T cell priming is a critical event in the initiation of the immune response to vaccination since it deeply influences both the magnitude and the quality of the immune response induced. CD4+ T cell priming, required for the induction of high-affinity antibodies and immune memory, represents a key target for improving and modulating vaccine immunogenicity.

A major challenge in the study of in vivo T cell priming is due to the low frequency of antigen-specific T cells. This review discusses the current knowledge on antigen-specific CD4+ T cell priming in the context of vaccination, as well as the most advanced tools for the characterization of the in vivo T cell priming and the opportunities offered by the application of systems biology.

Keywords: T cell priming, vaccination, CD4+ T cells, mucosal immunity, adoptive transfer, MHC class II tetramers

INTRODUCTION

T cell priming is an essential event for the induction of the adaptive immune response to vaccination. T cell priming is influenced by the type of vaccine formulation (antigen, adjuvant, delivery system), the dose and the route of administration. The characterization of T cell priming induced by a vaccination strategy is therefore critical in order to develop optimal prime-boost combinations capable of eliciting the type of immune response required to fight a specific pathogen.

The efficacy of most preventive vaccines relies on antibody response to block pathogen infection and generation of immune memory cells capable of rapid and effective reactivation following pathogen re-exposure (1, 2). In this context, primary activation of T-helper cells that are required for the induction of high-affinity antibodies and immune memory is essential (2). Furthermore, CD4+ T cell priming has been shown to be an early predictor of vaccine immunogenicity in humans (3, 4).

A limitation in the study of in vivo T cell priming is due to the low frequency of antigen-specific T cells. This has been overcome by the application of technologies such as adoptive transfer of transgenic antigen-specific T cells into recipient mice and major histocompatibility complexes (MHCs) class II tetramers (5, 6). It is particularly attractive to also consider systems biology approaches that have been recently applied to vaccinology to model T cell priming and develop tools to predict vaccine responsiveness and efficacy (7–9).

Here we review the current knowledge on antigen-specific CD4+ T cell priming in the context of prophylactic vaccination. Immunological events following primary vaccination by systemic and mucosal routes and their relevance for the rational development of prime-boost strategies are addressed. Moreover, the methodologies for studying in vivo CD4+ T cell priming and the potential of applying systems biology for its modeling are discussed.

IMMUNE MECHANISMS OF CD4+ T CELL PRIMING

CD4+ T cell priming represents a key step in the vaccination process due to the close relationship between CD4+ T cells and both long-term humoral immunity and protective antibodies. CD4+ T cell priming is influenced by several factors such as the local pro-inflammatory environment, the nature and the dose of the antigen, the vaccine formulation including the type of adjuvant and the route of immunization (10, 11). A schematic representation of the T cell priming event in the context of vaccination is reported in Figure 1. Generation of primed T-helper cells requires contact between antigen-bearing dendritic cells (DCs) and specific CD4+ T cells within the T zone of the lymph node (LN) closest to the site of vaccination (2, 12). The process of CD4+ T cell priming begins when naïve cells, that constantly transit between the circulatory and lymphatic systems, bind their T cell antigen receptors (TCRs) to foreign peptides loaded on MHCs class II molecules presented by antigen presenting cells (APCs), thus leading to T cell proliferation (13). Antigen persistence and duration of peptide presentation by APCs influence the magnitude of the primary T cell response (14, 15). The very early interaction between antigen-specific T cells and peptide-MHC-bearing APCs within the LN has been described with static and dynamic imaging methods and movies (13, 16, 17). Interaction between APCs and antigen-specific naïve T cells takes place within the first 8–20 h and is dependent on the presence of the antigen (13). Activated T cells begin to proliferate and finally, in a later and antigen-independent phase, they expand and differentiate into various functionally defined subsets.
of effector cells that, depending on the nature of the cytokine milieu generated by innate cells, express specific master transcription factors (18, 19). Polarization of the distinct effector T cell subsets is indeed regulated by the strength of antigenic stimulation, as well as by the cytokines present during priming (20). These polarizing cytokines are derived from the APCs, the responding T cells or bystander cells. Effector T cells can be emigrant lymphocytes such as Th1, Th2, or Th17 that exit the LNs and move to inflamed tissues, regulatory cells (Treg), or T follicular helper (Tfh) cells that relocate to B-T cell borders and interfollicular regions (21–23). Tfh cells are specialized to regulate multiple stages of antigen-specific B cell immunity through cognate cell contact and the secretion of cytokines (21). In the extra-follicular reaction, some antigen-primed B cells, after cognate contact of Tfh cells, undergo a process of rapid differentiation in short-lived plasma cells producing low-affinity antibodies such as IgM and IgG that appear in serum at low concentration a few days after immunization (2, 21). Interaction of Tfh cells with B cells drives the
formation of germinal center (GC) a dynamic micro-anatomical structure that supports the generation of B cell activation, antibody class switch recombination and affinity maturation (22, 24). Th17 cells that localize in GCs are referred to as GC-Th17 cells. A fraction of B cells matured during the GC reaction acquires the capacity to migrate toward long-term survival niches located within the bone marrow (BM) from where they can release vaccine antibodies for extended periods. Another fraction includes class-specific affinity-matured memory B cells that are able to rapidly expand and differentiate into plasma cells after antigen re-challenge (25).

Upon primary activation most of the antigen-experienced CD4+ T cells are short-lived and undergo apoptotic contraction leaving only a small fraction of competent memory precursor cells to migrate into the BM where they differentiate into long-lived memory cells. The frequency of memory T cells reflects therefore the magnitude of the initial T cell expansion and of its subsequent contraction. Two types of memory T cells have been identified based on their phenotype and function (26). Effector memory T cells (Tem) are circulating or tissue-resident cells and exert their immediate effector function after antigen encounter and mediate site-specific protection, while central memory T cells (Tcm) preferentially traffic through T cell areas of secondary LNs and BM and have a high proliferative potential (26). Their role is to recognize antigens transported by activated DCs into LNs and to rapidly undergo massive proliferation generating a delayed, but very large, wave of effector cells (26, 27). During a primary response memory T cell subsets are also generated. These cells are retained within draining lymphoid sites together with antigen-specific memory B cells and persistent complexes of peptide-MHC II (28). During a booster immunization, vaccine antigens restimulate memory T and B cells that rapidly activate a secondary immune response.

CD4+ T CELL PRIMING IN VACCINATION

In the context of vaccination strategies, T cell priming can be evaluated as a target for improving the immune response during vaccination as well as a tool for modulating the quality of the immune response. The nature and the dose of the vaccine antigen, the adjuvant or the vaccine delivery used, the route of immunization and the local environment are all factors that deeply affect the primary activation of CD4+ T cells (10, 11).

The development of distinct effector CD4+ T cell subsets is determined to a great extent by cytokines present during the T cell priming event that act as powerful polarizing factors (10). APCs express toll-like receptors (TLRs) that recognize distinct and highly conserved pathogen-associated molecules, thus activating a signaling cascade that dramatically impacts the quality and the quantity of the T cell response. This has encouraged the use of TLRs ligands as promising adjuvants (10, 29–33), that can influence the effector fate of antigen-specific primed CD4+ T cells (10). CD4+ T cell priming has been studied for characterizing the mechanism of action of adjuvants such as alum (34), the CpG ODN (35), the lipopolysaccharide (36) or its derivative-like monophosphoryl lipid A (37), cholera toxin (38), or its B subunit (CTB) (39, 40).

Another aspect to consider is the selection of the route of administration of the vaccine that affects the quality and the localization of the T cell response (41, 42). CD4+ T cell priming following immunization by different mucosal routes has been characterized in the murine model (35, 38, 43–45) as discussed in the next section. Recently, we have also demonstrated that the route used for priming, but not for booster immunization, influences the skewing of the CD4+ T effector response toward Th1 or Th2 with a stronger Th1 polarization upon nasal administration compared to the systemic one (46).

The development of vaccination approaches aimed at enhancing Th1 primary response is particularly attractive. The interaction of T-B cells is stabilized by adhesion molecules, such as the signaling lymphocytosis activation molecule (SLAM) family, that initiate intracellular signaling via recruitment of specific adapters such as the SLAM/associated protein (SAP) family (47). Targeted manipulation of the SAP/SLAM family has been employed recently as strategy for shaping and strengthening the immune response during vaccination (47, 48). The employment of a nanoparticle delivery system has also recently been shown to promote robust GC formation and enhance the expansion of vaccine antigen-specific Tfh cells leading to an enhanced humoral response (49).

The role of CD4+ T cells in developing durable functional neutralizing antibody responses, via Tfh cells, is considered of key importance for the development of vaccines against pathogens for which no vaccine is currently available, such as HIV (50). Despite the central role of T-helper cells in vaccine immunity, the specific contribution of HIV-specific CD4+ T cells in HIV infection is largely unknown and these cells have mostly been excluded from HIV vaccine design strategies because they can be infected by the virus itself (50). Strikingly, in simian immunodeficiency virus (SIV)-infected non-human primates, Tfh cells did not seem to be preferentially infected by the virus, and their frequency in LNs correlated with the magnitude of the SIV-specific IgG response, the avidity of the SIV-specific antibodies and the generation of the GCs (51).

Studies of H5N1 influenza vaccination of healthy adults have shown an increase in the frequency of virus-specific CD4+ T cells measured 22 days after the first dose. This increase predicted a rise in neutralizing antibody concentrations after boosting as well as their maintenance 6 months later (3, 52), thus suggesting that primary CD4+ T cell response can be considered a predictor marker of the secondary immune response. Similarly, CD4+ T cell expansion has shown to predict neutralizing antibody response to monovalent inactivated influenza A H1N1 vaccine (4).

MUCOSAL CD4+ T CELL PRIMING

Targeting mucosal sites by vaccination is an important goal considering that over 90% of infections occur at or through mucosal surfaces. The induction of mucosal immune responses requires the presence of a mucosa-associated lymphoid tissue that provides a continuous source of B and T cells to mucosal effector sites (53). A schematic representation of T cell priming in different mucosal sites following mucosal vaccination is reported in Figure 2. Inductive sites for mucosal immunity consist of organized mucosa-associated lymphoid tissue as well as local and regional draining LNs, whereas the effector sites include different compartments mainly consisting of the lamina propria of various mucose (54). Inductive sites in the gastro-intestinal and respiratory tracts have been well defined, and are composed by aggregated lymphoid tissues (gut-, nasal-, and bronchial-associated lymphoid
tissues, respectively) and mucosa-associated LNs (mesenteric and mediastinal LNs). On the contrary, the vaginal mucosa is devoid of histologically demonstrable organized mucosa-associated lymphoid tissue and the role of inductive site is played directly by draining iliac LNs (55). Moreover, antigen-uptake across the vaginal mucosa barrier and immune responses in the genital tract are greatly regulated and influenced by the hormonal state and estrus phase (56). Female genital tract has therefore some unique features that should be taken in consideration in the development of a vaccination strategy.

By using the adoptive transfer system (described in the next section), our laboratory has deeply analyzed the CD4+ T cell priming following nasal and vaginal immunization in the mouse model. Intranasal immunization with the recombinant vaccine vector Streptococcus gordonii (57–62), elicited an early clonal expansion of antigen-specific CD4+ T cells in the nasal-associated lymphoid tissue (NALT), and cervical and mediastinal LNs 3 days after immunization (43–45). Proliferated T cells were CD44hiCD45RBlo and expressed CD69 molecule within the early cell generations (44). Divided T cells disseminated in the respiratory, genital, and intestinal tracts (43) where they become detectable 5 days after priming. Similar results of antigen-specific clonal expansion and dissemination were observed immunizing with soluble ovalbumin (OVA) plus the adjuvant CpG ODN (35, 46). We also demonstrated that homing of nasally primed T cells into distal peripheral LNs was CD62L-dependent, while entry into mesenteric LNs depended on both CD62L and α4β7 expression (35) (Figure 2).

T cell priming was also studied following vaginal immunization in hormone synchronized mice, showing a very efficient activation of CD4+ T cells (38, 63). Antigen-specific CD4+ T cell clonal expansion was indeed detected in iliac LNs, and proliferated T cells disseminated toward distal LNs and spleen, similarly to what observed following nasal immunization (38). These data show that vaginal immunization is efficient in eliciting CD4+ T priming despite the absence of an organized mucosa-associated inductive site in the genital tract (Figure 2).

**PRIME-BOOST APPROACH**

Characterization of the magnitude and quality of the T cell priming elicited by a vaccine formulation is critically important for the rational development of prime-boost vaccine combinations. An interesting approach to vaccination is indeed the heterologous prime-boost strategy that primes the immune system to a target antigen delivered by a vector and then selectively boosts the secondary response only to the vaccine antigen by using a different
vaccine formulation. The heterologous prime-boost approach is specifically aimed at the generation and enrichment of high avidity T cells specific for the target antigen (64). Boosting with a different vector carrying the same antigen has been shown to be more efficient in inducing the immune responses compared to boosting with the same vector. The heterologous prime-boost approach is currently exploited in human studies aimed at developing vaccines against pathogens such as HIV (65), tuberculosis (66), and malaria (67). Furthermore, mucosal and parenteral routes can be combined in a vaccination prime-boost strategy to induce immune responses in both the local and systemic compartments. This approach has shown to be as strong or stronger than those resulting from homologous mucosal or parenteral vaccination alone (68–71). Recently, we have demonstrated that the polarization of CD4+ T effector cells is affected by the route used for priming but not for boosting, while local effector responses are mainly dependent on booster route (46).

Recent studies in the mouse model have also assessed the role of peptide-based priming on the subsequent B cell response elicited by whole protein boosting (72) or by infection with the pathogen (73) or with an attenuated viral vaccine (74). These studies showed that CD4+ T cell help is quite selective for the subsequent antibody production. CD4+ T cells specific for an epitope provide the appropriate help mainly to the protein-specific B cells, indicating a deterministic linkage between antibodies and CD4+ T cell responses (73, 74), even if discordant results have been recently reported (75).

Understanding the priming mechanisms of a vaccine formulation and optimizing its priming properties is therefore of critical relevance for the informed design of next generation prime-boost strategies.

TOOLS TO STUDY T CELL PRIMING
Antigen-specific primary activation has been mostly analyzed in animal models, within LNs draining the inoculation site or in the spleen. Primed CD4+ T cells can be detected in draining LNs within a few days after immunization, with a peak after 5–7 days (13, 43, 76). In humans, primed T cells can be studied in peripheral blood starting from 7 days following vaccination (4).

Several procedures have been employed to characterize antigen-specific primed T cells, including assays of helper cell activity using carrier/hapten systems (77), and the commonly used proliferation and cytokine production assays. These methods measure functional parameters as a read-out for T cells which react to the specific antigen challenge in vitro. A major limitation of these assays is that the phenotypic and functional properties of the reactive cells may be altered by the in vitro antigenic restimulation (78).

To overcome this limitation, technologies such as the adoptive transfer of TCR-transgenic T cells in mice (79) and, more recently, MHC class II tetramers (6) have been developed to allow the ex vivo analysis of primed T cells (see below). A summary of the most used assays for studying T cell priming in human and animal studies, with their main advantages and disadvantages, is reported in Table 1.

ADOPTIVE TRANSFER OF TRANSGENIC ANTIGEN-SPECIFIC T CELLS
In order to overcome the limitation of the low frequency of antigen-specific T cells in vivo, Jenkins and colleagues developed the adoptive transfer model of antigen-specific transgenic T cells into recipient mice (79). This system largely increases the number of antigen-specific naïve CD4+ T cells in vivo by employing TCR-transgenic mice that express a TCR specific for a defined peptide/MHC complex on most T cells, and thus allows the ex vivo analysis of their clonal expansion following antigenic stimulation (3). In order to track their proliferation, transgenic T lymphocytes are labeled with the vital dye 5-(and -6)-carboxyfluorescein diacetate succinimidyl ester (CFSE) (80) and then injected intravenously into immunocompetent recipient mice. Following vaccine administration, the T cell proliferation in the secondary lymphoid organs can be studied by flow cytometric analysis of CFSE dilution in the single-cell generations. The adoptive transfer method has proven to be a powerful tool for studying T cell primary responses to parenteral and mucosal immunization (35, 38–40, 43–45, 81–86), the role of the microenvironment for initiating T cell response in secondary lymphoid tissues (87) and the impact of aging on cellular immunity (88). Transgenic mice extensively used for studying the development of CD4+ T cell primary activation following immunization include DO11.10 (89) and OT-II (90) strains that contain rearranged TCR-Vα and -Vβ genes in the germline DNA encoding a TCR specific for chicken OVA peptide233–242 bound to I-A molecules in a context of H-2d and H-2b haplotype, respectively. Other transgenic models have been developed, such as SM1 RAG-2 deficient mice, that allow the visualization of Salmonella flagellin-specific CD4+ T cell responses (91), SMARTA transgenic mice, that produce CD4+ T cells expressing Va2 and Vb8.3 TCR specific for the lymphocytic choriomeningitis virus (LCMV) epitope gp61–80 (92), and Ag85B241–255 TCR-transgenic mice that allow to characterize tuberculosis-specific immune response (86).

Despite the important results obtained with this system, it has the limitation that the high number of naïve antigen-specific T cells transferred into recipient mice alters the physiologic conditions and can influence the immune response observed. Moreover, the study of the antigen-specific primary response is limited by the availability of the transgenic mouse strains with the TCR specific for a given model antigen.

MHC CLASS II TETRAMERS
The limitations of the methodology described above have been overcome with the development of MHC-peptide complexes. In 1996, the first work describing the use of a peptide-MHC class I complex for the identification and characterization of antigen-specific T lymphocytes was published (93). Initially developed for the study of CD8+ T cells, the technology has been extended to the class II system in the context of CD4+ T cells (6, 94) and has been applied to the study of human and murine T cells (95–97). This tool has provided an invaluable way to monitor T cell mediated immune responses and quantify the development of an antigen-dependent response. The technology allows identification of antigen-specific T cells based on the specificity of their surface TCR for particular MHC-peptide complexes. Since the affinity of TCR for a single peptide-loaded MHC molecule is generally low, multimerization of the peptide-MHC complexes is necessary for achieving much higher avidities for the TCR (93, 97). Today, the most prevalent multimer used consists of biotin-labeled
peptide-MHC complexes bound to streptavidin molecules forming tetravalent structures (96). The peptide of interest can be covalently linked to the β-chain of the MHC molecule for the generation of MHC molecule-peptide complexes or it can be loaded on empty soluble class II molecules (95). Tetramer technology offers the advantage of phenotyping the antigen-specific cells by combining surface marker labeling and allows for the simultaneous detection of different antigen-specific CD4+ T cells by using multiple tetramers conjugated to different fluorescent molecules. The major limitations in the use of tetramers are that immunodominant peptides have to be predefined and that humans have very diverse HLA class II molecules. Moreover, the low frequency of antigen-specific CD4+ T cells in blood (generally 1/3000–30000) and the low avidity of TCR-MHC-peptide complex recognition are challenging issues for the tetramer technology. One strategy developed to overcome the first problem is the selection of the tetramer-positive cells by sorting with magnetic beads, so the antigen-specific population could be enriched as much as 10,000-fold (76, 98).

By using distinct MHC class II tetramers, Jenkins and colleagues have analyzed in the mouse model the primary response of CD4+ T cells specific for three different peptides [the protein Flic427-441 of Salmonella typhimurium, the OVA323-339, and the 2W1S variant of I-Eα proteins52-68 (2W)] following intravenous immunization and correlated the primary response to the frequency of the respective naïve population size by combining the tetramer staining to the magnetic bead enrichment (76). Since the frequency of the naïve pool of 2W-specific CD4+ T cells in C57BL/6 mice, was the highest among the three peptides assessed, this peptide has been selected for its expression on Listeria monocytogenes and Leishmania major in order to track, by mean of the 2W-MHC class II tetramer, the peptide-specific T cell primary response following acute infection (99, 100). MHC class II tetramers have been used for identifying CD4+ T cell epitopes of Porphyromonas gingivalis proteins following oral infection of mice and as a tool for tracking and phenotyping specific effector and memory CD4+ T cells (101). In other murine studies, the magnitude and quality of the CD4+ T cell response induced by oral immunization with lipid-formulated BCG has been analyzed by using Mycobacterium tuberculosis Ag85B280–294-specific MHC class II tetramers, and compared with that induced by the subcutaneous immunization with BCG (102).

**SYSTEMS BIOLOGY APPROACH FOR STUDYING T CELL PRIMING**

Mathematical and computational modeling can be employed as tool for integrating experimental data into a quantitative analysis of immune responses to antigens. Application of systems biology in vaccinology, named systems vaccinology, has recently been proposed as new powerful tool to model and characterize immune responses to vaccination and to predict vaccine immunogenicity and efficacy (8, 9). Systems vaccinology aims to model the immunological network, from molecules to cells to tissues, in order to predict vaccine immunogenicity. The identification of molecular signatures induced early after vaccination which correlate with and predict the later development of protective immune responses, represents a strategy to prospectively determine vaccine efficacy. Systems biology approaches provide a detailed level of investigation to better and fully analyze the network of interactions within vaccine-specific innate and adaptive immunity. All this information is expected to high impact on rational vaccine

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**Table 1 | Methods used for studying antigen-specific T cell priming in humans and animals.**

| Analysis | Assay | Cellular function | Technical methodology | Advantages | Disadvantages |
|----------|-------|-------------------|-----------------------|------------|--------------|
| In vitro | Proliferation | Cell proliferation | 3H-TdR incorporation, Limiting dilution assay, Colorimetric assays | Wide response not restricted to single epitope; high sensitivity | Restimulation and expansion in vitro; use of radioisotopes |
| Cytokine release | Cytokine secretion | ELISPOT | Phenotypic analysis | Detection of rare specific T cells | Restimulation and expansion in vitro; use of radioisotopes; labor intensive |
| Ex vivo | Adoptive transfer | Cell proliferation | FACS staining | No restimulation; phenotypic analysis of cell generations; localization of labeled cells | High specificity | Few transgenic mouse strains available; altered physiological condition; laborious procedure |
| MHC II tetramers | Enumeration of Ag-specific cells | FACS staining | No restimulation; analysis in physiological condition; phenotypic analysis; rapid analysis; independent from T cell function; high specificity | Peptides have to be predefined; complicated manufacturing; restricted to single epitope specificities |

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*aOnly in mice.*
development, providing molecular prediction markers of vaccine immunogenicity, uncovering new correlates of vaccine efficacy, as well as guiding the design of new vaccine formulations and prime-boost strategies (7).

Systems biology represents therefore an attractive tool for studying T cell priming and modeling the initiation of the immune response following vaccination and predicting the priming properties of different vaccine formulations. The application of mathematical models can indeed be highly relevant to analyze antigen-specific CD4 T cell primary clonal expansion, based on the dilution of the CFSE dye. Quantitative analysis of T cell proliferation through mathematical models has been previously employed for in vitro studies of lymphocyte proliferation (103–105). On the contrary, the application to in vivo analysis raises several difficulties, mainly due to the fact that a LN is not an “isolated” site but is part of the complex immunological system.

Our group has recently employed a Multi-type Galton–Watson branching process with immigration (63, 106) to model in vivo CD4 T cell priming and estimate the probabilities of a cell to enter in division, rest in quiescence or migrate/dye. This model has been successfully applied to analyze CD4 T cell priming in mice immunized by different mucosal routes, such as vaginal or nasal, and has allowed the estimation of the probability of CD4 T cells to enter into division within the draining LNs (63). Ongoing work is focused on modeling lymphocyte trafficking within the lymphatic systems, including both draining and distal LNs and spleen, in order to obtain further quantitative information and generate a model capable of predicting the amount and distribution of primed CD4 T cells.

CONCLUDING REMARKS
CD4 T cell priming is an early biomarker of vaccine immunogenicity that should be considered as a critical parameter in the evaluation of vaccination strategies. The advances in understanding CD4 T cell priming and the availability of latest generation technologies for its study open the way for its use in the rational design of vaccine formulations and prime-boost combinations. CD4 T cell priming can be also considered an important biomarker for early prediction of vaccine immunogenicity and individual responsiveness to vaccination. The application of systems biology and mathematical modeling to the study of CD4 T cell priming offers further opportunities to identify early signatures of vaccine immunogenicity and guide the design of next generation vaccines.

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REFERENCES
1. Plotkin SA. Correlates of protection induced by vaccination. Clin Vaccine Immunol (2010) 17:1055–65. doi:10.1128/CVI.00131-10
2. Siegrist CA. Vaccine immunology. In: Plotkin AA, Orenstein WA, Offit PA editors. Vaccines. Philadelphia: Elsevier Inc (2008), p. 17–36.
3. Galli G, Medini D, Gorgogni E, Zedda L, Bardelli M, Malzone C, et al. Adjuvanted H5N1 vaccine induces early CD4 T cell response that predicts long-term persistence of protective antibody levels. Proc Natl Acad Sci U S A (2009) 106:3877–82. doi:10.1073/pnas.0813390106
4. Nayak JL, Fitzgerald TR, Richards KA, Yang H, Treanor JJ, Sant AJ. CD4 T cell expansion predicts neutralizing antibody responses to monovalent, inactivated 2009 pandemic influenza A(H1N1) virus subtype H1N1 vaccine. J Infect Dis (2013) 207:297–305. doi:10.1093/infdis/jis684
5. Pape KA, Kearney ER, Khoruts A, Mondonio A, Merica R, Chen ZM, et al. Use of adoptive transfer of T-cell-antigen-receptor-transgenic T cell for the study of T-cell activation in vivo. Immunol Rev (1997) 156:67–78. doi:10.1111/j.1600-065X.1997.tb00959.x
6. McHeyzer-Williams MG, Altman JD, Davis MM. Enumeration and characterization of memory cells in the TH compartment. Immunol Rev (1996) 150:5–21. doi:10.1111/j.1600-065X.1996.tb00693.x
7. Buonaguro L, Paludrnan B. Immunogenomics and systems biology of vaccines. Immunol Rev (2011) 239:197–208. doi:10.1111/j.1600-065X.2010.00971.x
8. Li S, Nakaya HI, Kazmin DA, Oh JZ, Paludrnan B. Systems biological approaches to measure and understand vaccine immunity in humans. Semin Immunol (2013) 25(3):209–18. doi:10.1016/j.smim.2013.05.003
9. Paludrnan B, Li S, Nakaya HI. Systems vaccinology. Immunity (2010) 33:516–29. doi:10.1016/j.immuni.2010.06.006
10. Jelley-Gibbs DM, Strutt TM, McKinstry KK, Swain SL. Influencing the fates of CD4 T cells on the path to memory: lessons from influenza. Immunity Cell Biol (2008) 86:343–52. doi:10.1038/icb.2008.13
11. Lanzavecchia A, Sallusto F. Understanding the generation and function of memory T cell subsets. Curr Opin Immunol (2005) 17:326–32. doi:10.1016/j.coi.2005.04.010
12. Lanzavecchia A, Sallusto F. Regulation of T cell immunity by dendritic cells. Cell (2001) 106:263–6. doi:10.1016/S0092-8674(01)00455-X
13. Mempel TR, Henrickson S, von Andrian UH. T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. Nature (2004) 427:154–9. doi:10.1038/nature02238
14. Obst R, van Santen HM, Mathis D, Benoist C. Antigen persistence is required throughout the expansion phase of a CD4 T cell response. J Exp Med (2005) 201:1555–65. doi:10.1084/jem.20042521
15. Itano AA, McSorley SJ, Reinhardt RL, Eht BD, Ingulli E, Rudensky AY, et al. Distinct dendritic cell populations sequentially present antigen to CD4 T cells and stimulate different aspects of cell-mediated immunity. Immunity (2003) 19:47–57. doi:10.1016/S1074-7613(03)00175-4
16. Germain RN, Jenkins MK. In vivo antigen presentation. Curr Opin Immunol (2004) 16:120–5. doi:10.1016/j.coi.2003.11.001
17. Catron DM, Itano AA, Pape KA, Mueller DL, Jenkins MK. Visualizing the first 50 hr of the primary immune response to a soluble antigen. Immunity (2004) 21:341–7. doi:10.1016/j.immuni.2004.08.007
18. Yamane H, Paul WE. Early signaling events that underlie fate decisions of naive CD4 T cells toward distinct T-helper cell subsets. Immunity Rev (2013) 252:12–23. doi:10.1111/imr.12032
19. Nakayamada S, Takahashi H, Kanno Y, O’Shea JJ. Helper T cell diversity and plasticity. Curr Opin Immunol (2012) 24:297–302. doi:10.1016/j.coi.2012.01.014
20. Fazileau N, McHeyzer-Williams LJ, Rosen H, McHeyzer-Williams MG. The function of follicular helper T cells is regulated by the strength of T cell antigen receptor binding. Nat Immunol (2009) 10:375–84. doi:10.1038/ni.1704
21. McHeyzer-Williams M, Okitsu S, Wang N, McHeyzer-Williams L. Molecular programming of B cell memory. Nat Rev Immunol (2012) 12:24–34. doi:10.1038/nri3128
22. Crotty S. Follicular helper CD4 T cells (Tfh). Annu Rev Immunol (2011) 29:621–63. doi:10.1146/annurev-immunol-031210-101400
23. Ma CS, Deenick EK, Batten M, Tangye SG. The origins, function, and regulation of T follicular helper cells. J Exp Med (2012) 209:1241–53. doi:10.1083/jem.20120994
24. Pepper M, Jenkins MK. Origins of CD4(+) effector and central memory T cells. Nat Immunol (2011) 12:467–71. doi:10.1038/ni.2038
25. Radvbruch A, Muehlinghaus G, Luger EO, Inamine A, Smith KG, Dörner T, et al. Competence and competition: the challenge of becoming a long-lived plasma cell. Nat Rev Immunol (2006) 6:741–50. doi:10.1038/nri1886

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26. Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subset: function, generation, and maintenance. Annu Rev Immunol (2004) 22:763. doi:10.1146/annurev.immunol.22.012703.104702
27. Reinhaert RL, Khoruts A, Merica R, Zell T, Jenkins MK. Visualizing the generation of memory CD4 T cells in the whole body. Nature (2001) 410:101–5. doi:10.1038/35065111
28. Fazilleau N, Eisenbraun MD, Malherbe L, Ebright JN, Pogue-Caley RR, McAleer JP, Vella AT. Educating CD4 T cells with vaccine adjuvants: lessons from lipopolysaccharide. Immunol Rev (2006) 206:249–53. doi:10.1111/j.0105-2896.2006.06348.x
29. van Duin D, Medzhitov R, Shaw AC. Triggering TLR signaling in vaccination. Trends Immunol (2006) 27:49–55. doi:10.1016/j.it.2005.11.005
30. Barr TA, Brown S, Ryan G, Zhao J, Gray D. TLR-mediated stimulation of APC: different profile and distribution of antigen specific T cells induced by intranasal administration of mice. PLoS One (2011) 6:e19346. doi:10.1371/journal.pone.0019346
31. McAfee JP, Vella AT. Educating CD4 T cells with vaccine adjuvants: lessons from lipopolysaccharide. Trends Immunol (2010) 31:349–53. doi:10.1016/j.it.2010.08.005
32. Mata-Haro V, Cekic C, Martin M, Chilton PM, Casella CR, Mitchell TM. The vaccine adjuvant monophosphoryl lipid A as a TRIF-biased agonist of TLR4. Nat Immunol (2007) 8:753–61. doi:10.1038/nait472
33. Ciabattini A, Pettini E, Fiorino F, Medaglini D. Distribution of antigen-experienced CD4+ T cells in nasal mucosa-associated lymphoid tissue following intranasal immunization with recombinant Streptococcus gordonii. Infect Immun (2006) 74:2760–6. doi:10.1128/IAI.74.5.2760-2766.2006
34. Neutra MR, Kozlowski PA. Mucosal vaccines: the promise and the challenge. Nat Immunol (2004) 5:13–6. doi:10.1038/nri1278
35. Ciabattini A, Cuppone AM, Pozzi G, Medaglini D. Adoptive transfer of transgenic T cells to study mucosal adjuvants. Methods (2009) 49:340–5. doi:10.1016/j.ymeth.2009.03.026
36. Reinhardt RL, Khoruts A, Merica R, Zell T, Jenkins MK. Visualizing the generation of memory CD4 T cells in the whole body. Nature (2001) 410:101–5. doi:10.1038/35065111
37. Mata-Haro V, Cekic C, Martin M, Chilton PM, Casella CR, Mitchell TM. The vaccine adjuvant monophosphoryl lipid A as a TRIF-biased agonist of TLR4. Nat Immunol (2007) 8:753–61. doi:10.1038/nait472
38. Marks EM, Helgeby A, Andersson JO, Schön K, Lycke NY. CD4 T cell priming upon vaccination. Proc Natl Acad Sci U S A (2012) 109:1080–6. doi:10.1073/pnas.1126481109
39. Moon JJ, Sub H, Li AV, Ockenhouse CF, Yadava A, Irvine DJ. Enhancing humoral responses to a malaria antigen with nanoparticle vaccines that expand Tfh cells and promote germinal center induction. Proc Natl Acad Sci U S A (2012) 109:1469–74. doi:10.1073/pnas.1116869109
40. Medaglini D, Ciabattini A, Cuppone AM, Costa C, Ricci S, Costalonga M, et al. Comparative study of the SLAM family of receptors adapter EAT-2 as a novel strategy for enhancing beneficial immune responses to vaccine antigens. J Immunol (2011) 186:722–32. doi:10.4049/jimmunol.1002105
41. Petrovas C, Yamamoto T, Gerner MY, Boswell KL, Wloka K, Smith EC, et al. CD4 T follicular helper cell dynamics during SIV infection. J Clin Invest (2012) 122:3281–94. doi:10.1172/JCI63039
42. Spensieri F, Borgogni E, Zedda L, Bardelli M, Buricchi F, Volpini G, et al. Human circulating influenza-CD4+ ICO5L1+IL-21+ T cells expand after vaccination, exert help function, and predict antibody responses. Proc Natl Acad Sci U S A (2013) 110:14330–5. doi:10.1073/pnas.1311998110
43. Harrison AM, Medaglini D. Mucosal adjuvants. Curr HIV Res (2010) 8:330–5. doi:10.2174/1570162107911208695
44. McAfee JP, Vella AT. Educating CD4 T cells with vaccine adjuvants: lessons from lipopolysaccharide. Trends Immunol (2010) 31:349–53. doi:10.1016/j.it.2010.08.005
45. Mata-Haro V, Cekic C, Martin M, Chilton PM, Casella CR, Mitchell TM. The vaccine adjuvant monophosphoryl lipid A as a TRIF-biased agonist of TLR4. Science (2007) 316:1628–32. doi:10.1126/science.1153896
46. Ciabattini A, Pettini E, Fiorino F, Prota G, Pozzi G, Medaglini D. Distribution of antigen-experienced CD4+ T cells in nasal mucosa-associated lymphoid tissue following intranasal immunization in mice. PLoS One (2011) 6:e19346. doi:10.1371/journal.pone.0019346
47. McAfee JP, Vella AT. Educating CD4 T cells with vaccine adjuvants: lessons from lipopolysaccharide. Trends Immunol (2010) 31:349–53. doi:10.1016/j.it.2010.08.005
48. Reinhardt RL, Khoruts A, Merica R, Zell T, Jenkins MK. Visualizing the generation of memory CD4 T cells in the whole body. Nature (2001) 410:101–5. doi:10.1038/35065111
49. Hu J, Havenar-Daughton C, Croisy S. Modulation of SAP dependent T:B cell interactions as a strategy to improve vaccination. Curr Opin Virol (2013) 3:363–70. doi:10.1016/j.coiviro.2013.05.015
50. Aldhamen YA, Appledorn DM, Seregin SS, Liu CI, Schultz JD, Godbehere S, et al. Expression of the SLAM family of receptors adapter EAT-2 as a novel strategy for enhancing beneficial immune responses to vaccine antigens. J Immunol (2011) 186:722–32. doi:10.4049/jimmunol.1002105
51. Moon JJ, Sub H, Li AV, Ockenhouse CF, Yadava A, Irvine DJ. Enhancing humoral responses to a malaria antigen with nanoparticle vaccines that expand Tfh cells and promote germinal center induction. Proc Natl Acad Sci U S A (2012) 109:1080–6. doi:10.1073/pnas.1126481109
52. Brandtzaeg P, Pabst R. Let's go mucosal: communication on slippery ground. Trends Immunol (2005) 26:570–7. doi:10.1016/j.it.2004.09.005
53. Ciabattini A, Pettini E, Fiorino F, Prota G, Pozzi G, Medaglini D. Distribution of antigen-experienced CD4+ T cells in nasal mucosa-associated lymphoid tissue following intranasal immunization in mice. PLoS One (2011) 6:e19346. doi:10.1371/journal.pone.0019346
54. Postak V, Rochereau N, Genin C, Verrier B, Paul S. New insights in mucosal vaccine development. Vaccine (2012) 30:142–54. doi:10.1016/j.vaccine.2011.11.003
55. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al. Safety and efficacy of MV A85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. Lancet (2013) 381:121–8. doi:10.1016/S0140-6736(13)60774-7
67. Sheby SH, Duncan CJ, Elias SC, Biswas S, Collins KA, O’Hara GA, et al. Phase I clinical evaluation of the safety and immunogenicity of the *Plasmodium falciparum* blood-stage antigen AMA1 in ChAd63 and MVA vaccine vectors. *PLoS One* (2012) 7:e31208. doi:10.1371/journal.pone.0031208

68. McCluskie MJ, Weeratna RD, Payette PJ, Davis HL. Parenteral and mucosal prime-boost immunization strategies in mice with hepatitis B surface antigen and Cpg DNA. *FEBS Immunol Med Microbiol* (2002) 32:179–85. doi:10.1111/j.1574-695X.2002.tb00551.x

69. Glynn A, Roy CJ, Powell BS, Adamovicz LC, Freytag LC, Clements JD. Protection against aerosolized *Yersinia pestis* challenge following homologous and heterologous prime-boost with recombinant plague antigens. *Infect Immun* (2005) 73:5256–61. doi:10.1128/IAI.73.8.5256-5261.2005

70. Mapletoft J, Latimer L, Babiuk LA, van Drunen Littel-van den Hurk S. Immunization of mice with a bovine respiratory syncytial virus vaccine induces superior immunity and protection compared to those by subcutaneous delivery or combinations of intranasal and subcutaneous prime-boost strategies. *Clin Vaccine Immunol* (2010) 17:23–35. doi:10.1128/CVI.00520-09

71. Pattani A, McKay PF, Garland MJ, Carram RM, Migalska K, Cassidy CM, et al. Microneedle mediated intradermal delivery of adapted recombinant HIV-1 CN54gag140 effectively primes mucosal boost inoculations. *J Control Release* (2012) 162:529–37. doi:10.1016/j.jconrel.2012.07.039

72. Steede NK, Rust BJ, Hossain MM, Freytag LC, Robinson JE, Landry SJ. Shaping T cell – B cell collaboration in the response to human immunodeficiency virus type 1 envelope glycoprotein gp120 by peptide priming. *PLoS One* (2013) 8:e56748. doi:10.1371/journal.pone.0056748

73. Alam S, Knowelden ZAG, Sangster MY, Sant AJ. CD4 T cell help is limiting and selective during the primary B cell response to influenza infection. *J Virol* (Forthcoming, 2013). doi:10.1128/JVI.02077-13

74. Sette A, Moutaftsi M, Moynor-Quiroz J, McCaulsmond MM, Davies DH, John- ston RJ, et al. Selective CD4+ T cell help for antibody responses to a large viral pathogen: deterministic linkage of specificities. *Immunology* (2008) 28:847–58. doi:10.1007/s10765-008-0148-0

75. Yin L, Calvo-Calle JM, Cruz I, Newman FK, Ennis FA, Frey SE, Ennis FA, et al. *CD4+ T cells provide intermolecular help to generate robust antibody responses in vaccinia virus-vaccinated humans. J Immunol* (2013) 190:6023–33. doi:10.4049/jimmunol.1208318

76. Moon JJ, Chu HH, Pepper M, McSorley SJ, Jameson SC, Kedl RM, et al. Naïve CD4+ T cell frequency varies for different epitopes and predicts repertoire diversity and response magnitude. *Immunology* (2007) 127:203–13. doi:10.1016/j.immuni.2007.07.007

77. Dunkley ML, Husband AJ. Distribution and functional characteristics of antigen-specific helper T cells arising after Peyer's patch immunization. *J Immunol Methods* (1987) 102:43–52. doi:10.1016/0022-1759(87)90084-1

78. Scriba TJ, Purbhoo M, Day CL, Robinson N, Fidler S, Fox J, et al. Defective TCR expression in transgenic mice constructed using cDNA-based alpha- and beta-chain genes under the control of heterologous regulatory elements. *Immunol Cell Biol* (1998) 76:34–40. doi:10.1046/j.1440-1711.1998.00709.x

79. Murphy K, Heimberger AM, Loh DY. Induction by antigen of intrathymic generation of antigen-specific helper T cells. *Aging Cell* (2006) 5:4951–60. doi:10.1111/j.1474-9726.2006.00386.x

80. Loeffler JS, Maue AG, Eaton SM, Lantplier PA, Tighe M, Haynes L. The aged environment contributes to the age-related functional defects of CD4 T cells in mice. *Aging Cell* (2012) 11:732–40. doi:10.1111/j.1474-9726.2012.00303.x

81. Barnden MJ, Allison J, Heath WR, Carbone FR. *CD4+ TCR+ thymocytes in vivo* reveals a local mucosal response to a disemminated infection. *Immunology* (2002) 106:365–77. doi:10.1046/j.1365-217x.2002.02659.x

82. Osenius A, Bachmann MF, Zinkernagel RM, Hengartner H. Virus-specific MHC-class II-restricted TCR-transgenic mice: effects on humoral and cellular immune responses after viral infection. *Eur J Immunol* (1998) 28:390–400. doi:10.1002/1874-8961(199801)28:1<390::AID-JEMT390>3.3.CO;2-8

83. Malik SA, Minna JD, Masson JD, Mazuma S, Lees AM. Phenotypic analysis of antigen-specific T lymphocytes. *Science* (1996) 274:94–9. doi:10.1126/science.274.5284.94

84. Crawford F, Kozono H, White J, Marrack P, Kapppler J. Detection of antigen-specific T cells with multivalent soluble class II MHC-covalent peptide complexes. *Immunology* (1998) 98:675–82. doi:10.1046/j.1365-2671.1998.00852.5

85. Tullous SS, Stern LJ. Class II major histocompatibility complex tetramer staining: progress, problems, and prospects. *Immunology* (2008) 128:305–13. doi:10.1111/j.1365-2657.2007.02801.x

86. Nepom GT. MHC class II tetramers. *J Immunol* (2012) 188:2477–82. doi:10.4049/jimmunol.1102398

87. Cochran JR, Cameron TO, Stern LJ. The relationship of MHC-peptide binding and T cell activation probed using chemically defined MHC class II oligomers. *Immunology* (2000) 102:241–50. doi:10.1046/j.1365-2674.2000.01027.x

88. Scibba TJ, Parrboho M, Day CL, Robinson N, Frieder S, Fox J, et al. Ultrasensitive detection and phenotyping of CD4+ T cells with optimized HLA class II tetramer staining. *J Immunol* (2005) 175:6334–43.

89. Erikk JM, Rowe BH, Kaufman GM, Lai J, Kuchlak JB, Way SS. Selective priming and expansion of antigen-specific Foxp3+ CD4+ T cells during *Listeria monocytogenes* infection. *J Immunol* (2009) 182:3032–8. doi:10.4049/jimmunol.0803402

90. Pagau AJ, Peters NC, Debrabant A, Ribeiro-Gomes E, Peppier M, Karp CL, et al. Tracking antigen-specific CD4+ T cells throughout the course of chronic *Leishmania major* infection in resistant mice. *Eur J Immunol* (2013) 43:427–38. doi:10.1002/eji.201242715

91. Bittner-Eddy PD, Fischer LA, Costalonga M. Identification of gingipain-Z, a virulence determinant of *Porphyromonas gingivalis* in C57BL/6 mice. *Mol Oral Microbiol* (2013) 28:452–66. doi:10.1111/omi.12038

92. Ancellet LR, Aldwell FE, Rich FJ, Kirman JR. Oral vaccination with lipid- formulated BCG induces a long-lived, multifunctional CD4+ T cell memory immune response. *PloS One* (2012) 7:e34588. doi:10.1371/journal.pone.0034588

93. Yates A, Chan C, Strid J, Moon S, Callard R, George A, et al. Reconstruction of cell population dynamics using CFSE. *MCB* Bioinformatics (2007) 8:196. doi:10.1186/1471-2105-8:196

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