Journal of Clinical Virology 119 (2019) 44–52

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Review

Influenza virus-specific CD4+ and CD8+ T cell-mediated immunity induced by infection and vaccination

Janina M. Jansen, Thomas Gerlach, Husni Elbahesh, Guus F. Rimmelzwaan*, Giulietta Saletti*

University of Veterinary Medicine, Center for Emerging Infections and Zoonoses, Bünteweg 17, 30559 Hannover, Germany

ARTICLE INFO

Keywords:
Influenza virus
T cell immunity
Influenza vaccines

ABSTRACT

Influenza A and B virus infections are a major cause of respiratory disease in humans and are responsible for substantial morbidity and mortality worldwide. Vaccination against influenza mainly aims at the induction of virus neutralizing serum antibodies, which are an important correlate of protection provided that the antibodies match the strains causing the outbreaks antigenically. In addition, virus-specific T cells are known to contribute to protective immunity to influenza virus infections by limiting duration and severity of the disease. As the majority of virus-specific T cells recognize epitopes located in relatively conserved proteins, like the Nucleoprotein and Matrix 1 protein, they display a high degree of cross-reactivity with a wide range of influenza viruses, including newly emerging viruses of alternative subtypes. Advancing our understanding of influenza virus-specific T cell responses and their role in protective immunity against influenza will aid the rational design of novel vaccines that could induce robust, broad and long-lasting immune responses.

Here, we discuss the contribution of influenza virus-specific CD4+ and CD8+ T cells to protective immunity against influenza infection and the requirements and strategies for their induction by natural infection or vaccination, especially in children.

1. Introduction

Influenza viruses (IV) are respiratory pathogens that cause significant morbidity and mortality and represent a major global health problem. Every year, seasonal influenza virus infections account for about 5 million severe cases and 500.000 deaths worldwide [1]. Annual vaccination is recommended for populations at high risk of severe disease including children under 5 years of age, older adults over 60 years of age, patients with underlying diseases like cardiovascular, renal and respiratory conditions, immunocompromised subjects, as well as pregnant women [2,3].

IV are single-stranded RNA viruses with a segmented genome, which belong to the family of *Orthomyxoviridae*. The various IV (sub) types differ in the number of gene segments as well as in their host range. Of the three IV types infecting humans, Influenza C viruses are known to cause rather mild symptoms in adults whereas Influenza A (IAV) and B (IBV) viruses can cause severe disease [4,5]. The viral genome of IAV and IBV encodes various non-structural and structural proteins, including the two surface membrane glycoproteins hemagglutinin (HA) and neuraminidase (NA), which play a pivotal role in the initiation of infection and release of newly produced viral particles. Consequently, they are an important target for antibodies that can neutralize the virus or restrict virus replication. Because of selective pressure exerted by HA- and NA-specific antibodies, these proteins display a high degree of variation to evade recognition, also known as antigenic drift [6–9]. The more conserved internal proteins, such as the Matrix 1 (M1) protein, the Nucleoprotein (NP) and the polymerases (PB1, PB2, PA) are important targets recognized by virus specific CD4 and CD8 positive T cells, which contribute to protective immunity to (re)infection [10–12].

Vaccination is the most important measure to control influenza virus infections. The protection achieved by licensed vaccines (inactivated, live attenuated and recombinant HA) is primarily mediated by the induction of antibodies directed toward the HA and, to a lesser extent, the NA. The main target of the virus neutralizing (VN) antibodies is the head domain of the HA, especially the epitopes located in or in proximity of the receptor-binding site. By blocking binding of influenza virus to its sialic acid receptors on host respiratory epithelial cells, VN antibodies can provide sterilizing immunity if the vaccine strains match the epidemic strains antigenically. Antibodies toward a more conserved region of the HA (the stalk) or NA may also contribute to protective immunity [13–15]. NA-specific antibodies can limit viral spread by inhibiting the enzymatic activity of NA, whereas HA stalk specific antibodies contribute to clearance of virus-infected cells by

* Corresponding authors.
E-mail addresses: Guus.Rimmelzwaan@tiho-hannover.de (G.F. Rimmelzwaan), giulietta.saletti@tiho-hannover.de (G. Saletti).

https://doi.org/10.1016/j.jcv.2019.08.009
Received 12 June 2019; Received in revised form 20 August 2019; Accepted 21 August 2019

mediating antibody-dependent cell cytotoxicity (ADCC) and possibly by inhibition of membrane fusion, HA0 cleavage and viral egress from infected cells [16,17,18, reviewed in 19]. Influenza vaccines mainly induce strain-specific VN antibodies providing limited protection against antigenically mismatching strains and therefore the composition of the seasonal influenza vaccine needs to be updated annually in order to cope with the antigenic drift of epidemic strains [20,21]. Moreover, the emergence of novel viruses of alternative subtypes originating by reassortment of gene segments from two or more IAVs (antigenic shift), may cause pandemic outbreaks of influenza due to the virtual absence of pre-existing VN antibodies to the new viruses (e.g. of avian or swine origin) in the human population [22].

In contrast to humoral immunity, that in general affords narrow strain-specific protection, cell mediated immunity (CMI) is more cross-reactive and can protect against infection with IV of various subtypes (so-called heterosubtypic immunity) and drift variants [10,23–29]. T cells recognize viral peptides bound to the major histocompatibility complex (MHC) expressed on the surface of the infected or antigen presenting cells (APCs). Upon engagement of the T cells receptor (TCR) by viral antigen and further signaling such as co-stimulation (e.g. via CD28) and cytokines, naïve T cells are activated and proliferate. After resolution of the infection, most of the differentiated T cells die by apoptosis and the remaining cells form a pool of virus-specific memory T cells that can readily respond to a subsequent infection. CD4+ and CD8+ memory T cells are characterized by distinct homing capacity, phenotype, and effector functions. They are traditionally divided into central memory (T_{CM}) or effector memory (T_{EM}) subsets, depending on the expression of several surface markers (e.g., CD62 L, CCR7, CD45RA, CD45RO,CD27 and CD28) [30]. T_{CM} express high levels of CD62 L and CCR7 and are usually found in the lymph nodes and tonsils. The T_{EM} are characterized by a low surface expression of CD62 L and CCR7, are able to migrate to peripheral tissues, have a lower proliferative capacity but exert a more potent effector function when compared to the T_{CM} [31–33].

The contribution of influenza virus-specific CD4+ and CD8+ T cells to protective immunity has been reported in several human studies [27,34–36]. CD4+ T cells have various functions. They provide “help” to B cells and thus contribute to the generation and maintenance of the germinal center and production of high-affinity, class-switched antibodies. Furthermore, CD4+ T cells support CD8+ T cell priming, expansion and establishment of a pool of long-lived memory cells and, like CD8+ T cells, can lyse virus-infected cells to some extent [37–42]. In addition, both CD4+ and CD8+ T cells are an important source of pro- (e.g. IFN-γ) and anti-inflammatory (e.g. IL10) cytokines and their role is described in the next paragraph. Importantly, virus-specific CD8+ T cells contribute to heterosubtypic immunity by recognizing epitopes located in the relatively conserved internal antigens, such as M1, NP and polymerases [24,26,28,43–52]. Currently used inactivated vaccines induce CD4+ T cells and antibodies, but induce CD8+ T cell responses inefficiently [53–55]. In contrast, live-attenuated influenza vaccines (LAIV) induce both CD4 and CD8 positive T cells as well as a local immune response. In this regard, recent studies have emphasized the importance of tissue resident memory T cells (T_{RM}) in protective immunity towards influenza virus infection [56,57].

Considering the importance of T cells in inducing broadly protective immunity to influenza virus infections, novel vaccination strategies able to induce virus-specific T cell responses, especially CD8+ T cells, are of special interest [reviewed in [58]]. In this review, we discuss the contribution of systemic and local influenza virus-specific CD4+ and CD8+ T cells to protection against influenza infection and vaccination strategies able to induce a long-lasting cellular immunity (Fig. 1).

2. Contribution of T cells to protective immunity to influenza virus infection

2.1. CD4+ T cells

Various animal and human studies have demonstrated that CD4+ T cells play an important role in protection against IV infections. In mice, it was shown that CD4+ T cells are able to protect from infection with a low virus dose, independent of B cells and CD8+ T cells [59]. In experimentally IV-infected humans, pre-existing CD4+ T cells, but not CD8+ T cells were associated with a lower virus shedding and less severe disease outcome [35].

CD4+ T cells or helper T cells, can be divided in several subsets based on their phenotype and cytokine expression profiles (T follicular helper cells, Th1, Th2, Th9, Th17 and Th22) [60,61]. CD4+ T cell differentiation initially relies on cognate interaction between the TCR and APCs that display viral peptide fragments in association with MHC class-II. Production of virus-neutralizing antibodies by B cells is acknowledged as the best correlate of protection to IV infection and T follicular helper cells (T_{FH}) play an important role in their production [62]. T_{FH} contribute to the generation and maintenance of the germinal center reaction and induction of long-lived memory B cells [63–65]. T_{FH} are characterized by chemokine receptor CXCR5 expression which is responsible for their localization in B cell follicles in secondary lymphoid organs [66]. Their differentiation is influenced by the cytokine milieu, strength of the TCR signal and subset of dendritic cells (DC) involved in the cognate interaction [67–69]. For instance, activation of specific DC subsets and T_{FH} by selected adjuvants like MF59 may favor the induction of specific and long-lasting protective antibody responses [70–74]. Recently, circulating precursors of the T_{FH} cells have been found in animals and humans and their presence correlated with magnitude and quality of IV-specific antibodies [74–79]. Moreover, the T_{FH} found in circulation upon vaccination are clonally related to the germinal center-derived cells, highlighting the link between these two T_{FH} subsets [80,81]. The antigen specificity of the CD4+ T cells assisting B cells appears to be critical to achieve an optimal boosting capacity and it has been suggested that the most effective help is obtained when B cell and CD4+ T cell specificity are matched [82–84].

CD4+ T cells are also important for the induction and maintenance of virus-specific CD8+ T cell-mediated immunity [85–88]. They regulate the recruitment of CD8+ T cells into the lymph nodes, their migration to the site of infection and the contact with viral antigen presented by resident DCs leading to activation of virus-specific CD8+ T cells [89–92]. It is also known that CD4+ Th1 cells secreting IFN-γ are essential for promoting the development of CD8+ T cell memory and their long-term maintenance [87, reviewed in 93–95]. It has been shown that CD4+ T cells generated at the time of priming activate molecular pathways that limit exhaustion of the IV-specific memory CD8+ T cells, which therefore can rapidly respond to a subsequent re-infection [96]. Moreover, CD4+ T cell help is crucial for CD8+ T cells acquiring killing capacity and being able to migrate by expressing specific homing receptors [97]. In addition to promoting anti-viral immune responses by secreting IFN-γ, CD4+ T cells can acquire cytotoxic functions and eliminate infected cells by using cytokine-mediated and perforin/granzyme-dependent mechanisms [35,41,98–101]. An inverse correlation between the frequency of CD4+ T cells expressing perforin, granzymes and IFN-γ and disease severity was observed in humans after experimental IV infection [35]. In addition to perforin and granzyme B, the expression of the class-I restricted T cell associated molecule (CRTAM) and the Natural Killer (NK) cell marker (NKG2C/E) might facilitate the identification and quantitation of CD4+ T cells with cytotoxic properties [102,103]. IV-specific cytotoxic CD4+ T cells are primarily induced at the site of infection or vaccination and it was shown that intranasal delivery of vaccine induces higher frequencies of these cells in the respiratory tract than parenteral delivery [35,41,101]. Although the cytotoxic CD4+ T cells resemble the CD8+ T cells in

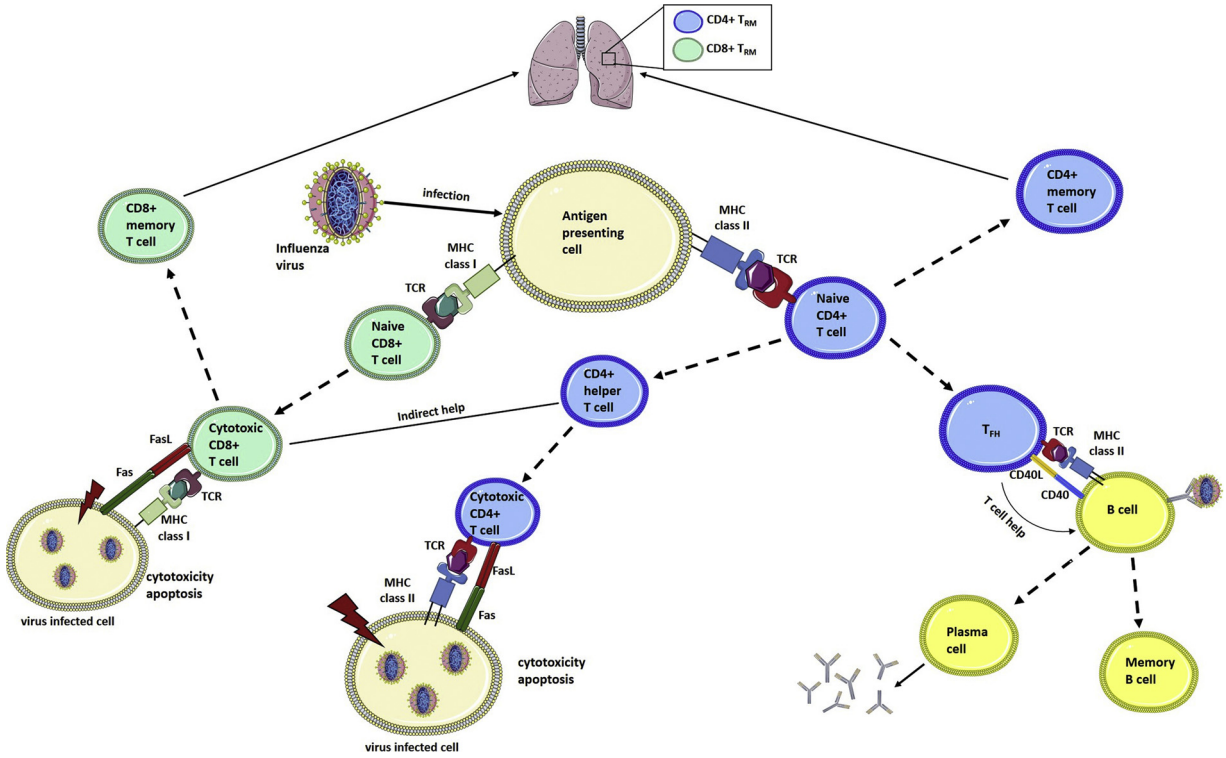

Fig. 1. Schematic representation of the cellular and humoral immune responses induced by influenza virus infection.
Influenza virus infection of antigen presenting cells leads to intracellular degradation of viral proteins producing peptide fragments, some of which are loaded onto MHC class I or class II molecules. The peptides presented by MHC class I or class II can be recognized by respectively CD8+ or CD4+ naïve T cells bearing TCRs of appropriate specificity. Upon engagement of the TCR by viral antigen and further signaling such as co-stimulation (e.g. via CD28; not shown) and cytokines (not shown), naïve T cells are activated. The activated T cells proliferate and differentiate into effector and memory T cells (dotted lines). The effector cells contribute to viral clearance, predominantly by recognizing and eliminating virus-infected cells and secreting pro-inflammatory and anti-viral cytokines. The memory T cells can be readily activated by a subsequent encounter with the cognate antigen and, upon further differentiation, exert their effector functions. The T_{FH} cells deliver help to B cells that differentiate into memory B cells and plasma cells that secrete high affinity antibodies against the target antigen. In the lung, locally produced mediators promote the development of lung CD4+ and CD8+ tissue resident memory T cells. The figure is an oversimplified schematic and was designed using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License: https://smart.servier.com/.
TCRs, T cell Receptors; TRM Tissue resident memory T cell; TFH T follicular helper T cells, MHC major histocompatibility complex.

terms of function, it is not yet clear if these two cytotoxic subsets have distinct or overlapping roles in protective immunity to influenza.

In humans, IV-specific CD4+ T cells recognize a variety of viral antigens including the HA and internal proteins [10,35,104–108]. Like IV-specific CD8+ T cells (see below), CD4+ T cells display a high degree of cross-reactivity with various IVs [28,35]. For example, CD4+ T cells that recognize conserved epitopes and that cross-react with avian and H1N1pdm09 virus have been reported [27,105,106,109–111].

It is known that single mutations within T cell epitopes can alter the MHC-II avidity and TCR binding, which are possible ways for the virus to evade recognition by CD4+ T cells [112–115]. Indeed, a single amino acid substitution identified in the HA of IV was associated with escape from recognition by IV-specific CD4+ T cells and not from recognition by antibodies [116]. Because $CD4^+$ T cells are critical for inducing and regulating effective T and B cell responses to IVs, full understanding of the respective CD4+ T cell population regarding their specificity, functions and frequency would aid rationale design of vaccines that induce protective CD4+ T cell responses.

2.2. CD8+ T cells

Animal studies have provided insight into the role of the CD8+ T cells in protective immunity against IV infections [25,117–119], reviewed in [120] and data are consistent with those reported later in human studies. Back in 1983, *McMichael et al.* had already demonstrated an inverse correlation between the IV-specific lytic activity of PBMCs and the extent of viral shedding after experimental infection of human volunteers that did not have antibodies to the challenge strain [121]. During the pandemic of 2009, it was shown that the frequency of pre-existing IV-specific CD8+ T cells inversely correlated with disease severity caused by infection with the H1N1pdm09 virus [34]. Furthermore, subjects infected with a H7N9 virus who showed a more rapid recovery from severe disease had early and prominent H7N9-specific CD8+ T cell responses compared to subjects with a prolonged recovery time [36].

Virus-specific CD8+ T cells are activated upon engagement of their TCR with peptide-MHC class-I complex. The main function of CD8+ cytotoxic T Lymphocytes (CTL) is to recognize and kill virus-infected cells by releasing perforin and granzyme B and inducing apoptosis via Fas/Fas ligand interaction [38,40] thus prevent more progeny virus being produced. Furthermore, activated CD8+ T cells produce pro-inflammatory cytokines like IFN-γ that inhibit viral replication [reviewed in 122].

IV-specific CD8+ T cells are mainly directed against the internal viral proteins [reviewed in [123]], which explains the high cross-reactivity of CTLs with IVs of different subtypes. Therefore, the induction of IV-specific CTL responses is considered a promising vaccination strategy for the induction of broadly protective immunity [25,124–127]. Due to the polymorphic nature of the HLA molecules, the set of peptides presented to T cells varies considerably between individuals. Consequently, there is heterogeneity in the specificity and magnitude of virus-specific T cell responses [128]. During infection, only a restricted number of the generated epitopes are immunogenic

and the response to these epitopes is usually observed in subjects expressing the same HLA alleles [reviewed in 12,129,130]. Despite the fact that the NP and M1 protein are relatively conserved, some CTL epitopes display variation [131]. It has been shown that amino acid substitutions at an anchor residue may result in a complete loss of epitope presentation whereas mutations within the TCR contact residues of the epitope may affect the recognition by specific CD8+ T cells [43,52,131–134]. Some epitopes remain conserved because mutations are not tolerated without loss of viral fitness [132,135,136] despite selective pressure against them exerted by T cells. One example of such an epitope is the $M1_{58-66}$ located in the M1 protein. Despite being immunodominant and presented by HLA-A*0201, an HLA- allele with high prevalence, this epitope is highly conserved, even across different subtypes of IAV. Most likely, the functional constraints are explained by the presence of an overlapping nuclear export domain [137].

In addition to mutations within epitopes, extra-epitopic amino acid residues that influence CD8+ T cell recognition have been identified [50]. Of interest, it was found that amino acid residues located outside the $M1_{58-66}$ epitope had an impact on the activation and lytic activity of $M1_{58-66}$ specific T cells. When the extra-epitopic residues were derived from M1 protein of a human IV then T cell activation was reduced compared to those derived from an avian IV. It was hypothesized that the reduced recognition of the epitope might reflect immune adaptation of human IV's, which was exemplified by better replicative capacity of human IV in the presence of $M1_{58-66}$ specific T cells [50].

Because IAV-specific CD8+ T cells preferentially recognize epitopes located in conserved proteins, they also contribute to heterosubtypic immunity. CD8+ T cells induced after infection with seasonal IVs cross-react with avian and swine IVs [24,26–28,138]. Recently, it was found that memory CD8+ T cells specific for epitopes located in PB1 cross-react with IAV, IBV and ICV, suggesting that they offer a degree of heterotypic immunity [139]. Thus, the identification of conserved epitopes could aid the development of a vaccine that affords broad and long-lasting protection against various IVs based on the induction of cross-reactive T cell responses. Studies in mice showed that CTLs induced by IAV infection are relatively long lived and persist in the lung after infection [47,140–144]. In humans, IAV-specific CD8+ T cells can be found in peripheral blood over a time-period of > 13 years [48] and have been found in the lung as well (see below) [145].

2.3. T_{RM} cells

During IV infection, some of the virus-specific T cells differentiate into Tissue resident memory T cells (T_{RM}) cells, which is regulated by the activity of certain transcription factors like Blimp-1 [146]. Active T_{RM} cells contribute to local protective immunity against reinfection with the same or heterologous viruses by remaining at the site of the primary infection [57,147,148]. T_{RM} cells, characterized by the expression of CD69 and CD103, can effectively eliminate virus-infected cells in the lung, thus limiting the progression of the disease [149].

Both CD4+ and CD8+ T cells are known to form T_{RM} cells, although the CD8+ T_{RM} subset has been better characterized in recent years because of their prominent role in heterosubtypic immunity. Upon recognizing virus-infected cells, the T_{RM} readily respond by killing the infected cells by producing anti-viral cytokines and promoting the recruitment of immune cells from the peripheral blood [150–155]. It has been reported that T_{RM} cells are derived from T Effector Memory (T_{EM}) cells rather than T Central Memory (T_{CM}) cells and some studies suggested that the conversion towards T_{RM} cells requires the presence of the antigen in lung tissues [147,156–158]. The local environment plays an important role in converting T_{EM} cells into T_{RM} cells. It has been found that IL-15, TGF-β and the activity of the transcription factors Blimp-1 and Homolog of Blimp-1 (Hobit) are important for the induction and maintenance of T_{RM} cells. On the other hand, downregulation of Eomes has been tied to T_{RM} conversion [146,147,159–161].

Studies in mice showed that T_{RM} in the upper respiratory tract (UTR) persist for a long time and were able to prevent spread of IV to the lung [162]. Additionally, vaccination with LAIV induced long-lasting T_{RM} cells in the lung and provided heterosubtypic protection [163]. The longevity of T_{RM} in mice was only found to last up to 7 months only. This might be a result of an imbalance between apoptosis of T_{RM} cells and conversion into new T_{RM} cells, since it was found that the ability of circulating CD8+ T cells to convert into T_{RM} cells declines over time. Of interest, this loss of T_{RM} cells correlated with a loss of protection [57,158]. The longevity of T_{RM} cells and heterosubtypic immunity was extended when mice were repeatedly exposed to the antigen [164]. Human studies showed that influenza-specific T_{RM} cells in the lung are highly proliferative, polyfunctional and display various TCR specificities, suggesting that these cells are important for an effective T cell response and protective immunity acting locally [165–167]. However, the lifespan of the human T_{RM} has not been reported yet.

3. Influenza vaccination strategies and management of immunity to influenza viruses

As mentioned above, seasonal influenza viruses display extensive antigenic drift, which necessitates annual updates of the vaccine composition and the selection of vaccine strains that match the epidemic strains antigenically. The selection of vaccine strains is based on antigenic characterization of newly emerging epidemic strains and epidemiological data. "Antigenic cartography" was developed as a tool to aid interpretation of the antigenic characterization data [8]. Using pre- and post-vaccination serum samples from human vaccinees, antibody landscapes were constructed which revealed that the use of antigenically advanced vaccine strains would not result in mismatching antibody response against virus from previous antigenic clusters, because these antibody responses were "back-boosted". It was therefore argued that the choice of novel antigenically distinct vaccine strains should have preference, even when these strains did not predominate the season in which the strain selection was made [168].

Difficulties in predicting the circulating IBV strains have prompted the development of quadrivalent influenza vaccines containing two IBV strains belonging to two antigenically distinct lineages, in addition to the two IAV strains of the current H1N1 and H3N2 subtypes [reviewed in [169–171]]. Of note, also Influenza B virus-specific CD8+ T cells display a high degree of cross-reactivity and the majority recognize viruses of both lineages [172].

Currently used seasonal influenza vaccines are inactivated (split or subunit) vaccines produced in embryonated chicken eggs or MDCK cells, recombinant HA protein produced in insect cells or cold- adapted (or temperature sensitive) live-attenuated vaccines (LAIV), which are administered intranasally.

In contrast to IIV, the LAIVs induce a multifaceted responses that include humoral mucosal antibodies (secretory IgA), as well as cellular immunity, especially CD8+ T cells [173]. The induction of T cell immunity by LAIV resides in the capacity of this vaccine to mimic a natural infection by replicating in the upper respiratory tract.

Replication in antigen presenting cells leads to de novo synthesis of viral proteins in the cytosol, consequently resulting in endogenous antigen processing and MHC class I restricted presentation of viral peptides to virus-specific CD8+ T cells. The LAIV also promotes T_{FH} proliferation in nasal-associated lymphoid tissue (NALT), which is crucial for an efficient antibody production [174]. In this regard, LAIV induces higher number of cross-reactive plasmablasts compared to IIV [175].

Interestingly, LAIV were more efficacious than IIV in young children and the virus-specific immunity was maintained up to one year in most pediatric study subjects [176–179]. The superior efficacy of LAIV in children seems to correlate with the induction of local and systemic T

cell responses [53,180–183].

Another consideration to use LAIV in children preferably is that the annual use of IIV from an early age (> 6 months) onward may prevent productive infection and consequently the induction of virus-specific CD8+ T cell responses. This was demonstrated in a mouse model, ferrets and in two groups of children that received annual vaccination with IIV or not [55,184–186]. In the animal models, the lack of infection-induced (cross-reactive) virus-specific CD8+ T cells caused by prior vaccination against a seasonal IV strain left mice and ferrets more susceptible to infection with highly pathogenic IV of the H5N1 subtype. Therefore, it was hypothesized that the use of LAIV in children should be preferred since this vaccine not only protects against seasonal influenza, but most likely also induces heterosubtypic immunity [187]. Accordingly, it was shown that children vaccinated with LAIV were protected against a drifted H3N2 strain that was not contained in the vaccine, highlighting the potential of LAIV to induce cross-reactive immunity [188].

Furthermore, the CD4+ T cell response to IIV in immunologically naïve children that had not been previously exposed to influenza virus, was reduced regarding IFN-γ production and breadth of viral antigen reactivity compared to that of young adults [189]. These data suggest that early immunological imprinting by IIV may skew the repertoire of virus-specific CD4+ T cells.

Although virus-specific T cells are appreciated for their role in protective immunity against influenza virus, it requires robust and standardized assays to quantitate their numbers, specificity and functionality to establish the correlates of protection and to define minimum thresholds of protection. Considering the high polymorphism of the human leucocyte antigens and consequently the T cell repertoires in individual subjects, this remains a challenge. Potentially, repertoire and RNAseq analysis at the single cell level may improve our understanding or the protective capacity of virus-specific T cell responses [190,191].

Funding

This work was funded by the Alexander von Humboldt Foundation in the framework of the Alexander von Humboldt Professorship endowed by the German Federal Ministry of Education and Research.

CRediT authorship contribution statement

Janina Jansen: Writing - original draft. **Thomas Gerlach:** Writing - review & editing. **Husni Elbahesh:** Writing - review & editing. **Guus F. Rimmelzwaan:** Conceptualization, Writing - review & editing, Supervision. **Giulietta Saletti:** Conceptualization, Writing - original draft, Supervision.

Declaration of Competing Interest

None of the authors declares a conflict of interest.

References

[1] WHO, WHO - Influenza Fact Sheet Burden of disease Vol. 2019 (2019).
[2] W. Putri, D.J. Muscatello, M.S. Stockwell, A.T. Newall, Economic burden of seasonal influenza in the United States, Vaccine 36 (2018) 3960–3966.
[3] WHO, Fact Sheet - Influenza (Seasonal) Vol. 2019 (2019).
[4] C.J. Murray, A.D. Lopez, B. Chin, D. Feehan, K.H. Hill, Estimation of potential global pandemic influenza mortality on the basis of vital registry data from the 1918–20 pandemic: a quantitative analysis, Lancet 368 (2006) 2211–2218.
[5] S.-W. Yoon, R.J. Webby, R.G. Webster, Evolution and ecology of influenza a viruses, in: R.W. Compans, M.B.A. Oldstone (Eds.), Influenza Pathogenesis and Control, Vol. I Springer International Publishing, 2014, pp. 359–375.
[6] B.F. Koel, D.F. Burke, T.M. Bestebroer, S. van der Vliet, G.C.M. Zondag, G. Vervaet, E. Skepner, N.S. Lewis, M.I.J. Spronken, C.A. Russell, et al., Substitutions near the receptor binding site determine major antigenic change during influenza virus evolution, Science 342 (2013) 976–979.
[7] B.F. Koel, R. Mögling, S. Chutinimitkul, P.L. Fraaij, D.F. Burke, S. van der Vliet, E. de Wit, T.M. Bestebroer, G.F. Rimmelzwaan, A.D. Osterhaus, et al., Identification of amino acid substitutions supporting antigenic change of influenza A(H1N1)pdm09 viruses, J. Virol. 89 (2015) 3763–3775.
[8] D.J. Smith, A.S. Lapedes, J.C. de Jong, T.M. Bestebroer, G.F. Rimmelzwaan, A.D.M.E. Osterhaus, R.A.M. Fouchier, Mapping the antigenic and genetic evolution of influenza virus, Science 305 (2004) 371–376.
[9] M.R. Sandbulte, K.B. Westgeest, J. Gao, X. Xu, A.I. Klimov, C.A. Russell, D.F. Burke, D.J. Smith, R.A.M. Fouchier, M.C. Eichelberger, Discordant antigenic drift of neuraminidase and hemagglutinin in H1N1 and H3N2 influenza viruses, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 20748–20753.
[10] E. Assarsson, H.H. Bui, J. Sidney, Q. Zhang, J. Glenn, C. Oseroff, I.N. Mbawuike, J. Alexander, M.J. Newman, H. Grey, et al., Immunomic analysis of the repertoire of T-cell specificities for influenza A virus in humans, J. Virol. 82 (2008) 12241–12251.
[11] A.C. Hayward, L. Wang, N. Goonetilleke, E.B. Fragaszy, A. Bermingham, A. Copas, O. Dukes, E.R. Millett, I. Nazareth, J.S. Nguyen-Van-Tam, et al., Natural t cell-mediated protection against seasonal and pandemic influenza. Results of the flu watch cohort study, Am. J. Respir. Crit. Care Med. 191 (2015) 1422–1431.
[12] A. Wahl, F. Schafer, W. Bardet, R. Buchli, G.M. Air, W.H. Hildebrand, HLA class I molecules consistently present internal influenza epitopes, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 540–545.
[13] G.S. Tan, P.E. Leon, R.A. Albrecht, I. Margine, A. Hirsh, J. Bahl, F. Krammer, Broadly-reactive neutralizing and non-neutralizing antibodies directed against the H7 influenza virus hemagglutinin reveal divergent mechanisms of protection, PLoS Pathog. 12 (2016) e1005578-e1005578.
[14] F. Krammer, P. Palese, Influenza virus hemagglutinin stalk-based antibodies and vaccines, Curr. Opin. Virol. 3 (2013) 521–530.
[15] D. Corti, A.L. Suguitan Jr., D. Pinna, C. Silacci, B.M. Fernandez-Rodriguez, F. Vanzetta, C. Santos, C.J. Luke, F.J. Torres-Velez, N.J. Temperton, et al., Heterosubtypic neutralizing antibodies are produced by individuals immunized with a seasonal influenza vaccine, J. Clin. Invest. 120 (2010) 1663–1673.
[16] S. Jegaskanda, E.R. Job, M. Kramski, K. Laurie, G. Isitman, R. de Rose, W.R. Winnall, I. Stratov, A.G. Brooks, P.C. Reading, et al., Cross-reactive influenza-specific antibody-dependent cellular cytotoxicity antibodies in the absence of neutralizing antibodies, J. Immunol. 190 (2013) 1837–1848.
[17] R.D. de Vries, N.J. Nieuwkoop, M. Pronk, E. de Bruin, G. Leroux-Roels, E.G.W. Huijskens, R.S. van Binnendijk, F. Krammer, M.P.G. Koopmans, G.F. Rimmelzwaan, Influenza virus-specific antibody dependent cellular cytoxicity induced by vaccination or natural infection, Vaccine 35 (2017) 238–247.
[18] R.D. de Vries, N.J. Nieuwkoop, F.R.M. van der Klis, M.P.G. Koopmans, F. Krammer, Rimmelzwaan GF: primary human influenza B virus infection induces cross-lineage hemagglutinin stalk-specific antibodies mediating antibody-dependent cellular cytoxicity, J. Infect. Dis. 217 (2018) 3–11.
[19] S.R. Christensen, S.A. Toulmin, T. Griesman, L.E. Lamerato, J.G. Petrie, E.T. Martin, A.S. Monto, S.E. Hensley, Assessing the protective potential of H1N1 influenza virus hemagglutinin head and stalk antibodies in humans, J. Virol. 93 (2019) e02134–02118.
[20] V.N. Petrova, C.A. Russell, The evolution of seasonal influenza viruses, Nat. Rev. Microbiol. 16 (2018) 60.
[21] H. Xie, X.F. Wan, Z. Ye, E.P. Plant, Y. Zhao, Y. Xu, X. Li, C. Finch, N. Zhao, T. Kawano, et al., H3N2 mismatch of 2014-15 northern hemisphere influenza vaccines and head-to-head comparison between human and ferret antisera derived antigenic maps, Sci. Rep. 5 (2015) 15279.
[22] D. Vijaykrishna, L.L.M. Poon, H.C. Zhu, S.K. Ma, O.T.W. Li, C.L. Cheung, G.J.D. Smith, J.S.M. Peiris, Y. Guan, Reassortment of pandemic H1N1/2009 influenza A virus in swine, Science 328 (2010) 1529-1529.
[23] G.T. Belz, W. Xie, P.C. Doherty, Diversity of epitope and cytokine profiles for primary and secondary influenza a virus-specific CD8+ T cell responses, J. Immunol. 166 (2001) 4627–4633.
[24] C.E. van de Sandt, J.H. Kreijtz, G. de Mutsert, M.M. Geelhoed-Mieras, M.L. Hillaire, S.E. Vogelzang-van Trierum, A.D. Osterhaus, R.A. Fouchier, G.F. Rimmelzwaan, Human cytotoxic T lymphocytes directed to seasonal influenza A viruses cross-react with the newly emerging H7N9 virus, J. Virol. 88 (2014) 1684–1693.
[25] J.W. Yewdell, J.R. Bennink, G.L. Smith, B. Moss, Influenza A virus nucleoprotein is a major target antigen for cross-reactive anti-influenza A virus cytotoxic T lymphocytes, Proc. Natl. Acad. Sci. U. S. A. 82 (1985) 1785–1789.
[26] J.H.C.M. Kreijtz, G. de Mutsert, C.A. van Baalen, R.A.M. Fouchier, A.D.M.E. Osterhaus, G.F. Rimmelzwaan, Cross-recognition of avian H5N1 influenza virus by human cytotoxic T-lymphocyte populations directed to human influenza A virus, Pathogenesis Immun. 82 (2008) 5161–5166.
[27] L.Y. Lee, L.A. Ha do, C. Simmons, M.D. de Jong, N.V. Chau, R. Schumacher, Y.C. Peng, A.J. McMichael, J.J. Farrar, G.L. Smith, et al., Memory T cells established by seasonal human influenza A infection cross-react with avian influenza A (H5N1) in healthy individuals, J. Clin. Invest. 118 (2008) 3478–3490.
[28] M.L. Hillaire, S.E. van Trierum, J.H. Kreijtz, R. Bodewes, M.M. Geelhoed-Mieras, N.J. Nieuwkoop, R.A. Fouchier, T. Kuiken, A.D. Osterhaus, G.F. Rimmelzwaan, Cross-protective immunity against influenza pH1N1 2009 viruses induced by seasonal influenza A (H3N2) virus is mediated by virus-specific T-cells, J. Gen. Virol. 92 (2011) 2339–2349.
[29] J. Jameson, J. Cruz, M. Terajima, F.A. Ennis, Human CD8+ and CD4+ T lymphocyte memory to influenza a viruses of swine and avian species, J. Immunol. 162 (1999) 7578–7583.
[30] S.N. Mueller, T. Gebhardt, F.R. Carbone, W.R. Heath, Memory T cell subsets, migration patterns, and tissue residence, Annu. Rev. Immunol. 31 (2013) 137–161.

[31] S.C. Jameson, D. Masopust, Understanding subset diversity in T cell memory, Immunity 48 (2018) 214–226.
[32] F. Sallusto, D. Lenig, R. Förster, M. Lipp, A. Lanzavecchia, Two subsets of memory T lymphocytes with distinct homing potentials and effector functions, Nature 401 (1999) 708–712.
[33] M.M. Hufford, T.S. Kim, J. Sun, T.J. Braciale, The effector T cell response to influenza infection, Curr. Top. Microbiol. Immunol. 386 (2015) 423–455.
[34] S. Sridhar, S. Begom, A. Bermingham, K. Hoschler, W. Adamson, W. Carman, T. Bean, W. Barclay, J.J. Deeks, A. Lalvani, Cellular immune correlates of protection against symptomatic pandemic influenza, Nat. Med. 19 (2013) 1305–1312.
[35] T.M. Wilkinson, C.K. Li, C.S. Chui, A.K. Huang, M. Perkins, J.C. Liebner, R. Lambkin-Williams, A. Gilbert, J. Oxford, B. Nicholas, et al., Preexisting influenza-specific CD4+ T cells correlate with disease protection against influenza challenge in humans, Nat. Med. 18 (2012) 274–280.
[36] Z. Wang, Y. Wan, C. Qiu, S. Quinones-Parra, Z. Zhu, L. Loh, D. Tian, Y. Ren, Y. Hu, X. Zhang, et al., Recovery from severe H7N9 disease is associated with diverse response mechanisms dominated by CD8(+) T cells, Nat. Commun. 6 (2015) 6833.
[37] J. Spitaels, K. Roose, X. Saelens, Influenza and memory t cells: how to awake the force, Vaccines 4 (2016).
[38] D.J. Topham, R.A. Tripp, P.C. Doherty, CD8+ T cells clear influenza virus by perforin or Fas-dependent processes, J. Immunol. 159 (1997) 5197–5200.
[39] L. Xu, H. Yoon, M.Q. Zhao, J. Liu, C.V. Ramana, R.I. Enelow, Cutting edge: pulmonary immunopathology mediated by antigen-specific expression of TNF-α by antiviral CD8+ T cells, J. Immunol. 173 (2004) 721–725.
[40] G.E. Price, L. Huang, R. Ou, M. Zhang, D. Moskophidis, Perforin and Fas cytolytic pathways coordinately shape the selection and diversity of CD8+-T-cell escape variants of influenza virus, J. Virol. 79 (2005) 8545–8559.
[41] J.A. Juno, D. van Bockel, S.J. Kent, A.D. Kelleher, J.J. Zaunders, C.M. Munier, Cytotoxic CD4 T cells-friend or foe during viral infection? Front. Immunol. 8 (2017) 19.
[42] A. Takeuchi, T. Saito, CD4 CTL, a cytotoxic subset of CD4(+) t cells, their differentiation and function, Front. Immunol. 8 (2017) 194-194.
[43] E.G.M. Berkhoff, A.C.M. Boon, N.J. Nieuwkoop, R.A.M. Fouchier, K. Sintnicolaas, Osterhaus ADME, G.F. Rimmelzwaan, A mutation in the HLA-B*2705-restricted NP 383-391 epitope affects the human influenza A virus-specific cytotoxic T-lymphocyte response in vitro, Pathogenesis Immun. 78 (2004) 5216–5222.
[44] A.C.M. Boon, G. de Mutsert, R.A.M. Fouchier, A.D.M.E. Osterhaus, G.F. Rimmelzwaan, The hypervariable immunodominant NP418-426 epitope from the influenza A virus nucleoprotein is recognized by cytotoxic T lymphocytes with high functional avidity, J. Virol. 80 (2006) 6024–6032.
[45] A.C.M. Boon, G. de Mutsert, D. van Baarle, D.J. Smith, A.S. Lapedes, R.A.M. Fouchier, K. Sintnicolaas, A.D.M.E. Osterhaus, G.F. Rimmelzwaan, Recognition of homo- and heterosubtypic variants of influenza A viruses by human CD8+ T lymphocytes, J Immunol. 172 (2004) 2453–2460.
[46] M.L.B. Hillaire, S.E. Vogelzang-van Trierum, J.H.C.M. Kreijtz, G. de Mutsert, R.A.M. Fouchier, A.D.M.E. Osterhaus, G.F. Rimmelzwaan, Human T-cells directed to seasonal influenza A virus cross-react with 2009 pandemic influenza A (H1N1) and swine-origin triple-reassortant H3N2 influenza viruses, J Gen Virol. 94 (2013) 583–592.
[47] Kreijtz JHCM, R. Bodewes, J.M.A. van den Brand, G. de Mutsert, C. Baas, G. van Amerongen, R.A.M. Fouchier, A.D.M.E. Osterhaus, G.F. Rimmelzwaan, Infection of mice with a human influenza A/H3N2 virus induces protective immunity against lethal infection with influenza A/H5N1 virus, Vaccine 27 (2009) 4983–4989.
[48] C.E. van de Sandt, M.L. Hillaire, M.M. Geelhoed-Mieras, A.D. Osterhaus, R.A. Fouchier, Rimmelzwaan GF: human influenza a virus-specific CD8+ T-cell response is long-lived, J. Infect. Dis. 212 (2015) 81–85.
[49] C.E. van de Sandt, J.H. Kreijtz, M.M. Geelhoed-Mieras, N.J. Nieuwkoop, M.I. Spronken, D.A. van de Vijver, R.A. Fouchier, A.D. Osterhaus, G.F. Rimmelzwaan, Differential recognition of influenza a viruses by M158-66 epitope-specific CD8+ T cells is determined by extraepitopic amino acid residues, J. Virol. 90 (2016) 1009–1022.
[50] C.E. van de Sandt, M.R. Pronk, C.A. van Baalen, R.A.M. Fouchier, G.F. Rimmelzwaan, Variation at extra-epitopic amino acid residues influences suppression of influenza virus replication by M158-66 epitope-specific CD8(+) T lymphocytes, J. Virol. 92 (2018).
[51] C.E. van de Sandt, K.A. Sagong, M.R. Pronk, T.M. Bestebroer, M.I. Spronken, M.P.G. Koopmans, R.A.M. Fouchier, G.F. Rimmelzwaan, H1N1pdm09 influenza virus and its descendants lack extra-epitopic amino acid residues associated with reduced recognition by M158-66-specific CD8+ T cells, J. Infect. Dis. 218 (2018) 581–585.
[52] J.T.M. Voeten, T.M. Bestebroer, N.J. Nieuwkoop, R.A.M. Fouchier, A.D.M.E. Osterhaus, G.F. Rimmelzwaan, Antigenic drift in the influenza A virus (H3N2) nucleoprotein and escape from recognition by cytotoxic T lymphocytes, J. Virol. 74 (2000) 6800–6807.
[53] X.S. He, T.H. Holmes, C. Zhang, K. Mahmood, G.W. Kemble, D.B. Lewis, C.L. Dekker, H.B. Greenberg, A.M. Arvin, Cellular immune responses in children and adults receiving inactivated or live attenuated influenza vaccines, J. Virol. 80 (2006) 11756–11766.
[54] B.D. Forrest, M.W. Pride, A.J. Dunning, M.R.Z. Capeding, T. Chotpitayasunondh, J.S. Tam, R. Rappaport, J.H. Eldridge, W.C. Gruber, Correlation of cellular immune responses with protection against culture-confirmed influenza virus in young children, Clin. Vaccine Immunol. 15 (2008) 1042–1053.
[55] R. Bodewes, J.H. Kreijtz, C. Baas, M.M. Geelhoed-Mieras, G. de Mutsert, G. van Amerongen, J.M. van den Brand, R.A. Fouchier, A.D. Osterhaus, G.F. Rimmelzwaan, Vaccination against human influenza A/H3N2 virus prevents the induction of heterosubtypic immunity against lethal infection with avian influenza A/H5N1 virus, PLoS One 4 (2009) e5538.
[56] A. Pizzolla, T.H.O. Nguyen, J.M. Smith, A.G. Brooks, K. Kedzierska, W.R. Heath, P.C. Reading, L.M. Wakim, Resident memory CD8(+) T cells in the upper respiratory tract prevent pulmonary influenza virus infection, Sci. Immunol. 2 (2017).
[57] T. Wu, Y. Hu, Y.T. Lee, K.R. Bouchard, A. Benechet, K. Khanna, L.S. Cauley, Lung-resident memory CD8 T cells (TRM) are indispensable for optimal cross-protection against pulmonary virus infection, J. Leukoc. Biol. 95 (2014) 215–224.
[58] H. Elbahesh, G. Saletti, T. Gerlach, G.F. Rimmelzwaan, Broadly protective influenza vaccines: design and production platforms, Curr. Opin. Virol. 34 (2019) 1–9.
[59] K.K. McKinstry, T.M. Strutt, Y. Kuang, D.M. Brown, S. Sell, R.W. Dutton, S.L. Swain, Memory CD4+ T cells protect against influenza through multiple synergizing mechanisms, J. Clin. Invest. 122 (2012) 2847–2856.
[60] S.L. Swain, K.K. McKinstry, T.M. Strutt, Expanding roles for $CD4^+$ T cells in immunity to viruses, Nat. Rev. Immunol. 12 (2012) 136–148.
[61] A.J. Sant, A.T. DiPiazza, J.L. Nayak, A. Rattan, K.A. Richards, CD4 T cells in protection from influenza virus: viral antigen specificity and functional potential, Immunol. Rev. 284 (2018) 91–105.
[62] P. Devarajan, M.C. Jones, O. Kugler-Umana, A.M. Vong, J. Xia, S.L. Swain, Pathogen recognition by CD4 effectors drives key effector and most memory cell generation against respiratory virus, Front. Immunol. 9 (2018) 596-596.
[63] C.G. Vinuesa, M.A. Linterman, D. Yu, I.C. MacLennan, Follicular helper T cells, Annu. Rev. Immunol. 34 (2016) 335–368.
[64] C.G. de Vinuesa, M.C. Cook, J. Ball, M. Drew, Y. Sunners, M. Cascalho, M. Wabl, G.G. Klaus, I.C. MacLennan, Germinal centers without T cells, J. Exp. Med. 191 (2000) 485–494.
[65] M. Stebegg, S.D. Kumar, A. Silva-Cayetano, V.R. Fonseca, M.A. Linterman, L. Graca, Regulation of the germinal center response, Front. Immunol. 9 (2018) 2469-2469.
[66] D. Breitfeld, L. Ohl, E. Kremmer, J. Ellwart, F. Sallusto, M. Lipp, R. Förster, Follicular B helper T cells express Cxc chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production, J. Exp. Med. 192 (2000) 1545–1552.
[67] S. Keck, M. Schmaler, S. Ganter, L. Wyss, S. Oberle, E.S. Huseby, D. Zehn, C.G. King, Antigen affinity and antigen dose exert distinct influences on CD4 T-cell differentiation, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 14852–14857.
[68] N. Fazilleau, L.J. McHeyzer-Williams, H. Rosen, M.G. McHeyzer-Williams, The function of follicular helper T cells is regulated by the strength of T cell antigen receptor binding, Nat. Immunol. 10 (2009) 375–384.
[69] V. Krishnamoorthy, S. Kannanganat, M. Maienschein-Cline, S.L. Cook, J. Chen, N. Bahroos, E. Sievert, E. Corse, A. Chong, R. Sciammas, The IRF4 gene regulatory module functions as a read-write integrator to dynamically coordinate T helper cell fate, Immunity 47 (2017) 481–497 e487.
[70] D.T. O'Hagan, MF59 is a safe and potent vaccine adjuvant that enhances protection against influenza virus infection, Expert Rev. Vaccines 6 (2007) 699–710.
[71] A.L. Wilkins, D. Kazmin, G. Napolitani, E.A. Clutterbuck, B. Pulendran, C.-A. Siegrist, A.J. Pollard, AS03- and MF59-adjuvanted influenza vaccines in children, Front. Immunol. 8 (2017).
[72] R. Cioncada, M. Maddaluno, H.T.M. Vo, M. Woodruff, S. Tavarini, C. Sammicheli, M. Tortoli, A. Pezzicoli, E. De Gregorio, M.C. Carroll, et al., Vaccine adjuvant MF59 promotes the intranodal differentiation of antigen-loaded and activated monocyte-derived dendritic cells, PLoS One 12 (2017) e0185843-e0185843.
[73] B. Mastelic Gavillet, C.S. Eberhardt, F. Auderset, F. Castellino, A. Seubert, J.S. Tregoning, P.-H. Lambert, E. de Gregorio, G. Del Giudice, C.-A. Siegrist, MF59 mediates its B cell adjuvanticity by promoting t follicular helper cells and thus germinal center responses in adult and early life, J. Immunol. 194 (2015) 4836.
[74] F. Spensieri, E. Siena, E. Borgogni, L. Zedda, R. Cantisani, N. Chiappini, F. Schiavetti, D. Rosa, F. Castellino, E. Montomoli, et al., Early rise of blood T follicular helper cell subsets and baseline immunity as predictors of persisting late functional antibody responses to vaccination in humans, PLoS One 11 (2016) e0157066.
[75] J. He, L.M. Tsai, Y.A. Leong, X. Hu, C.S. Ma, N. Chevalier, X. Sun, K. Vandenberg, S. Rockman, Y. Ding, et al., Circulating precursor CCR7(lo)PD-1(hi) CXCR5(+) CD4(+) T cells indicate Tfh cell activity and promote antibody responses upon antigen reexposure, Immunity 39 (2013) 770–781.
[76] S.E. Bentebibel, S. Khurana, N. Schmitt, P. Kurup, C. Mueller, G. Obermoser, A.K. Palucka, R.A. Albrecht, A. Garcia-Sastre, H. Golding, et al., ICOS(+)PD-1(+)CXCR3(+) T follicular helper cells contribute to the generation of high-avidity antibodies following influenza vaccination, Sci. Rep. 6 (2016) 26494.
[77] M. Koutsakos, A.K. Wheatley, L. Loh, E.B. Clemens, S. Sant, S. Nussing, A. Fox, A.W. Chung, K.L. Laurie, A.C. Hurt, et al., Circulating TFH cells, serological memory, and tissue compartmentalization shape human influenza-specific B cell immunity, Sci. Transl. Med. 10 (2018).
[78] P. Devarajan, B. Bautista, A.M. Vong, K.K. McKinstry, T.M. Strutt, S.L. Swain, New insights into the generation of CD4 memory may shape future vaccine strategies for influenza, Front. Immunol. 7 (2016) 136.
[79] C.S. Ma, E.K. Deenick, M. Batten, S.G. Tangye, The origins, function, and regulation of T follicular helper cells, J. Exp. Med. 209 (2012) 1241–1253.
[80] R.S. Herati, A. Muselman, L. Vella, B. Bengsch, K. Parkhouse, D. Del Alcazar, J. Kotzin, S.A. Doyle, P. Tebas, S.E. Hensley, et al., Successive annual influenza vaccination induces a recurrent oligoclonotypic memory response in circulating T follicular helper cells, Sci. Immunol. 2 (2017).
[81] A. Heit, F. Schmitz, S. Gerdts, B. Flach, M.S. Moore, J.A. Perkins, H.S. Robins, A. Aderem, P. Spearman, G.D. Tomaras, et al., Vaccination establishes clonal relatives of germinal center T cells in the blood of humans, J. Exp. Med. 214 (2017)

2139–2152.
[82] J.L. Nayak, T.F. Fitzgerald, K.A. Richards, H. Yang, J.J. Treanor, A.J. Sant, CD4+ T-cell expansion predicts neutralizing antibody responses to monovalent, inactivated 2009 pandemic influenza A(H1N1) virus subtype H1N1 vaccine, J. Infect. Dis. 207 (2013) 297–305.
[83] J.L. Nayak, K.A. Richards, H. Yang, J.J. Treanor, A.J. Sant, Effect of influenza A(H5N1) vaccine prepandemic priming on CD4+ T-cell responses, J. Infect. Dis. 211 (2015) 1408–1417.
[84] S. Alam, Z.A.G. Knowlden, M.Y. Sangster, A.J. Sant, CD4 T cell help is limiting and selective during the primary B cell response to influenza virus infection, J. Virol. 88 (2014) 314–324.
[85] J.C. Sun, M.J. Bevan, Defective CD8 T cell memory following acute infection without CD4 T cell help, Science 300 (2003) 339–342.
[86] D.J. Shedlock, H. Shen, Requirement for CD4 T cell help in generating functional CD8 T cell memory, Science 300 (2003) 337–339.
[87] J.M. Riberdy, J.P. Christensen, K. Branum, P.C. Doherty, Diminished primary and secondary influenza virus-specific CD8(+) T-cell responses in CD4-depleted Ig(-/-) mice, J. Virol. 74 (2000) 9762–9765.
[88] G.T. Belz, D. Wodarz, G. Diaz, M.A. Nowak, P.C. Doherty, Compromised influenza virus-specific CD8(+)-T-cell memory in CD4(+)-T-cell-deficient mice, J. Virol. 76 (2002) 12388–12393.
[89] H. Beuneu, Z. Garcia, P. Bousso, Cutting edge: cognate CD4 help promotes recruitment of antigen-specific CD8 T cells around dendritic cells, J. Immunol. 177 (2006) 1406–1410.
[90] S.R. Bennett, F.R. Carbone, F. Karamalis, J.F. Miller, W.R. Heath, Induction of a CD8+ cytotoxic T lymphocyte response by cross-priming requires cognate CD4+ T cell help, J. Exp. Med. 186 (1997) 65–70.
[91] Y. Kumamoto, L.M. Mattei, S. Sellers, G.W. Payne, A. Iwasaki, CD4+ T cells support cytotoxic T lymphocyte priming by controlling lymph node input, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 8749–8754.
[92] B.J. Laidlaw, N. Zhang, H.D. Marshall, M.M. Staron, T. Guan, Y. Hu, L.S. Cauley, J. Craft, S.M. Kaech, CD4+ T cell help guides formation of CD103+ lung-resident memory CD8+ T cells during influenza viral infection, Immunity 41 (2014) 633–645.
[93] M.J. Bevan, Helping the CD8+ T-cell response, Nat. Rev. Immunol. 4 (2004) 595.
[94] B.J. Laidlaw, J.E. Craft, S.M. Kaech, The multifaceted role of CD4(+) T cells in CD8(+) T cell memory, Nat. Rev. Immunol. 16 (2016) 102–111.
[95] B. Rocha, C. Tanchot, Towards a cellular definition of CD8+ T-cell memory: the role of CD4+ T-cell help in CD8+ T-cell responses, Curr. Opin. Immunol. 16 (2004) 259–263.
[96] J.G. Cullen, H.A. McQuilten, K.M. Quinn, M. Olshansky, B.E. Russ, A. Morey, S. Wei, J.E. Prier, N.L. La Gruta, P.C. Doherty, et al., CD4(+) T help promotes influenza virus-specific CD8(+) T cell memory by limiting metabolic dysfunction, Proc. Natl. Acad. Sci. U. S. A. 116 (2019) 4481–4488.
[97] T. Ahrends, A. Spanjaard, B. Pilzecker, N. Babala, A. Bovens, Y. Xiao, H. Jacobs, J. Borst, CD4(+) t cell help confers a cytotoxic T cell effector program including coinhibitory receptor downregulation and increased tissue invasiveness, Immunity 47 (2017) 848–861 e845.
[98] D.M. Brown, A.T. Lampe, A.M. Workman, The differentiation and protective function of cytolytic CD4 T cells in influenza infection, Front. Immunol. 7 (2016) 93.
[99] D.M. Brown, A.M. Dilzer, D.L. Meents, S.L. Swain, CD4 T cell-mediated protection from lethal influenza: perforin and antibody-mediated mechanisms give a one-two punch, J. Immunol. 177 (2006) 2888–2898.
[100] D.M. Brown, C. Kamperschroer, A.M. Dilzer, D.M. Roberts, S.L. Swain, IL-2 and antigen dose differentially regulate perforin- and FasL-mediated cytolytic activity in antigen specific CD4+ T cells, Cell. Immunol. 257 (2009) 69–79.
[101] L. Hua, S. Yao, D. Pham, L. Jiang, J. Wright, D. Sawant, A.L. Dent, T.J. Braciale, M.H. Kaplan, J. Sun, Cytokine-dependent induction of CD4+ T cells with cytotoxic potential during influenza virus infection, J. Virol. 87 (2013) 11884–11893.
[102] A. Takeuchi, S. Badr Mel, K. Miyauchi, C. Ishihara, R. Onishi, Z. Guo, Y. Sasaki, H. Ike, A. Takumi, N.M. Tsuji, et al., CRTAM determines the CD4+ cytotoxic T lymphocyte lineage, J. Exp. Med. 213 (2016) 123–138.
[103] N.B. Marshall, A.M. Vong, P. Devarajan, M.D. Brauner, Y. Kuang, R. Nayar, E.A. Schutten, C.H. Castonguay, L.J. Berg, S.L. Nutt, et al., NKG2C/E marks the unique cytotoxic CD4 T cell subset, ThCTL, generated by influenza infection, J. Immunol. 198 (2017) 1142–1155.
[104] J.A. Babon, J. Cruz, L. Orphin, P. Pazoles, M.D. Co, F.A. Ennis, M. Terajima, Genome-wide screening of human T-cell epitopes in influenza A virus reveals a broad spectrum of CD4(+) T-cell responses to internal proteins, hemagglutinins, and neuraminidases, Hum. Immunol. 70 (2009) 711–721.
[105] K.A. Richards, F.A. Chaves, A.J. Sant, Infection of HLA-DR1 transgenic mice with a human isolate of influenza a virus (H1N1) primes a diverse CD4 T-cell repertoire that includes CD4 T cells with heterosubtypic cross-reactivity to avian (H5N1) influenza virus, J. Virol. 83 (2009) 6566–6577.
[106] K.A. Richards, J. Nayak, F.A. Chaves, A. DiPiazza, Z.A. Knowlden, S. Alam, J.J. Treanor, A.J. Sant, Seasonal influenza can poise hosts for CD4 T-cell immunity to H7N9 avian influenza, J. Infect. Dis. 212 (2015) 86–94.
[107] M. Savic, J.L. Dembinski, Y. Kim, G. Tunheim, R.J. Cox, F. Oftung, B. Peters, S. Mjaaland, Epitope specific T-cell responses against influenza A in a healthy population, Immunology 147 (2016) 165–177.
[108] L. Chen, D. Zanker, K. Xiao, C. Wu, Q. Zou, W. Chen, Immunodominant CD4+ T-cell responses to influenza A virus in healthy individuals focus on matrix 1 and nucleoprotein, J. Virol. 88 (2014) 11760–11773.
[109] K.M. Gostic, M. Ambrose, M. Worobey, J.O. Lloyd-Smith, Potent protection against H5N1 and H7N9 influenza via childhood hemagglutinin imprinting, Science 354 (2016) 722–726.
[110] S. Alam, A.J. Sant, Infection with seasonal influenza virus elicits CD4 T cells specific for genetically conserved epitopes that can be rapidly mobilized for protective immunity to pandemic H1N1 influenza virus, J. Virol. 85 (2011) 13310–13321.
[111] J.T. Weinfurter, K. Brunner, S.V. Capuano 3rd, C. Li, K.W. Broman, Y. Kawaoka, T.C. Friedrich, Cross-reactive T cells are involved in rapid clearance of 2009 pandemic H1N1 influenza virus in nonhuman primates, PLoS Pathog. 7 (2011) e1002381.
[112] C.A. Lazarski, F.A. Chaves, S.A. Jenks, S. Wu, K.A. Richards, J.M. Weaver, A.J. Sant, The kinetic stability of MHC class II:peptide complexes is a key parameter that dictates immunodominance, Immunity 23 (2005) 29–40.
[113] C.A. Lazarski, F.A. Chaves, A.J. Sant, The impact of DM on MHC class II-restricted antigen presentation can be altered by manipulation of MHC-peptide kinetic stability, J. Exp. Med. 203 (2006) 1319–1328.
[114] A.J. Sant, F.A. Chaves, S.A. Jenks, K.A. Richards, P. Menges, J.M. Weaver, C.A. Lazarski, The relationship between immunodominance, DM editing, and the kinetic stability of MHC class II:peptide complexes, Immunol Rev. 207 (2005) 261–278.
[115] C.E. Van de Sandt, J.H.C.M. Kreijtz, G.F. Rimmelzwaan, Evasion of influenza a viruses from innate and adaptive immune responses, Viruses 4 (2012).
[116] E.G.M. Berkhoff, M.M. Geelhoed-Mieras, M. Jonges, D.J. Smith, R.A.M. Fouchier, A.D.M.E. Osterhaus, G.F. Rimmelzwaan, An amino acid substitution in the influenza A virus hemagglutinin associated with escape from recognition by human virus-specific CD4+ T-cells, Virus Res. 126 (2007) 282–287.
[117] B.S. Bender, T. Croghan, L. Zhang, P.A. Small Jr., Transgenic mice lacking class I major histocompatibility complex-restricted T cells have delayed viral clearance and increased mortality after influenza virus challenge, J. Exp. Med. 175 (1992) 1143–1145.
[118] P.C. Doherty, D.J. Topham, R.A. Tripp, Establishment and persistence of virus-specific CD4+ and CD8+ T cell memory, Immunol Rev. 150 (1996) 23–44.
[119] M.A. Wells, P. Albrecht, F.A. Ennis, Recovery from a viral respiratory infection. I. Influenza pneumonia in normal and T-deficient mice, J Immunol. 126 (1981) 1036–1041.
[120] A.F. Altenburg, G.F. Rimmelzwaan, R.D. de Vries, Virus-specific T cells as correlate of (cross-)protective immunity against influenza, Vaccine 33 (2015) 500–506.
[121] A.J. McMichael, F.M. Gotch, D.W. Dongworth, A. Clark, C.W. Potter, Declining T-cell immunity to influenza, 1977–82, Lancet 2 (1983) 762–764.
[122] J.H. Kreijtz, R.A. Fouchier, G.F. Rimmelzwaan, Immune responses to influenza virus infection, Virus Res. 162 (2011) 19–30.
[123] E.B. Clemens, C. van de Sandt, S.S. Wong, L.M. Wakim, S.A. Valkenburg, Harnessing the power of T cells: the promising hope for a universal influenza vaccine, Vaccines (Basel) 6 (2018).
[124] F. Gotch, A. McMichael, G. Smith, B. Moss, Identification of viral molecules recognized by influenza-specific human cytotoxic T lymphocytes, J. Exp. Med. 165 (1987) 408–416.
[125] J. Jameson, J. Cruz, F.A. Ennis, Human cytotoxic T-lymphocyte repertoire to influenza A viruses, J. Virol. 72 (1998) 8682–8689.
[126] A.J. McMichael, F.M. Gotch, G.R. Noble, P.A. Beare, Cytotoxic T-cell immunity to influenza, N. Engl. J. Med. 309 (1983) 13–17.
[127] M. Wang, K. Lamberth, M. Harndahl, G. Roder, A. Stryhn, M.V. Larsen, M. Nielsen, C. Lundegaard, S.T. Tang, M.H. Dziegiel, et al., CTL epitopes for influenza A including the H5N1 bird flu; genome-, pathogen-, and HLA-wide screening, Vaccine 25 (2007) 2823–2831.
[128] A.C.M. Boon, G. de Mutsert, Y.M.F. Graus, R.A.M. Fouchier, K. Sintnicolaas, A.D.M.E. Osterhaus, G.F. Rimmelzwaan, 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. 76 (2002) 582–590.
[129] J.W. Yewdell, J.R. Bennink, Immunodominance in major histocompatibility complex class I-restricted T lymphocyte responses, Annu. Rev. Immunol. 17 (1999) 51–88.
[130] E. Grant, C. Wu, K.-F. Chan, S. Eckle, M. Bharadwaj, Q.M. Zou, K. Kedzierska, W. Chen, Nucleoprotein of influenza A virus is a major target of immunodominant CD8+ T-cell responses, Immunol Cell Biol. 91 (2013) 184–194.
[131] H.M. Machkovech, T. Bedford, M.A. Suchard, J.D. Bloom, Positive selection in CD8+ T-cell epitopes of influenza virus nucleoprotein revealed by a comparative analysis of human and swine viral lineages, J. Virol. 89 (2015) 11275–11283.
[132] E.G.M. Berkhoff, E. de Wit, M.M. Geelhoed-Mieras, A.C.M. Boon, J. Symons, R.A.M. Fouchier, A.D.M.E. Osterhaus, G.F. Rimmelzwaan, Functional constraints of influenza A virus epitopes limit escape from cytotoxic T lymphocytes, Pathogenesis Immun. 79 (2005) 11239–11246.
[133] G.F. Rimmelzwaan, A.C.M. Boon, J.T.M. Voeten, E.G.M. Berkhoff, R.A.M. Fouchier, A.D.M.E. Osterhaus, Sequence variation in the influenza A virus nucleoprotein associated with escape from cytotoxic T lymphocytes, Virus Res. 103 (2004) 97–100.
[134] E.G.M. Berkhoff, M.M. Geelhoed-Mieras, R.A.M. Fouchier, A.D.M.E. Osterhaus, G.F. Rimmelzwaan, Assessment of the extent of variation in influenza A virus cytotoxic T-lymphocyte epitopes by using virus-specific CD8+ T-cell clones, J. Gen. Virol. 88 (2007) 530–535.
[135] G.F. Rimmelzwaan, E.G.M. Berkhoff, N.J. Nieuwkoop, R.A.M. Fouchier, A.D.M.E. Osterhaus, Functional compensation of a detrimental amino acid substitution in a cytotoxic-T-lymphocyte epitope of influenza a viruses by comutations, J. Virol. 78 (2004) 8946–8949.
[136] G.F. Rimmelzwaan, E.G.M. Berkhoff, N.J. Nieuwkoop, D.J. Smith, R.A.M. Fouchier, A.D.M.E. Osterhaus, Full restoration of viral fitness by multiple compensatory co-mutations in the nucleoprotein of influenza A virus cytotoxic T-

lymphocyte escape mutants, J. Gen. Virol. 86 (2005) 1801–1805.
[137] S. Cao, X. Liu, M. Yu, J. Li, X. Jia, Y. Bi, L. Sun, G.F. Gao, W. Liu, A nuclear export signal in the matrix protein of Influenza A virus is required for efficient virus replication, J. Virol. 86 (2012) 4883–4891.
[138] R. Bodewes, Kreijtz JHCM, G. van Amerongen, M.L.B. Hillaire, S.E. Vogelzang-van Trierum, N.J. Nieuwkoop, P. van Run, T. Kuiken, R.A.M. Fouchier, A.D.M.E. Osterhaus, et al., Infection of the upper respiratory tract with seasonal influenza A(H3N2) virus induces protective immunity in ferrets against infection with A(H1N1)pdm09 virus after intranasal, but not intratracheal, inoculation, J. Virol. 87 (2013) 4293–4301.
[139] M. Koutsakos, P.T. Illing, T.H.O. Nguyen, N.A. Mifsud, J.C. Crawford, S. Rizzetto, A.A. Eltahla, E.B. Clemens, S. Sant, B.Y. Chua, et al., Human CD8+ T cell cross-reactivity across influenza A, B and C viruses, Nat. Immunol. 20 (2019) 613–625.
[140] S.A. Valkenburg, V. Venturi, T.H.Y. Dang, N.L. Bird, P.C. Doherty, S.J. Turner, M.P. Davenport, K. Kedzierska, Early priming minimizes the age-related immune compromise of CD8+ T cell diversity and function, PLoS Pathog. 8 (2012) e1002544.
[141] K.J. Flynn, G.T. Belz, J.D. Altman, R. Ahmed, D.L. Woodland, P.C. Doherty, Virus-specific CD8+ T cells in primary and secondary influenza pneumonia, Immunity 8 (1998) 683–691.
[142] K. Kedzierska, V. Venturi, K. Field, M.P. Davenport, S.J. Turner, P.C. Doherty, Early establishment of diverse T cell receptor profiles for influenza-specific CD8+ CD62Lhi memory T cells, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 9184–9189.
[143] R.J. Hogan, L.S. Cauley, K.H. Ely, T. Cookenham, A.D. Roberts, J.W. Brennan, S. Monard, D.L. Woodland, Long-term maintenance of virus-specific effector memory CD8+ T cells in the lung airways depends on proliferation, J Immunol. 169 (2002) 4976–4981.
[144] J.H.C.M. Kreijtz, R. Bodewes, G. van Amerongen, T. Kuiken, R.A.M. Fouchier, A.D.M.E. Osterhaus, G.F. Rimmelzwaan, Primary influenza A virus infection induces cross-protective immunity against a lethal infection with a heterosubtypic virus strain in mice, Vaccine 25 (2007) 612–620.
[145] G.J. de Bree, E.M.M. van Leeuwen, T.A. Out, H.M. Jansen, R.E. Jonkers, R.A.W. van Lier, Selective accumulation of differentiated CD8+ T cells specific for respiratory viruses in the human lung, J. Exp. Med. 202 (2005) 1433–1442.
[146] F.M. Behr, N.A.M. Kragten, T.H. Wesselink, B. Nota, R.A.W. van Lier, D. Amsen, R. Stark, P. Hombrink, K.P.J.M. van Gisbergen, Blimp-1 rather than hobit drives the formation of tissue-resident memory CD8(+) T cells in the lungs, Front. Immunol. 10 (2019) 400-400.
[147] L.M. Wakim, J. Smith, I. Caminschi, M.H. Lahoud, J.A. Villadangos, Antibody-targeted vaccination to lung dendritic cells generates tissue-resident memory CD8 T cells that are highly protective against influenza virus infection, Mucosal Immunol. 8 (2015) 1060–1071.
[148] A. Pizzolla, L.M. Wakim, Memory T cell dynamics in the lung during influenza virus infection, J. Immunol. 202 (2019) 374–381.
[149] S.N. Mueller, L.K. Mackay, Tissue-resident memory T cells: local specialists in immune defence, Nat. Rev. Immunol. 16 (2016) 79–89.
[150] J.R. Teijaro, D. Turner, Q. Pham, E.J. Wherry, L. Lefrançois, D.L. Farber, Cutting edge: tissue-retentive lung memory CD4 T cells mediate optimal protection to respiratory virus infection, J. Immunol. (Baltimore, Md. 1950) 187 (2011) 5510–5514.
[151] D.L. Turner, K.L. Bickham, J.J. Thome, C.Y. Kim, F. D'Ovidio, E.J. Wherry, D.L. Farber, Lung niches for the generation and maintenance of tissue-resident memory T cells, Mucosal Immunol. 7 (2014) 501–510.
[152] T. Wu, Y. Hu, Y.-T. Lee, K.R. Bouchard, A. Benechet, K. Khanna, L.S. Cauley, Lung-resident memory CD8 T cells (TRM) are indispensable for optimal cross-protection against pulmonary virus infection, J. Leukoc. Biol. 95 (2014) 215–224.
[153] S. Ariotti, M.A. Hogenbirk, F.E. Dijkgraaf, L.L. Visser, M.E. Hoekstra, J.-Y. Song, H. Jacobs, J.B. Haanen, T.N. Schumacher, Skin-resident memory CD8+ T cells trigger a state of tissue-wide pathogen alert, Science 346 (2014) 101–105.
[154] L.K. Mackay, A.T. Stock, J.Z. Ma, C.M. Jones, S.J. Kent, S.N. Mueller, W.R. Heath, F.R. Carbone, T. Gebhardt, Long-lived epithelial immunity by tissue-resident memory T (TRM) cells in the absence of persisting local antigen presentation, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 7037–7042.
[155] J.M. Schenkel, K.A. Fraser, V. Vezys, D. Masopust, Sensing and alarm function of resident memory CD8$^+$ T cells, Nat. Immunol. 14 (2013) 509–513.
[156] S. Takamura, H. Yagi, Y. Hakata, C. Motozono, S.R. McMaster, T. Masumoto, M. Fujisawa, T. Chikaishi, J. Komeda, J. Itoh, et al., Specific niches for lung-resident memory CD8+ T cells at the site of tissue regeneration enable CD69-independent maintenance, J. Exp. Med. 213 (2016) 3057–3073.
[157] L.M. Wakim, N. Gupta, J.D. Mintern, J.A. Villadangos, Enhanced survival of lung tissue-resident memory CD8(+) T cells during infection with influenza virus due to selective expression of IFITM3, Nat. Immunol. 14 (2013) 238–245.
[158] B. Slütter, N. Van Braeckel-Budimir, G. Abboud, S.M. Varga, S. Salek-Ardakani, J.T. Harty, Dynamics of influenza-induced lung-resident memory T cells underlie waning heterosubtypic immunity, Sci. Immunol. 2 (2017).
[159] L.K. Mackay, M. Minnich, N.A. Kragten, Y. Liao, B. Nota, C. Seillet, A. Zaid, K. Man, S. Preston, D. Freestone, et al., Hobit and Blimp1 instruct a universal transcriptional program of tissue residency in lymphocytes, Science 352 (2016) 459–463.
[160] L.K. Mackay, E. Wynne-Jones, D. Freestone, D.G. Pellicci, L.A. Mielke, D.M. Newman, A. Braun, F. Masson, A. Kallies, G.T. Belz, et al., T-box transcription factors combine with the cytokines TGF-beta and IL-15 to control tissue-resident memory T cell fate, Immunity 43 (2015) 1101–1111.
[161] T.M. Strutt, K. Dhume, C.M. Finn, J.H. Hwang, C. Castonguay, S.L. Swain, K.K. McKinstry, IL-15 supports the generation of protective lung-resident memory CD4 T cells, Mucosal Immunol. 11 (2018) 668–680.
[162] A. Pizzolla, T.H.O. Nguyen, J.M. Smith, A.G. Brooks, K. Kedzieska, W.R. Heath, P.C. Reading, L.M. Wakim, Resident memory CD8(+) T cells in the upper respiratory tract prevent pulmonary influenza virus infection, Sci. Immunol. 2 (2017).
[163] K.D. Zens, J.K. Chen, D.L. Farber, Vaccine-generated lung tissue-resident memory T cells provide heterosubtypic protection to influenza infection, JCI Insight 1 (2016).
[164] N. Van Braeckel-Budimir, S.M. Varga, V.P. Badovinac, J.T. Harty, Repeated antigen exposure extends the durability of influenza-specific lung-resident memory CD8(+) T cells and heterosubtypic immunity, Cell Rep. 24 (2018) 3374–3382 e3373.
[165] A. Pizzolla, T.H. Nguyen, S. Sant, J. Jaffar, T. Loudovaris, S.I. Mannering, P.G. Thomas, G.P. Westall, K. Kedzierska, L.M. Wakim, Influenza-specific lung-resident memory T cells are proliferative and polyfunctional and maintain diverse TCR profiles, J. Clin. Invest. 128 (2018) 721–733.
[166] P. Hombrink, C. Helbig, R.A. Backer, B. Piet, A.E. Oja, R. Stark, G. Brasser, A. Jongejan, R.E. Jonkers, B. Nota, et al., Programs for the persistence, vigilance and control of human CD8+ lung-resident memory T cells, Nat. Immunol. 17 (2016) 1467.
[167] A.E. Oja, B. Piet, C. Helbig, R. Stark, D. van der Zwan, H. Blaauwgeers, E.B.M. Remmerswaal, D. Amsen, R.E. Jonkers, P.D. Moerland, et al., Trigger-happy resident memory CD4+ T cells inhabit the human lungs, Mucosal Immunol. 11 (2017) 654.
[168] J.M. Fonville, S.H. Wilks, S.L. James, A. Fox, M. Ventresca, M. Aban, L. Xue, T.C. Jones, N.M.H. Le, Q.T. Pham, et al., Antibody landscapes after influenza virus infection or vaccination, Science (New York, N.Y.) 346 (2014) 996–1000.
[169] P.C. Soema, R. Kompier, J.P. Amorij, G.F. Kersten, Current and next generation influenza vaccines: formulation and production strategies, Eur. J. Pharm. Biopharm. 94 (2015) 251–263.
[170] J.T. Treanor, F.R. Albano, D.C. Sawlwin, A. Graves Jones, J. Airey, N. Formica, V. Matassa, J. Leong, Immunogenicity and safety of a quadrivalent inactivated influenza vaccine compared with two trivalent inactivated influenza vaccines containing alternate B strains in adults: a phase 3, randomized noninferiority study, Vaccine 35 (2017) 1856–1864.
[171] C.S. Ambrose, M.J. Levin, The rationale for quadrivalent influenza vaccines, Hum. Vaccin. Immunother. 8 (2012) 81–88.
[172] C.E. van de Sandt, Y. Dou, S.E. Vogelzang-van Trierum, K.B. Westgeest, M.R. Pronk, A.D.M.E. Osterhaus, R.A.M. Fouchier, G.F. Rimmelzwaan, M.L.B. Hillaire, Influenza B virus-specific CD8+ T-lymphocytes strongly cross-react with viruses of the opposing influenza B lineage, J. Gen. Virol. 96 (2015) 2061–2073.
[173] K.G. Mohn, I. Smith, H. Sjursen, R.J. Cox, Immune responses after live attenuated influenza vaccination, Hum. Vaccin. Immunother. 14 (2018) 571–578.
[174] A. Aljurayyan, S. Puksuriwong, M. Ahmed, R. Sharma, M. Krishnan, S. Sood, K. Davies, D. Rajashekar, S. Leong, P.S. McNamara, et al., Activation and induction of antigen-specific t follicular helper cells play a critical role in live-attenuated influenza vaccine-induced human mucosal anti-influenza antibody response, J. Virol. 92 (2018).
[175] S. Sasaki, T.H. Holmes, R.A. Albrecht, A. García-Sastre, C.L. Dekker, X.-S. He, H.B. Greenberg, Distinct cross-reactive B-cell responses to live attenuated and inactivated influenza vaccines, J. Infect. Dis. 210 (2014) 865–874.
[176] K.G. Mohn, G. Bredholt, K.A. Brokstad, R.D. Pathirana, H.J. Aarstad, C. Tondel, R.J. Cox, Longevity of B-cell and T-cell responses after live attenuated influenza vaccination in children, J. Infect. Dis. 211 (2015) 1541–1549.
[177] C.S. Ambrose, M.J. Levin, R.B. Belshe, The relative efficacy of trivalent live attenuated and inactivated influenza vaccines in children and adults, Influenza Other Respir. Viruses 5 (2011) 67–75.
[178] S. Ashkenazi, A. Vertruyen, J. Arístegui, S. Esposito, D.D. McKeith, T. Klemola, J. Biolek, J. Kühr, T. Bujnowski, D. Desgrandchamps, et al., Superior relative efficacy of live attenuated influenza vaccine compared with inactivated influenza vaccine in young children with recurrent respiratory tract infections, Pediatr. Infect. Dis. J. 25 (2006) 870–879.
[179] R.B. Belshe, K.M. Edwards, T. Vesikari, S.V. Black, R.E. Walker, M. Hultquist, G. Kemble, E.M. Connor, Live attenuated versus inactivated influenza vaccine in infants and young children, N. Engl. J. Med. 356 (2007) 685–696.
[180] R.B. Belshe, S.L. Toback, T. Yi, C.S. Ambrose, Efficacy of live attenuated influenza vaccine in children 6 months to 17 years of age, Influenza Other Respir. Viruses 4 (2010) 141–145.
[181] J. Rhorer, C.S. Ambrose, S. Dickinson, H. Hamilton, N.A. Oleka, F.J. Malinoski, J. Wittes, Efficacy of live attenuated influenza vaccine in children: a meta-analysis of nine randomized clinical trials, Vaccine 27 (2009) 1101–1110.
[182] D.F. Hoft, E. Babusis, S. Worku, C.T. Spencer, K. Lottenbach, S.M. Truscott, G. Abate, I.G. Sakala, K.M. Edwards, C.B. Creech, 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. 204 (2011) 845–853.
[183] D.C. Powers, L.F. Fries, B.R. Murphy, B. Thumar, M.L. Clements, In elderly persons live attenuated influenza A virus vaccines do not offer an advantage over inactivated virus vaccine in inducing serum or secretory antibodies or local immunologic memory, J. Clin. Microbiol. 29 (1991) 498–505.
[184] R. Bodewes, J.H. Kreijtz, M.L. Hillaire, M.M. Geelhoed-Mieras, R.A. Fouchier, A.D. Osterhaus, G.F. Rimmelzwaan, Vaccination with whole inactivated virus vaccine affects the induction of heterosubtypic immunity against influenza virus A/H5N1 and immunodominance of virus-specific CD8+ T-cell responses in mice, J. Gen. Virol. 91 (2010) 1743–1753.
[185] R. Bodewes, J.H. Kreijtz, M.M. Geelhoed-Mieras, G. van Amerongen, R.J. Verburgh, S.E. van Trierum, T. Kuiken, R.A. Fouchier, A.D. Osterhaus,

G.F. Rimmelzwaan, Vaccination against seasonal influenza A/H3N2 virus reduces the induction of heterosubtypic immunity against influenza A/H5N1 virus infection in ferrets, J. Virol. 85 (2011) 2695–2702.
[186] R. Bodewes, P.L. Fraaij, M.M. Geelhoed-Mieras, C.A. van Baalen, H.A. Tiddens, A.M. van Rossum, F.R. van der Klis, R.A. Fouchier, A.D. Osterhaus, G.F. Rimmelzwaan, Annual vaccination against influenza virus hampers development of virus-specific CD8(+) T cell immunity in children, J. Virol. 85 (2011) 11995–12000.
[187] R. Bodewes, J.H.C.M. Kreijtz, G.F. Rimmelzwaan, Yearly influenza vaccinations: a double-edged sword? Lancet Infect. Dis. 9 (2009) 784–788.
[188] R.B. Belshe, W.C. Gruber, P.M. Mendelman, I. Cho, K. Reisinger, S.L. Block, J. Wittes, D. Iacuzio, P. Piedra, J. Treanor, et al., Efficacy of vaccination with live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine against a variant (A/Sydney) not contained in the vaccine, J. Pediatr. 136 (2000) 168–175.
[189] I. Shannon, C.L. White, A. Murphy, X. Qiu, J.J. Treanor, J.L. Nayak, Differences in the influenza-specific CD4 T cell immunodominance hierarchy and functional potential between children and young adults, Sci. Rep. 9 (2019) 791-791.
[190] S. Sant, L. Grzelak, Z. Wang, A. Pizzolla, M. Koutsakos, J. Crowe, T. Loudovaris, S.I. Mannering, G.P. Westall, L.M. Wakim, et al., Single-cell approach to influenza-specific CD8(+) T cell receptor repertoires across different age groups, tissues, and following influenza virus infection, Front. Immunol. 9 (2018) 1453-1453.
[191] Y. Steuerman, M. Cohen, N. Peshes-Yaloz, L. Valadarsky, O. Cohn, E. David, A. Frishberg, L. Mayo, E. Bacharach, I. Amit, et al., Dissection of influenza infection in vivo by single-cell RNA sequencing, Cell Syst. 6 (2018) 679–691 e674.