millions of individuals who had been exposed to both seasonal flu or seasonal flu vaccine and novel H1N1 would have been hospitalized due to the reaction, an event that did not, in fact, come about. Of course, one might also consider the source: if seasonal flu vaccine were considered "dangerous" the market for the new H1N1 vaccine could potentially increase, whereas if TIV were protective, individuals who had been previously vaccinated might not opt for the new vaccine, resulting in a smaller market for the H1N1 vaccine that companies (and governments) had spent millions to produce.

A contrarian view

Those that support the concept of cross-protective immune response due to T cell memory point out that, even in the absence of antibody responses, cellular immune response has been shown to provide effective protective immunity in animal models [Epstein *et al.* original paper published in 1998 (v); follow up study published this year: (vi)]. Both cross-reactive CTL and helper T cells have been identified by a number of investigators; cross-reactive T cell responses have already been demonstrated between circulating strains of influenza and epidemic strains (such as H5N1) in the absence of cross-reactive antibodies [vii,viii,ix]. Continuing along this line of thinking, T cell responses to cross-reactive epitopes might have contributed to diminished incidence of influenza-like illnesses among older adults as was reported during the current pandemic.

Association between T cell response and protection from influenza

The role of TIV or prior H1N1 exposure in protection against novel H1N1 is supported by murine studies that show that cross-reactive T cell epitopes are involved in attenuation of disease and protection against influenza. For example: (1) The rate of viral clearance depends on the presence of CD4+ T cells [x]; (2) Cytotoxic T cells are required for viral clearance [xi,xii]; (3) T cell help is required for high specific IgG antibody titers [xiii]; (4) Vaccine efficacy is improved when cross-reactive helper T cell populations are present from prior infection and/or vaccination [xiv]; and, (5) Memory helper T (Th) cells specific to a previous influenza strain contribute to cross-strain antibody responses [xv]. Memory T cells to conserved epitopes have also been shown to confer protection to heterologous infection [xvi,xvii]. CTL response has also been associated with viral clearance and reduced symptom severity [xviii,xix]. And finally, some non-responsiveness to conventional influenza vaccines may be linked to HLA, suggesting that T cell responses are a critical component of protective immunity in humans [xx].

Role of humoral immunity

It cannot be disputed that humoral immunity is the primary means of resistance to and recovery from influenza virus infection [xxi,xxii]. Humoral antibodies specific for the HA and NA antigens are dependent on cognate T cell help. Secretory IgA and IgM provide protection against the establishment of initial infection, however, IgG neutralizes newly replicating virus once infection has been established. In addition, IgG prevents viral pathology in the lung (murine models). Several neutralizing epitopes have been identified in HA, including HA 92-105, 127-133 and 183-195 of influenza virus H3N2. Antigenic variation does occur within these B cell epitopes. However despite this variation, heterosubtypic protection has been observed in humans [xxiii,xxiv] and the mechanisms (T cells come to mind) have been under study.

T cells help improve antibody titers

Because antibody titers are Th cell dependent, the activation of Th cells is critically important to the magnitude and kinetics of the host antiviral immune response [xii]. In the absence of functional (memory) CD4 T cells, the rate of viral clearance upon secondary infection slows considerably, beyond the degree seen in the primary response [xxv,xxvi]. Thus antibodies may be unable to protect against variant challenge viruses whereas T cells responding to conserved epitopes may act to mitigate severe disease, as could have been the case during the most recent outbreak of pandemic flu. Similarly, the high mortality rate of young adults that occurred during the "Spanish flu" could have been due, in part, to their lack of cross-reactive memory T cells; this deficiency may have lead to delays in humoral immune responses and thus more severe disease (higher viral loads) and increased likelihood of death [an idea discussed with Dr. Greg Poland, see also ref. xxvii].

The evidence

If T cell response was critical to lowering active case rates, it makes sense that the T cell epitopes between H1N1 would be conserved. A number of groups have therefore performed in silico, in vitro and in vivo studies to determine whether T cell epitopes were conserved between novel H1N1 and TIV, using as a departure point the A/California/04/2009 (H1N1) sequence published in March 2009 [xxviii].

A team led by De Groot and Martin took the first steps needed to test the hypothesis and reported in August 2009 that highly conserved, potentially cross-reactive epitopes were present in both conventional TIV and novel H1N1 [xxix]. They found greater than 50% conservation of helper T and CTL epitopes between novel H1N1 and TIV HA for selected HLA molecules. Nine highly promiscuous helper T cell epitopes of the sixteen contained in the S-OIV H1N1 HA sequence (56%) were 100% conserved in the 2008–2009 influenza vaccine strain H1N1 HA; 81% were either identical or had one conservative amino acid substitution. Similarly, fifty percent of predicted CTL epitopes found in novel H1N1 HA were also found in TIV HA sequences.

In addition, Sette and others performed immunoinformatics analysis and in vitro validation, publishing their report in November, 2009 and showing a great deal of cross-reactivity and confirming the epitopes selected by Martin and De Groot [xxx]. Xing and Cardona have also identified cross-conserved epitopes, using as a departure point the list of epitopes in IEDB [xxxi].

"Immunotypic similarity"

During the evaluation of the novel H1N1 vaccine by CSL in Australia, "even in subjects with no measurable antibodies at baseline, a single dose of vaccine elicited a robust immune response" [xxxii]. The authors attributed this dramatic result to "immunotypic similarity" between the 2009 H1N1 virus and recent seasonal strains, both H1N1 and H3N2, alluding to but not mentioning the potential for the existence of cross-reactive T cell epitopes.

Antibody Evidence

A further study published in the same issue of NEJM by Hancock *et al.* analyzed stored-serum samples from trials of seasonal TIV predating the current pandemic [ii]. They showed the presence of cross-reactive antibodies to 2009 H1N1 in selected older adults and that vaccination with the seasonal vaccine correlated with a doubling of cross-reactive antibodies titers in those subjects. Where IgG antibodies are present, wouldn't T memory cells, cross reactive for conserved epitopes, be present too?

1977 Swine Flu Prequel

Similarly, studies of H1N1 vaccine performed during the previous swine influenza outbreak, in 1977, demonstrated that individuals born before 1957 (and who had been previously exposed to H1N1 viruses) required only a single dose of vaccine to achieve protective levels of antibody, while those born after 1957 (i.e., never previously exposed to H1N1) required two doses [xxxiii]. This lack of dependency on boosting for protective antibody titers suggests that memory helper T cells generated by infection pre-1957 may have been cross-reactive with the 1977 vaccine. The CSL study cited above [xxxii] is a modern-day replicate of this earlier study.

Seasonal flu vaccine-vaccinated individuals not hospitalized for H1N1

The presence of cross-reactive T cell responses might also explain why 2009 H1N1 ran an inverted course – sparing older people, who were less ill with H1N1 but also – if the hypothesis is correct - making them the main reservoirs of transmissible infection, rather than children as is usually the case.

As it turns out, this contrarian view is supported by an important case study from Mexico, which showed that adults who had prior TIV were less likely to fall ill with H1N1. While their T cell response (in the absence of a cross-reactive humoral immune response) may not have provided complete protection against novel H1N1 infection, the severity of the illness appears to have been reduced, leading to a lower hospitalization rate compared to unvaccinated adults in the same older age groups. TIV vaccination effectiveness against laboratory confirmed cases of influenza A/H1N1 was 73% (95% confidence interval 34% to 89%) [xxxiv].

T time

Thus four studies (the CDC study, the Hancock *et al.* study, the CSL study and the publication from Mexico) suggest that a cross-reactive helper T cell response (due to prior exposure), in addition to circulating memory B cells (in the case of the Hancock study and CSL studies), may have contributed to the development of protective antibody titers and protection against clinical disease (in Dominguez-Cherit et al.). In all cases, cross-reactive T cell epitopes may have contributed to protection, but unfortunately, no direct measure of T cell help was performed.

These studies clearly show, that due to vaccination or exposure, T cell responses to cross-reactive epitopes may have the capacity to attenuate the course of novel H1N1-induced disease, in the absence of cross-reactive antibody response. Should additional studies prove the hypothesis to be correct, the results should have broad implications for the public health response to H1N1 influenza and future influenza epidemics.

Conclusion

The authors hope that the evidence that cellular immunity and cross-reactive T cell epitopes played an important role in protection against widespread H1N1 in older adults will deserve greater emphasis in future epidemics, and will lead to support for the development of alternative approaches to “priming” human immune responses against emergent infections, with the aim of diminishing the number of individuals who develop clinical symptoms following infection. The evidence does not support monolithic emphasis on humoral immunity, particularly when the current pandemic is viewed through the “retrospectoscope”.

We are certainly not alone in calling for improving flu vaccines. According to Bob Graham and Jim Talent (writing for the Washington Post [xxxv), “For generations, the United States

has neglected to nurture the technologies and systems needed to respond to emergencies related to disease. Nowhere has this been more evident than in the response to H1N1… To make flu vaccine, we rely on a 60-year-old production method based on chicken eggs. It is safe but slow and has led to long lines at clinics and shortages of vaccine. It is not just that priority groups have been left unprotected. We learned last month that this method leads to multiple manufacturing issues, such as the recall of 800,000 children's vaccine doses, due to diminished potency."

In the context of future epidemics – for influenza and other pathogens, new approaches to vaccine design clearly deserve consideration. Improved protective measures might be developed based on immunization with T cell epitope peptides – or pseudoproteins based on T cell epitopes - even though this would not confer sterilizing immunity. As demonstrated by the rapidity of the immunoinformatics analyses performed for H1N1 [28-30], most of which were completed within weeks of the publication of the genome, this type of intervention is likely to be more rapid than full scale vaccine replacement and may not be as expensive as developing an entirely new flu vaccine. The concept is supported by current laboratory and clinical evidence, and given the opportunity for further development, might in fact save more lives – perhaps the most important barometer of success for politicians, policy makers and vaccine developers alike, as we prepare for the next pandemic flu.

Acknowledgments

The authors acknowledges that there is a potential conflict of interest related to their relationship with EpiVax and attests that the work contained in this research report is free of any bias that might be associated with the commercial goals of the company.

Abbreviations / Acronyms

MHC	Major Histocompatibility Complex
HA	Hemagglutinin
NA	Neuraminidase
TIV	Trivalent Inactivated Virus Vaccine
LAIV	Live attenuated influenza vaccine
CTL	cytotoxic T lymphocyte
Th cell	T helper cell

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