T-cell immunity against the A(H1N1) 2009 pandemic virus

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The sudden emergence of the novel reassortant A(H1N1) 2009 influenza virus led to rapid global spread, due to minimal pre-existing antibody levels in those born after 1950. Memory T cells specific for more conserved viral peptides elicit broad immunity and can promote more rapid recovery. However, mutations within T-cell immunogenic peptides occur, although less commonly than at antibody-binding sites. Comparison of human T-cell peptides between the pandemic H1N1 2009 and seasonal strains showed 50–70% conservation, depending on the particular virus protein and influenza strains. Experimental analysis demonstrated cross-recognition of some T-cell epitopes (for example, HLA-A2$^+$M1$_{58\text{-}66}$), although there was also evidence of immune escape by other immunodominant peptides (for example, NP$_{418\text{-}426}$ presented by the HLA-B7 family). Non-conserved T-cell regions of A(H1N1) 2009 highly resembled those derived from H1N1-1918 rather than recent seasonal viruses, reflecting protein conservation (in the parent swine virus) from influenza strains circulating early in the 20th century. As a consequence, individuals with HLA types presenting variable T-cell peptides had diminished pre-existing T-cell memory towards the A(H1N1) 2009 virus.

Conservation of T-cell peptides between H1N1 2009 and seasonal influenza A viruses

The A(H1N1) 2009 influenza virus is a newly emerged reassortant with HA, NA and NS components from the classical North American swine lineage, PA and PB2 from the avian North American lineage, NA and NP from the Eurasian swine lineage and PB1 from a seasonal H3N2 human strain[1]. Classical swine-lineage influenza A viruses have been maintained in pigs at least since 1918, while seasonal H1N1 viruses evolved in the human population over the past century. The minimal level of pre-existing antibody immunity to A(H1N1) 2009 in those under age 60 led to the fast, global dissemination of this novel 2009 virus throughout the human population. Since influenza-specific T cells are commonly directed at peptides derived from more conserved internal proteins of the virus (Figure 1), it was of interest to determine the extent to which pre-existing T-cell immunity might provide some measure of protection against the newly emerged A(H1N1) 2009 influenza.

Virus-specific CD8$^+$ T cells function to clear infected cells presenting viral peptides bound to class I major histocompatibility complex (MHC-I) glycoproteins on the surface of infected cells. Comparison of T-cell peptide sequences between H1N1 2009 (obtained from NCBI) and seasonal viruses circulating between 1988 and 2008 (retrieved from the Immune Epitope Database) showed more conservation between A(H1N1) 2009 and recent seasonal strains for T-cell peptides (51%; 111 out of 217) than for the antigenic regions seen by antibodies (31%)[1]. This reflects the fact that the immunogenic T-cell peptides are

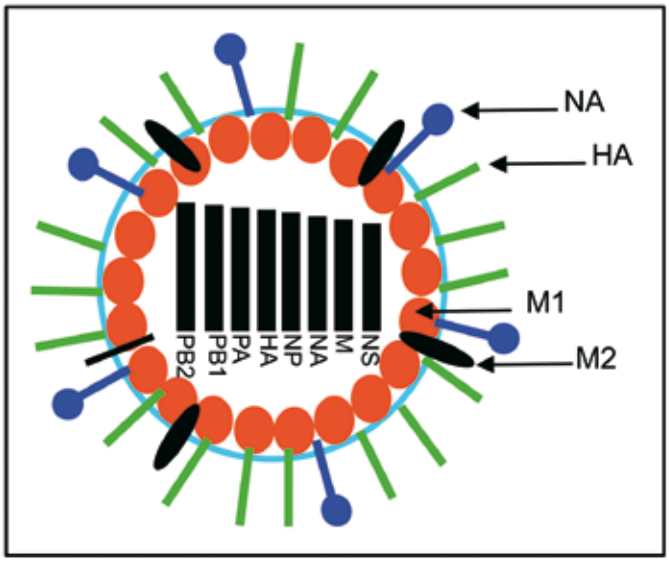

Figure 1. Diagram of an influenza virus. Influenza-specific antibodies recognise predominantly surface glycoproteins (HA and NA), while T cells are commonly directed at peptides derived from more conserved internal proteins of the virus (mainly NP, M1, PB1).

derived mainly from internal components of the virus while neutralising antibodies recognise the surface HA or NA proteins. Furthermore, the immunogenic determinants targeted by "killer" $CD8^+$ T cells had a greater level of sequence similarity (69%) than those recognised (41%) by the $CD4^+$ T "helpers"[1] that tend to see longer peptides complexed with MHC-II.

Our study compared the two most immunogenic influenza proteins, NP and M1 (Figure 1), in the first A(H1N1) 2009 virus sequenced within the Australasian region (A/Auckland/1/2009) to those of seven influenza viruses that emerged between 1918 and 2007. We found ~70% conservation across 73 different $CD8^+$ and $CD4^+$ T-cell epitopes (Table 1). Further sequencing of A(H1N1) 2009 isolates from recently infected patients over five months showed a total conservation of T-cell peptides across different H1N1 2009 isolates (Table 1). As at least some variations within influenza-specific T-cell peptides appear to be driven by immune selection pressure, lack of antigenic variants within T-cell peptides suggest minimal immune pressure on the newly emerged pandemic virus.

Cross-reactive immunity of human PBMCs to A(H1N1) 2009 and seasonal viruses.

Analysis using PBMCs from healthy adult donors who had not been infected with A(H1N1) 2009 established that some level of pre-existing T-cell immunity towards the pandemic virus had been elicited by recent exposure to seasonal influenza strains[1-5] (Table 2). When PBMCs were cultured with peptide pools that target $CD4^+$ or $CD8^+$ T cells, the pre-existing memory cells produced IFN-γ after exposure to conserved or non-conserved seasonal and pandemic A(H1N1) 2009-derived epitopes[1,4]. Evidence of memory to A(H1N1) 2009 was further confirmed for $M1_{58}$-specific $CD8^+$ T cells in HLA-$A2^+$ individuals and $CD4^+$ T cells in HLA-$DR*0401^+$ donors[3,4]. As $M1_{58-66}$ is one of the most conserved influenza-derived T-cell peptides (since 1918)[6], cross-reactivity with A(H1N1) 2009 is not surprising. Both HLA-A2 and HLA-DR*0401 alleles are widely distributed within the human population (25–50% and 12–40%, respectively); thus a high proportion of individuals previously infected with seasonal influenza viruses would have some level of pre-existing T-cell memory towards the A(H1N1) 2009 virus. Sharing of 52% for $CD4^+$ T-cell peptides was also obtained by a bioinformatics approach[7]. As the recall of T-cell memory can limit disease severity, epitope sharing with seasonal strains could have contributed to the generally mild outcome of influenza A(H1N1) 2009 infection.

Our analysis of the pandemic A(H1N1) 2009 virus[8] focused on the more variable and immunodominant $NP_{418-426}$ peptide presented by the widely distributed MHC-I B7 allelic family[9,10]. To compare recognition of the pandemic A(H1N1) 2009 and seasonal NP_{418} variants, PBMCs from individuals not infected with A(H1N1) 2009 were cultured with NP_{418} variants derived from

Table 1. Conservation of T-cell peptides between A(H1N1) 2009 and seasonal viruses.

	Nucleoprotein			Matrix 1		
T-cell epitopes	# T-cell epitopes	Average # conserved between H1N1 2009 and 6 seasonal strains	# conserved between 10 recently emerging H1N1 2009 strains*	# T-cell epitopes	Average # conserved between H1N1 2009 and 6 seasonal strains	# conserved between recently emerging H1N1 2009 strains*
CD8+	18	13 (72.2%)	18 (100%)	14	11 (78.6%)	14 (100%)
CD4+	21	15 (71.4%)	21 (100%)	20	12 (60%)	20 (100%)
Total	39	28 (71.8%)	39 (100%)	34	23 (67.6%)	34 (100%)

*H1N1 2009 strains received by the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne, Australia in Jul–Nov
Comparison between H1N1 2009 and seasonal viruses for ^ for M1 and ~ NP analysis included:
A/Auckland/1/2009 (H1N1 2009)
A/Brisbane/10/2007 (H3N2 seasonal 2009 vaccine)^~
A/Solomon Islands/3/2006 (H1N1 seasonal 2009 vaccine)^~
A/Memphis/51/1983 (H1N1)^~
A/Victoria/3/1975 (H3N2)~
A/Victoria/3/Hong Kong/14/1974^
A/Puerto Rico/8/1934 (H1N1)~
A/Brevig Mission/1/1918^ (H1N1)~

Table 2. Relevant publications assessing human T-cell immunity towards A(H1N1) 2009.

Reference	Cells used	HLA restriction	Method of detection	Pre-existing T-cell immunity	Novel responses towards H1N1-2009
Greenbaum *et al.*, 2009	PBMCs from healthy individuals not infected with H1N1-2009	NA	*ex vivo* IFN-γ ELISPOT and ICS for $CD4^+$ and $CD8^+$ T-cell responses	Comparable CD4 and CD8 responses to seasonal and H1N1-2009 strains	NA
Gras *et al.*, 2010	PBMCs from healthy individuals not infected with H1N1-2009 PBMCs from patients hospitalised with H1N1-2009	HLA-B*0702 HLA-B*3501 HLA-B*3503	IFN-γ production by ICS following 10d *in vitro* culture with NP_{418} variants (1918-2009)	Lack of cross-reactive responses between seasonal and H1N1-2009 strains	Novel $CD8^+$ T cells detected towards H1N1-2009; cross-reactive with H1N1 1918
Tu *et al.*, 2010	PBMCs from healthy individuals not infected with H1N1-2009 PBMCs from healthy individuals vaccinated with 2008–2009 inactivated trivalent vaccine	HLA-A*0201	Following 10d *in vitro* culture, IFN-γ^+CD8^+ cells used in cytotoxicity assay against virus-infected or peptide-pulsed targets	Bulk CTL cross-reactive responses between seasonal and H1N1-2009 strains; $M1_{58}$ cross-reactive between seasonal and H1N1-2009	NA
Gras, Kedzierski and Valkenburg *et al,* 2010	PBMCs from healthy individuals not infected with H1N1-2009	HLA-DR*0401	H^3 thymidine incorporation after 14d *in vitro* culture IFN-γ ELISPOT. Dual tetramer stain after 14d *in vitro* culture and *ex vivo*	Cross-reactive $CD4^+$ responses detected to non-conserved NA, HA, MP and NP peptides	$CD4^+$ T-cell cross-reactivity between H1N1-2009 and seasonal strains by dual pMHC II tetramer straining
Agrati *et al.*, 2010	PBMCs from healthy individuals not infected with H1N1-2009 PBMCs from patients hospitalised with H1N1-2009 infection	NA	Cytokine bead array, phenotypic mAb stain, IFN-γ production after 24hr mitogenic stimulation	NA	Severe disease associated with T-cell anergy, higher % of CM CD4, CD95 expression, lower IFN-α and MCP-1
Richards *et al.*, 2010	PBMCs from healthy individuals not infected with H1N1-2009	NA	*ex vivo* IFN-γ ELISPOT with peptide pools and virus	Cross-reactive responses between seasonal and H1N1-2009 peptides across HA, NA, NP, MP, and PB1	NA
Subbramanian *et al.*, 2010	PBMCs from healthy (young and older) individuals not infected with H1N1-2009	NA	IFN-γ ELISPOT after 7d *in vitro* culture to CD4 HA peptides	Cross-reactive responses between conserved (C terminal) seasonal and H1N1-2009 HA peptides, responses to HA variants (N terminal) lower magnitude	Novel responses to CD4 HA variants (N terminal) inferred from data
Duvvuri *et al.*, 2010	NA – bioinformatics approach Alleles of interest derived from healthy individuals not infected with H1N1-2009 from previous studies	DRB1*0101 (Subalelles: DRB1*0401,DRB1*0404 DRB1*0701,DRB1*1501 also binding: A*0101 A*0201,A*0301,A*2402)	HLA-DR and peptide binding affinities predicted by NETMHCIIPAN (an online server), and BMC Epitope Conservancy Tool	52% CD4 cross-reactivity estimated for HA CD4 responses	

viruses isolated between 1918 and 2009. Though there was a high degree of cross-reactivity for the NP_{418} seasonal variants, there was no pre-existing $CD8^+$ T-cell memory for 2009-NP_{418} (Table 2). However, strong $CD8^+$ T-cell responses to A(H1N1) 2009-NP_{418} were found in patients hospitalised with A(H1N1) 2009 infection who subsequently recovered. Furthermore, $CD8^+$ T cells specific for the 2009 variants were cross-reactive with T-cell peptides derived from the 1918 pandemic virus. This provided evidence that the 1918 pandemic virus, like A(H1N1) 2009, would have primed effector $CD8^+$ T cells. Strikingly, analysis of T-cell responses from patients of different HLA types who were hospitalised with very severe clinical symptoms showed minimal (SV and KK, unpublished) or impaired[11] T-cell responses towards a number of epitopes, suggesting that humans also show the immunosuppressive effect observed in severe influenza infections in mice[12].

Overall, though there was pre-existing $CD8^+$ T cell-mediated immunity directed towards the conserved (for example, $M1_{58}$) and some variable peptides[1-4], differences in peptide sequence from seasonal strains meant that the newly emerged A(H1N1) 2009 virus failed to recall memory T-cell responses directed at prominent human epitopes like $B7^+NP_{418}$[8]. Thus, a considerable proportion of the human population had levels of T-cell memory that were lower for A(H1N1) 2009 than for the repeated, seasonal influenza A virus challenges over the preceding years. For the future, we need to develop a better understanding of cross-reactivity between different pandemic and seasonal strains, and to unravel the mechanisms underlying the minimal T-cell responsiveness observed in some severely affected, hospitalised patients.

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