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Harnessing Cross-Reactive CD8$^+$ T_{RM} Cells for Long-Standing Protection Against Influenza A Virus

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Introduction

As of today, influenza A virus infection is very much a health issue for the human population. Over a 100 years have passed since the 1918 influenza pandemic that caused the deaths of tens of millions of people over the world, but despite this we have not been able to develop an efficient vaccine that can protect the population. Since they first became available, >60 years ago, commercial vaccines all primarily induce an antibody response targeting the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA).

The vaccine is the most effective method of protecting the population against influenza infection available today, nevertheless the U.S. Centers for Disease Control and Prevention estimated the efficiency to be ranging between 20% and 60% over the last years (12,33,40,48,60). The major underlying reason for this is the ever-evolving influenza A virus. Genetic drift results in changes of the surface proteins, making it impossible for antibodies to recognize and neutralize newly evolved virus. As a result, there is an annual need for re-evaluation and possibly re-formulation of the vaccine in an attempt to match the circulating strains. An alternative to the antibody-mediated immunity, targeting the variable HA and NA, is to induce cellular immunity targeting the highly conserved internal influenza proteins.

Nineteen years ago from Peter C. Doherty's laboratory, Christensen *et al.* published a study illustrating the capacity and critical role of CD8 T cells in heterosubtypic protection, an article that has become one of the cornerstones when discussing the important role of CD8 T cells in cross protection against respiratory viruses (8). The data presented in that article clearly demonstrated that by generating a large number of influenza-specific CD8 T cells, protection against influenza A challenge was improved, not only against homosubtypic strains, but also against heterosubtypic influenza strains where seroimmunity was lacking. Specifically, the T cell immune response was boosted by re-infection with serologically different influenza strains that shared the same CD8 T cell nucleoprotein (NP) epitope. When an influenza-specific CD8 T cell population was present before challenge, limited expansion of the CD8 T cell population was required, thus not only protecting against the virus infection but also limiting immunopathology. The article also illustrated that pre-existing cellular immunity have limitations. Despite high numbers of cross-reactive CD8 T cells, present as early as 1-day postchallenge, viral titers did not differ from groups with no T cell immunity. This pinpointed another important fact, the CD8 T cell response will first be activated after the influenza virus has infected its host. Since this publication in 2000, substantial effort has been put into understanding the basic foundations necessary for designing a vaccine generating a long-lasting cross-protective CD8 T cell population.

Memory CD8 T Cells

At the time point of the Christensen's article, memory T cells were considered to fall into two main categories, central memory or effector memory T cells. Central memory T cells, T_{CM}, are located in the secondary lymphoid organs and the blood and are CD62L$^+$ CCR7$^+$ cells. Effector memory cells, T_{EM}, are circulating CD62L$^-$ CCR7$^-$ cells surveying the blood and peripheral tissues. For a long period of time, all memory T cells were categorized into these two populations. However, since then groups studying memory T cells found that a population of the effector memory T cells were residing in the peripheral tissues and did not reenter the circulation (17,32). This memory population was

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coined tissue-resident memory T cells (T_{RM}) and have been identified in various tissue since including lungs, liver, intestine, and female reproductive tract and is identified by a high expression of CD69, and in many tissues also CD103 and CD49a (13,19,31,43).

The crucial role that T_{RM} cells play in cellular immunity against respiratory infections has become clear over the last decade. Several decades ago, early work on lung airway T cells unknowingly provided some of the first descriptions of tissue-resident memory CD8 T cells and their importance for protective immunity to heterosubtypic influenza challenge. One of these first studies by Christensen *et al.* that investigated airway memory T cells found that a single intraperitoneal priming regimen was inferior to an intraperitoneal-prime and intranasal-boost regimen for protection against a heterosubtypic influenza challenge (8). Although this defect was originally attributed to differences in the number of circulating influenza-specific memory CD8 T cells between vaccination regimens, armed with current knowledge it is likely that the lack of influenza-specific memory CD8 T cells in the respiratory tract after intraperitoneal priming was also a major contributing factor (35,41,47). Another early study provided the first evidence of a $CD69^+$ ''resting'' influenza-specific memory CD8 T cell population in the airways that was highly prevalent on day 50 postinfection, but gradually disappeared over the next 3–4 months (30). It was not until the formal identification of T_{RM} that the correlation between the gradual loss of influenza-specific memory CD8 T cells from the airways observed by Marshall *et al.* (30), and the gradual decline in protective cellular immunity to heterosubtypic influenza infection, could be fully appreciated (27).

More recent studies have employed parabiosis and/or intravital labeling techniques to greatly expand our knowledge of the T_{RM} population in the lung (1,21). Virus-specific lung T_{RM} are essential for heterosubtypic immunity against influenza viruses, and the gradual decline in immunity over time parallels a gradual loss in lung T_{RM} (56). Lung T_{RM} can be divided into two distinct subsets based on their localization in the interstitium or the airways. These unique microenvironments lead to different phenotypic and functional traits of interstitial and airway T_{RM}, but both subsets contribute to antiviral immunity (36,58). Transcriptional analysis of lung T_{RM} showed these cells have a similar core genetic signature to T_{RM} in other peripheral tissues, such as the skin, gut, and genital tract (23,29). However, unlike T_{RM} in other sites, the requirements for establishment of T_{RM} in the lung, and the longevity of these cells in the tissue, are unique. Several reports have shown the generation of lung T_{RM} requires that activated effector T cells re-encounter their specific antigen in the lung tissue (35,41,47). This has important implications for vaccination strategies, as intramuscular (i.m.) or subcutaneous vaccines that fail to traffic antigen to the lungs are unlikely to generate airway or interstitial T_{RM} in sufficient numbers for cellular immune protection.

Another critical difference between T_{RM} in the lungs versus other tissues is the gradual decay of the T_{RM} pool in both the airways and the interstitium. Although the mechanisms driving this loss are not fully understood, lung T_{RM} showed increased apoptosis under steady-state conditions compared to circulating T_{EM} (44). In addition, airway T_{RM} were more prone to cell death than interstitial T_{RM} due to the limited nutrients available in the airway environment (Uddbäck et al, in preparation; and S. Hayward et al, in preparation). Although these findings would suggest that vaccines designed to generate lung T_{RM} would offer only transient protection, several reports have described mechanisms that can improve the longevity of lung T_{RM}. Repeated boosting of virus-specific cells resulted in decreased apoptosis and prolonged maintenance of lung T_{RM} under steady-state conditions (51). It has also been shown that lung T_{RM} preferentially reside in areas of tissue repair within the lung, and a more natural pathogen exposure history may generate or maintain these niches to support T_{RM} persistence (47). Developing a better understanding of the interplay between lung T_{RM} and their local environment will be essential for developing strategies to improve their longevity.

Choosing a Target

Apart from understanding T cell memory populations and how the cellular immunity will provide cross protection, it is crucial during vaccine design to select the right targets for the immune response. Long-lasting influenza-specific memory T cells have been found in peripheral blood from human blood donors up to at least 13 years after the influenza infection (52). These long-lived T cells were still functional and produced interferon gamma (IFNγ) upon *ex vivo* stimulation. While all of the internal proteins of influenza are highly conserved relative to the surface protein HA and NA (42), not all will serve as good targets for inducing an immune response. NP, polymerase acid (PA), polymerase basic protein 1 (PB1), PB2, and matrix protein 1 (M1) have been researched as potential targets with varying results.

NP is by far the most investigated target as the $CD8^+$ T cell response to influenza A infections in both mice and humans is primarily dominated by an NP-specific response (2,55). High number of NP-specific T cells can be found in spleen, mediastinal lymph node, airways, and lungs after a cleared influenza infection in mice, which provides researchers with a tool that at least in part is translatable to a human response. As Christensen *et al.* (8) illustrated in their article from 2000, this influenza specific population, when boosted to sufficient numbers, can provide protection against subsequent infection with heterosubtypic influenza strains. For a vaccine, viral vectors have been a popular approach used to induce a protective NP-specific response. Adenovirus and modified vaccinia virus Ankara (MVA) encoding the NP gene have both been successful at generating a NP-specific T cells response (15,49). The NP-specific cells generated in these studies were capable of protecting mice against both homosubtypic and heterosubtypic challenge, also in the absence of B cells. Due to the compiling studies clearly demonstrating NP as a frontrunner in the selection of relevant targets, NP will likely be included in the case of future T cell inducing vaccine. However, T cells are not infallible and if the majority of the CD8 T cell response is directed toward a single epitope that is mutated between influenza strains, T cells will fail to recognize and clear the virus (49). Still, the risk of this happening is much lower compared to when targeting HA and

NA, as the NP gene is highly conserved between influenza A strains. To some extent the high degree of conservation of the internal proteins between influenza strains can be explained by functional constraint (4). Moreover, using mathematical modeling and mapping CD8 T cell NP epitopes in humans, Li *et al.* illustrated that the conservation is likely dependent on several aspects, including the fact that polymorphism of the human major histocompatibility complex-I gene restricts the advantage of a mutated strain to only a fraction of the human population carrying the relevant MHC-I alleles, and shows that other epitopes can compensate when infection occurs with mutated variants of the influenza virus (26). The possibility of escape variants of the NP gene nevertheless stresses the need to investigate other target genes as a way to increase the breadth of the vaccine induced response and avoid immune escape.

In a 2017 study from our research group, the immune response generated in response to vaccination with an adenoviral vector expressing PB1, a relatively poorly investigated target, was analyzed (50). We found that by linking the PB1 gene to invariant chain in a nonreplicating adenovirus (AdIiPB1) (18), vaccination induced high numbers of CD8 memory T cells that produced cytokines including IFNγ in response to *ex vivo* stimulation with $PB1_{703–711}$ peptide (3). However, despite the high number of PB1-specific memory T cells in C57BL/6 mice, AdIiPB1 vaccinated subjects only displayed 50% survival upon lethal challenge. The PB1-specific T cells generated showed low cytolytic capacity *in vivo* and after further investigation we found that the $PB1_{703–711}$ peptide-MHC complex had low stability over time, resulting in a very high concentration of peptide being required for activation of the T cells. This correlates with a previous study by Peter C. Doherty and Stephen Turners group demonstrating the connection between peptide-MHC stability and CD8 T cell activation (10). Despite evidence that intra nasal priming route could improve the immunity to low avidity targets, also published by Peter C. Doherty in collaboration with Katherine Kedzierskas group, protective capacities by the T cells generated by AdIiPB1 vaccination in C57BL/6 mice was not sufficient despite an intra nasal priming route (53). These results emphasized that many factors apart from cell numbers need to be considered when choosing a vaccine target. In addition to this, AdIiPB1 was less efficient at inducing high number of CD8 T cells in the lung and airways compared to adenoviruses expressing other genes such as NP and PA (Unpublished data). Further, a DNA vaccine expressing PB1 induced an immune response that could provide homosubtypic protection in Balb/C mice; however, cross-protective capacities of the immunity generated were never investigated (22). Interestingly though, PB1 epitopes were identified as among the most prevalent in humans in a study from Assarsson *et al.* in 2008, and PB1 still has potential as a target, in particular if combined with other genes (2). Despite generating a significant response in mice, few PA epitopes are described in humans and PA has been poorly investigated as a target for an influenza vaccine. Encoded by an adenovirus, immunization with PA can generate an immune response and protect against influenza challenge in both C57BL/6 and Balb/C mice (Article in preparation). The PA-specific response observed in mice is not directly translatable to humans; however, it can still be used to understand differences in different antigen-specific responses. In addition, to broaden protection and reduce chance of immune escape by mutations, PA is of interest.

M1 epitopes have also been identified at high frequencies in the human population and M1 has therefore been investigated as a vaccine target, both as a stand-alone but mainly in combination with NP (7,28,45). One of the most successful trials have been a heterologous prime boost strategy where first immunization occurs with a chimpanzee adenovirus vector encoding NP+M1 fusion protein and a conserved part of the HA stalk (cH13/14), followed by boosting with a MVA vector encoding the same targets; using this approach vaccination have shown promising results in mice and ferrets (34). Importantly, the MVA vaccine both with and without the addition of the AdNP+M1 has also been well tolerated in humans and significantly increased numbers of circulating? Memory T cells (5,9). Notably, in these studies the vaccines were administered i.m., the most common administration route in humans. In one initial study using the MVA+Ad vaccine regime in mice, an intranasal route was used for boosting with Ad after i.m. priming with MVA, and this induced higher number of antigen-specific cells in the BAL of mice, compared to a i.m. + i.m. delivery (24). However, localized cellular immunity in response to the MVA+Ad vaccination regime has not been further implemented or investigated down the line and, as will be discussed below, it is likely that vaccination by the i.m. route, though established and practical, will not result in the most optimal protection possible.

Local Delivery

For a vaccine to generate the intended response, vaccination route needs to be carefully considered. Vaccination must not only deliver the vaccine in a safe manner for patients, but also be practical. Therefore, unnecessary painful routes or complicated regimes must be carefully evaluated before taken into use. As lung T_{RM} cells are gaining increased attention, and compiling studies illustrates their importance for optimal cross protection in respiratory infection (36,56), a lot of recent immunization strategies have focused on the ability to induce a T_{RM} population and how to make this population stable over time. As previously mentioned, the majority of data indicate that airway and lung interstitium T_{RM} cells require local antigen for establishment, which means that the vaccine need to be administered locally into the airways (46). Intranasal vaccination with an AdNP have successfully generated an influenza-specific CD8 T cells response in the lungs that was able to provide cross protection against different influenza A strains (20). However, it was never investigated how long the induced immunity lasted as viral challenge was performed 3 weeks after vaccination at the latest. Moreover, virus-like particles including epitopes from HA and M1 have also been used in intranasal vaccination to generate localized cross-reactive CD8 T cells, but again protection has been poorly investigated beyond a few months post-vaccination (14).

The live attenuated influenza virus (LAIV) FluMist, is the only commercially available vaccine that is delivered locally into the airways in the form of a nose spray, demonstrating that intranasal application is possible also in

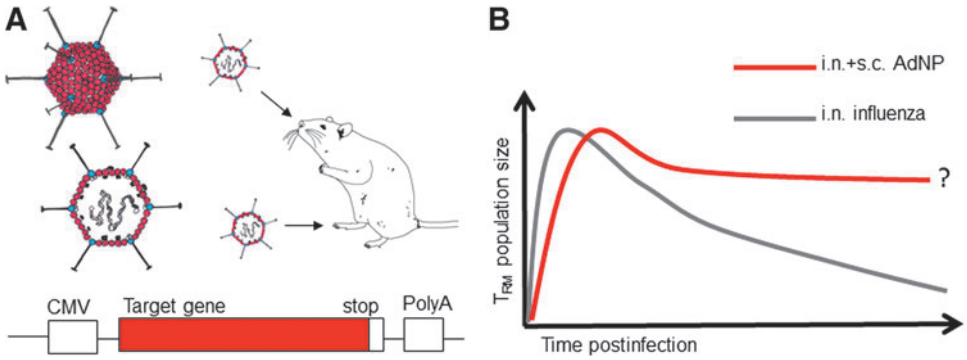

FIG. 1. The adenovirus as a vaccine vector. **(A)** Illustration of an adenovirus vector and the dual immunization strategy. **(B)** Representative illustration of T_{RM} population dynamics over time after influenza infection and AdNP immunization. CMV; cytomegalovirus promotor; i.n. intranasal; s.c. subcutaneous; T_{RM}, tissue-resident memory T cells.

humans. Even though the primary goal of FluMist has been to induce a B cell and antibody mediated immune response, it has also been investigated as a way to increase CD8 T cells with promising results (16,57). In addition, a PR8 LAIV has been studied and when administered intranasally, it has been found able to establish a CD8 T cell population in the lungs of mice, which was crucial for clearance of a heterosubtypic influenza challenge (54).

We showed, in 2016, that by immunizing with AdNP both intranasally and subcutaneously we could induce long-lasting immunity in mice (Fig. 1) (49). By combining the two vaccination routes, both a strong localized and central immune response was induced. The generated memory T cell populations were cross protective and protection lasted for at least 8 months. When the phenotype of the airway population was analyzed, a substantial part of the antigen-specific airway population expressed residency markers CD69 and CD103. Recently, we have been exploring the underlying reasons for this long-lasting protection, and we demonstrated that persistent antigen after adenovirus vaccination can maintain a lung T_{RM} population long term (Fig. 2) (Article in preparation). Inflation, the concept that a small amount of persistent antigen can stimulate a T cell population without causing T cell exhaustion, has recently been gaining substantial attention and also how inflation can maintain the T_{RM} population in the lungs and airways (6). Except for adenovectors, intranasal vaccination with Murin Cytomegalovirus (MCMV) vectors has recently been explored as a method for generating and maintaining a T_{RM} population by inflation long term (37,38,59). However, as discussed earlier, cells in the lung and particularly the airways have a high rate of apoptosis and the time limit of the inflation process and the effect on the T_{RM} population long term in the lungs has not been thoroughly studied. If and when the persistent antigen in the lungs is cleared, unless the population has permanently differentiated, the antigen-specific T_{RM} cells will likely be lost within months. However, it has been suggested that, using a doxycycline-regulated adenovirus, the majority of programming occurs early after priming and if the antigen is removed later than 60 days after priming it has little or no effect on the memory population (11).

Moving Forward

To obtain heterosubtypic immunity against influenza infections, memory T cells are highly likely to play an important role. T cell immunity will, however, not provide sterile immunity and to provide optimal protection, a

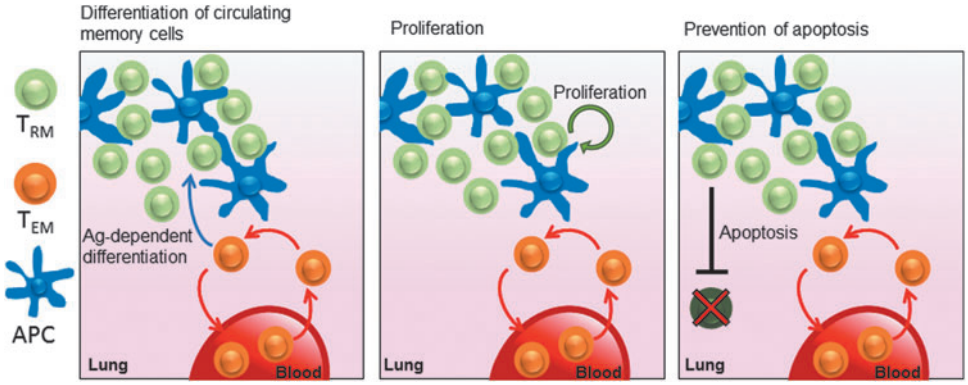

FIG. 2. Mechanisms of how persistent antigen may maintain a lung T_{RM} population T_{RM} population dynamics in the lung of i.n.+s.c. AdNP immunized mice. Persistent antigen (represented by the APC) in the lungs after AdNP immunization may maintain the population through differentiation of circulating T_{EM} cells surveying the peripheral tissue (*left*), by antigen-driven proliferation (*middle*), or by prevention of apoptosis (*right*), or by any combination of these possibilities. APC, antigen-presenting cells.

vaccine likely also has to induce an antibody response. Several studies have been investigating a combination of T and B cell targets, with the MVA vector currently coming out as the frontrunner. One of the greatest challenges with a CD8 T cell inducing influenza vaccine is how to maintain a large enough CD8 T cells population in the respiratory system to have long-lasting protection. As discussed earlier, the antigen-specific population located in the airways is under particular stress, causing them to undergo apoptosis at a high rate. Several studies point to the importance of local antigen for the formation and maintenance of T_{RM} and it is possible that extended exposure by persistent antigen or repeated antigen exposure can increase the survival of the T_{RM} population (51). It is reasonable to hypothesize that repeated antigen exposure and persistent antigen cause a similar result, namely an extended life span of the T_{RM} population. How long the T_{RM} population can be maintained in the lungs of humans still remains to be investigated and there is currently no solution to the high apoptosis rate of T_{RM} population. One issue of translating mouse studies to humans is that most of murine studies are performed in mice with no pre-existing immunological memory toward influenza, and vaccination is usually not followed by any irrelevant infections. Both these scenarios are likely to occur in humans and to influence the formation and maintenance of the human lung T_{RM} population and therefore needs to be further investigated.

Methods to improve the T_{RM} population by increasing the size, extending the life span and thereby heterosubtypic immunity has been explored by including adjuvant genes important for resident memory T cell formation and survival in the vaccines (25,39). However, these approaches have used self-molecules, 4-1BB and interleukin-1β, and the adverse effects needs to be carefully evaluated. Moreover, if the high apoptosis rate of the lung T_{RM} population observed in multiple studies cannot be prevented, no matter how large a population we start out with, eventually the cells, and heterosubtypic protection, will be lost. Either scientist need to figure out a way to make the airway environment less stressful for the cells and reduce apoptosis, an unlikely scenario as this could affect other basic or immunological functions in the lung, or the T_{RM} population needs to be replenished based on persistent antigen that can pull circulating T_{EM} into the T_{RM} population. If neither of these options will be possible, perhaps a universal one-time influenza vaccine is too much to ask for?

Author Disclosure Statement

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References

1. Anderson KG, Sung H, Skon CN, *et al.* Cutting edge: intravascular staining redefines lung CD8 T cell responses. J Immunol 2012;189:2702–2706.
2. 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;82:12241–12251.
3. Belz GT, Xie W, and Doherty PC. Diversity of epitope and cytokine profiles for primary and secondary influenza a virus-specific CD8+ T cell responses. J Immunol 2001;166: 4627–4633.
4. Berkhoff EG, de Wit E, Geelhoed-Mieras MM, *et al.* Functional constraints of influenza A virus epitopes limit escape from cytotoxic T lymphocytes. J Virol 2005;79: 11239–11246.
5. Berthoud TK, Hamill M, Lillie PJ, *et al.* Potent CD8+ T-cell immunogenicity in humans of a novel heterosubtypic influenza A vaccine, MVA-NP+M1. Clin Infect Dis 2011; 52:1–7.
6. Bolinger B, Sims S, O'Hara G, *et al.* A new model for CD8+ T cell memory inflation based upon a recombinant adenoviral vector. J Immunol 2013;190:4162–4174.
7. Boon AC, de Mutsert G, Graus YM, *et al.* The magnitude and specificity of influenza A virus-specific cytotoxic T-lymphocyte responses in humans is related to HLA-A and -B phenotype. J Virol 2002;76:582–590.
8. Christensen JP, Doherty PC, Branum KC, and Riberdy JM. Profound protection against respiratory challenge with a lethal H7N7 influenza A virus by increasing the magnitude of CD8(+) T-cell memory. J Virol 2000;74:11690–11696.
9. Coughlan L, Sridhar S, Payne R, *et al.* Heterologous two-dose vaccination with simian adenovirus and poxvirus vectors elicits long-lasting cellular immunity to influenza virus A in healthy adults. EBioMedicine 2018;29:146–154.
10. Denton AE, Wesselingh R, Gras S, *et al.* Affinity thresholds for naive CD8+ CTL activation by peptides and engineered influenza A viruses. J Immunol 2011;187:5733–5744.
11. Finn JD, Bassett J, Millar JB, *et al.* Persistence of transgene expression influences CD8+ T-cell expansion and maintenance following immunization with recombinant adenovirus. J Virol 2009;83:12027–12036.
12. Gaglani M, Pruszynski J, Murthy K, *et al.* Influenza vaccine effectiveness against 2009 pandemic influenza A(H1N1) virus differed by vaccine type during 2013–2014 in the United States. J Infect Dis 2016;213:1546–1556.
13. Gebhardt T, Wakim LM, Eidsmo L, *et al*. Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. Nat Immunol 2009;10:524–530.
14. Hemann EA, Kang SM, and Legge KL. Protective CD8 T cell-mediated immunity against influenza A virus infection following influenza virus-like particle vaccination. J Immunol 2013;191:2486–2494.
15. Hessel A, Savidis-Dacho H, Coulibaly S, *et al.* MVA vectors expressing conserved influenza proteins protect mice against lethal challenge with H5N1, H9N2 and H7N1 viruses. PLoS One 2014;9:e88340.
16. Hoft DF, Babusis E, Worku S, *et al.* Live and inactivated influenza vaccines induce similar humoral responses, but only live vaccines induce diverse T-cell responses in young children. J Infect Dis 2011;204:845–853.
17. Hogan RJ, Usherwood EJ, Zhong W, *et al.* Activated antigen-specific CD8+ T cells persist in the lungs following

recovery from respiratory virus infections. J Immunol 2001;166:1813–1822.
18. Holst PJ, Sorensen MR, Mandrup Jensen CM, *et al*. MHC class II-associated invariant chain linkage of antigen dramatically improves cell-mediated immunity induced by adenovirus vaccines. J Immunol 2008;180:3339–3346.
19. Jiang X, Clark RA, Liu L, *et al*. Skin infection generates non-migratory memory CD8+ T(RM) cells providing global skin immunity. Nature 2012;483:227–231.
20. Kim SH, Kim JY, Choi Y, *et al*. Mucosal vaccination with recombinant adenovirus encoding nucleoprotein provides potent protection against influenza virus infection. PLoS One 2013;8:e75460.
21. Klonowski KD, Williams KJ, Marzo AL, *et al*. Dynamics of blood-borne CD8 memory T cell migration in vivo. Immunity 2004;20:551–562.
22. Kosik I, Krejnusova I, Praznovska M, Polakova K, and Russ G. A DNA vaccine expressing PB1 protein of influenza A virus protects mice against virus infection. Arch Virol 2012;157:811–817.
23. Kumar BV, Ma W, Miron M, *et al.* Human tissue-resident memory T cells are defined by core transcriptional and functional signatures in lymphoid and mucosal sites. Cell Rep 2017;20:2921–2934.
24. Lambe T, Carey JB, Li Y, *et al.* Immunity against heterosubtypic influenza virus induced by adenovirus and MVA expressing nucleoprotein and matrix protein-1. Sci Rep 2013;3:1443.
25. Lapuente D, Storcksdieck Genannt Bonsmann M, Maaske A, *et al.* IL-1beta as mucosal vaccine adjuvant: the specific induction of tissue-resident memory T cells improves the heterosubtypic immunity against influenza A viruses. Mucosal Immunol 2018;11:1265–1278.
26. Li ZT, Zarnitsyna VI, Lowen AC, *et al.* Why are CD8 T cell epitopes of human influenza A virus conserved? J Virol 2019;93:e01534-18.
27. Liang S, Mozdzanowska K, Palladino G, and Gerhard W. Heterosubtypic immunity to influenza type A virus in mice. Effector mechanisms and their longevity. J Immunol 1994; 152:1653–1661.
28. Liu J, Wu B, Zhang S, *et al.* Conserved epitopes dominate cross-CD8+ T-cell responses against influenza A H1N1 virus among Asian populations. Eur J Immunol 2013;43: 2055–2069.
29. Mackay LK, Minnich M, Kragten NA, *et al.* Hobit and Blimp1 instruct a universal transcriptional program of tissue residency in lymphocytes. Science 2016;352:459–463.
30. Marshall DR, Turner SJ, Belz GT, *et al.* Measuring the diaspora for virus-specific CD8+ T cells. Proc Natl Acad Sci U S A 2001;98:6313–6318.
31. Masopust D, Choo D, Vezys V, *et al.* Dynamic T cell migration program provides resident memory within intestinal epithelium. J Exp Med 2010;207:553–564.
32. Masopust D, Vezys V, Marzo AL, and Lefrancois L. Preferential localization of effector memory cells in nonlymphoid tissue. Science 2001;291:2413–2417.
33. McLean HQ, Thompson MG, Sundaram ME, *et al.* Influenza vaccine effectiveness in the United States during 2012–2013: variable protection by age and virus type. J Infect Dis 2015;211:1529–1540.
34. McMahon M, Asthagiri Arunkumar G, Liu WC, *et al.* Vaccination with viral vectors expressing chimeric hemagglutinin, NP and M1 antigens protects ferrets against influenza virus challenge. Front Immunol 2019;10:2005.
35. McMaster SR, Wein AN, Dunbar PR, *et al.* Pulmonary antigen encounter regulates the establishment of tissue-resident CD8 memory T cells in the lung airways and parenchyma. Mucosal Immunol 2018;11:1071–1078.
36. McMaster SR, Wilson JJ, Wang H, and Kohlmeier JE. Airway-resident memory CD8 T cells provide antigen-specific protection against respiratory virus challenge through rapid IFN-gamma production. J Immunol 2015; 195:203–209.
37. Morabito KM, Ruckwardt TJ, Bar-Haim E, *et al.* Memory inflation drives tissue-resident memory CD8(+) T cell maintenance in the lung after intranasal vaccination with murine cytomegalovirus. Front Immunol 2018;9:1861.
38. Morabito KM, Ruckwardt TR, Redwood AJ, *et al*. Intranasal administration of RSV antigen-expressing MCMV elicits robust tissue-resident effector and effector memory CD8+ T cells in the lung. Mucosal Immunol 2017;10:545–554.
39. Moraes TJ, Lin GH, Wen T, and Watts TH. Incorporation of 4-1BB ligand into an adenovirus vaccine vector increases the number of functional antigen-specific CD8 T cells and enhances the duration of protection against influenza-induced respiratory disease. Vaccine 2011;29: 6301–6312.
40. Ohmit SE, Thompson MG, Petrie JG, *et al.* Influenza vaccine effectiveness in the 2011-2012 season: protection against each circulating virus and the effect of prior vaccination on estimates. Clin Infect Dis 2014;58:319–327.
41. Pizzolla A, Nguyen THO, Smith JM, *et al.* Resident memory CD8(+) T cells in the upper respiratory tract prevent pulmonary influenza virus infection. Sci Immunol 2017;2:eaam6970.
42. Powell TJ, Strutt T, Reome J, *et al.* Priming with cold-adapted influenza A does not prevent infection but elicits long-lived protection against supralethal challenge with heterosubtypic virus. J Immunol 2007;178: 1030–1038.
43. Schenkel JM, Fraser KA, Vezys V, and Masopust D. Sensing and alarm function of resident memory CD8(+) T cells. Nat Immunol 2013;14:509–513.
44. Slutter B, Van Braeckel-Budimir N, Abboud G, *et al*. Dynamics of influenza-induced lung-resident memory T cells underlie waning heterosubtypic immunity. Sci Immunol 2017;2:eaag2031.
45. Sui Z, Chen Q, Fang F, Zheng M, and Chen Z. Cross-protection against influenza virus infection by intranasal administration of M1-based vaccine with chitosan as an adjuvant. Vaccine 2010;28:7690–7698.
46. Takamura S, Roberts AD, Jelley-Gibbs DM, *et al*. The route of priming influences the ability of respiratory virus-specific memory CD8+ T cells to be activated by residual antigen. J Exp Med 2010;207:1153–1160.
47. Takamura S, Yagi H, Hakata Y, *et al.* Specific niches for lung-resident memory CD8+ T cells at the site of tissue regeneration enable CD69-independent maintenance. J Exp Med 2016;213:3057–3073.
48. Treanor JJ, Talbot HK, Ohmit SE, *et al.* Effectiveness of seasonal influenza vaccines in the United States during a season with circulation of all three vaccine strains. Clin Infect Dis 2012;55:951–959.
49. Uddback IE, Pedersen LM, Pedersen SR, *et al.* Combined local and systemic immunization is essential for durable T-cell mediated heterosubtypic immunity against influenza A virus. Sci Rep 2016;6:20137.

50. Uddback IE, Steffensen MA, Pedersen SR, *et al*. PB1 as a potential target for increasing the breadth of T-cell mediated immunity to Influenza A. Sci Rep 2016;6:35033.
51. Van Braeckel-Budimir N, Varga SM, Badovinac VP, and Harty JT. Repeated antigen exposure extends the durability of influenza-specific lung-resident memory CD8(+) T cells and heterosubtypic immunity. Cell Rep 2018;24:3374.e3–3382.e3.
52. van de Sandt CE, Hillaire ML, Geelhoed-Mieras MM, *et al*. Human influenza A virus-specific CD8+ T-cell response is long-lived. J Infect Dis 2015;212:81–85.
53. Venturi V, Davenport MP, Swan NG, Doherty PC, and Kedzierska K. Consequences of suboptimal priming are apparent for low-avidity T-cell responses. Immunol Cell Biol 2012;90:216–223.
54. Wang Z, Kedzierski L, Nuessing S, *et al.* Establishment of memory CD8+ T cells with live attenuated influenza virus across different vaccination doses. J Gen Virol 2016;97:3205–3214.
55. Wu C, Zanker D, Valkenburg S, *et al.* Systematic identification of immunodominant CD8+ T-cell responses to influenza A virus in HLA-A2 individuals. Proc Natl Acad Sci U S A 2011;108:9178–9183.
56. Wu T, Hu Y, Lee YT, *et al.* Lung-resident memory CD8 T cells (TRM) are indispensable for optimal cross-protection against pulmonary virus infection. J Leukoc Biol 2014;95:215–224.
57. Zens KD, Chen JK, and Farber DL. Vaccine-generated lung tissue-resident memory T cells provide heterosubtypic protection to influenza infection. JCI Insight 2016;1:85832.
58. Zhao J, Zhao J, Mangalam AK, *et al.* Airway memory CD4(+) T cells mediate protective immunity against emerging respiratory coronaviruses. Immunity 2016;44:1379–1391.
59. Zheng X, Oduro JD, Boehme JD, *et al.* Mucosal CD8+ T cell responses induced by an MCMV based vaccine vector confer protection against influenza challenge. PLoS Pathog 2019;15:e1008036.
60. Zimmerman RK, Nowalk MP, Chung J, *et al.* 2014–2015 Influenza vaccine effectiveness in the United States by vaccine type. Clin Infect Dis 2016;63:1564–1573.

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