Do Memory CD4 T Cells Keep Their Cell-Type Programming: Plasticity versus Fate Commitment?

Complexities of Interpretation due to the Heterogeneity of Memory CD4 T Cells, Including T Follicular Helper Cells

Shane Crotty^{1,2,3}

¹Division of Vaccine Discovery, La Jolla Institute for Allergy and Immunology, La Jolla, California 92037

²Scripps Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery (CHAVI-ID), La Jolla, California 92037

³Department of Medicine, Division of Infectious Diseases, University of California, San Diego, La Jolla, California 92093

Correspondence: shane@lji.org

Plasticity is the ability of a cell type to convert to another cell type. There are multiple effector CD4 T-cell subtypes, including T_H1, T_H2, T_H17, T_H1*, CD4 CTL, T_H9, and T_{FH} cells. It is commonly thought that a CD4 T cell can readily show full plasticity—full conversion from one differentiated cell—and this propensity to plasticity is possessed by memory CD4 T cells. However, there remains no direct demonstration of in vivo–generated resting memory CD4 T-cell conversion to a different subtype on secondary antigen challenge in vivo in an intact animal at the single-cell level. What has been clearly shown is that CD4 T cells possess extraordinary capacity for phenotypic heterogeneity, but that is a distinct property from plasticity. Heterogeneity is diversity of the resting memory CD4 T-cell population, not conversion of a single differentiated cell into another subtype. Apparently, plasticity at the population level can be accomplished by either mechanism, as heterogeneity of CD4 T-cell subpopulations could affect large shifts in subtype distribution at the overall population level via differential exponential expansion and death.

GREAT DEBATES

What are the most interesting topics likely to come up over dinner or drinks with your colleagues? Or, more importantly, what are the topics that *don't* come up because they are a little too controversial? In ***Immune Memory and Vaccines: Great Debates***, Editors Rafi Ahmed and Shane Crotty have put together a collection of articles on such questions, written by thought leaders in these fields, with the freedom to talk about the issues as they see fit. This short, innovative format aims to bring a fresh perspective by encouraging authors to be opinionated, focus on what is most interesting and current, and avoid restating introductory material covered in many other reviews.

The Editors posed 13 interesting questions critical for our understanding of vaccines and immune memory to a broad group of experts in the field. In each case, several different perspectives are provided. Note that while each author knew that there were additional scientists addressing the same question, they did not know who these authors were, which ensured the independence of the opinions and perspectives expressed in each article. Our hope is that readers enjoy these articles and that they trigger many more conversations on these important topics.

Editors: Shane Crotty and Rafi Ahmed

Additional Perspectives on Immune Memory and Vaccines: Great Debates available at www.cshperspectives.org

Copyright © 2018 Cold Spring Harbor Laboratory Press; all rights reserved; doi: 10.1101/cshperspect.a032102

Cite this article as *Cold Spring Harb Perspect Biol* 2018;10:a032102

S. Crotty

Here, plasticity is defined as the conversion of a single cell possessing a well-characterized CD4 T-cell type into a cell no longer possessing that phenotype and instead possessing a different well-characterized CD4 T-cell phenotype. For example, conversion of a memory T_H1 cell ($T\text{-bet}^+IFN-\gamma^+CXCR5^-Bcl6^-$) into a T_{FH} cell ($Bcl6^+CXCR5^+T\text{-bet}^-IFN-\gamma^-$) would be plasticity. Separately, heterogeneity within a well-characterized CD4 T-cell population is defined here as a collection of varied phenotypes ($<100\%$ of the cell population) linked by a shared core phenotype. For example, heterogeneity among T_H1 cells can be observed by flow cytometry or mass cytometry by defining T_H1 cells as $T\text{-bet}^+IFN-\gamma^+$ cells and then observing fractions of the population expressing tumor necrosis factor (TNF), or interleukin (IL)-2, or Blimp1, or IL-10, or Eomes, etc. As another example, heterogeneity among T_H2 cells can be observed by flow cytometry or mass cytometry by defining T_H2 cells as $GATA3^{hi}$ cells and then observing fractions of the population expressing IL-5, IL-4, IL-13, CRT_{H2} , CCR4, or IL-10, etc. As another example, heterogeneity among germinal center (GC) T_{FH} cells can be observed by flow cytometry or mass cytometry by defining GC T_{FH} cells as $Bcl6^+CXCR5^+$ cells and then observing fractions of the population expressing CXCL13, IL-21, IL-4, or CXCR3, etc. (Crotty 2014; Vinuesa et al. 2016). Heterogeneity at the whole population level further includes the range of differentiated CD4 T-cell subtypes present, including T_H1 , T_H2 , T_H17 , T_H1^* , CD4 CTL, T_H9 , and T_{FH} cells, and perhaps even some form of “unbiased” T_H0 -type cells. Both plasticity and heterogeneity must be described based on analyses at the single-cell level.

Reports of T-cell program plasticity are unconvincing when the data are population-level changes in phenotypes. Such results can easily be the outcome of outgrowth of a minor cell population to become the dominant cell population, or vice versa, particularly given the exponential proliferation that T cells are capable of. Also unconvincing are the relevance of reports of cell program plasticity for which the central experiments are cell transfers into new hosts, particularly new hosts that are

severely immunocompromised (e.g., T-cell-deficient mice). Such experiments show that CD4 T-cell plasticity can occur under extreme conditions, but the experiments have no demonstrated relevance to what CD4 T cells actually do or experience in an intact animal. In contrast, if transferred cells do maintain stability, those results are more credible, because they show stability of cell identity even when exposed to nonphysiological stresses. Apparent plasticity of differentiated CD4 T cells in vitro is generally not convincing, both because the in vitro experiments lack demonstrated in vivo relevance and because the experiments are performed at the cell population level, masking the impact of outgrowth of minor cell populations. The strictest criterion for demonstration of plasticity is the use of a lineage marker reporter transgenic mouse, tracking, over time, cells marked irreversibly. Such an experiment directly establishes the transcriptional history of a given cell. Many lineage-tracking experiments have been performed on nTregs, making use of *Foxp3*-IRES-GFP/YFP/RFP-Cre-based designs (Rubtsov et al. 2008, 2010; Zhou et al. 2009; Miyao et al. 2012). The central conclusions from the two later studies with more sophisticated modified *Foxp3* gene reporter constructs was that $Foxp3^+$ nTregs are very stable, with almost no plasticity (Rubtsov et al. 2010; Miyao et al. 2012). In contrast, substantial gene-expression heterogeneity could be observed in conditions of stress and while still maintaining core $Foxp3^+$ nTreg programming. Still, the stability conclusions drawn from such studies are not necessarily directly transferrable for antigen-specific CD4 T-cell responses and CD4 T-cell memory, because nTregs develop their initial programming during thymic development.

STABILITY DURING A PRIMARY RESPONSE

There are no lineage marker reporter mouse studies showing plasticity of T_H1 , T_H2 , T_H17 , or T_{FH} cells during a primary immune response in an intact animal. Thus, excluding thymic-derived Tregs, there is no definitive evidence of physiologically relevant CD4 T-cell plasticity during a primary immune response. Cell-trans-

Do Memory CD4 T Cells Keep Their Cell-Type Programming?

fer experiments have attempted to address stability or plasticity of antigen-specific CD4 T cells during a primary immune response. We observed that T_{FH} and T_{H1} cells during a viral infection establish largely irreversible cell fates by 72 h postinfection, based on cell transfers of virus-specific T_{H1} or T_{FH} cells from virally infected mice into time-matched virally infected mice (Choi et al. 2013). Similar pronounced cell-fate commitment results were independently reported using a protein immunization and an RFP-Bcl6 reporter mouse strain when transferring $CXCR5^{-}Bcl6^{-}$ or $CXCR5^{+}Bcl6^{+}$ cells at day 7 postinfection (Liu et al. 2012). Plasticity of T_{H1} and T_{H2} cells to become T_{FH} cells has been reported; however, those experiments used in vitro-generated T_{H1} and T_{H2} cells transferred into mice (Liu et al. 2012) or in vitro polarized cells then repolarized under different in vitro conditions (Lu et al. 2011). It is almost certainly the case that there is a window of time early during effector CD4 T-cell differentiation in a primary immune response when a given CD4 T cell possesses pluripotency, simultaneously expresses lineage-defining transcription factors (e.g., Bcl6 and T-bet and ROR γ t) (Nakayamada et al. 2011; Oestreich et al. 2012), and maintains the capacity to respond to different extrinsic signals and subsequently commit to one differentiated cell type (e.g., T_{FH} or T_{H1} or T_{H17}) (DuPage and Bluestone 2016). Thus, simple questions regarding durable stability versus plasticity must be assessed after that point, which is nontrivial to accomplish.

STABILITY DURING TRANSITION FROM EFFECTOR CELL TO MEMORY CELL

The transition from an effector CD4 T cell to a central memory CD4 T cell appears to also be a transition from a cell with a highly polarized gene-expression program to a cell with a less polarized gene-expression program. This may be key to understanding the apparent plasticity of memory CD4 T cells, discussed below.

Based on single-cell transfer studies in mouse model systems, most CD4 T-cell clones are capable of generating memory cells (Tubo et al. 2016), and a given individual CD4 T-cell

clone can differentiate into multiple different CD4 T-cell types (e.g., T_{FH} and T_{H1}) as they divide during a primary immune response (Tubo et al. 2013). Furthermore, those effector cells can then develop into memory T_{FH} and T_{H1} cells in frequencies comparable with the frequencies of T_{FH} and T_{H1} cells generated by that clone during the effector phase of the CD4 T-cell response (Tubo et al. 2016). Human T-cell receptor (TCR) sequencing clonotype analysis of antigen-specific human memory CD4 T cells has shown that a given TCR sequence can be found in T_{H1} , T_{H2} , and T_{H17} antigen-specific central memory cells (Becattini et al. 2015), consistent with the mouse model observation.

During a primary immune response, it has been observed that T_{FH} cells can have gene expression of other T-cell differentiation programs. In the context of a mouse with an acute lymphocytic choriomeningitis virus (LCMV) infection, the mantle T_{FH} cells (mT_{FH} , outside of GCs) and GC T_{FH} cells express T-bet and interferon γ (IFN- γ) at substantially higher amounts than naïve CD4 T cells (Johnston et al. 2009; Yusuf et al. 2010; Ray et al. 2015). In our first paper on T_{FH} cells, we stated “it is notable that T-bet and IFN- γ were still expressed in the T_{FH} in vivo, although at lower levels than in T_{H1} /non- T_{FH} LCMV-specific CD4 T cells. These observations are consistent with a model in which T_{FH} cells follow their own differentiation pathway but are not an isolated lineage and can show partial characteristics of T_{H1} / T_{H2} polarization depending on environmental conditions.” Similar observations have been made for simian immunodeficiency virus (SIV) infection of rhesus macaques (Iyer et al. 2015). Given that both LCMV and SIV infections are extreme T_{H1} -biased immune responses, the presence of T_{H1} gene expression by T_{FH} cells in LCMV, and SIV immune response may represent uncommon exceptions. In support of that concept, human tonsillar GC T_{FH} cells expressing T_{H1} , T_{H2} , or T_{H17} cytokines are rarely observed (Ma et al. 2009; Yu et al. 2009; Dan et al. 2016; Havenar-Daughton et al. 2016). For example, <1% of GC T_{FH} cells produce IL-17 (Yu et al. 2009; Wong et al. 2015; Dan et al. 2016). Mouse GC T_{FH} cells rarely produce IL-

S. Crotty

13, even under very strong T_H2 polarizing helminth infection conditions (Liang et al. 2012), or house dust mite sensitization (Ballesteros-Tato et al. 2016) (T_{FH} cells normally express IL-4 as part of canonical T_{FH} programming, distinct and independent of T_H2 programming, and thus T_{FH} expression of IL-4 is not an indication of any T_H2 gene programming [Crotty 2011]). Furthermore, Bcl6 represses many T_H1 , T_H2 , and T_H17 genes and can prevent T_H1 differentiation (Johnston et al. 2009; Oestreich et al. 2012; Hatzi et al. 2015). A counterargument can now be made that cytokine expression by intracellular cytokine staining is insufficiently sensitive to determine whether a GC T_{FH} cell may possess T_H1 , or T_H2 , or T_H17 gene expression, because GC T_{FH} cells are intrinsically stingy cytokine producers, and intracellular cytokine staining missed $\sim 98\%$ of human or macaque antigen-specific GC T_{FH} cells (Dan et al. 2016; Haveran-Daughton et al. 2016). Thus, single-cell RNAseq of GC T_{FH} cells may be required to better understand whether GC T_{FH} cells with partial T_H1 , T_H2 , or T_H17 heterogeneity characteristics are common or rare.

Considering the process from the opposite direction, it is clear that human memory T_{FH} cells can show certain phenotypic markers commonly associated with T_H1 , T_H2 , or T_H17 cells (discussed more in the next section). What is the ontogeny of those cells? One possibility is that T_{FH} cells can be imprinted with a fractional amount of T_H1 gene programming by an antigen-presenting cell during the early stages of T-cell priming in response to a T_H1 pathogen, and, although that gene-expression program is efficiently squelched by Bcl6 in the effector mT_{FH} and GC T_{FH} progeny of that cell, a partial T_H1 program remains imprinted (Fig. 1). As a GC T_{FH} cell transitions into a memory cell, it loses expression of Bcl6 protein (Kitano et al. 2011; Liu et al. 2012; Choi et al. 2013; Hale et al. 2013; Locci et al. 2013; Ise et al. 2014), thus derepressing a range of genes, including *Ccr7* and *Il7ra* (Fig. 1) (Kitano et al. 2011; Choi et al. 2013). One possibility is that the loss of Bcl6 protein as the GC T_{FH} cell transitions into a memory T_{FH} cell can allow a partial T_H1 program imprinted at the time of T-cell priming to

then become derepressed in some cells in the absence of Bcl6 protein, resulting in the central memory T_{FH} cell acquiring a partially mixed T_{FH}/T_H1 phenotype (Fig. 1, model 1). Such an event may be considered “imprinted partial plasticity” because the phenotype of the cell would be dependent on the initial signals it received during T-cell priming, even if the gene-expression program was kept silent for long periods of time. If the cell were to subsequently become a T_H1 cell and lose T_{FH} characteristics, that would be “imprinted full plasticity.” This concept has not been directly tested.

A second possibility (model 2) is that a GC T_{FH} cell that does not have any T_H1 , T_H2 , or T_H17 gene expression or imprinting may be induced to activate a partial T_H1 , T_H2 , or T_H17 gene-expression program if there are T_H1 , T_H2 , or T_H17 differentiation inductive signals still present in the environment when the GC T_{FH} cell is transitioning to become a memory cell and losing Bcl6 protein expression (Fig. 1). Such an event would be CD4 T-cell plasticity, termed “de novo partial plasticity” here, to distinguish it from imprinted plasticity.

Alternatively, a substantial proportion of memory T_{FH} cells may be generated very early during an immune response derived from mantle T_{FH} cells (mT_{FH}) without going through a GC T_{FH} cell stage. Evidence for such a pathway comes from studies of *Sh2d1a*^{-/-} mice and humans, which have CD4 T cells that can differentiate into mT_{FH} cells but not GC T_{FH} cells and have evidence of T_{FH} cell memory (He et al. 2013). If memory T_{FH} cells are generated via such a pathway in immunocompetent mice and humans, it is plausible that memory T_{FH} cells derived from mT_{FH} cells may be less polarized than memory T_{FH} cells derived from GC T_{FH} cells, with less fixed-fate programming, and therefore may be more likely to have mixed attributes of T_{FH} and T_H1 or other programs (model 3). As is the case for models 1 and 2, this concept has also not been directly tested.

Given the observations and models described above, the presence of CXCR3⁺ CXCR5⁺ memory CD4 T cells in human peripheral blood is consistent with differentiation models based on heterogeneity, imprinted

Model 1. Imprinted partial plasticity

Diagram illustrating the differentiation and fate of T_H1 cells in a T_H1 inflammatory environment.

The diagram shows a sequence of cell states: mT_{FH}, GC T_{FH}, T_{FH}, Memory T_{FH}, and 2° T_{FH}. A dashed green circle highlights the initial stages (mT_{FH} to T_{FH}).

An arrow points from the T_{FH} stage to a T_H1 inflammatory environment (orange cloud). Inside this environment, a T_H1 cell (orange) transitions to a T_{FH}1 cell (orange). The T_{FH}1 cell then transitions to a Memory T_{FH}1 cell (orange). The Memory T_{FH}1 cell can further transition to 2° T_{FH}1 cells (orange) or 2° T_H1 cells (orange).

The 2° T_{FH}1 cells are labeled with CXCR5⁺ Bcl6⁺ IFN-γ CXCR3⁺ T-bet⁻. The 2° T_H1 cells are labeled with CXCR5⁻ Bcl6⁻ IFN-γ CXCR3⁺ T-bet^H.

The diagram also shows the effect of Ag (antigen) on the transition from Memory T_{FH} to 2° T_{FH} and from Memory T_{FH}1 to 2° T_{FH}1 and 2° T_H1.

Cite this article as *Cold Spring Harb Perspect Biol* 2018;10:a032102

S. Crotty

partial plasticity, or de novo partial plasticity. CXCR3 is expressed by T_H1 cells and is a direct target of T-bet, and the $CXCR3^+ CXCR5^+$ memory CD4 T cells in human peripheral blood cells are almost all capable of producing IFN- γ on stimulation (Morita et al. 2011; Bentebibel et al. 2013; Locci et al. 2013; Obeng-Adjei et al. 2015). Thus, $CXCR3^+ CXCR5^+$ memory CD4 T cells could have potentially derived from $CXCR3^+$ GC T_{FH} cells or m T_{FH} cells (Iyer et al. 2015), or $CXCR3^-$ GC T_{FH} cells or m T_{FH} cells that were exposed to a T_H1 environment while transitioning to memory T_{FH} cells.

CD4 T-cell biology usually does not fit tidy single pathway models; heterogeneity of phenotypes and differentiation patterns are common, as this is likely important to confound pathogen immune evasion strategies (Crotty 2012). Thus, a new report is surprising, but perhaps should not have been. Development of T_H2 cells (IL-5 $^+$ IL-13 $^+$ CXCR5 $^-$) in the lungs in response to a second exposure to house dust mite antigens was observed to be dependent on effector T_{FH} cells, with multiple lines of evidence pointing to differentiation of GC T_{FH} cells ($CXCR5^+PD-1^{hi}$ IL-21 $^+$) into T_H2 cells (Ballesteros-Tato et al. 2016). These appeared to be fully differentiated active GC T_{FH} cells, to the best of the ability of the authors to sort a pure cell population, with the previously stated caveats. In contrast, a different group, using a different IL-21 reporter mouse, did not observe T_{FH} cells to be precursors to T_H2 cells (IL-33R $^+$ IL-5 $^+$ IL-13 $^+$) in a similar house dust mite model (Coquet et al. 2015), but they did not gate on CXCR5 $^+$ cells for the cell sorts. The two groups also transferred cells at different times after the primary antigen exposure, which may result in tracking cells at different points of cell-fate commitment. When $T_{FH} \rightarrow T_H2$ plasticity occurred, the T_{FH} cells were taken 6 d after the primary immunizations (Ballesteros-Tato et al. 2016). In neither study were resting memory CD4 T cells used (cells without activation marker expression taken at >30 d after the last antigen exposure). Lineage-tracking models that do not depend on cell transfers are likely to be the only means of resolving such disparate observations.

MEMORY CD4 T-CELL PHENOTYPE HETEROGENEITY AND STABILITY DURING RESTING MEMORY

Resting memory CD4 T cells appear to be largely stable over time by major lineage-defining phenotypic markers, as shown for antigen-specific T_{FH} , T_H1 , and T_H2 cells in mouse models (Harrington et al. 2008; Hale et al. 2013; Hondowicz et al. 2016; Tubo et al. 2016). Human data support the same conclusion but the antigen-specific data are limited (Locci et al. 2013; Bancroft et al. 2016; Da Silva Antunes et al. 2017). No longitudinal data on antigen-specific resting memory CD4 T-cell phenotypes from individual human donors are available at single-cell flow-cytometric resolution, which is much needed to demonstrate memory CD4 T-cell subset stability.

T_H17 cells may be an exception to memory. There remains little evidence showing clear demonstration of in vivo-generated T_H17 memory cells in mice. T_H17 memory was absent in intact mice in one longitudinal antigen-specific CD4 T-cell study (Pepper et al. 2010). A later paper reported T_H17 memory, but it depended on transfer of in vitro-generated T_H17 cells (Muranski et al. 2011). Although an *Il17a* lineage marking reporter mouse has been available for many years (Hirota et al. 2011), there is a lack of publications on in vivo-generated T_H17 memory. In humans, presence of antigen-experienced T_H17 cells to *Candida albicans* has been clearly demonstrated (Zielinski et al. 2012); however, recurrent or continual exposure was not excluded, and a resting memory T_H17 phenotype (e.g., Ki67 $^-$) was not shown for *C. albicans*-specific cells. Thus, although the existence of resting stable memory T_H17 cells seems biologically reasonable and there is indirectly supportive literature (Lindström et al. 2012; McGeachy 2013), data showing antigen-specific resting memory T_H17 cells with single-cell analysis are currently quite limited.

Although there has been evidence of heterogeneity within CD4 T-cell subsets, going back to early descriptions of T_H1 and T_H2 cells (e.g., T_H2 cells producing some or all of IL-4, IL-5,

Do Memory CD4 T Cells Keep Their Cell-Type Programming?

IL-13, and IL-10), the vastness of the dimensional space of memory T-cell phenotypic heterogeneity was first made evident by the human memory CD8 T-cell mass spectrometry study of Newell and Davis (Newell et al. 2012). There was such phenotypic variety of memory CD8 T cells to influenza and cytomegalovirus (CMV) that the authors did not even attempt to put a number on the total range of memory CD8 T-cell phenotypes observed; instead, the diversity was best calculated as large spaces of phenotypic variation in three-dimensional principal component analysis (PCA) plots (Newell et al. 2012). Subsequent mass spectrometry analysis of human CD4 T cells has shown even more heterogeneity (Wong et al. 2015), consistent with the diversity of T_H1 , T_H2 , T_H17 , T_{FH} , T_H9 , T_H1^* , CD4 CTL, and iTreg biology. Importantly, phenotypic diversity in human memory CD4 T cells is seen even at the level of individual TCR clonotypes (Becattini et al. 2015). One example of heterogeneity is the presence or absence of PD-1 expression by resting memory T_{FH} cells (Locci et al. 2013). Heterogeneity is clearly present among chemokine receptor expression by memory CD4 T cells. A population of $CCR6^+$ $CXCR5^+$ resting memory CD4 T cells is present in human peripheral blood, and it has been suggested those cells represent “ T_{FH17} ” memory cells (Morita et al. 2011). However, $CCR6$ expression is not specific to T_H17 cells and does not correlate well with T_H17 programming in many cases. One report found no IL-17a expression by stimulated $CCR6^+$ $CXCR5^+$ memory CD4 cells in single-cell analysis (Wong et al. 2015). Therefore, most $CCR6$ expression by memory T_{FH} cells may be unrelated to T_H17 biology and simply reflective of preferential chemotaxis needs. A similar situation exists for “ T_{FH2} ” memory cells. “ T_{FH2} ” memory CD4 T cells in human peripheral blood are commonly defined only on the basis of negative markers ($CXCR3^-$ $CCR6^-$) (Morita et al. 2011; Jacquemin et al. 2015), based on the assumption that all T_{FH} memory cells must have a T_H1 , T_H2 , or T_H17 bias (which has certainly not been demonstrated at the single-cell level for GC T_{FH} cells or resting memory T_{FH} cells); thus, the concept of T_{FH2} cells remains poorly

defined. Given that T_{FH} cells produce IL-4 in a T_{FH} -specific manner (independent of the T_H2 program) (Crotty 2011), identification of a T_{FH2} cell requires demonstration of a resting memory $CXCR5^+$ CD4 T cell capable of producing IL-5 and/or IL-13 at the single-cell level—a criterion not reached in the current literature. Such cells may exist, but they have not been shown directly, and they may be a minor fraction of the $CXCR3^-$ $CCR6^-$ memory T_{FH} cells. There is clearly vast phenotypic heterogeneity among resting memory CD4 T cells, including resting memory T_{FH} cells. However, at the level of cytokine production, it is still unknown how rare memory T_{FH} cells with cytokine production attributes of other CD4 T-cell subsets are, except for overlap between T_H1 and T_{FH} programs in memory CD4 T cells, which has been clearly shown by multiple groups.

PLASTICITY WHEN MEMORY CD4 T CELLS ARE RECALLED?

A first study using an IL-21-GFP reporter mouse observed extensive plasticity of IL-21-GFP⁺ $CXCR5^+$ T_{FH} or IL-21-GFP⁺ $CXCR5^+$ cells after transfer into new hosts, with <50% of the memory cells maintaining $CXCR5$ expression, and a majority of the cells observed after recall by influenza infection were $CXCR5^-$ (Lüthje et al. 2012). A newer IL-21 fluorescent protein reporter mouse model observed robust stability of IL-21⁺ $CXCR5^+$ T_{FH} CD4 T cells after transfers into new hosts, but did not test memory time points (Weinstein et al. 2016). In the context of an acute LCMV infection, memory CD4 T cells appear to largely maintain their T_H1 or T_{FH} programming upon 2° response. TCR transgenic memory CD4 T cells with a resting T_H1 phenotype all became effector T_H1 cells ($T\text{-bet}^{\text{hi}}$ $Bcl6^{\text{lo}}$ $CXCR5^-$ Granzyme B⁺) when transferred into a new host that was then infected with LCMV. In the same study, the majority of memory CD4 T cells with a resting T_{FH} phenotype became effector T_{FH} and GC T_{FH} cells on rechallenge ($T\text{-bet}^{\text{lo}}$ $Bcl6^+$ $CXCR5^+$ Granzyme B⁻). A fraction of the memory T_{FH} cells did lose $CXCR5$ in

S. Crotty

the 2° response, but because those experiments depended on cell transfers, it is unknown whether all of the memory T_{FH} cells would have maintained their T_{FH} program on 2° challenge under physiological conditions in an intact animal where they were allowed to maintain their normal localization. It is, of course, also formally possible that the memory cells would have showed even more plasticity if studying in an intact host. Another longstanding challenge with sorted cells that have high proliferative capacity such as lymphocytes is that even a 1% CXCR5[−] contamination of sorted cells could subsequently expand extensively during the exponential proliferation following 2° challenge, thus confounding cell-fate interpretations of cell-transfer experiments.

Resting central memory CD4 T cells show less polarized features than actively responding effector cells. Resting central memory T_H1 or T_{FH} cells have less active transcription and protein expression of many signature features of activated T_H1 cells or activated GC T_{FH} cells. For example, central memory T_H1 cells express low levels of T-bet compared with effector cells, and resting memory T_{FH} cells express Bcl6 but at levels indistinguishable from other central memory cells. Intuitively, one expects such cells to be prone to plasticity. Programmed chromatin modifications may prevent plasticity. Memory T_{FH} cells rapidly up-regulate Bcl6 in vivo on restimulation (Ise et al. 2014). It is unknown whether memory T_{FH} cells with mixed T_{FH}–T_H1 phenotypes (CXCR3⁺CXCR5⁺) differentiate into T_{FH} (CXCR3[−]CXCR5⁺Bcl6⁺T-bet[−]), T_{FH}1 (CXCR3⁺CXCR5⁺Bcl6⁺T-bet⁺), and/or conventional T_H1 cells (CXCR3⁺CXCR5[−]Bcl6[−]T-bet⁺) in vivo in an intact mouse or human (Fig. 1). Evidence of plasticity was not observed in humans at the level of the overall CD4 T-cell response to pertussis, wherein the whole-cell pertussis vaccine and the acellular pertussis vaccine elicit predominantly T_H1 and T_H2 polarizing responses, respectively, and reimmunization with the acellular pertussis vaccine elicits a CD4 T-cell recall response with the same T_H1 or T_H2 characteristics for whichever vaccine the individual was initially immunized (Bancroft et al. 2016).

CONCLUDING REMARKS

There remains no direct demonstration of in vivo-generated resting memory CD4 T-cell conversion to a different subtype on secondary antigen challenge in vivo in an intact animal at the single-cell level. Lineage tracing experiments are needed to directly test whether plasticity occurs and, more importantly, how common or rare the process is under physiological conditions. Unfortunately, proper lineage-tracking genetic models are difficult to generate for T_{FH}, T_H17, and T_H2 cells. The lineage-defining transcription factors for T_{FH}, T_H17, and T_H2 cells are Bcl6, RORγT, and GATA3. GATA3 is constitutively expressed by all CD4 T cells; T_H2 cells are defined by high GATA3 expression. Thus, a conventional GATA3 lineage reporter would mark all T cells. Therefore, development of a high-fidelity T_H2 lineage genetic marker is a difficult challenge. A successful T_H2 lineage genetic marker would probably need to depend on transcription from a gene or locus other than GATA3. Bcl6 is highly expressed by thymocytes, and thus a Bcl6 lineage reporter mouse constructed based on standard designs would be expected to mark most T cells. RORγT is also expressed by thymocytes and, thus, an RORγT lineage reporter mouse based on standard designs would be expected to mark most T cells. Therefore, a successful T_{FH} or T_H17 lineage genetic marker would probably need to depend on transcription from a gene or locus other than GATA3; IL-17a is one candidate for T_H17 cells (Hirota et al. 2011). CXCR5 may be a good candidate for T_{FH} cells. As for T_H1 cells, a T-bet lineage reporter should be useful, but a key caveat is that transient expression of T-bet early on by a naïve CD4 T cell after activation is common and may have no influence on the future history of the cell. The same caveat applies for all constitutively active lineage marker systems, and contributed to controversy over the ontogeny of “exTregs.” Transient expression of Foxp3 by some cells may result in erroneous conclusions on the basis of very transient Cre expression (Rubtsov et al. 2008; Zhou et al. 2009; Miyao et al. 2012). This concern may be

Do Memory CD4 T Cells Keep Their Cell-Type Programming?

avoided by using estrogen-responsive Cre protein (Rubtsov et al. 2010).

As an alternative to lineage-tracking genetic markers, single-cell transfers into infection-matched hosts may be the best test of stability versus plasticity in a recall response. Such experiments would need to show that transferred cells (not using single-cell transfers) would localize in the new host to the same regions of lymph node (LN) and spleen as untransferred antigen-specific resting memory CD4 T cells of that subtype (e.g., proper microanatomical localization of memory T_{FH} cells posttransfer).

In the end, it is unknown whether the appearance of plasticity by memory CD4 T cells at the population level is predominantly because of heterogeneity and outgrowth of subpopulations or predominantly attributable to plasticity.

It remains possible that true memory CD4 T-cell plasticity may be primarily of interest for purely academic reasons, as a memory recall response with apparent plasticity at the whole-cell population level could presumably be accomplished via rapid outgrowth of a very minor population of memory cells. Because essentially all in vivo CD4 T-cell responses involve a mixture of subtypes (T_H1, T_H2, T_H CTL, and T_{FH}, for example), that scenario is plausible.

ACKNOWLEDGMENTS

This work is funded by the National Institutes of Health (NIH) National Institute of Allergy and Infectious Diseases (NIAID) R01 (S.C.). Thanks to Daniel DiToro for helpful discussions.

REFERENCES

- Ballesteros-Tato A, Randall TD, Lund FE, Spolski R, Leonard WJ, León B. 2016. T follicular helper cell plasticity shapes pathogenic T helper 2 cell-mediated immunity to inhaled house dust mite. *Immunity* **44**: 259–273.
- Bancroft T, Dillon MBC, Da Silva Antunes R, Paul S, Peters B, Crotty S, Lindestam Arlehamn CS, Sette A. 2016. Th1 versus Th2 T cell polarization by whole-cell and acellular childhood pertussis vaccines persists upon re-immunization in adolescence and adulthood. *Cell Immunol* **304**–**305**: 35–43.
- Becattini S, Latorre D, Mele F, Foglierini M, De Gregorio C, Cassotta A, Fernandez B, Kelderman S, Schumacher TN, Corti D, et al. 2015. Functional heterogeneity of human memory CD4⁺ T cell clones primed by pathogens or vaccines. *Science* **347**: 400–406.
- Bentebibel SE, Lopez S, Obermoser G, Schmitt N, Mueller C, Harrod C, Flaño E, Mejias A, Albrecht RA, Blankenship D, et al. 2013. Induction of ICOS⁺CXCR3⁺CXCR5⁺ TH cells correlates with antibody responses to influenza vaccination. *Sci Transl Med* **5**: 176ra32.
- Choi YS, Yang JA, Yusuf I, Johnston RJ, Greenbaum J, Peters B, Crotty S. 2013. Bcl6 expressing follicular helper CD4 T cells are fate committed early and have the capacity to form memory. *J Immunol* **190**: 4014–4026.
- Coquet JM, Schuijs MJ, Smyth MJ, Deswarte K, Beyaert R, Braun H, Boon L, Karlsson Hedestam GB, Nutt SL, Hamad H, et al. 2015. Interleukin-21-producing CD4⁺ T cells promote type 2 immunity to house dust mites. *Immunity* **43**: 318–330.
- Crotty S. 2011. Follicular helper CD4 T cells (T_{FH}). *Annu Rev Immunol* **29**: 621–663.
- Crotty S. 2012. The 1-1-1 fallacy. *Immunol Rev* **247**: 133–142.
- Crotty S. 2014. T follicular helper cell differentiation, function, and roles in disease. *Immunity* **41**: 529–542.
- Dan JM, Lindestam Arlehamn CS, Weiskopf D, da Silva Antunes R, Havenar-Daughton C, Reiss SM, Brigger M, Bothwell M, Sette A, Crotty S, et al. 2016. A cytokine-independent approach to identify antigen-specific human germinal center T follicular helper cells and rare antigen-specific CD4⁺ T cells in blood. *J Immunol* **197**: 983–993.
- Da Silva Antunes R, Paul S, Sidney J, Weiskopf D, Dan JM, Phillips E, Mallal S, Crotty S, Sette A, Arlehamn CSL. 2017. Definition of human epitopes recognized in tetanus toxoid and development of an assay strategy to detect ex vivo tetanus CD4⁺ T cell responses. *PLoS ONE* **12**: e0169086.
- DuPage M, Bluestone JA. 2016. Harnessing the plasticity of CD4⁺ T cells to treat immune-mediated disease. *Nat Rev Immunol* **16**: 149–163.
- Hale JS, Youngblood B, Latner DR, Mohammed AUR, Ye L, Akondy RS, Wu T, Iyer SS, Ahmed R. 2013. Distinct memory CD4⁺ T cells with commitment to T follicular helper- and T helper 1-cell lineages are generated after acute viral infection. *Immunity* **38**: 805–817.
- Harrington LE, Janowski KM, Oliver JR, Zajac AJ, Weaver CT. 2008. Memory CD4 T cells emerge from effector T-cell progenitors. *Nature* **452**: 356–360.
- Hatzi K, Nance JP, Kroenke MA, Bothwell M, Haddad EK, Melnick A, Crotty S. 2015. BCL6 orchestrates Tfh cell differentiation via multiple distinct mechanisms. *J Exp Med* **212**: 539–553.
- Havenar-Daughton C, Reiss SM, Carnathan DG, Wu JE, Kendric K, Torrents de la Peña A, Kasturi SP, Dan JM, Bothwell M, Sanders RW, et al. 2016. Cytokine-independent detection of antigen-specific germinal center T follicular helper cells in immunized nonhuman primates using a live cell activation-induced marker technique. *J Immunol* **197**: 994–1002.
- He J, Tsai LM, Leong YA, Hu X, Ma CS, Chevalier N, Sun X, Vandenberg K, Rockman S, Ding Y, et al. 2013. Circulating precursor CCR7^{lo}PD-1^{hi} CXCR5⁺ CD4⁺ T cells indicate Tfh cell activity and promote antibody responses upon antigen reexposure. *Immunity* **39**: 770–781.

S. Crotty

- Hirota K, Duarte JH, Veldhoen M, Hornsby E, Li Y, Cua DJ, Ahlfors H, Wilhelm C, Tolaini M, Menzel U, et al. 2011. Fate mapping of IL-17-producing T cells in inflammatory responses. *Nat Immunol* **12**: 255–263.
- Hondowicz BD, An D, Schenkel JM, Kim KS, Steach HR, Krishnamurthy AT, Keitany GJ, Garza EN, Fraser KA, Moon JJ, et al. 2016. Interleukin-2-dependent allergen-specific tissue-resident memory cells drive asthma. *Immunity* **44**: 155–166.
- Ise W, Inoue T, McLachlan JB, Kometani K, Kubo M, Okada T, Kurosaki T. 2014. Memory B cells contribute to rapid Bcl6 expression by memory follicular helper T cells. *Proc Natl Acad Sci* **111**: 11792–11797.
- Iyer SS, Gangadhara S, Victor B, Gomez R, Basu R, Hong JJ, Labranche C, Montefiori DC, Villinger F, Moss B, et al. 2015. Codelivery of envelope protein in alum with MVA vaccine induces CXCR3-biased CXCR5⁺ and CXCR5[−] CD4⁺ T cell responses in rhesus macaques. *J Immunol* **195**: 994–1005.
- Jacquemin C, Schmitt N, Contin-Bordes C, Liu Y, Narayanan P, Seneschal J, Maurouard T, Dougall D, Davizon ES, Dumortier H, et al. 2015. OX40 ligand contributes to human lupus pathogenesis by promoting T follicular helper response. *Immunity* **42**: 1159–1170.
- Johnston RJ, Poholek AC, DiToro D, Yusuf I, Eto D, Barnett B, Dent AL, Craft J, Crotty S. 2009. Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. *Science* **325**: 1006–1010.
- Kitano M, Moriyama S, Ando Y, Hikida M, Mori Y, Kurosaki T, Okada T. 2011. Bcl6 protein expression shapes pregerminal center B cell dynamics and follicular helper T cell heterogeneity. *Immunity* **34**: 961–972.
- Liang HE, Reinhardt RL, Bando JK, Sullivan BM, Ho IC, Locksley RM. 2012. Divergent expression patterns of IL-4 and IL-13 define unique functions in allergic immunity. *Nat Immunol* **13**: 58–66.
- Lindenstrøm T, Woodworth J, Dietrich J, Aagaard C, Andersen P, Agger EM. 2012. Vaccine-induced th17 cells are maintained long-term postvaccination as a distinct and phenotypically stable memory subset. *Infect Immun* **80**: 3533–3544.
- Liu X, Yan X, Zhong B, Nurieva RI, Wang A, Wang X, Martin-Orozco N, Wang Y, Chang SH, Esplugues E, et al. 2012. Bcl6 expression specifies the T follicular helper cell program in vivo. *J Exp Med* **209**: 1841–1852.
- Locci M, Havenar-Daughton C, Landais E, Wu J, Kroenke MA, Arlehamn CL, Su LF, Cubas R, Davis MM, Sette A, et al. 2013. Human circulating PD-1⁺CXCR3-CXCR5⁺ memory Tfh cells are highly functional and correlate with broadly neutralizing HIV antibody responses. *Immunity* **39**: 758–769.
- Lu KT, Kanno Y, Cannons JL, Handon R, Bible P, Elkhouloun AG, Anderson SM, Wei L, Sun H, O'Shea JJ, et al. 2011. Functional and epigenetic studies reveal multistep differentiation and plasticity of in vitro-generated and in vivo-derived follicular T helper cells. *Immunity* **35**: 622–632.
- Lüthje K, Kallies A, Shimohakamada Y, Belz GT, Light A, Tarlinton DM, Nutt SL. 2012. The development and fate of follicular helper T cells defined by an IL-21 reporter mouse. *Nat Immunol* **13**: 491–498.
- Ma CS, Suryani S, Avery DT, Chan A, Nanan R, Santner-Nanan B, Deenick EK, Tangye SG. 2009. Early commitment of naïve human CD4⁺ T cells to the T follicular helper (T_{FH}) cell lineage is induced by IL-12. *Immunol Cell Biol* **87**: 590–600.
- McGeachy MJ. 2013. Th17 memory cells: Live long and proliferate. *J Leukoc Biol* **94**: 921–926.
- Miyao T, Floess S, Setoguchi R, Luche H, Fehling HJ, Waldmann H, Huehn J, Hori S. 2012. Plasticity of Foxp3⁺ T cells reflects promiscuous Foxp3 expression in conventional T cells but not reprogramming of regulatory T cells. *Immunity* **36**: 262–275.
- Morita R, Schmitt N, Bentebibel SE, Ranganathan R, Bourdery L, Zurawski G, Foucat E, Dullaers M, Oh S, Sabzghabaei N, et al. 2011. Human blood CXCR5⁺CD4⁺ T cells are counterparts of T follicular cells and contain specific subsets that differentially support antibody secretion. *Immunity* **34**: 108–121.
- Muranski P, Borman ZA, Kerkar SP, Klebanoff CA, Ji Y, Sanchez-Perez L, Sukumar M, Reger RN, Yu Z, Kern SJ, et al. 2011. Th17 cells are long lived and retain a stem cell-like molecular signature. *Immunity* **35**: 972–985.
- Nakayamada S, Kanno Y, Takahashi H, Jankovic D, Lu KT, Johnson TA, Sun HW, Vahedi G, Hakim O, Handon R, et al. 2011. Early Th1 cell differentiation is marked by a Tfh cell-like transition. *Immunity* **35**: 919–931.
- Newell EW, Sigal N, Bendall SC, Nolan GP, Davis MM. 2012. Cytometry by time-of-flight shows combinatorial cytokine expression and virus-specific cell niches within a continuum of CD8⁺ T cell phenotypes. *Immunity* **36**: 142–152.
- Obeng-Adjei N, Portugal S, Tran TM, Yazew TB, Skinner J, Li S, Jain A, Felgner PL, Doumbo OK, Kayentao K, et al. 2015. Circulating Th1-cell-type Tfh cells that exhibit impaired B cell help are preferentially activated during acute malaria in children. *Cell Rep* **13**: 425–439.
- Oestreich KJ, Mohn SE, Weinmann AS. 2012. Molecular mechanisms that control the expression and activity of Bcl-6 in TH1 cells to regulate flexibility with a T_{FH}-like gene profile. *Nat Immunol* **13**: 405–411.
- Pepper M, Linehan JL, Pagán AJ, Zell T, Dileepan T, Cleary PP, Jenkins MK. 2010. Different routes of bacterial infection induce long-lived TH1 memory cells and short-lived TH17 cells. *Nat Immunol* **11**: 83–89.
- Ray JP, Staron MM, Shyer JA, Ho P-C, Marshall HD, Gray SM, Laidlaw BJ, Araki K, Ahmed R, Kaech SM, et al. 2015. The interleukin-2-mTORC1 kinase axis defines the signaling, differentiation, and metabolism of T helper 1 and follicular B helper T cells. *Immunity* **43**: 690–702.
- Rubtsov YP, Rasmussen JP, Chi EY, Fontenot J, Castelli L, Ye X, Treuting P, Siewe L, Roers A, Henderson WR, et al. 2008. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. *Immunity* **28**: 546–558.
- Rubtsov YP, Niec RE, Josefowicz S, Li L, Darce J, Mathis D, Benoist C, Rudensky AY. 2010. Stability of the regulatory T cell lineage in vivo. *Science* **329**: 1667–1671.
- Tubo NJ, Pagán AJ, Taylor JJ, Nelson RW, Linehan JL, Ertelt JM, Huseby ES, Way SS, Jenkins MK. 2013. Single naïve CD4⁺ T cells from a diverse repertoire produce different effector cell types during infection. *Cell* **153**: 785–796.

Do Memory CD4 T Cells Keep Their Cell-Type Programming?

- Tubo NJ, Fife BT, Pagán AJ, Kotov DI, Goldberg MF, Jenkins MK. 2016. Most microbe-specific naïve CD4⁺ T cells produce memory cells during infection. *Science* **351**: 511–514.
- Vinuesa CG, Linterman MA, Yu D, MacLennan ICM. 2016. Follicular helper T cells. *Annu Rev Immunol* **34**: 335–368.
- Weinstein JS, Herman EI, Lainez B, Licona-Limon P, Esplugues E, Flavell R, Craft J. 2016. T_{FH} cells progressively differentiate to regulate the germinal center response. *Nat Immunol* **17**: 1197–1205.
- Wong MT, Chen J, Narayanan S, Lin W, Anicete R, Kiaang HTK, De Lafaille MAC, Poidinger M, Newell EW. 2015. Mapping the diversity of follicular helper T cells in human blood and tonsils using high-dimensional mass cytometry analysis. *Cell Rep* **11**: 1822–1833.
- Yu D, Rao S, Tsai LM, Lee SK, He Y, Sutcliffe EL, Srivastava M, Linterman M, Zheng L, Simpson N, et al. 2009. The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. *Immunity* **31**: 457–468.
- Yusuf I, Kageyama R, Monticelli L, Johnston RJ, DiToro D, Hansen K, Barnett B, Crotty S. 2010. Germinal center T follicular helper cell IL-4 production is dependent on signaling lymphocytic activation molecule receptor (CD150). *J Immunol* **185**: 190–202.
- Zhou X, Bailey-Bucktrout SL, Jeker LT, Penaranda C, Martínez-Llordella M, Ashby M, Nakayama M, Rosenthal W, Bluestone JA. 2009. Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells in vivo. *Nat Immunol* **10**: 1000–1007.
- Zielinski CE, Mele F, Aschenbrenner D, Jarrossay D, Ronchi F, Gattorno M, Monticelli S, Lanzavecchia A, Sallusto F. 2012. Pathogen-induced human TH17 cells produce IFN- γ or IL-10 and are regulated by IL-1 β . *Nature* **484**: 514–518.

Cold Spring Harbor Perspectives in Biology

Do Memory CD4 T Cells Keep Their Cell-Type Programming: Plasticity versus Fate Commitment?: Complexities of Interpretation due to the Heterogeneity of Memory CD4 T Cells, Including T Follicular Helper Cells

Shane Crotty

Cold Spring Harb Perspect Biol 2018; doi: 10.1101/cshperspect.a032102 originally published online April 21, 2017

Subject Collection [Immune Memory and Vaccines: Great Debates](#)

Is There Natural Killer Cell Memory and Can It Be Harnessed by Vaccination?: Can Natural Killer and CD8 T Cells Switch Jobs?

Christine A. Biron and Marcus Altfeld

Is There Natural Killer Cell Memory and Can It Be Harnessed by Vaccination?: Vaccination Strategies Based on NK Cell and ILC Memory

Megan A. Cooper, Todd A. Fehniger and Marco Colonna

Is It Possible to Develop Cancer Vaccines to Neoantigens, What Are the Major Challenges, and How Can These Be Overcome?: Neoantigens as Vaccine Targets for Cancer

Haydn T. Kissick

Is It Possible to Develop Cancer Vaccines to Neoantigens, What Are the Major Challenges, and How Can These Be Overcome?: Neoantigens: Nothing New in Spite of the Name

Olivera J. Finn and Hans-Georg Rammensee

Is There Natural Killer Cell Memory and Can It Be Harnessed by Vaccination?: NK Cell Memory and Immunization Strategies against Infectious Diseases and Cancer

Joseph C. Sun and Lewis L. Lanier

Is There Natural Killer Cell Memory and Can It Be Harnessed by Vaccination?: Natural Killer Cells in Vaccination

Harold R. Neely, Irina B. Mazo, Carmen Gerlach, et al.

Is It Possible to Develop Cancer Vaccines to Neoantigens, What Are the Major Challenges, and How Can These Be Overcome?: Targeting the Right Antigens in the Right Patients

Stephen P. Schoenberger

Which Dengue Vaccine Approach Is the Most Promising, and Should We Be Concerned about Enhanced Disease after Vaccination?: There Is Only One True Winner

Scott B. Halstead

For additional articles in this collection, see <http://cshperspectives.cshlp.org/cgi/collection/>

**All Modifications and
Oligo Types Synthesized**
Long Oligos • Fluorescent • Chimeric • DNA • RNA • Antisense

Oligo Modifications?
Your wish is our command.

Which Dengue Vaccine Approach Is the Most Promising, and Should We Be Concerned about Enhanced Disease after Vaccination?: The Challenges of a Dengue Vaccine

Gavin Screaton and Juthathip Mongkolsapaya

Which Dengue Vaccine Approach Is the Most Promising, and Should We Be Concerned about Enhanced Disease after Vaccination?: The Path to a Dengue Vaccine: Learning from Human Natural Dengue Infection Studies and Vaccine Trials

Aravinda M. de Silva and Eva Harris

Is It Possible to Develop a "Universal" Influenza Virus Vaccine?: Potential for a Universal Influenza Vaccine

James E. Crowe, Jr.

Is It Possible to Develop a "Universal" Influenza Virus Vaccine?: Outflanking Antibody Immunodominance on the Road to Universal Influenza Vaccination

Davide Angeletti and Jonathan W. Yewdell

Which Dengue Vaccine Approach Is the Most Promising, and Should We Be Concerned about Enhanced Disease after Vaccination?: Questions Raised by the Development and Implementation of Dengue Vaccines: Example of the Sanofi Pasteur Tetravalent Dengue Vaccine

Bruno Guy

Which Dengue Vaccine Approach Is the Most Promising, and Should We Be Concerned about Enhanced Disease after Vaccination?: The Risks of Incomplete Immunity to Dengue Virus Revealed by Vaccination

Stephen S. Whitehead and Kanta Subbarao

Is It Possible to Develop a "Universal" Influenza Virus Vaccine?: Immunogenetic Considerations Underlying B-Cell Biology in the Development of a Pan-Subtype Influenza A Vaccine Targeting the Hemagglutinin Stem

Sarah F. Andrews, Barney S. Graham, John R. Mascola, et al.

Is It Possible to Develop a "Universal" Influenza Virus Vaccine?: Potential Target Antigens and Critical Aspects for a Universal Influenza Vaccine

Florian Krammer, Adolfo García-Sastre and Peter Palese

For additional articles in this collection, see <http://cshperspectives.cshlp.org/cgi/collection/>

All Modifications and Oligo Types Synthesized

Oligo Modifications?
Your wish is our command.

Long Oligos • Fluorescent • Chimeric • DNA • RNA • Antisense