Vaccine-induced T cell protection from influenza viruses

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KEY MESSAGES

1. T cell–activating vaccines provide robust protection against diverse influenza viruses, which is not possible by current inactivated influenza vaccines.
2. Wyeth/IL-15/5flu is a universal anti-influenza vaccine.
3. CD4 and CD8 T cells act together for protection from influenza viruses.

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Introduction

Influenza causes yearly seasonal epidemics, worldwide pandemics, and occasional outbreaks from cross species transmission, despite availability of vaccines and antiviral drugs. A diverse array of influenza viruses circulates between different species, reassort and drift antigenically over time, yet certain immune targets remain relatively constant across diverse strains of influenza viruses.[1] Vaccines that target such conserved regions enable universal immunity against influenza. It is essential to improve influenza vaccines to provide broadly reactive immune protection from pandemic and outbreak influenza viruses to pre-arm the immune system. Virus-specific $CD4^+$ and $CD8^+$ T cells responses kill virus-infected cells, coordinate local innate immune responses, and react against different strains and subtypes of influenza secondary to conservation of immune targets. A vaccine designed to stimulate T cell immunity towards heterosubtypic strains of influenza is generated.[2] This multivalent vaccinia virus-based H5N1 influenza vaccine (Wyeth/IL-15/5flu) expresses five H5N1-derived influenza proteins (HA, NA, M1, M2, and NP), in combination with the immune stimulatory cytokine interleukin-15 (IL-15).[2] We assessed the breadth of immunity generated by the vaccine in a mouse model with challenge of seasonal, pandemic, and highly lethal avian influenza viruses.[3] Vaccinated mediated protection was dependent on T cell recruitment, especially engagement of CD4 T cells, as a cornerstone of the vaccine immune response.

Methods

Vaccination and infection of mice

Female BALB/c ($H\text{-}2^d$) mice (6-8 weeks of age) were primed twice 3 weeks apart via the subcutaneous route with 10^7 plaque-forming units in 100 μL PBS of vaccinia Wyeth/IL-15/5flu, Wyeth/mutIL-15/5flu, Wyeth, or PBS alone, and then challenged with influenza virus 3 weeks later. For influenza challenge, mice were anaesthetised and infected intranasally with 25 μL of H7N9 (A/Shanghai/2/2013), HPAI H7N7 (A/Netherlands/219/2003), mouse-adapted H3N2 (A/Hong Kong/1/68-MA20C), or pandemic H1N1 (A/California/04/2009). All experiments involving H7N9 and HPAI H7N7 viruses were conducted in a BSL3 laboratory. All animal studies were approved by the institutional animal ethics committee. Mice were monitored for survival, weight loss, and symptom severity (n=5 per group). Infected lungs were harvested at day 7 (n=3) for viral loads by standard TCID50 assay on MDCK cells.

Intracellular cytokine staining for influenza-specific T cells

Lymphocytes from vaccinated mice or human PBMC (resting or restimulated with vaccine) were stimulated in vitro with low pathogenic H5N2 virus (to represent vaccine-specific T cell responses) for 6 hours and then in the presence of BFA for 12 hours. CD4 and CD8 T cell IFNγ accumulation was measured by intracellular cytokine staining and flow cytometry.

Selective depletion of T cell subsets

Vaccinated mice were depleted of $CD4^+$ T cells, $CD8^+$ T cells, or both prior to influenza infection. Mice were treated with GK1.5 (anti-CD4) and 2.43 (anti-CD8) or isotype control (IgG2b) monoclonal antibodies (BioXCell) at 100 μg intraperitoneally at days -4, -2, 0, and 3.[4] Depletion was confirmed at day -1 at >98%, compared with isotype-treated mice and non-depleted mice. Untouched magnetically purified CD4 or CD8 T cells were isolated from

the spleens of naïve mice by MagCellect kit (R&D). Naïve T cells (1×10^6 cells per mouse) were given intravenously to T cell–depleted recipient BALB/c as indicated (Fig 2). Mice were then challenged with H3N2. Mice were monitored for survival, weight loss, and symptom severity (n=5 per group). Infected lungs were harvested at day 7 (n=3) for viral loads by standard TCID50 assay on MDCK cells.

PBMC vaccine restimulation

Human PBMCs were isolated from Red Cross buffy packs by density gradient centrifugation and stored in liquid nitrogen. PBMCs were restimulated with ultraviolet-inactivated Wyeth/IL-15/5flu virus for 10 days in vitro in the presence of IL-2 (10U/mL). At day 10, matching un-restimulated PBMCs from the same donors were used for baseline comparisons. CD4 and CD8 T cell IFNγ production was assessed by intracellular cytokine staining following stimulation with Wyeth/IL-15/5flu, Wyeth, H5N2 viruses (MOI 2) or PBS. Samples were acquired by flow cytometry.

Results

Wyeth/IL-15/5flu, a live replicating vaccinia virus encoding five H5N1-derived influenza proteins and IL-15 cytokine as a molecular adjuvant, is a robust universal anti-influenza vaccine. Lethal influenza challenge of vaccinated BALB/c (Fig 1) and B6 mice (data not shown) had 100% survival, whereas unvaccinated mice all succumbed to infection. Increased survival of vaccinated mice was accompanied by reduced peak viral loads at day 3 (data not shown), early viral clearance by day 7 after infection, improved weight recovery, and reduced symptom severity.

Protection of vaccinated mice correlated with significantly elevated cross-reactive H5-specific T cells at the site of infection. Influenza-specific CD4 and CD8 T cells were substantially increased in the lungs, spleen, and lymph nodes of vaccinated mice. Passive transfer of T cell subsets from vaccinated mice to naïve recipient mice showed reduced viral loads and increased survival (data not shown), whereas serum transfer was unable to confer any level of protection. Therefore, to determine the contribution of T cell subsets to vaccine-mediated protection, selective depletion of T cell subsets was performed at the time of vaccination or challenge in combination with naïve T cell transfer (Fig 2).

Despite CD8 T cells having a role in protection from influenza infection, our vaccine continued to

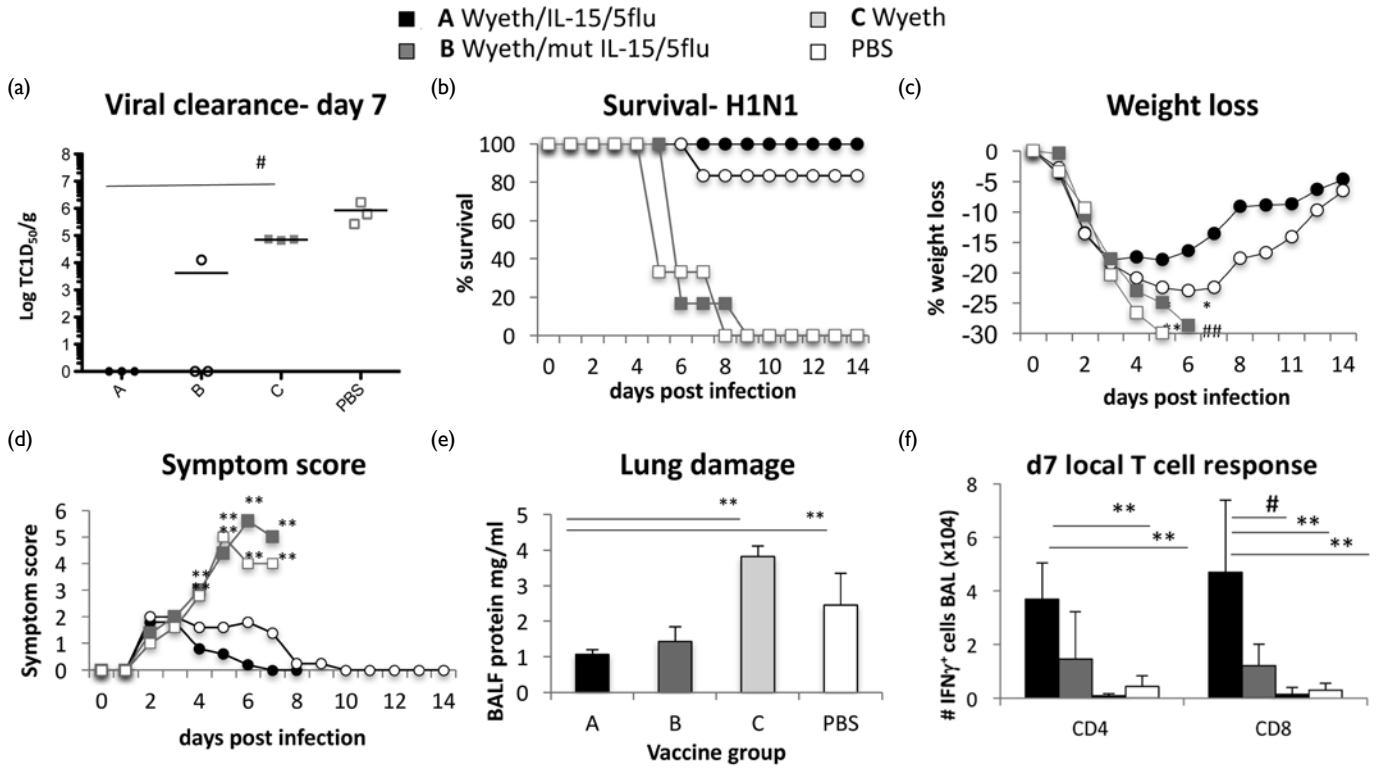

FIG 1. Wyeth/IL-15/5flu reduces viral load, morbidity, and mortality against heterologous challenge. BALB/c mice were vaccinated with Wyeth/IL-15/5flu, Wyeth/mut-IL-15/5flu, Wyeth (control vaccine vector), or PBS twice 3 weeks apart and then challenged with H7N9, H7N7, H3N2, or H1N1. (a) Lung viral titres by standard MDCK TCID50 assay (H3N2) at day 7. Mice were monitored daily for (b) survival, (c) weight loss, and (d) symptoms of H1N1 influenza infection. (e) Lung damage was assessed by total protein concentration in the BAL in a standard BCA assay. (f) Cells isolated by BAL wash of the lungs from day 7 after H3N2 infection were stimulated H5N2 virus (MOI2) for 6 hours, and 12 hours with BFA. IFNγ production by CD4 and CD8 T cells was assessed by ICS and samples acquired by flow cytometry. # P<0.05, * P<0.01, ## P<0.005, ** P<0.001 versus A vaccine group.

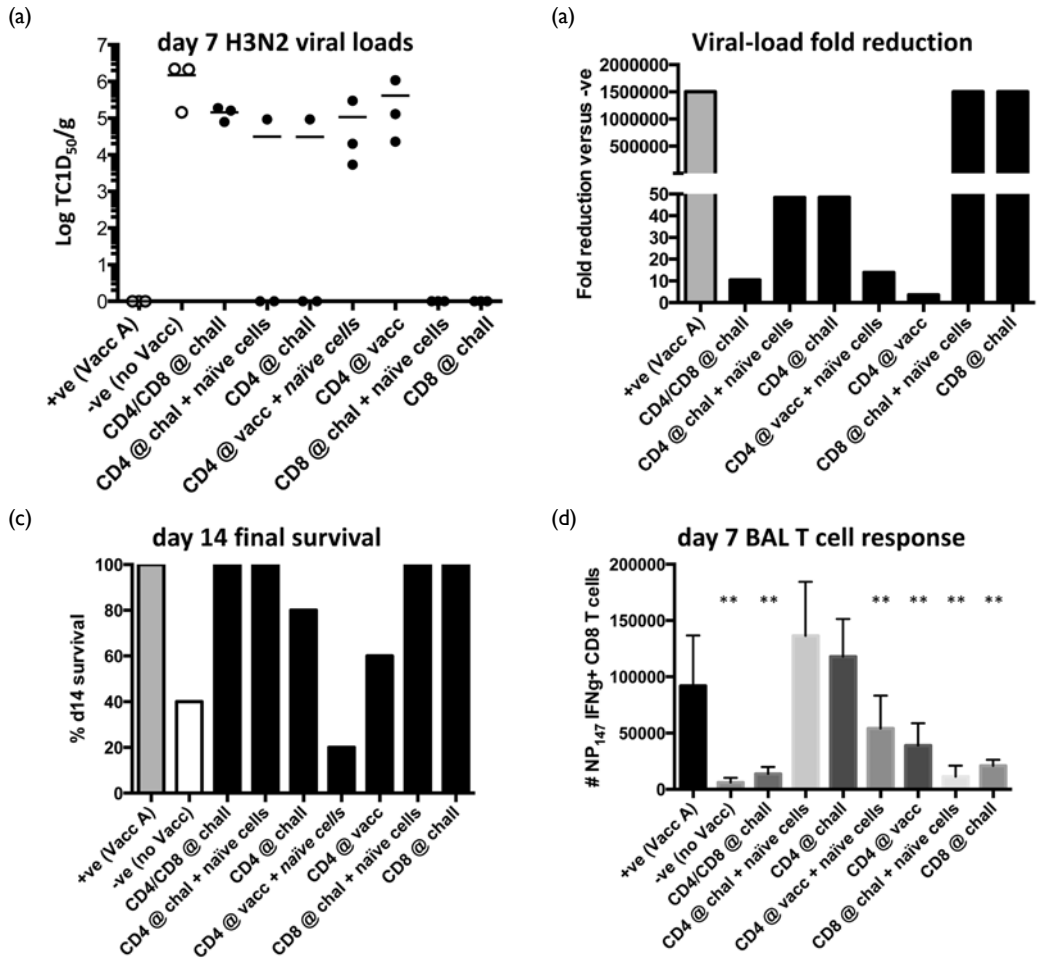

FIG 2. T cell depletion impairs CD4 help for memory and CD4 T cells are needed at challenge for protection. Mice were depleted for CD4+CD8 at challenge, CD4 at challenge or vaccination, or CD8 at challenge. Prior to infection, selected mice were given naïve splenocytes intravenously (10 days after the final depletion). Mice were infected with H3N2. (a) Lung viral load was determined at day 7 (n=3). (b) Fold reduction in viral load was compared with negative controls, and (c) monitored for survival at day 14 (n=5). (d) BAL NP147-specific CD8 T cells were enumerated from day 7 after H3N2 infection (n=3).

confer protection in CD8 depleted mice. Conversely, CD4 and CD4+CD8 depletion at the time of challenge had elevated viral loads and reduced survival (Fig 2). However, depletion of CD4 T cell subsets at the time of vaccination impaired the formation of CD8 T cell memory and resulted in further increased viral loads. CD4 and CD8 T cells act in synergy for vaccine-mediated protection, and CD4 T cells are essential for protection.

Our vaccine was universally immunostimulatory, providing protection in different mouse strains, reduced viral loads in immune-compromised nude and SCID mice (data not shown), and further stimulated human PBMCs to generate increased H5-specific CD4 T cell responses in vitro (Fig 3). Restimulation of human PBMCs with ultraviolet-inactivated Wyeth/IL-15/5flu resulted in proliferation and expansion of vaccinia-specific and H5-specific T cells, especially H5-specific CD4 T cells, which showed a significant increase from baseline memory levels in paired uncultured samples.

Discussion

T cell–activating vaccines are broadly protective against influenza infection and are a promising approach for clinical development. The breadth of T cell–mediated vaccine protection covered diverse strains, subtypes, and highly lethal avian influenza viruses. T cells from vaccinated mice were necessary for protection from influenza infection, especially CD4 T cells for their helper functions formation of CD8 T cell memory and antibodies; however, CD4 T cells themselves were necessary at the time of vaccination to mediate protection from lethal infection. New roles are emerging of the pivotal roles of CD4 T cells play during vaccination and infection responses.[5]

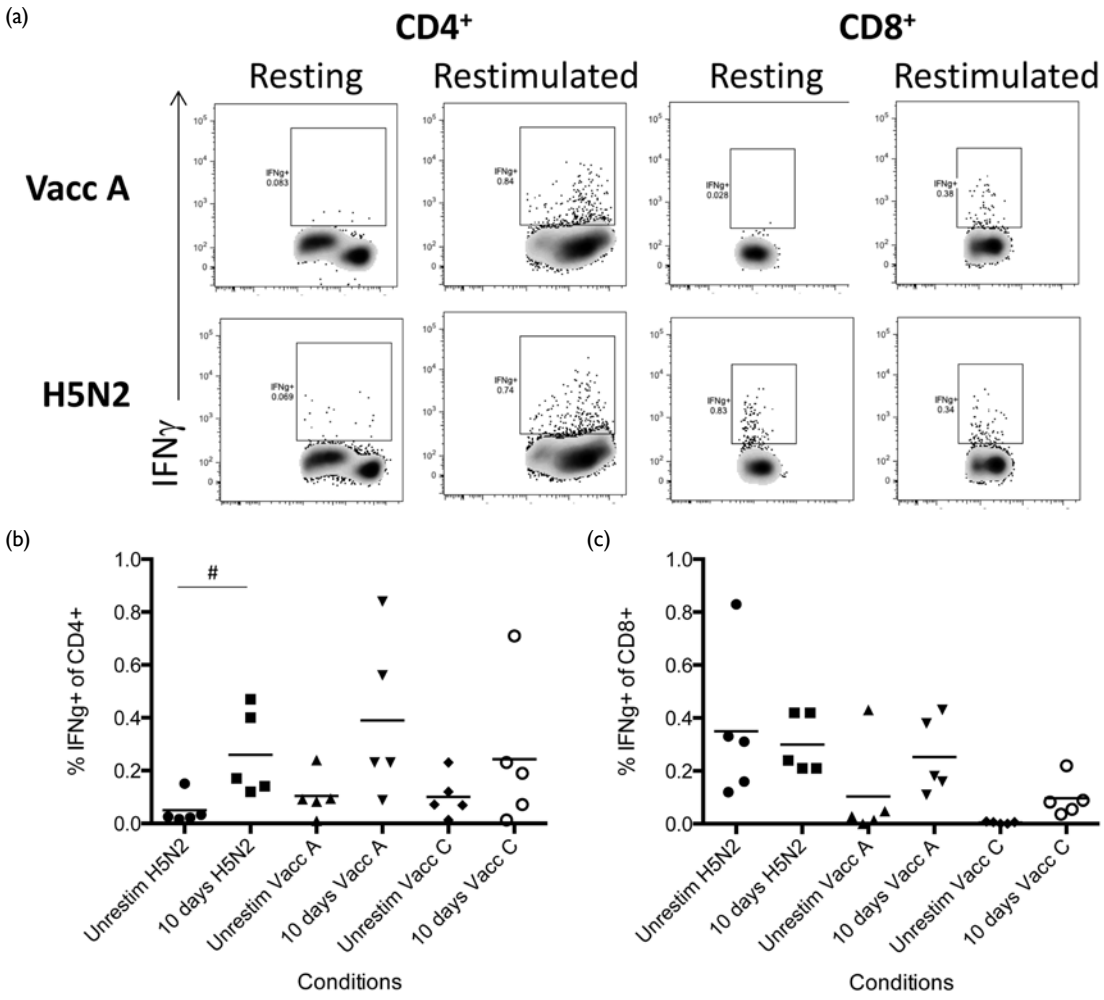

FIG 3. Human PBMCs restimulated with vaccinia A have increased H5-specific CD4+ T cells. PBMCs (n=5 donors) were restimulated with ultraviolet-inactivated vaccinia A virus for 10 days in the presence of IL-2. At day 10, cells were stimulated with H5N2 (MOI4), Wyeth/IL-15/5flu, Wyeth, or PBS (negative control). Direct ex vivo restimulation of resting CD4 and CD8 T cells from matched donors was also performed. CD4 and CD8 T cells were assessed for IFNγ production by intracellular cytokine staining. (a) Representative FACS plots of IFNγ CD4 and CD8+ T cells, (b) Individual donors CD4 T cells, and (c) CD8 T cells.

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Results from this study have been published in:
(1) Valkenburg SA, Li OT, Mak PW, et al. IL-15 adjuvanted multivalent vaccinia-based universal influenza vaccine requires CD4+ T cells for heterosubtypic protection. Proc Natl Acad Sci U S A 2014;111:5676-81.
(2) Valkenburg SA, Li OTW, Li A, et al. Protection by universal influenza vaccine is mediated by memory CD4 T cells. Vaccine 2018;36:4198-206.

References

1. Assarsson E, Bui HH, Sidney J, et al. Immunomic analysis of the repertoire of T-cell specificities for influenza A virus in humans. J Virol 2008;82:12241-51.
2. Poon LL, Leung YH, Nicholls JM, et al. Vaccinia virus-based multivalent H5N1 avian influenza vaccines adjuvanted with IL-15 confer sterile cross-clade protection in mice. J Immunol 2009;182:3063-71.
3. Valkenburg SA, Li OT, Mak PW, et al. IL-15 adjuvanted multivalent vaccinia-based universal influenza vaccine requires CD4+ T cells for heterosubtypic protection. Proc Natl Acad Sci U S A 2014;111:5676-81.
4. Guo H, Santiago F, Lambert K, Takimoto T, Topham DJ. T cell-mediated protection against lethal 2009 pandemic H1N1 influenza virus infection in a mouse model. J Virol 2011;85:448-55.
5. McKinstry KK, Strutt TM, Kuang Y, et al. Memory CD4+ T cells protect against influenza through multiple synergizing mechanisms. J Clin Invest 2012;122:2847-56.