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B cells, not dendritic cells, prime the dominant CD4+ Tfh response to *Plasmodium* infection

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Abstract

B cells, not dendritic cells, prime the dominant CD4+ Tfh response to *Plasmodium* infection

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CD4+ T follicular helper (Tfh) cells dominate the acute response to a blood-stage *Plasmodium* infection and provide signals to direct B cell differentiation and protective antibody expression. We studied antigen-specific CD4+ Tfh cells responding to *Plasmodium* infection in order to understand the generation and maintenance of the Tfh response. We discovered that a dominant, phenotypically stable, CXCR5+ Tfh population emerges within the first four days of infection and results in a CXCR5+ CCR7+ Tfh/central memory T cell response that persists well after parasite clearance. We also found that CD4+ T cell priming by B cells was both necessary and sufficient to generate this Tfh-dominant response, whereas priming by conventional dendritic cells was dispensable. This study provides important insights into the development of CD4+ Tfh cells during *Plasmodium* infection and highlights the heterogeneity of antigen-presenting cells involved in CD4+ T cell priming.

Dedication

For my sister Hazel, without whom I would not have survived the middle years of my PhD.

For my very soon-to-be husband Jeremy, in the pursuit of a double-doctor household.

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Chapter 1: Introduction

1.1: Induction of the adaptive immune response

The immune system consists of a critical network of molecules, cells, and barriers that has evolved to protect organisms from potentially dangerous microbes, called pathogens. Upon exposure, host organisms can develop disease or experience tissue damage as a result of the pathogen's presence. This can occur through a variety of mechanisms including, but not limited to, the dissemination of toxins interacting with host cells, the replacement of a beneficial/required commensal species, or the sequestration of essential nutrients. Vertebrates have developed a two-tiered immune system equipped to protect against pathogens that breach the barrier provided by the skin and mucosal surfaces.

The first tier, the innate immune system, is composed of surveilling cells and circulating molecules that quickly recognize common, invariant motifs expressed by broad classes of pathogens. This system can induce inflammatory responses and the complement cascade to neutralize the invading pathogen. In contrast, the adaptive immune system utilizes specialized white blood cells, primarily T cells and B cells, which express finely-tuned epitope-specific receptors produced through a somatic recombination process, called the T cell receptor (TCR) and B cell receptor (BCR), respectively. Activation of these receptors triggers pathogen-specific immune responses, resulting in the production of molecules, such as cytokines and antibodies, in an effort to clear the pathogen. Uniquely, T cells and B cells can form long-lasting "memory" populations that can be re-activated to more quickly respond to instances of reinfection. Furthermore, these cells have the ability to form tissue-resident populations to respond to reinfections at a localized site, without needing to involve the lymphocytes in lymphoid organs. Together, the innate and adaptive immune systems provide broad, rapid protection against pathogens and a pathogen-specific response that can be recalled throughout the life of the host organism to prevent illness and tissue damage.

Activated CD4+ T cells act as a key mediator between the innate immune cells that have detected a pathogen and activated B cells that need signals to guide the development of their response, a mechanism known as “T cell help” (Claman et al., 1966; Miller and Mitchell, 1968; Mitchell and Miller, 1968; Nossal et al., 1968). Naive CD4+ T cells are activated by antigen presenting cells (APCs), which present antigens that are derived from proteins processed by the cell’s proteasomes and loaded onto the major histocompatibility complex class II (MHC II) molecule (Blum et al., 2013). The TCR binds specific complexes of peptide-loaded MHC molecules and forms an immune synapse, a membrane structure that also facilitates the ligation of a variety of membrane-bound co-stimulatory molecules and the acquisition of secreted cytokines that impact the differentiation of the CD4+ T cell (Grakoui et al., 1999). Activated CD4+ T cells can broadly differentiate into two main populations with categorically distinct functions: effector T cells (Teff) and T follicular helper cells (Tfh) (Choi et al., 2011; DiToro et al., 2018; Johnston et al., 2009). Teff cells tend to circulate out of the lymphoid tissue to the site of infection/injury (Iezzi et al., 2001). Teff cells will also produce a specific set of cytokines promoted by fate-determining transcription factors (T-bet, GATA3, RORγt, etc.) These transcription factors are expressed in response to signals T cells receive during activation and the broad class of pathogen to which they are responding. The effector cytokines produced can be directly microbicidal, activate macrophages, and/or tune the types of antibodies that are produced by activated B cells (Ding et al., 1988; Huang et al., 1993; Stuehr and Marletta, 1987; Taub and Cox, 1995).

Another subset of CD4+ T cells provides signals specifically to B cells activated by the same epitope or “cognate” B cells (Garside et al., 1998; Lanzavecchia, 1985; Van den Eertwegh et al., 1993). Upon the observation that these cells co-localize with B cells at the T-B border in lymphoid organs due to the expression of the homing chemokine receptor CXCR5, they were named T follicular helper (Tfh) cells (Breitfeld et al., 2000; Kim et al., 2001; Schaerli et al., 2000). Tfh cell programming was later found to be dependent on the effects of the transcription

factors TCF1 and Bcl6, which directly antagonize Blimp1, an essential factor for Th1 effector differentiation (Johnston et al., 2012; Johnston et al., 2009; Oestreich et al., 2012; Poholek et al., 2010; Wu et al., 2015; Yu et al., 2009). These transcription factors, among other factors and events, regulate the expression of various Tfh costimulatory molecules, including CD28, ICOS, CD40L, OX40, PD-1, and SLAM family receptors, whose ligands are expressed by activated B cells (Gaspal et al., 2005; Haynes et al., 2007; Linterman et al., 2009; Liu et al., 2015; Qi et al., 2008; Rolf et al., 2010; Watanabe et al., 2017). When cognate B cells confer these signals with TCR stimulation, they reinforce the Tfh phenotype and can promote their transition to germinal center (GC) Tfh (Kerfoot et al., 2011). Together these findings led the field to consider the Teff and Tfh populations to be completely distinct subsets. However, a more nuanced understanding is emerging that allows for biased Tfh responses according to the type of helper response induced, based primarily on reports of Tfh cells expressing both Bcl6 and a Teff-defining factor, like T-bet, GATA3, or CXCR3 (Fahey et al., 2011; Iyer et al., 2015; Marshall et al., 2011; Morita et al., 2011; Obeng-Adjei et al., 2015; Pepper et al., 2011; Velu et al., 2016). While these cells (Tfh1, Tfh2, etc.) have primarily been described in circulation, they have also been indicated in lymphoid tissues, usually in the context of chronic infection. The factors driving the development of these biased Tfh cells remain unclear but may rely on the degree of inflammation found in lymphoid tissues.

Many factors dictate the bifurcation of Teff and Tfh from recently activated CD4+ T cells during T cell priming, including TCR signaling, cytokine signaling, costimulation, and the antigen presenting cell (APC) providing these factors. Although the cell subset frequencies vary with different infections, endogenous, epitope-specific, polyclonal cells responding to either bacterial or viral infections tend to generate relatively equal proportions of Tfh cells and Teff cells at the population level due to heterogeneity within the naïve CD4+ T cell repertoire (Tubo et al., 2013). The division of labor between the Teff and Tfh functions is generally evident within the first 5-10 days after infection and is thought to be driven initially by dendritic cell (DC) priming, followed by

interactions with B cells (Hale et al., 2013; Pepper et al., 2011). Prolonged or persistent TCR stimulation promotes Tfh differentiation during priming, which typically occurs when a dendritic cell presents antigen to a naïve CD4+ T cell (Baumjohann et al., 2013; Deenick et al., 2010; Fahey et al., 2011). This increased dwell time acts independently of inherent TCR signal strength on the CD4+ T cell fate decision (Keck et al., 2014). IRF4 is a critical factor in the differentiation of various CD4+ T cell subsets downstream of TCR signaling by interpreting TCR signal strength during antigen presentation (Huber and Lohoff, 2014). IRF4 coordinates either increased Blimp1 and other Teff gene induction when a cell receives high-strength TCR stimulation or increased Bcl6 and Tfh gene induction if that strength of signal is low/intermediate (Krishnamoorthy et al., 2017). This work has led to the development of two different models of Tfh development based on TCR affinity and dwell time: 1. lower TCR affinity results in signaling events that lead to Bcl6 induction and the antagonism of Teff programming (Krishnamoorthy et al., 2017); and 2. high TCR affinity indicated by increased dwell time prevents signals like IL-2 from inducing Teff programming (Tubo et al., 2013; DiToro et al., 2018)). Further downstream, activated CD4+ T cells receiving IL-6 and IL-21 activate STAT3 to induce BCL6 expression (Nurieva et al., 2008). IL-6 also activates STAT3 to downregulate CD25 (IL-2Ra) expression, favoring Tfh formation. Relatedly, the absence of IL-2 signaling biases Tfh formation rather than promoting Teff differentiation and occurs through STAT3-mediated downregulation of CD25 (IL-2Ra) (Choi et al., 2013).

Antigen presenting cells also provide costimulatory signals to direct CD4+ T cell differentiation, but the signals required for Tfh formation differ between the initial contact with a DC and a Tfh cell’s subsequent contact with a B cell that promotes the maintenance of the phenotype. During the early priming, DCs necessarily provide B7-CD28 costimulation along with peptide-MHC (Watanabe et al., 2017). PI3K signaling downstream of ICOS ligation from DCs is critical in the formation of Tfh cells (Choi et al., 2011; Rolf et al., 2010), but it is also an important signal from B cells for the maintenance of the phenotype (Nurieva et al., 2008; Xu et

al., 2013). Recent work has been able to account for the effects of multiple signals together, which is critical to a physiological understanding of this process as their interplay undoubtedly influences cell fate decisions. IL-2 producing cells are fated to form Tfh cells and this programming is linked to cells with the highest TCR signal strength (DiToro et al., 2018), which has been shown by others to be directly due to increased dwell times. Understanding the kinetics and relative contributions of additional signals, like costimulation, in concert with the signals a T cell receives during priming and differentiation will allow for a greater ability to manipulate the formation of very specific T cell responses.

Tfh cells that have engaged B cells at the T-B border can migrate into structures in B cell follicles called germinal centers (GCs) (Haynes et al., 2007; Schwickert et al., 2011), where somatic hypermutation and isotype class-switching primarily occur (Berek et al., 1991; Jacob et al., 1991; Muramatsu et al., 2000), to participate in selection of affinity matured-GC B cells (Allen et al., 2007; Liu et al., 2015). GC-derived B cells can form plasma cells, which secrete antibodies at very high rates (Paus et al., 2006; Phan et al., 2006), or they can form memory B cells, primed to respond to a secondary challenge (Ridderstad and Tarlinton, 1998; Takahashi et al., 2001). GC Tfh cells produce the cytokines IL-4 and IL-21 to regulate the physical cycling of GC B cells between the dark and light zones, which regulates the balance between affinity maturation and selection, respectively (McHeyzer-Williams et al., 2015; Shulman et al., 2014; Victora et al., 2010). Tfh cells can also help B cells at extrafollicular sites, partially through IL-21 production, leading these B cells to primarily form short-lived plasma cells (Lee et al., 2011). The resulting fine-tuned humoral response is considered the ultimate goal of the immune response as it leads to long-lived B cell populations and high affinity antibodies with long persistence to protect the body from reinfection.

1.2: Generation of memory CD4+ T cells by immunization and infection

The generation of antibody responses is a powerful strategy against subsequent infections as they are generally a systemic mechanism for pathogen clearance. Memory T cell

responses, on the other hand, act locally either in lymphoid or in non-lymphoid tissue. In the case of CD4+ helper T cells, the memory populations are broadly distinguished by their functions during restimulation: production of microbicidal factors or providing T cell help to B cells. Protection against pathogens that are best controlled with the invocation of T-dependent antibody responses, such as *Plasmodium*, relies on the synergy of memory T and B cell populations (Langhorne et al., 1990; McDonald and Phillips, 1978).

Following a primary immune response, the majority (~90%) of Teff cells will undergo apoptosis (Williams et al., 2008), while a small population of Teff cells becomes the T effector memory (Tem) population (Harrington et al., 2008). These cells lack expression of CCR7 and CD62L, allowing them to circulate out of lymphoid tissue, surveil non-lymphoid tissues, and quickly produce antimicrobial cytokines when restimulated – more quickly than lymphoid tissue-localized memory CD4+ T cells (Reinhardt et al., 2001; Sallusto et al., 1999). This lymphoid tissue-patrolling memory CD4+ T cell population, or T central memory (Tcm) cells, are seeded by the Tfh population (DiToro et al., 2018; Fairfax et al., 2015; Pepper et al., 2010). The Tcm is considered to be a less terminally differentiated population of cells, relative to Tem, that can develop into Teff or Tfh cells upon restimulation, based on the examination of functional responses (Pepper et al., 2011) or the expression of effector cell markers, like Blimp1 (Kallies et al., 2009). However, recent work examining single-cell transcriptomics suggests that the multipotency previously attributed to Tcm cells is actually due to heterogeneity in the potential fates of restimulated CXCR5+ memory cells. Ciucci et al. demonstrated distinct transcriptional signatures within the Tfh compartment, with the memory precursor subset exhibiting early expression of the transcription factor Thpok (Ciucci et al., 2019). Thpok antagonizes Blimp1-associated exhaustion programming, therefore preserving the cell's effector function potential upon restimulation (Ciucci et al., 2019). This work suggests there are early indicators of memory fates in CD4+ T cells that can be observed during the peak of the primary response.

Regardless of the mechanism of memory development, the rapid emergence of Teff and Tfh populations formed from restimulated memory CD4+ T cell populations enables the immune system to both directly combat the pathogen and re-trigger the B cell response more quickly than a naïve CD4+ T cell's response would (London et al., 2000; Rogers et al., 2000). However, the importance of the generation of a Tcm population is especially evident in systems where T-dependent memory B cell responses are critical for rapid pathogen clearance. While some memory B cell populations can be reactivated independently of T cells (Bernasconi et al., 2002; Richard et al., 2008; Zuccarino-Catania et al., 2014), a large body of work demonstrates that Tcm cells are critical for a rapid response from memory B cells (Ise et al., 2014; Kim et al., 2001; MacLeod et al., 2011; Schaerli et al., 2000; Weber et al., 2012), particularly for the formation of rapidly antibody-secreting plasmablasts (Krishnamurty et al., 2016). The reactivation of the memory B cell seems to be dependent on the ability of the memory B cell to present antigen to cognate Tcm cells, according to studies conducted in both mice and humans (Chevalier et al., 2011; Shimoda et al., 2006). This presentation by B cells induces Bcl6 expression in the T cell, reinitiating the Tfh programming (Ise et al., 2014).

The human CD4+ T cell memory compartments share many of the same general characteristics and functions as the murine counterparts, largely guided by their chemokine receptor expression (Fritsch et al., 2005; Sallusto et al., 1999). Just as in mice, Tem cells are found in nonlymphoid tissues, whereas Tcm cells are observed in the circulation and tonsils (Campbell et al., 2001). Early human studies demonstrated that CD4+ memory T cells undergo self-renewal as indicated by their decreased telomere length compared to naïve CD4+ T cells (Weng et al., 1995). More recently, others demonstrated that Tem cells have a more rapid turnover rate compared to Tcm cells, which may be due to intrinsic differences in their programming (Gattinoni et al., 2011; Macallan et al., 2004). Understanding the relative importance of these two populations and then how to best maintain critical populations for

secondary immune responses on a pathogen-specific basis can dramatically change how vaccination is approached.

1.3: General characteristics of *Plasmodium* infection and adaptive immune response

Parasites of the *Plasmodium* genus are the causative agent of the disease malaria. These protozoa are carried by female *Anopheles* mosquitoes which transmit the sporozoite stage of the parasite to mammalian hosts during blood feeding. Once in the skin, sporozoites that are not trapped and killed by skin-resident macrophages quickly travel to the liver and invade hepatocytes. Within the hepatocyte, the sporozoite matures to the merozoite stage. While restricted to the liver, infection with *Plasmodium* parasites is clinically asymptomatic (Hopp and Sinnis, 2015; Prudêncio et al., 2006). Merozoites emerge once fully matured and travel to the bloodstream where they establish infection in red blood cells (RBCs) and expand exponentially via asexual reproduction (Aly et al., 2009). Blood-stage infection is clinically characterized by fever, headache, and chills and in severe cases progresses to include severe anemia, respiratory distress, and cerebral malaria (WHO, 2018). Malaria is a treatable and preventable disease that developed areas of the world have eliminated the transmission of by using interventions such as insecticides and bed netting. However, under-resourced areas of the world, especially sub-Saharan Africa and Southeast Asia, have yet been unable to do so (WHO, 2018). This has led the field to pursue vaccination strategies informed by the natural immune response to curb the incidence of malaria transmission and, importantly, severe malaria cases.

Clearance of *Plasmodium* parasites in both murine and human infection depends significantly on the adaptive immune system. Antibody production is critical for clearance of both human- and murine-tropic strains of the blood-stage parasite (Cohen et al., 1961; Crompton et al., 2010; Hirunpetcharat et al., 1997; Moss et al., 2012; Riley et al., 1992). CD4+ T cells are an important component of this response, likely due to their role in eliciting T cell-dependent antibodies (Cigel et al., 2003; Langhorne et al., 1990; McDonald and Phillips, 1978). This CD4+

T cell response is critical during the primary immune response, while the memory cells that form contribute to clearance of parasites during a secondary challenge (Gwyer Findlay et al., 2014; Langhorne et al., 1990; McDonald and Phillips, 1978; Vinetz et al., 1990). Humans living in malaria-endemic areas fail to develop a recall response that quickly clears the parasite until they have endured several malaria seasons. Therefore, young children are at great risk of developing severe malaria symptoms (Langhorne et al., 2008). This non-sterilizing immunity eventually builds so that by young adulthood, individuals who have been repeatedly exposed to the parasite continue to transmit the disease, but no longer exhibit clinical symptoms while infected (Mueller et al., 2013). This phenomenon is dependent on continued seasonal exposure, otherwise this basic immunity to disease is lost (Färnert et al., 2015).

Efforts to study the memory CD4+ T cell response to *Plasmodium* infection have largely demonstrated a lack of memory maintenance or diversity of memory responses. Acute blood-stage *Plasmodium* infection in immunologically intact mice results in sterile immunity to a reinfection initiated soon after the primary infection (Murphy, 1980). However, this period of sterilizing immunity to blood-stage parasites in mice is not lifelong, perhaps due in part to a declining CD4+ memory T cell compartment (Freitas do Rosário et al., 2008; Murphy, 1980). This work raises questions about the formation and maintenance of memory cells in this model, which could potentially illuminate failures of the development of the human memory response to malaria as well. Several studies have demonstrated that the acute CD4+ T cell response to a blood-stage *Plasmodium* infection in both humans and mice is dominated by CD4+ Tfh cells, followed by the development of Tcm cells (Obeng-Adjei et al., 2015; Pérez-Mazliah et al., 2015; Silva et al., 2013). Prior reports examining the acute CD4+ T cell response also suggests that the Th1-biased Tfh response that is generated during *Plasmodium* infection does not provide adequate B cell help (Obeng-Adjei et al., 2015; Ryg-Cornejo et al., 2016). Based on our knowledge of the function and fate of Tfh cells, understanding how this biased acute response impacts the quality and the longevity of the memory CD4+ T cell response and how it interacts

with responding B cells will be critical for developing a long-lasting vaccination strategy that withstands the effects of natural infection in endemic areas.

1.4: Outstanding questions

There are various factors contributing to the difficulty in generating an effective vaccine against *Plasmodium*, including the complexity of the unicellular organism, the variations across species, and the multi-stage life cycle, among others. Some of the most effective vaccination endeavors have utilized attenuated sporozoites or administered prophylactic antimalarial drugs alongside sporozoite inoculation and have demonstrated protection in the context of controlled human malaria infections (Clyde et al., 1973; Lyke et al., 2017; Mordmüller et al., 2017; Roestenberg et al., 2009; Seder et al., 2013). Unfortunately, these strategies have not been feasible as a mass vaccination program, due to the maintenance conditions of the sporozoites, the administration route, the requirement of the co-administration of other drugs, and/or the sheer number of sporozoites needed per individual. Other strategies that have shown promise in pre-clinical studies and in malaria-naïve humans have been disappointingly unsuccessful in human studies in malaria-endemic areas, indicating that the effects of natural infection on the immune response need to be better understood to develop a vaccination strategy that will not be altered by natural infection in endemic regions (Ogutu et al., 2009; Wykes et al., 2005). Of these efforts, the top malaria vaccine candidate is RTS,S, which when tested on children in malaria-endemic areas only provided 30-50% of exposed individuals with short-lived responses (The RTS, 2012). Recent work demonstrates the potential for this vaccine to stimulate antigen-specific Tem and Tcm populations and suggests that both populations could play roles in promoting parasite clearance and providing B cell help (Moncunill et al., 2017). The failure to generate sterilizing immunity when exposed to natural infection even when forming a potentially effective memory T cell repertoire suggests a defective immune response during infection. To this end, the work presented here examines the development of an endogenous antigen-specific memory CD4+ T cell response – in particular the factors influencing the formation of

memory CD4+ T cells induced by *Plasmodium* infection – in order to effectively promote the generation of a useful immune response.

Chapter 2: Characterization of the acute and memory CD4+ T cell responses during *Plasmodium* infection

2.1: Introduction: CD4+ T cell responses during *Plasmodium* infection

The acute CD4+ T cell response to *Plasmodium* infection has been characterized as a Th1-biased response, with effector T cells producing type 1 cytokines like IFNy and TNFa (Amante and Good, 1997; Su and Stevenson, 2000; Zander et al., 2016; Zander et al., 2017). These powerful cytokines can have significant off-target pathological effects, leading to disruption of the splenic architecture (Cadman et al., 2008), and need to be countered by IL-10 or TGF-b (Couper et al., 2008; Li et al., 1999; Niikura et al., 2010; Omer et al., 2003; Omer and Riley, 1998; Sanni et al., 2004). Th1 cells have been identified as the primary source of IL-10 during *Plasmodium* infection in an IL-27-dependent manner and can be co-expressed with inflammatory cytokines (Freitas do Rosário et al., 2012; Villegas-Mendez et al., 2016). The development and functional potential of the Tfh response in malaria remains unclear. While Tfh1 cells have been described in malaria infection (Obeng-Adjei et al., 2015; Wikenheiser et al., 2018), the Tfh population generated has been reported to be not useful in providing B cell help (Butler et al., 2011; Ryg-Cornejo et al., 2016). Specifically, the magnitude of the Tfh response does not correlate with *Plasmodium*-specific antibody responses generated (Obeng-Adjei et al., 2015). Additionally, the waning of the CD4+ T cell response following *Plasmodium* infection has been documented in a global manner, first through loss of immunity to rechallenge and later through the global loss of the cells (Freitas do Rosário et al., 2008; Murphy, 1980). These approaches, however, do not follow the interactions that *Plasmodium*-specific cells experience. This led us to develop a system to interrogate the development of an antigen-specific CD4+ memory T cell response to *Plasmodium* infection in mice.

2.2: Results

2.2a: Epitope-specific CD4+ T cells exhibit limited expansion in response to *Plasmodium* infection

To study an antigen-specific CD4+ T cell response to *Plasmodium yoelii* infection, we used a transgenic parasite expressing a well-characterized CD4+ T cell epitope derived from the glycoprotein of lymphocytic choriomeningitis virus (LCMV), $GP_{66\text{-}80}$ (*Py-GP66*) (Dow et al., 2008; Hahn et al., 2018; Zander et al., 2017). This allowed us to use the previously generated $GP_{66\text{-}77}$:I-Ab tetramer and magnetic bead enrichment to examine rare, antigen-specific cells responding early in infection (Moon et al., 2007). To gain an understanding of the kinetics of the response, we first compared GP66+ cells in LCMV and *Py-GP66* infections at several timepoints (**Fig. 2.1A, B**). Comparable numbers of CD44+ GP66+ cells emerged from the naïve precursor population of ~100 cells as early as 4 days post infection with either *Py-GP66* or LCMV. However, whereas this reached a peak of approximately 4,000 cells at 12 days post-infection with *Py-GP66,* it expanded to ~80,000 cells 12 days after LCMV infection (17-fold higher), consistent with previous reports (MacLeod et al., 2008; Nelson et al., 2015; Whitmire et al., 2006) (**Fig. 2.1B**). Furthermore, in LCMV, the numbers of GP66+ cells contracted with viral clearance and then stabilized to maintain a population of long-lived memory cells, as previously described (Corbo-Rodgers et al., 2012; Hondowicz et al., 2018; Matloubian et al., 1994). During *Plasmodium* infection, however, the number of CD44+ GP66+ cells continued to decline (**Fig. 2.1B**). We analyzed the contraction of GP66+ cells from the peak at 12 days post-infection to 150 days post-infection by nonlinear regression (dashed lines). The slopes of the lines (31.15 for LCMV and -7.001 for *Py-GP66*) demonstrate that the contraction/memory phases have statistically different kinetics. As the differences in these responses begins early in the induction of the adaptive immune responses to these infections, we sought to examine how early events during the initiation of the GP66+ response may differentially impact the resulting expansion and maintenance of the cells in the context of these two infections.

Differences in the number of GP66+ cells in the two infections could arise due to multiple factors that affect how antigen is perceived at these early timepoints. Changes in antigen load can alter clonal expansion and the degree of T cell contraction (Park et al., 2008); differences in TCR signal strength can alter the transcriptional events downstream of TCR stimulation (Iwata et al., 2017); and alterations in co-stimulatory signals can sustain the proliferative burst (Howland et al., 2000; McAdam et al., 2000). T cell responsiveness during priming can be compared by measuring the responder frequency and proliferative capacity, which are associated with perception of antigen and the maintenance of the proliferative burst (Gudmundsdottir et al., 1999). To address these possibilities, we adoptively transferred CFSE-labeled TCR transgenic CD4+ T cells specific for the GP66 epitope (SMARTA) (Oxenius et al., 1998) into congenically-marked WT recipients infected with LCMV or *Py-GP66* one day post-transfer. We found that in both infections, GP66+ SMARTA cells undergo at least 7 rounds of proliferation, based on the dilution of CFSE (**Fig. 2.1C**). We calculated both the responder frequency (the fraction of input SMARTA cells that proliferated at least once) and the proliferative capacity (the number of daughter cells generated per input SMARTA precursor cell) (Gudmundsdottir et al., 1999). There was no difference in the responder frequency between the LCMV and *Py-GP66* infections, indicating comparable access to antigen in both infections. However, there was a marked decrease in the proliferative capacity of the GP66+ SMARTA cells during *Plasmodium* infection, suggesting differential interpretation of co-stimulation (**Fig. 2.1D**). These data demonstrate that the conditions required to sustain cell division may be altered at an early stage in the response to *Plasmodium* infection and raise the possibility that this is a phenotypically stable phenotype imprinted early in the adaptive immune response

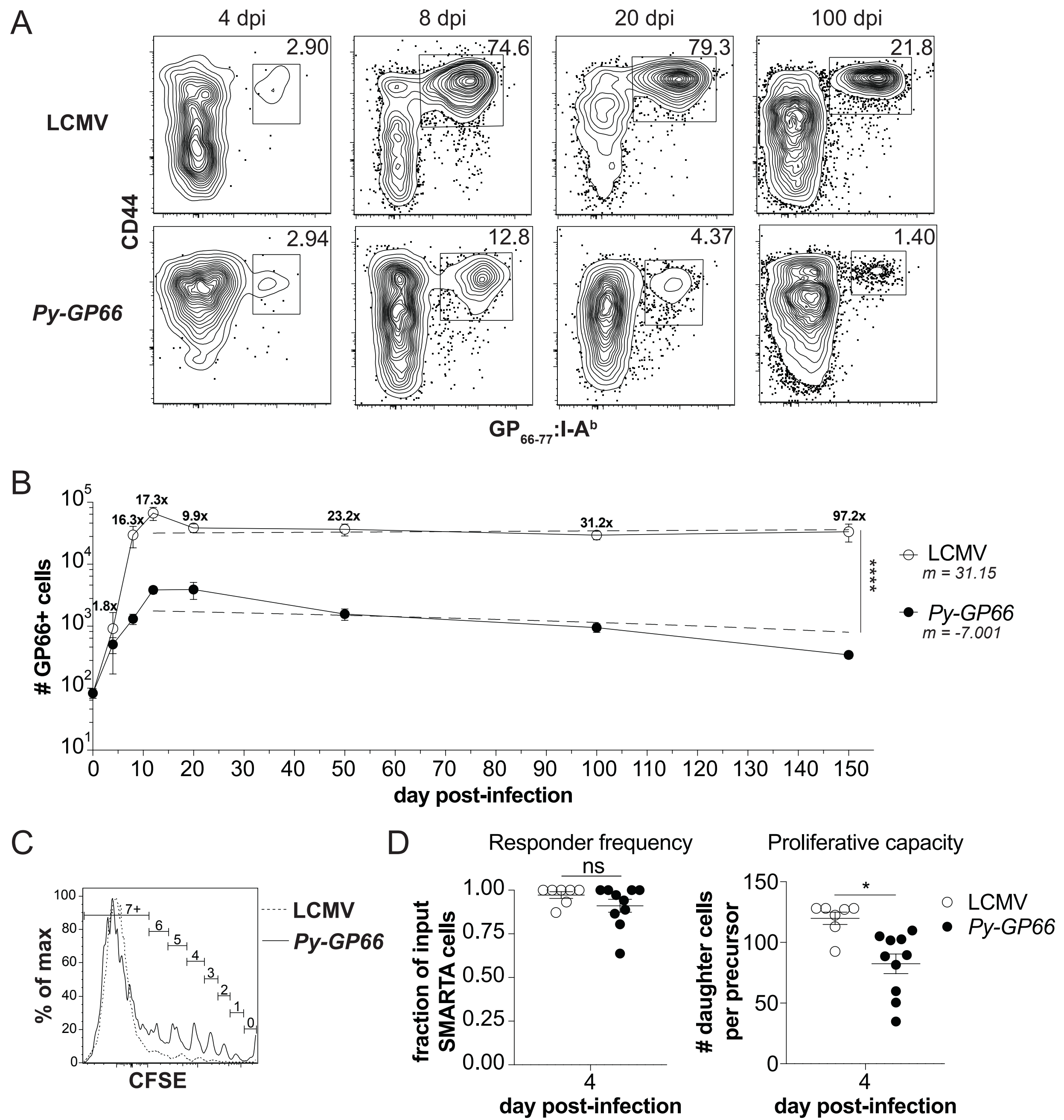

Figure 2.1: Expansion of epitope-specific CD4+ T cells in response to *Plasmodium* infection

A. At acute and late time points post-infection with LCMV or *Py-GP66*, the activation and expansion of GP66+ CD4+ T cells from secondary lymphoid organs were assessed by flow cytometry. Plots are gated on dump- CD3+ CD8- CD4+ cells. **B.** Graph shows the number of

GP66+ cells from both infections over time. The fold change in the cell number from *Py-GP66* infection is indicated above the LCMV curve. Data are pooled from 4-8 mice from each cohort at each timepoint from two independent experiments. A nonlinear regression from the peak at day 12 to day 150 was performed for each infection and is indicated by the dashed lines. The slope of the *Py-GP66* line is -7.001 (-17.75 to 3.747) and the slope of the LCMV line is 31.15 (-170.7 to 233). **C.** CFSE-labeled GP66-specific transgenic CD4+ T cells (SMARTA) were transferred to WT mice and infected with LCMV or *Py-GP66*. Number of cell divisions are indicated on gates. Both histograms are gated on SMARTA+ CD44+ GP66+ T cells. **D.** Responder frequency and proliferative capacity of SMARTA+ CD44+ GP66+ cells were calculated as previously described (Gudmundsdottir et al., 1999). Data are pooled from 7-10 mice per cohort from two independent experiments and were analyzed by unpaired *t*-test. For B. and D., data are shown as mean ± SEM. *p < 0.05 ****p < 0.0001.

2.2b: Epitope-specific CD4+ T cells express CXCR5 at both acute and memory timepoints after infection

To gain a greater understanding of how the differentiation of these two epitope-specific populations occurs, we also examined the phenotype of the GP66+ CD4+ T cells in the context of the two infections. Current models suggest that a two-step process initiated by priming via a dendritic cell (DC) followed by subsequent interactions with B cells results in Tfh differentiation (Lu et al., 2011; Poholek et al., 2010). The initial interactions between DCs and CD4+ T cells are thought to drive an early bifurcation of activated CD4+ T cells into a Tfh or Teff pathway based on their ability to express CXCR5 or the IL-2 Rα chain, respectively (Chang et al., 2014; Pepper et al., 2011; Tubo et al., 2013). Tfh cells that up-regulate BCL6 and CXCR5 migrate to the T-B border of secondary lymphoid organs where the Tfh phenotype is maintained by interactions with B cells (Cannons et al., 2010; Choi et al., 2011; Deenick et al., 2010; Glatman

Zaretsky et al., 2009; Haynes et al., 2007; Johnston et al., 2009; Nurieva et al., 2008; Nurieva et al., 2009; Poholek et al., 2010; Qi et al., 2008; Salek-Ardakani et al., 2011; Watanabe et al., 2017). We therefore first determined the kinetics of activated GP66+ CD4+ T cell differentiation in the two infections to understand how early the Tfh phenotype emerges. In uninfected animals, GP66+ cells did not express CXCR5, as expected (**Fig. 2.2A**) (Tubo et al., 2013). At four days post-infection, we could consistently find an expanded population of CD44+ GP66+ T cells in both infections. In LCMV-infected mice only a small proportion of GP66+ CD4+ T cells expressed CXCR5 at this timepoint; yet the vast majority (~80%) of GP66+ CD4+ T cells responding to *Plasmodium* already expressed CXCR5 (**Fig. 2.2A**). The global CD44+ CD4+ T cell population demonstrates a similar disparity (**Fig. 2.3A, B**). Approximately half of the cells responding to LCMV exhibited a Tfh phenotype by day 8, as previously described (Tubo et al., 2013; Wu et al., 2015), though the frequency of CXCR5+ CD44+ GP66+ cells continued to increase until day 20. In contrast, CD44+ GP66+ cells induced by *Plasmodium* infection maintained a predominant CXCR5+ Tfh phenotype all timepoints observed (**Fig. 2.2A, B**). We confirmed that the CXCR5+ CD4+ cells formed in *Plasmodium* infection were bona fide Th1 Tfh cells through analysis of the fate-determining transcription factors for this subset, T-bet and BCL6 (Cannons et al., 2010). While it was difficult to perform these studies on the low numbers of GP66+ cells at day 4, by day 8 post-infection with *Plasmodium*, the majority of GP66+ cells expressed high levels of BCL6 and lower levels of T-bet, consistent with a Th1 Tfh phenotype (**Fig. 2.3C, D**) (Obeng-Adjei et al., 2015). Together, these data demonstrate that the same epitope-specific population can adopt different frequencies of Tfh versus Teff phenotypes with different kinetics in response to different infections. These data further suggest that some aspect of *Plasmodium* infection commits the GP66+ cells to a stable Tfh phenotype very early in infection.

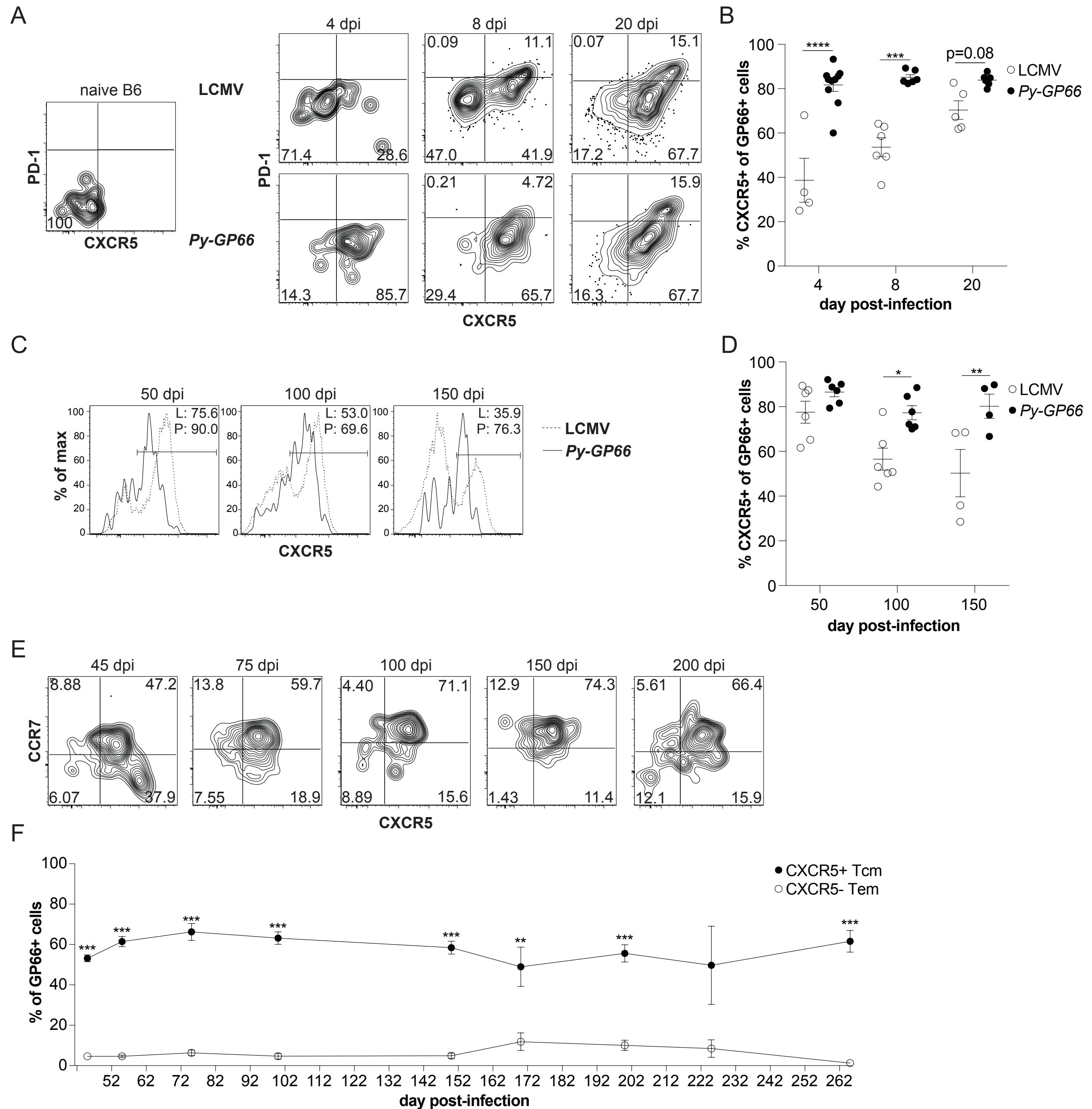

Figure 2.2: Plasmodium-specific CD4+ T cells express CXCR5 at both acute and memory timepoints

A. Representative flow plots of antigen-specific CD4+ T cells isolated from secondary lymphoid organs at naive or acute time points after LCMV or *Py-GP66* infection. Plots are gated on dump-CD3+ CD8- CD4+ CD44+ GP66+ cells. **B.** Summary data of the percent of CXCR5+ GP66+ cells shown in 2A. Data are pooled from 4-8 mice per time point from at least two independent

experiments and were analyzed by two-way ANOVA. **C.** Representative flow plots of GP66-specific CD4+ T cells at memory time points after LCMV or *Py-GP66* infection. **D.** Summary data of the percent of CXCR5+ GP66+ cells shown in Fig. 2C. Data are pooled from 4-6 mice per time point from at least two independent experiments and were analyzed by two-way ANOVA. **E.** Representative flow plots from GP66+ CD4+ T cells at memory time points after *Py-GP66*. CCR7+ T central memory (Tcm) cells and CCR7- T effector memory (Tem) populations are shown. GC Tfh cells are included the CCR7- CXCR5+ population (Haynes et al., 2007). **F.** Summary data of the percent of the Tcm and the Tem populations from Fig. 2E. Data are pooled from 3-10 mice per time point from at least two independent experiments and were analyzed by unpaired *t*-tests. For B., D., and F., data are shown as mean ± SEM. *p < 0.05 **p < 0.01 ***p < 0.001 ****p < 0.0001.

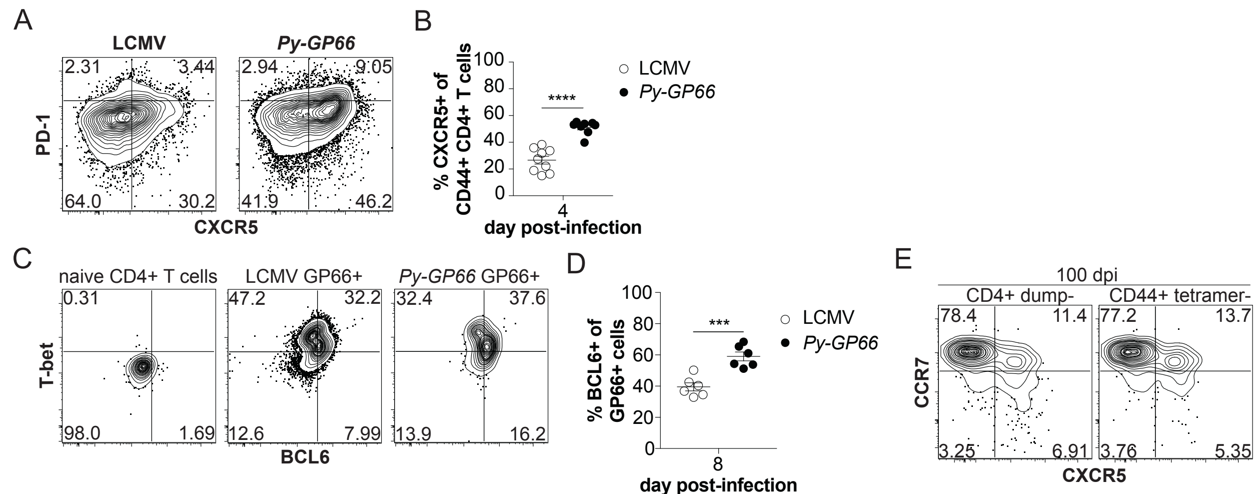

Figure 2.3: The expression of Tfh and Tcm markers in naïve cells

A. Representative flow plots of CD44+ antigen non-specific cells from 4 days post-infection with LCMV or *Py-GP66* in WT mouse. Cells are gated on dump- CD3+ CD8- CD4+ CD44+ cells. **B.** Summary data of the percent of CXCR5+ cells from Fig. S1A. Data are pooled from 8-9 mice per cohort and are representative of two independent experiments. **C.** Representative plots of naive cells (gated on dump- CD3+ CD8- CD4+ CD44-) and the LCMV and *Py-GP66* plots

(gated on dump- CD3+ CD8- CD4+ CD44+ GP66+) show T-bet and Bcl-6 expression 8 days post-infection with LCMV and *Py-GP66* infection. **D.** Summary data of the percent Bcl-6+ GP66+ cells from Fig. S1C. Data are pooled from 6 mice per cohort and are representative of two independent experiments. **E.** Plots are gated on dump- CD3+ CD8- CD4+ cells and dump- CD3+ CD8- CD4+ CD44+ GP66- cells, respectively, following 100 days *Py-GP66* infection. Representative of 6 mice from at least two independent experiments. Data were analyzed by unpaired *t*-test. For B. and D., data are shown as mean ± SEM. ***p < 0.001. ****p < 0.0001.

We next assessed the stability of this early Tfh population by analyzing the GP66+ CD4+ T cells at later timepoints. We hypothesized that the early Tfh differentiation would result in a later central memory population, as previously described in Th1 bacterial and viral infections (DiToro et al., 2018; Pepper et al., 2011). Fifty days after either infection, the majority of CD4+ CD44+ GP66+ cells maintained a CXCR5+ Tfh phenotype, but at later timepoints, the frequency of CXCR5+ cells decreased in the LCMV-infected mice, while *Plasmodium*-infected mice maintained approximately 80% CXCR5+ cells (**Fig. 2.2C, D**). Of note, the level of CXCR5 expression, as determined by mean fluorescence intensity (MFI), on the CD44+ GP66+ CXCR5+ cells after LCMV infection was consistently higher than after *Plasmodium* infection at all the timepoints tested, despite the frequency of total CXCR5+ cells being generally higher in response to *Plasmodium* infection than LCMV infection (**Fig. 2.2C, D**). In order to determine the types of memory cells that form in response to *Plasmodium* infection, we also assessed markers that distinguish central and effector memory cells (CCR7 and CD62L) (Sallusto et al., 1999). Gates were drawn using the expression of CCR7 and CXCR5 on global CD4+ T cells and global CD4+ CD44+ T cells (**Fig 2.3E**). We demonstrate that GP66+ memory T cells following *Plasmodium* infection largely adopted a Tcm (CCR7+ CXCR5+) phenotype (**Fig. 2.2E**). This skewing in the memory pool was observed at all timepoints examined up to 260 days post-infection (**Fig. 2.2F**). These data therefore demonstrate that the early differentiation

of CXCR5+ Tfh cells observed is maintained long-term as a CXCR5+ Tcm population, suggesting that this is a stable phenotype, imprinted early in the response to *Plasmodium* infection.

2.3: Discussion

The *Plasmodium*-specific CD4+ T cell population acquires a stable CXCR5+ Tfh phenotype at acute timepoints during infection that is maintained late into the memory response. As shown in previous bacterial and viral infections, this early Tfh cell population forms a prominent Tcm population (DiToro et al., 2018; Fairfax et al., 2015; Pepper et al., 2011). This is in contrast to other studies (Opata et al., 2015; Stephens and Langhorne, 2010) in which adoptively transferred, monoclonal TCR transgenic CD4+ T cells specific for a *Plasmodium* antigen predominantly formed a Tem population. This result may be due to the specificity of the monoclonal TCR transgenic population that was utilized, as monoclonal TCR transgenic populations do not represent the compendium of phenotypes seen in polyclonal endogenous antigen-specific populations (Tubo et al., 2013). Skewing of the polyclonal antigen-nonspecific CD4+ T cells to a BCL6+ Tfh phenotype has also been observed at early timepoints during *Plasmodium* infection, which further supports our findings (Obeng-Adjei et al., 2015; Pérez-Mazliah et al., 2015; Silva et al., 2013).

Chapter 3: Factors driving T follicular helper cell fate in *Plasmodium* infection

3.1: Introduction: The signals and antigen presenting cells driving Tfh differentiation

The inflammatory milieu caused by *Plasmodium* infection is known to drive a specific dysfunction of dendritic cells (DCs) where they exhibit inefficient antigen uptake, decreased antigen presentation, and increased apoptosis (Götz et al., 2017; Pinzon-Charry et al., 2013; Urban et al., 1999; Woodberry et al., 2012; Wykes et al., 2007). Recent work details that the cDC1 population promotes Teff differentiation, while cDC2 cells promote Tfh cells (Kotov et al.,

2019). It has also been described that cDC1 cells are increased in the context of *Plasmodium* infection (Guermonprez et al., 2013), but as they are likely highly dysfunctional, they would be unable to promote Teff differentiation. In cases where the DCs are unable to serve as the initial antigen presenting cell, B cells can fill that niche and can stimulate the formation of Tfh cells without the input of DC-derived signals (Barnett et al., 2014; Constant, 1999; Evans et al., 2000; Hong et al., 2018). Coupling this information with the data on the formation of *Plasmodium*-specific Tfh cells as early as day 4 post-infection (detailed in Chapter 2), we utilized genetically manipulated mice and biochemical ablation of costimulatory factors to determine the critical factors in driving the early differentiation of Tfh cells during *Plasmodium* infection.

3.2: Results

3.2a: The Tfh-skewed epitope-specific CD4+ T cell response is B cell-dependent

We focused on understanding the factors that could be driving this stable CXCR5+ Tfh/Tcm-skewed phenotype. As described above, Tfh differentiation is thought to be a two-step process depending on early priming by DCs and later maintenance of the Tfh phenotype by B cells. We therefore hypothesized that if we activated CD4+ T cells in the absence of B cells, we would observe no difference in the Tfh-like phenotype at day 4 but would see a loss of the Tfh phenotype at day 8 or later after *Plasmodium* infection. We tested this hypothesis by infecting WT and μMT mice, which lack mature B cells, with *Py-GP66* and examined the phenotype of GP66+ cells in each group at various timepoints. Interestingly, we observed that even as early as 4 days post-infection there was a significant loss of CXCR5 expression by GP66+ cells in the μMT mice (**Fig. 3.1A**). Later in infection, a small population of Tfh cells emerged, which was consistently half as frequent in the μMT mice that it was in WT mice (**Fig. 3.1A, B**). We also found that the number of GP66+ cells was consistently lower in μMT mice than WT mice during the early response to *Plasmodium* infection, and approximately 2 logs lower 4 days after infection (**Fig. 3.1B**). These data suggest that B cells play a crucial role in the CD4+ T cell

response to *Plasmodium* infection prior to day 4. We were unable to study these cells beyond day 15 due to fatality in the μMT mice resulting from high parasite burden (**Fig. 3.2A**) (Roberts et al., 1977; Weinbaum et al., 1976). Since μMT animals are known to have aberrant lymphoid organization (Chyou et al., 2011), we also depleted B cells in WT mice with anti-CD20 antibody administered on the day of and 3 days after infection with *Py-GP66*. We achieved 81% reduction of B cells in the spleen with this treatment compared to the isotype-treated control (**Fig. 3.2B**). At 4 days post-infection, we observed that transient depletion of B cells decreased the Tfh population by approximately 20% compared to the isotype-treated control (**Fig. 3.1C, D**). The B cell depletion resulted in a decrease in the number of GP66+ cells, though this was not significant. (**Fig. 3.1D**). These alterations in differentiation occurred without appreciable increase in the parasite burden in mice that lacked B cells compared to WT controls, suggesting the eventual generation of productive antibody responses in both groups of mice (**Fig. 3.2A, C**). These data support our hypothesis that B cells are critical for providing very early signals in the response to *Plasmodium* infection, and even partial depletion of B cells can affect the early differentiation of a predominant *Plasmodium*-specific Tfh response.

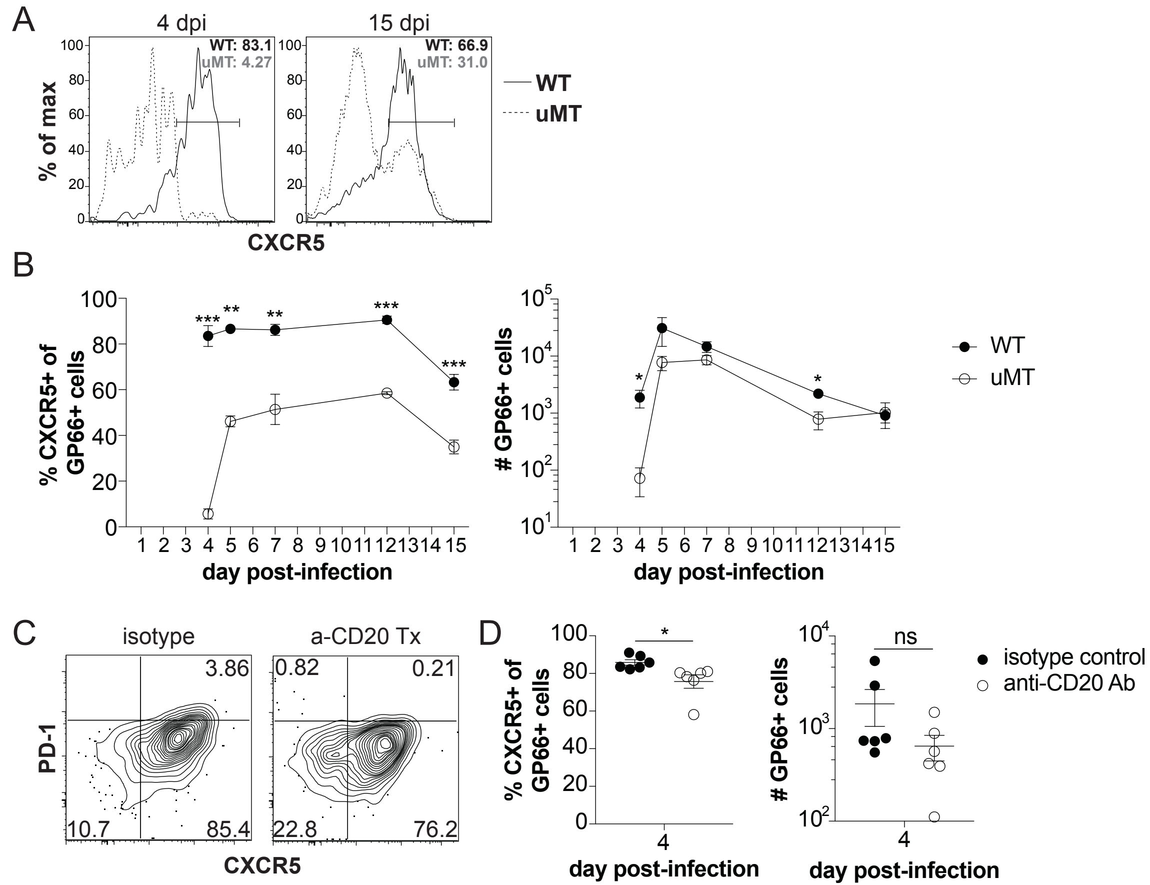

Figure 3.1: The Tfh-skewed epitope-specific CD4+ T cell response is B cell-dependent

A. Representative flow plots demonstrating CXCR5 expression on antigen-specific CD4+ T cells at acute time points after *Py-GP66* infection in WT and μMT mice. Plots are gated on dump- CD3+ CD8- CD4+ CD44+ GP66+ cells. **B.** Summary data of the percent of CXCR5+ GP66+ cells and number of GP66+ cells from Fig. 3.1A. Data are pooled from 3-6 mice per cohort from at least two independent experiments and were analyzed by unpaired *t*-tests. **C.** WT mice were treated with anti-CD20 antibody or isotype control immediately prior to infection and on day 3 post-infection. Representative flow plots of GP66+ cells from the secondary lymphoid organs of mice infected 4 days prior with *Py-GP66*. **D.** Summary data of the percent of CXCR5+ GP66+ cells and number of GP66+ cells from Fig. 3.1C. Data are pooled from 6 mice per cohort from two independent experiments and were analyzed by unpaired *t*-test. For B., D. and F., data are shown as mean ± SEM. *p < 0.05 **p < 0.01 ***p < 0.001.

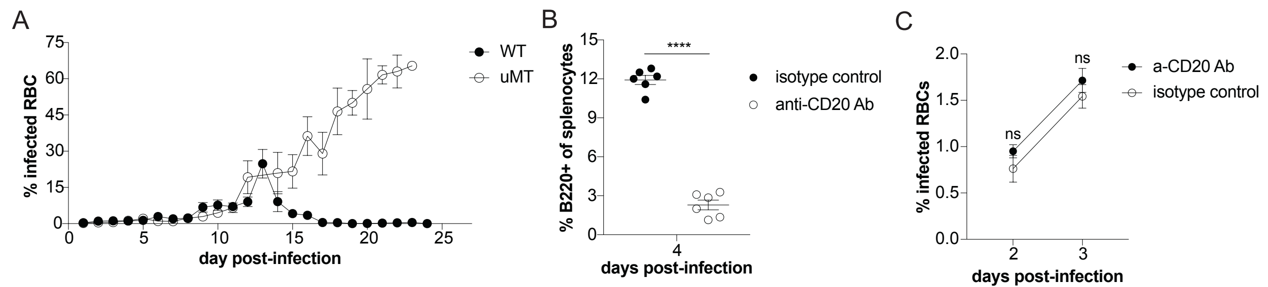

Figure 3.2: B cell depletion and the impact of B cell loss on parasite burden

A. Percent infected red blood cells in WT and μMT following *Py-GP66* infection. Data are pooled from 3-21 mice per cohort and are representative of over two independent experiments. **B.** Percent of B220+ B cells following anti-CD20 depletion and 4 days *Py-GP66* infection. Data are pooled from 6 mice per cohort and are representative of 2 independent experiments. **C.** Percent infected red blood cells in WT mice treated with anti-CD20 antibody or isotype control. Data are pooled from 6 mice per cohort and are representative of two independent experiments. Data were analyzed by two-way ANOVA. ****p < 0.0001.

3.2b: B cells, and not DCs, are necessary and sufficient to prime the epitope-specific CD4+ T cell response

CD4+ T cells interpret multiple signals from professional APCs during priming, including presentation of antigen on peptide:MHC complexes and ligand/receptor pair interactions referred to as co-stimulation. Many reports indicate that DCs serve as the initial APC for Tfh differentiation in various infections (Barnett et al., 2014; Cassell and Schwartz, 1994; Deenick et al., 2010; Itano et al., 2003). However, it has also been widely reported that DCs have dysfunctional responses during blood-stage *Plasmodium* infection in both mouse models and human studies, including a reduced capacity for antigen uptake and antigen presentation (Götz et al., 2017; Loughland et al., 2017; Pinzon-Charry et al., 2013; Urban et al., 1999; Woodberry et al., 2012; Wykes et al., 2007). We therefore tested whether DCs were involved in CD4+ T cell

priming during *Plasmodium* infection. To accomplish this, we utilized the Zbtb46-DTR (zDC-DTR) mouse model, in which administration of diphtheria toxin (DT) specifically depletes the conventional DC (cDC) compartment without affecting plasmacytoid DCs (pDC), macrophages, monocytes, or natural killer cells (Meredith et al., 2012). We achieved a 77% depletion of the cDC compartment in these mice 2 days after treatment (**Fig. 3.3A**) and found that in the absence of cDCs, GP66+ cells maintain a prominent Tfh skewing at day 4 post-infection (**Fig. 3.4A**). Furthermore, we observed no significant changes in the frequency of CXCR5+ cells or the number of GP66+ cells (**Fig. 3.4B**). Together these data demonstrate that cDCs are not necessary to activate *Plasmodium*-specific CD4+ T cells or to promote Tfh differentiation in the context of this infection. This contradicts previous studies identifying cDCs as the critical APC during *Plasmodium* infection (Ueffing et al., 2017; Voisine et al., 2010). However, these studies utilized CD11c as the cDC marker, which is also upregulated by activated B cells (Rubtsov et al., 2011; Sullivan et al., 2015; Weiss et al., 2009). Our approach allows for the dissection of the specific contribution of the cDCs and demonstrates that they are dispensable for activation of *Plasmodium*-specific CD4+ T cells.

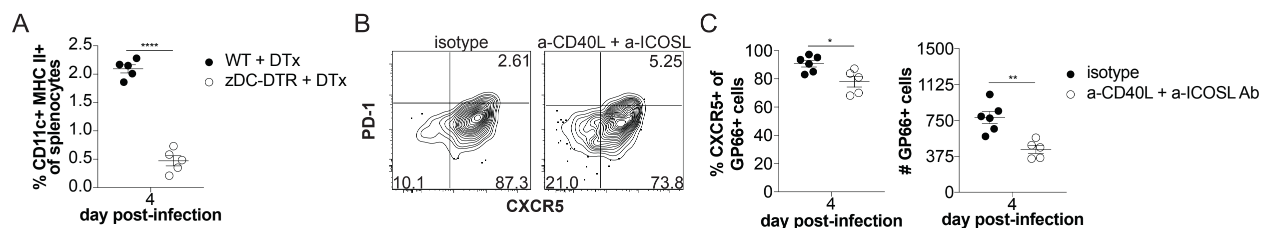

Figure 3.3: cDC depletion and the loss of Tfh cells in response to a-CD40L and a-ICOSL blockade

A. Percent of CD11c+ MHC II+ cells following diptheria toxin treatment (DTx) in WT and zDC-DTR mice. Data are pooled from 5 mice per cohort and are representative of two independent experiments. Data were analyzed by unpaired *t*-test. **B.** WT mice were treated with a-CD40L + a-ICOSL antibodies or isotype controls daily 0-3 days post-infection. Representative flow plots

from GP66+ cells 4 days post-infection with *Py-GP66*. **C.** Summary data of the percent of CXCR5+ GP66+ cells and number of GP66+ cells from Fig. 3.3A. Data are pooled from 5-6 mice per cohort and are representative of 2 independent experiments. Data were analyzed by unpaired *t*-test. *p < 0.05 **p < 0.01 ****p < 0.0001.

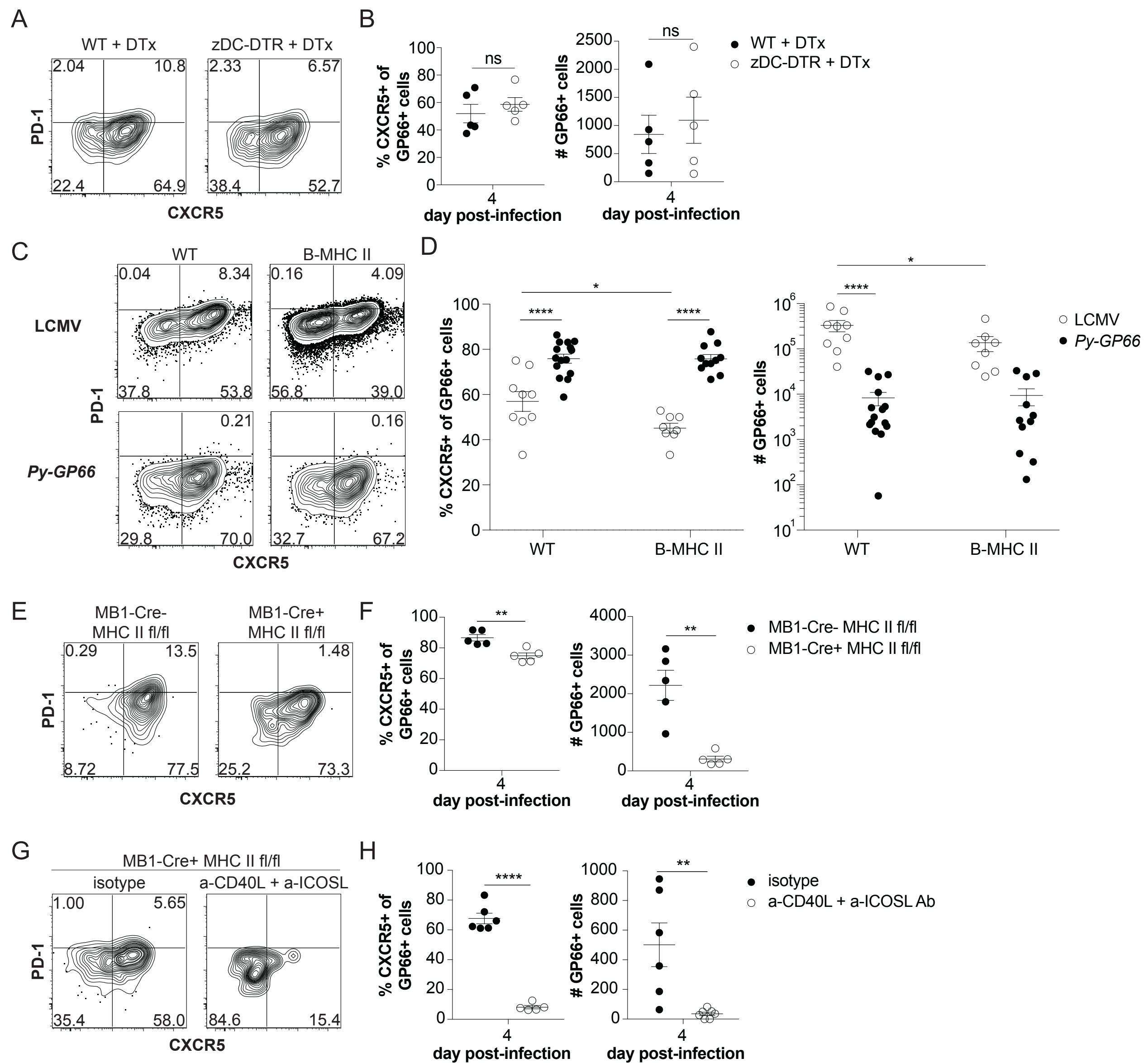

Figure 3.4: B cells are necessary and sufficient to prime the GP66+ CD4+ T cell response, while DCs are dispensable

A. Representative flow plots of GP66+ cells from 4 days post-infection with *Py-GP66* in WT and zDC-DTR mice. Both cohorts were treated with diphtheria toxin (DTx) -1 and 2 days post-infection. **B.** Summary data of the percent of CXCR5+ GP66+ cells and number of GP66+ cells in Fig. 4A. Data are pooled from 5 mice per cohort and are representative of two independent experiments. Data were analyzed by unpaired *t*-test. **C.** Representative flow plots showing transferred SMARTA+ GP66+ cells 4 days post-infection with LCMV or *Py-GP66* in WT and B-

MHC II mice. Plots are gated on dump- CD3+ CD8- CD4+ CD44+ GP66+ cells. **D.** Summary data of the percent of CXCR5+ SMARTA+ GP66+ cells and number of SMARTA+ GP66+ cells shown in Fig. 4C. Data are pooled from 8-15 mice per cohort and are representative of three independent experiments. Data were analyzed by two-way ANOVA. **E.** Representative flow plots of endogenous GP66+ CD4+ T cells from MB1-Cre- $MHC^{fl/fl}$ or MB1-Cre+ $MHC^{fl/fl}$ mice infected with *Py-GP66* 4 days prior. **F.** Summary data of the percent of CXCR5+ GP66+ cells and number of GP66+ cells shown in Fig. 4E. Data are representative of 5 mice per cohort from two independent experiments and were analyzed by unpaired *t*-test. **G.** MB1-Cre+ $MHC^{fl/fl}$ mice were treated with a-CD40L + a-ICOSL antibodies or isotype controls daily 0-3 days post-infection. Representative flow plots from GP66+ cells 4 days post-infection with *Py-GP66*. **H.** Summary data of the percent of CXCR5+ GP66+ cells and number of GP66+ cells from Fig. 4G. Data are pooled from 5-6 mice per cohort and are representative of two independent experiments. Data were analyzed by unpaired *t*-test. For B., D., F., and H., data are shown as mean ± SEM. *p < 0.05 **p < 0.01 ****p < 0.0001.

We next examined if alternatively, B cells could be priming CD4+ T cells in response to *Plasmodium* infection. Following activation, B cells upregulate MHC II and co-stimulatory molecules, demonstrating their capacity to prime naïve CD4+ T cells (Barnett et al., 2014; Constant, 1999; Hawrylowicz and Unanue, 1988; Hong et al., 2018; Krieger et al., 1985). Additionally, B cell priming has been demonstrated *in viv*o using mice that only express MHC II (I-Ab) on CD19+ B cells (B-MHC II). In B-MHC II mice, antigen presentation by B cells was shown to be sufficient to drive the expansion and formation of antigen-specific Tfh cells, not only in response to immunization (Constant, 1999), but also during viral infection (Barnett et al., 2014; Evans et al., 2000; Hong et al., 2018). We utilized these mice to determine if a similar phenomenon could occur in response to *Plasmodium* infection. Since B-MHC II mice do not develop a normal CD4+ T cell compartment, due to the lack of positive selection (Barnett et al., 2014), it is necessary to adoptively transfer CD4+ T cells and examine these. We transferred

TCR transgenic SMARTA CD4+ T cells specific for the GP66 epitope into WT or B-MHC II mice one day prior to infection with LCMV or *Py-GP66* and analyzed the differentiation of these cells 4 days post-infection. As expected, the absence of DC-derived priming hampered the expansion of GP66+ SMARTA cells responding to LCMV infection, but did not impact the expansion during *Plasmodium* infection. We observed a small decrease in the expression of CXCR5 at 4 days post-infection when cells were responding to LCMV infection. Remarkably, in response to *Plasmodium* infection, the GP66+ SMARTA cells in B-MHC II mice developed a prominent Tfh skewing that closely resembled their phenotype in WT controls (**Fig. 3.4C, D**). To perform the converse experiment, we generated MB1-Cre+ MHC II$^{fl/fl}$ mice to specifically delete the beta chain of the I-Ab MHC II molecule from B cells, thus preventing B cells from presenting antigen. Four days after infection with *Py-GP66*, there was a 10% decrease in Tfh frequency and a 7-fold reduction in the total number of GP66+ cells generated in mice lacking MHC class II on B cells (**Fig. 3.4E, F**). Together these data demonstrate that B cells play a significant role in directing the expansion of CD4+ T cells responding to *Plasmodium* infection, and perhaps reveal how phenotype and proliferation of CD4+ T cells may be directed distinctly by different APCs. These data also support more recent work demonstrating that antigen presentation by B cells alone is sufficient to drive the expansion and formation of prominent Tfh populations in the context of infection (Hong et al., 2018).

Since B cells can influence the differentiation of CD4+ T cells through both antigen presentation and co-stimulation, we also interrogated how co-stimulatory signals influence the differentiation of the Tfh phenotype in response to *Plasmodium* infection. B cells can provide an array of co-stimulatory signals, including CD40 and ICOSL, which are critical for driving the differentiation of Tfh cells (Cannons et al., 2010; Choi et al., 2011; Deenick et al., 2010; Glatman Zaretsky et al., 2009; Haynes et al., 2007; Johnston et al., 2009; Nurieva et al., 2008; Nurieva et al., 2009; Poholek et al., 2010; Qi et al., 2008; Salek-Ardakani et al., 2011; Watanabe et al., 2017). We therefore began by testing how CD40-CD40L and ICOS-ICOSL interactions direct

the differentiation of GP66+ cells. We infected WT and MB1-Cre+ MHC II$^{fl/fl}$ mice with *Py-GP66* and treated with anti-CD40L and anti-ICOSL antibodies daily for the first three days of infection and analyzed the responses at 4 days post-infection. Blocking interactions between these receptor-ligand pairs in WT mice resulted in a 12% decrease of CXCR5+ Tfh cells, as well as a 1.7-fold decrease in cell number (**Fig. 3.3B, C**). When this treatment was conducted in MB1-Cre+ MHC II$^{fl/fl}$ mice to determine if synergy might occur between antigen-dependent and -independent interactions, we observed a nearly 60% decrease in the frequency of CXCR5+ T cells, in addition to a 14-fold decrease in the total numbers of GP66+ cells (mean = 35.2 cells) generated in mice lacking B cell antigen presentation and co-stimulation compared to isotype-treated controls (**Fig. 3.4G, H**). Independently, the effects of removing antigen presentation by B cells or blocking co-stimulation had notable effects on the differentiation of Tfh cells. However, the dramatic reduction of both Tfh differentiation and antigen-specific cell expansion when both antigen presentation and co-stimulatory interactions between CD4+ T cells and B cells are blocked highlights the synergy of the multiple cues that determine CD4+ T cell fate. These data emphasize the importance of both co-stimulation and antigen presentation for CD4+ T cell fate during the priming phase of the immune response to *Plasmodium* infection.

3.3: Discussion

This work demonstrates that B cells can provide both peptide:MHC complexes and co-stimulatory signals required for priming CD4+ T cells generated during *Plasmodium* infection. Of interest, several studies have noted an increase in the circulating cDC1 population in humans following *Plasmodium* infection, coupled with a significant decrease of HLA-DR on the surface of these cells (Arama et al., 2011; Guermonprez et al., 2013; Urban et al., 2006). Others have reported a decrease in antigen uptake and antigen presentation by DCs in both mouse and human studies (Götz et al., 2017; Loughland et al., 2017; Pinzon-Charry et al., 2013; Urban et al., 1999; Woodberry et al., 2012; Wykes et al., 2007). Engwerda and colleagues described a specific dysfunction of the cDCs to prime IFNy-producing CD4+ Teff cells, as a result of type I

IFN signaling perceived by the cDCs during the first few days of blood-stage *Plasmdoium* infection (Haque et al., 2014). Although multiple DC subsets were susceptible to dysfunction in this inflammatory environment, cDCs in particular were suppressed and unable to promote Teff responses. There are likely multiple parasite-dependent mechanisms preventing cDC populations from generating a diverse acute CD4+ T cell response to *Plasmodium* infection. These findings together with our work support the hypothesis that while Teff cell-promoting cDCs are increased in number, they are significantly hampered in their functionality. This then results in the B cell population being the professional APC population poised to prime CD4+ T cells and likely due to the costimulation provided by the presenting B cell, the CD4+ T cell is fated to form a Tfh cell.

Chapter 4: Concluding Remarks

Our data support a model in which B cells serve as the primary APC during blood-stage *Plasmodium* infection. The distinctive role for B cells in this context is likely influenced by the diminished DC function (Götz et al., 2017; Loughland et al., 2017; Pinzon-Charry et al., 2013; Urban et al., 1999; Woodberry et al., 2012; Wykes et al., 2007) and/or the splenic disruption associated with this infection (Cadman et al., 2008). Recent work has demonstrated that cDC1 cells are important promoters of the effector CD4+ T cell populations, while the cDC2 population promotes Tfh cells (Kotov 2019). These findings are consistent with previous findings and our work, suggesting that while effector T cell-promoting cDCs are increased in number, they are dysfunctional, resulting in promotion of Tfh differentiation.

Additionally, the architectural changes that have been described in the spleen during *Plasmodium* infection of both mice and humans are characterized by the presence of B cells outside of the follicle and a blurring of the red and white pulp areas (Achtman et al., 2003; Alves et al., 2015; Cadman et al., 2008; Urban et al., 2005). Recent work detailing the specialization of various DC subsets and their localization in the lymphoid tissue may provide indications of

additional mechanisms at play. Li et al. described the positioning of IL-2 quenching dendritic cells at the interface of the T cell zone and the B cell follicle – where activated CD4+ T cells will search for cognate B cells and receive signals promoting the Tfh phenotype. With the disruption of the T-B border in *Plasmodium* infection, we hypothesize that these IL-2 quenching dendritic cells which express both membrane and soluble CD25 may have these Tfh-promoting effects at sites deep in the T cell zone and distal to the T-B border (Li et al., 2016). The suppressed DC function, the membrane-bound and soluble IL-2 sinks, and the loss of well-defined B and T cell areas likely allow CD4+ T and B cells to freely associate and exchange signals that would normally only be provided at the T-B border, resulting in the depressed cell numbers and skewed Tfh differentiation very early in the response to *Plasmodium* infection. While it remains to be determined if the co-stimulatory signals that CD4+ T cells require to form Tfh cells are uniquely provided by B cells in this system, it is clear that conventional DCs are not critical to serve this function. Overall, the work here demonstrates that B cells play an important role early in the development of the CD4+ T cell response during *Plasmodium* infection prior to antibody production, through antigen presentation and likely co-stimulatory factors such as CD40 and ICOSL.

The mechanisms that are employed to skew activated T cells to a Tfh phenotype all provide potential points for therapeutic intervention. Future avenues of research can build off the knowledge presented here to understand how to optimize the CD4+ T cell response during natural infection. For decades, elevated levels of soluble CD25 have been reported during various chronic infections, including the two major strains of human *Plasmodium* (Deloron et al., 1989; Josimovic-Alasevic et al., 1988; Otterdal et al., 2018). In addition to the Tfh-promoting stimuli CD4+ T cells have been demonstrated to receive, they are also significantly hampered from differentiating into Teff cells. Preliminary data (**Fig. 4.1A, B**) indicates that transient administration of recombinant IL-2 with S4B6 (IL-2 antibody complex) early during infection to promote IL-2 signaling slows the development of the Tfh response and promotes better

maintenance of the magnitude of the antigen-specific cell population at a memory time point, even though the population is again predominantly Tfh.

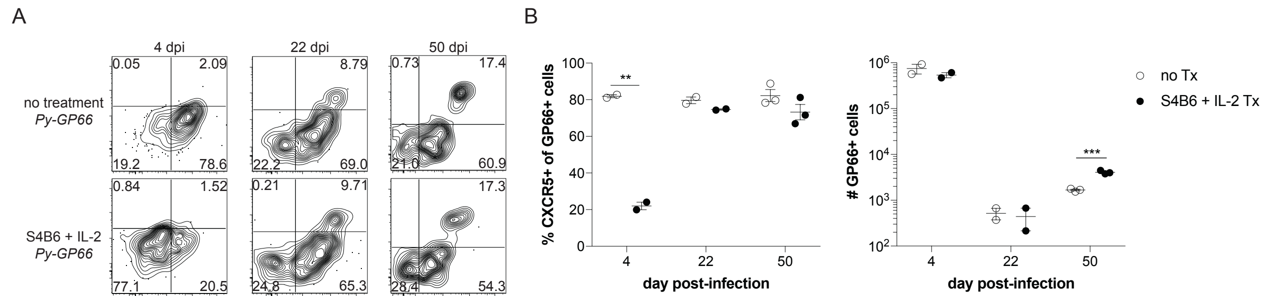

Figure 4.1: IL-2 signaling early in infection promotes acute Teff responses and preserves antigen-specific cell population magnitude

A. WT mice were untreated or treated with S4B6 antibody + IL-2 daily 0-3 days post-infection. Representative flow plots from GP66+ cells 4, 22, and 50 days post-infection with *Py-GP66*. **B.** Summary data of the percent of CXCR5+ GP66+ cells and number of GP66+ cells from Fig. 4.1A. Data are pooled from 2-3 mice per cohort and are representative of 1 experiment. Data were analyzed by unpaired *t*-test. For B. data are shown as mean ± SEM. **p < 0.01 ***p < 0.001.

Lastly, the B cell response to *Plasmodium* infection demonstrates some unusual characteristics. As with the memory CD4+ T cells that form, both IgM+ and class-switched memory B cells decline over time. However, there exists a more stable, non-somatically hypermutated IgD+ memory B cell population that is dependent on interactions with CD4+ T cells for its formation, but does not require cognate interactions (Krishnamurty et al., 2016, manuscript in preparation). It is unclear what T cell-derived factors could promote the generation of this population, but it could be the result of a B cell that received inappropriate T cell help signals. These cells could potentially serve to maintain a number of antigen-specific clones in the B cell repertoire, even as the highly mutated cells are lost.

The data presented here demonstrate the potential for B cells to serve not only as APCs to CD4+ T cells, but also as efficient inducers of the Tfh response. It provides the basis for a mechanism where in an inflammatory environment ill-suited for priming by dendritic cells, B cells fill the niche of the antigen presenting cell. In doing so, B cell antigen presentation drives a predominant CD4+ Tfh response during the acute immune response that fates the memory response to primarily Tcm cells. Understanding the combined effects of the early events during CD4+ T cell priming and their effects on the development of the memory CD4+ T cell population in the context of *Plasmodium* infection will allow the field to develop therapeutic targets to improve natural immunity to this parasitic infection.

Chapter 5: Materials and Methods

Mice

4- to 10-week old male C57BL/6, B6.129S2-Ighmtm1Cgn/J (μMT), B6.C(Cg)-Cd79a$^{tm1(cre)Reth}$/EhobJ (MB1-Cre), and B6.129X1-H2-Ab1^{tm1Koni}/J (I-A$^{b\ fl/fl}$) mice were purchased from the Jackson Laboratory. MB1-Cre$^{+/-}$ and I-A^{b} mice were crossed to generate MB1-Cre+ MHC II$^{fl/fl}$ mice; MB1-Cre- MHC II$^{fl/fl}$ littermates were used as controls in these experiments. Dr James Moon (Massachusetts General Hospital, Havard Medical School) provided B6;D2-TCR LCMV RAG-deficient (SMARTA) mice. Dr. Terri Laufer (University of Pennsylvania) provided CD19-Aßb (B-MHC II) mice (Barnett et al., 2014). Dr. Michael Gerner (University of Washington) provided B6(Cg)-Zbtb46$^{tm1(HBEGF)Mnz}$/J (zDC-DTR) mice (Meredith et al., 2012). All animals were bred and house in Animal Biosafety Level (ABSL)-2 conditions. Experiments were performed in accordance with the University of Washington Institutional Animal Care and Use Committee (IACUC) guidelines.

Parasite and viral infections

Plasmodium yoelii 17XNL-GP66 (*Py-GP66*) parasite (Hahn et al., 2018; Zander et al., 2017) was maintained as frozen blood stocks and passaged through donor mice. *Py-GP66* infections

in experimental mice were initiated by an intraperitoneal (IP) injection of 10^6 infected red blood cells (iRBC) obtained from donor mice. Parasitemia was determined by flow cytometry based on the percent of CD45- Ter119+ Hoescht+ cells, as previously described (Malleret et al., 2011). Dr. David Masopust (University of Minnesota) provided Lymphocytic choriomeningitis virus – Armstrong strain (LCMV). LCMV infections were initiated by IP injection of 10^5 plaque forming units (pfu) per mouse.

Antibody and diptheria toxin treatments

B cell depletion: For depletion of CD20+ B cells, mice were anesthetized with isofluorane and treated with anti-CD20 antibody (clone 18B12) (Biogen Idec) at 10mg/kg in phosphate-buffered saline (PBS) via intravenous (IV) injection. Control mice were treated with 10mg/kg of isotype control antibody (anti-c-Kit; clone 2B8) (Biogen Idec) in PBS via IV injection. Doses were administered at 0 and 3 days post-infection, according to the depletion kinetics (Dunn et al., 2007).

CD40-CD40L and ICOS-ICOSL signaling blockade: For the blockade of CD40-CD40L and ICOS-ICOSL signaling, mice were treated with 0.5mg anti-CD40L antibody (clone MR-1) (Bio X Cell) and 0.25mg anti-ICOSL antibody (clone HK5.3) (Bio X Cell) in PBS via IV injection. Control mice were treated with 0.5mg and/or 0.25mg of polyclonal Armenian hamster IgG and rat IgG2, respectively (Bio X Cell). Doses were administered daily from 0-3 days post-infection.

Diphtheria toxin: Diphtheria toxin (Sigma) was administered via intraperitoneal (IP) injection at 20ng/g at 1 day prior to infection and 2 days post-infection.

IL-2 + S4B6 treatment: For the enforced IL-2 signaling experiment, mice were treated with 0.5mg anti-IL-2 antibody (clone S4B6) and 0.015mg recombinant mouse IL-2. Doses were administered daily from 0-3 post-infection.

SMARTA cell transfer

CD4+ T cells were isolated from B6;D2-TCR LCMV RAG-deficient (SMARTA) mice by negative selection with the CD4+ T cell MACS isolation kit (Miltenyi Biotec) and were labeled with

carboxyfluorescein succinimidyl ester (CFSE) (Invitrogen). Recipient mice were anesthetized with isoflourane and received 10^5 CFSE-labeled CD4+ T cells via IV injection 1 day prior to injection.

Tetramer-specific cell enrichment

Single cell suspensions were prepared from the spleen and several lymph nodes (axial, brachial, cervical, inguinal, mesenteric, and periaortic) from experimental mice in PBS containing 2% heat-inactivated fetal bovine serum (FBS). Cells were stained with GP_{66-77}:I-A^b-APC tetramer (I-A^b/LCMV.GP66.DIYKGVYQFKSV - NIH Tetramer Core) (Dow et al., 2008) for 1 hour at room temperature, washed, and incubated with magnetic anti-APC beads (Miltenyi Biotec) for 30 minutes on ice. Tetramer-specific cells were enriched using LS MACS columns (Miltenyi Biotec), as previously described (Moon et al., 2007). The enriched cells were stained with antibodies specific surface molecules (detailed in **Table 1**). For intranuclear transcription factor staining, cells were fixed with eBioscience Foxp3/Transcription Factor Staining Buffer Set (ThermoFisher) and stained with antibodies for intranuclear molecules (detailed in **Table 1**). All samples were acquired on the LSR II cytometer (BD) and analyzed using the Flow Jo software (Tree Star, v9). All flow plots were gated on lymphocyte gate, singlets, B220- CD11b- CD11c- (dump-) CD3+ CD8- CD4+. Additional gating strategies are indicated in the figure legends.

Statistical analysis

The responder frequency and proliferative capacity were calculated as previously described (Gudmundsdottir et al., 1999). The results presented represent mean ± SEM. Statistical analyses were performed by nonlinear regression, unpaired *t*-test, or two-way ANOVA, as specified in the figure legends. Statistical tests were calculated using GraphPad Prism (v7).

Table 5.1: Flow cytometry antibodies and reagents

Marker	Clone	Fluorophore	Dilution	Company	Catalog No.
Bcl-6	K112-91	APC-Cy7	1:20	BD	563581
CCR7	4B12	Biotin	1:20	Biolegend	120104
CCR7	4B12	PerCP-Cy5.5	1:20	eBioscience	45-1971
CD3	145-2C11	PerCP-Cy5.5	1:100	BD	551163
CD4	RM4-5	APC	1:100	BD	554063
CD4	GK1.5	BV711	1:100	BD	563050
CD4	RM4-4	FITC	1:100	BD	553055
CD8a	53-6.7	BV510	1:100	BD	563068
CD8a	53-6.7	PE-Cy7	1:100	BD	552877
CD11b	M1/70	eFlour 450	1:100	eBioscience	48-0112
CD11b	M1/70	PE-CF594	1:100	BD	562287
CD11b	M1/70	PerCP-Cy5.5	1:100	BD	550993
CD11c	N418	eFlour 450	1:100	eBioscience	48-0114
CD11c	HL3	PE-CF594	1:100	BD	562454
CD11c	HL3	PerCP-Cy5.5	1:100	BD	560584
CD25	PC61	BV421	1:100	BD	562606
CD44	IM7	AF700	1:100	BD	560567
CD45R (B220)	RA3-6B2	BV421	1:100	BD	562922
CD45R (B220)	RA3-6B2	PerCP-Cy5.5	1:100	eBioscience	45-0452
CD45R (B220)	RA3-6B2	PE-CF594	1:100	BD	562290
CD62L	MEL-14	BV786	1:100	BD	564109
CD69	H1.2F3	FITC	1:100	eBioscience	11-0691
CXCR5	2G8	PE	1:20	BD	551959
CXCR5	2G8	PE-Cy7	1:20	BD	560617
F4/80	BM8	FITC	1:100	eBioscience	11-4801
PD-1	J43	PE-Cy7	1:100	eBioscience	25-9985
Tbet	eBio4B10	PE	1:100	eBioscience	12-5825
Streptavidin	-	APC-Cy7	1:100	BD	554063
Streptavidin	-	BV605	1:100	BD	563260

The reagents listed above were utilized in various combinations to visualize molecules expressed on the cell surface or intracellularly. The antibody clone, fluorophore, dilution and purchasing information are indicated.

Chapter 6: Abbreviations

APC: antigen presenting cell

BCR: B cell receptor

CFSE: carboxyfluorescein succinimidyl ester

DC: dendritic cell

DTx/DTR: diptheria toxin treatment/diptheria toxin receptor

GP66: glycoprotein 66-77

IP: intraperitoneal

IV: intravenous

iRBC: infected red blood cell

LCMV: lymphocytic choriomeningitis virus

LN: lymph nodes

MHC II: major histocompatibility complex class II

Py: *Plasmodium yoelii*

Tcm: T central memory

TCR: T cell receptor

Teff: T effector

Tem: T effector memory

Tfh: T follicular helper

Chapter 7: References

Achtman, A., M. Khan, I. MacLennan, and J. Langhorne. 2003. Plasmodium chabaudi chabaudi infection in mice induces strong B cell responses and striking but temporary changes in splenic cell distribution. *J Immunol* 171:317-324.

Allen, C.D., T. Okada, H.L. Tang, and J.G. Cyster. 2007. Imaging of germinal center selection events during affinity maturation. *Science* 315:528-531.

Alves, F.A., M. Pelajo-Machado, P.R. Totino, M.T. Souza, E.C. Gonçalves, M.P. Schneider, J.A. Muniz, M.A. Krieger, M.C. Andrade, C.T. Daniel-Ribeiro, and L.J. Carvalho. 2015. Splenic architecture disruption and parasite-induced splenocyte activation and anergy in Plasmodium falciparum-infected Saimiri sciureus monkeys. *Malar J* 14:128.

Aly, A.S., A.M. Vaughan, and S.H. Kappe. 2009. Malaria parasite development in the mosquito and infection of the mammalian host. *Annu Rev Microbiol* 63:195-221.

Amante, F.H., and M.F. Good. 1997. Prolonged Th1-like response generated by a Plasmodium yoelii-specific T cell clone allows complete clearance of infection in reconstituted mice. *Parasite Immunol* 19:111-126.

Arama, C., P. Giusti, S. Boström, V. Dara, B. Traore, A. Dolo, O. Doumbo, S. Varani, and M. Troye-Blomberg. 2011. Interethnic differences in antigen-presenting cell activation and TLR responses in Malian children during Plasmodium falciparum malaria. *PLoS One* 6:e18319.

Barnett, L.G., H.M. Simkins, B.E. Barnett, L.L. Korn, A.L. Johnson, E.J. Wherry, G.F. Wu, and T.M. Laufer. 2014. B cell antigen presentation in the initiation of follicular helper T cell and germinal center differentiation. *J Immunol* 192:3607-3617.

Baumjohann, D., S. Preite, A. Reboldi, F. Ronchi, K.M. Ansel, A. Lanzavecchia, and F. Sallusto. 2013. Persistent antigen and germinal center B cells sustain T follicular helper cell responses and phenotype. *Immunity* 38:596-605.

Berek, C., A. Berger, and M. Apel. 1991. Maturation of the immune response in germinal centers. *Cell* 67:1121-1129.

Bernasconi, N.L., E. Traggiai, and A. Lanzavecchia. 2002. Maintenance of serological memory by polyclonal activation of human memory B cells. *Science* 298:2199-2202.

Blum, J.S., P.A. Wearsch, and P. Cresswell. 2013. Pathways of antigen processing. *Annu Rev Immunol* 31:443-473.

Breitfeld, D., L. Ohl, E. Kremmer, J. Ellwart, F. Sallusto, M. Lipp, and R. Förster. 2000. Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. *J Exp Med* 192:1545-1552.

Cadman, E.T., A.Y. Abdallah, C. Voisine, A.M. Sponaas, P. Corran, T. Lamb, D. Brown, F. Ndungu, and J. Langhorne. 2008. Alterations of splenic architecture in malaria are

induced independently of Toll-like receptors 2, 4, and 9 or MyD88 and may affect antibody affinity. *Infect Immun* 76:3924-3931.

Campbell, J.J., K.E. Murphy, E.J. Kunkel, C.E. Brightling, D. Soler, Z. Shen, J. Boisvert, H.B. Greenberg, M.A. Vierra, S.B. Goodman, M.C. Genovese, A.J. Wardlaw, E.C. Butcher, and L. Wu. 2001. CCR7 expression and memory T cell diversity in humans. *J Immunol* 166:877-884.

Cannons, J.L., H. Qi, K.T. Lu, M. Dutta, J. Gomez-Rodriguez, J. Cheng, E.K. Wakeland, R.N. Germain, and P.L. Schwartzberg. 2010. Optimal germinal center responses require a multistage T cell:B cell adhesion process involving integrins, SLAM-associated protein, and CD84. *Immunity* 32:253-265.

Cassell, D., and R. Schwartz. 1994. A quantitative analysis of antigen-presenting cell function: activated B cells stimulate naive CD4 T cells but are inferior to dendritic cells in providing costimulation. *J Exp Med* 180:1829-1840.

Chang, J.T., E.J. Wherry, and A.W. Goldrath. 2014. Molecular regulation of effector and memory T cell differentiation. *Nat Immunol* 15:1104-1115.

Chevalier, N., D. Jarrossay, E. Ho, D.T. Avery, C.S. Ma, D. Yu, F. Sallusto, S.G. Tangye, and C.R. Mackay. 2011. CXCR5 expressing human central memory CD4 T cells and their relevance for humoral immune responses. *J Immunol* 186:5556-5568.

Choi, Y.S., D. Eto, J.A. Yang, C. Lao, and S. Crotty. 2013. Cutting edge: STAT1 is required for IL-6-mediated Bcl6 induction for early follicular helper cell differentiation. *J Immunol* 190:3049-3053.

Choi, Y.S., R. Kageyama, D. Eto, T.C. Escobar, R.J. Johnston, L. Monticelli, C. Lao, and S. Crotty. 2011. ICOS receptor instructs T follicular helper cell versus effector cell differentiation via induction of the transcriptional repressor Bcl6. *Immunity* 34:932-946.

Chyou, S., F. Benahmed, J. Chen, V. Kumar, S. Tian, M. Lipp, and T.T. Lu. 2011. Coordinated regulation of lymph node vascular-stromal growth first by CD11c+ cells and then by T and B cells. *J Immunol* 187:5558-5567.

Cigel, F., J. Batchelder, J.M. Burns, D. Yañez, H. van der Heyde, D.D. Manning, and W.P. Weidanz. 2003. Immunity to blood-stage murine malarial parasites is MHC class II dependent. *Immunol Lett* 89:243-249.

Ciucci, T., M.S. Vacchio, Y. Gao, F. Tomassoni Ardori, J. Candia, M. Mehta, Y. Zhao, B. Tran, M. Pepper, L. Tessarollo, D.B. McGavern, and R. Bosselut. 2019. The Emergence and Functional Fitness of Memory CD4. *Immunity* 50:91-105.e104.

Claman, H.N., E.A. Chaperon, and R.F. Triplett. 1966. Thymus-marrow cell combinations. Synergism in antibody production. *Proc Soc Exp Biol Med* 122:1167-1171.

Clyde, D.F., H. Most, V.C. McCarthy, and J.P. Vanderberg. 1973. Immunization of man against sporozite-induced falciparum malaria. *Am J Med Sci* 266:169-177.

Cohen, S., I. McGregor, and S. Carrington. 1961. Gamma-globulin and acquired immunity to human malaria. *Nature* 192:733-737.

Constant, S.L. 1999. B lymphocytes as antigen-presenting cells for CD4+ T cell priming in vivo. *J Immunol* 162:5695-5703.

Corbo-Rodgers, E., K.R. Wiehagen, E.S. Staub, and J.S. Maltzman. 2012. Homeostatic division is not necessary for antigen-specific CD4+ memory T cell persistence. *J Immunol* 189:3378-3385.

Couper, K.N., D.G. Blount, M.S. Wilson, J.C. Hafalla, Y. Belkaid, M. Kamanaka, R.A. Flavell, J.B. de Souza, and E.M. Riley. 2008. IL-10 from CD4CD25Foxp3CD127 adaptive regulatory T cells modulates parasite clearance and pathology during malaria infection. *PLoS Pathog* 4:e1000004.

Crompton, P.D., M.A. Kayala, B. Traore, K. Kayentao, A. Ongoiba, G.E. Weiss, D.M. Molina, C.R. Burk, M. Waisberg, A. Jasinskas, X. Tan, S. Doumbo, D. Doumtabe, Y. Kone, D.L. Narum, X. Liang, O.K. Doumbo, L.H. Miller, D.L. Doolan, P. Baldi, P.L. Felgner, and S.K. Pierce. 2010. A prospective analysis of the Ab response to Plasmodium falciparum before and after a malaria season by protein microarray. *Proc Natl Acad Sci U S A* 107:6958-6963.

Deenick, E.K., A. Chan, C.S. Ma, D. Gatto, P.L. Schwartzberg, R. Brink, and S.G. Tangye. 2010. Follicular helper T cell differentiation requires continuous antigen presentation that is independent of unique B cell signaling. *Immunity* 33:241-253.

Deloron, P., J.P. Lepers, and P. Coulanges. 1989. Evolution of the levels of soluble interleukin-2 receptors during Plasmodium falciparum and P. vivax malaria. *J Clin Microbiol* 27:1887-1889.

Ding, A.H., C.F. Nathan, and D.J. Stuehr. 1988. Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. Comparison of activating cytokines and evidence for independent production. *J Immunol* 141:2407-2412.

DiToro, D., C.J. Winstead, D. Pham, S. Witte, R. Andargachew, J.R. Singer, C.G. Wilson, C.L. Zindl, R.J. Luther, D.J. Silberger, B.T. Weaver, E.M. Kolawole, R.J. Martinez, H. Turner, R.D. Hatton, J.J. Moon, S.S. Way, B.D. Evavold, and C.T. Weaver. 2018. Differential IL-2 expression defines developmental fates of follicular versus nonfollicular helper T cells. *Science* 361:

Dow, C., C. Oseroff, B. Peters, C. Nance-Sotelo, J. Sidney, M. Buchmeier, A. Sette, and B.R. Mothé. 2008. Lymphocytic choriomeningitis virus infection yields overlapping CD4+ and CD8+ T-cell responses. *J Virol* 82:11734-11741.

Dunn, R.M., E., R. Peach, and M. Kehry. 2007. Anti-mouse CD20 antibodies and uses thereof. In U.S.P.a.T. Office, editor Biogen Idec Inc, USA.

Evans, D.E., M.W. Munks, J.M. Purkerson, and D.C. Parker. 2000. Resting B lymphocytes as APC for naive T lymphocytes: dependence on CD40 ligand/CD40. *J Immunol* 164:688-697.

Fahey, L.M., E.B. Wilson, H. Elsaesser, C.D. Fistonich, D.B. McGavern, and D.G. Brooks. 2011. Viral persistence redirects CD4 T cell differentiation toward T follicular helper cells. *J Exp Med* 208:987-999.

Fairfax, K., B. Everts, E. Amiel, A. Smith, G. Schramm, H. Haas, G. Randolph, J. Taylor, and E. Pearce. 2015. IL-4-secreting secondary T follicular helper (Tfh) cells arise from memory T cells, not persisting Tfh cells, through a B cell-dependent mechanism. *J Immunol* 194:2999-3010.

Freitas do Rosário, A., S. Muxel, S. Rodríguez-Málaga, L. Sardinha, C. Zago, S. Castillo-Méndez, J. Alvarez, and M. D'Império Lima. 2008. Gradual decline in malaria-specific memory T cell responses leads to failure to maintain long-term protective immunity to Plasmodium chabaudi AS despite persistence of B cell memory and circulating antibody. *J Immunol* 181:8344-8355.

Freitas do Rosário, A.P., T. Lamb, P. Spence, R. Stephens, A. Lang, A. Roers, W. Muller, A. O'Garra, and J. Langhorne. 2012. IL-27 promotes IL-10 production by effector Th1 CD4+ T cells: a critical mechanism for protection from severe immunopathology during malaria infection. *J Immunol* 188:1178-1190.

Fritsch, R.D., X. Shen, G.P. Sims, K.S. Hathcock, R.J. Hodes, and P.E. Lipsky. 2005. Stepwise differentiation of CD4 memory T cells defined by expression of CCR7 and CD27. *J Immunol* 175:6489-6497.

Färnert, A., K. Wyss, S. Dashti, and P. Naucler. 2015. Duration of residency in a non-endemic area and risk of severe malaria in African immigrants. *Clin Microbiol Infect* 21:494-501.

Garside, P., E. Ingulli, R.R. Merica, J.G. Johnson, R.J. Noelle, and M.K. Jenkins. 1998. Visualization of specific B and T lymphocyte interactions in the lymph node. *Science* 281:96-99.

Gaspal, F.M., M.Y. Kim, F.M. McConnell, C. Raykundalia, V. Bekiaris, and P.J. Lane. 2005. Mice deficient in OX40 and CD30 signals lack memory antibody responses because of deficient CD4 T cell memory. *J Immunol* 174:3891-3896.

Gattinoni, L., E. Lugli, Y. Ji, Z. Pos, C.M. Paulos, M.F. Quigley, J.R. Almeida, E. Gostick, Z. Yu, C. Carpenito, E. Wang, D.C. Douek, D.A. Price, C.H. June, F.M. Marincola, M. Roederer, and N.P. Restifo. 2011. A human memory T cell subset with stem cell-like properties. *Nat Med* 17:1290-1297.

Glatman Zaretsky, A., J.J. Taylor, I.L. King, F.A. Marshall, M. Mohrs, and E.J. Pearce. 2009. T follicular helper cells differentiate from Th2 cells in response to helminth antigens. *J Exp Med* 206:991-999.

Grakoui, A., S.K. Bromley, C. Sumen, M.M. Davis, A.S. Shaw, P.M. Allen, and M.L. Dustin. 1999. The immunological synapse: a molecular machine controlling T cell activation. *Science* 285:221-227.

Gudmundsdottir, H., A.D. Wells, and L.A. Turka. 1999. Dynamics and requirements of T cell clonal expansion in vivo at the single-cell level: effector function is linked to proliferative capacity. *J Immunol* 162:5212-5223.

Guermonprez, P., J. Helft, C. Claser, S. Deroubaix, H. Karanje, A. Gazumyan, G. Darasse-Jèze, S.B. Telerman, G. Breton, H.A. Schreiber, N. Frias-Staheli, E. Billerbeck, M. Dorner, C.M. Rice, A. Ploss, F. Klein, M. Swiecki, M. Colonna, A.O. Kamphorst, M. Meredith, R. Niec, C. Takacs, F. Mikhail, A. Hari, D. Bosque, T. Eisenreich, M. Merad, Y. Shi, F. Ginhoux, L. Rénia, B.C. Urban, and M.C. Nussenzweig. 2013. Inflammatory Flt3l is essential to mobilize dendritic cells and for T cell responses during Plasmodium infection. *Nat Med* 19:730-738.

Gwyer Findlay, E., A. Villegas-Mendez, N. O'Regan, J.B. de Souza, L.M. Grady, C.J. Saris, E.M. Riley, and K.N. Couper. 2014. IL-27 receptor signaling regulates memory CD4+ T cell populations and suppresses rapid inflammatory responses during secondary malaria infection. *Infect Immun* 82:10-20.

Götz, A., M.S. Tang, M.C. Ty, C. Arama, A. Ongoiba, D. Doumtabe, B. Traore, P.D. Crompton, P. Loke, and A. Rodriguez. 2017. Atypical activation of dendritic cells by. *Proc Natl Acad Sci U S A* 114:E10568-E10577.

Hahn, W.O., N.S. Butler, S.E. Lindner, H.M. Akilesh, D.N. Sather, S.H. Kappe, J.A. Hamerman, M. Gale, W.C. Liles, and M. Pepper. 2018. cGAS-mediated control of blood-stage malaria promotes Plasmodium-specific germinal center responses. *JCI Insight* 3:

Hale, J., B. Youngblood, D. Latner, A. Mohammed, L. Ye, R. Akondy, T. Wu, S. Iyer, and R. Ahmed. 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.

Haque, A., S.E. Best, M. Montes de Oca, K.R. James, A. Ammerdorffer, C.L. Edwards, F. de Labastida Rivera, F.H. Amante, P.T. Bunn, M. Sheel, I. Sebina, M. Koyama, A. Varelias, P.J. Hertzog, U. Kalinke, S.Y. Gun, L. Rénia, C. Ruedl, K.P. MacDonald, G.R. Hill, and C.R. Engwerda. 2014. Type I IFN signaling in CD8- DCs impairs Th1-dependent malaria immunity. *J Clin Invest* 124:2483-2496.

Harrington, L.E., K.M. Janowski, J.R. Oliver, A.J. Zajac, and C.T. Weaver. 2008. Memory CD4 T cells emerge from effector T-cell progenitors. *Nature* 452:356-360.

Hawrylowicz, C.M., and E.R. Unanue. 1988. Regulation of antigen-presentation-I. IFN-gamma induces antigen-presenting properties on B cells. *J Immunol* 141:4083-4088.

Haynes, N.M., C.D. Allen, R. Lesley, K.M. Ansel, N. Killeen, and J.G. Cyster. 2007. Role of CXCR5 and CCR7 in follicular Th cell positioning and appearance of a programmed cell death gene-1high germinal center-associated subpopulation. *J Immunol* 179:5099-5108.

Hirunpetcharat, C., J.H. Tian, D.C. Kaslow, N. van Rooijen, S. Kumar, J.A. Berzofsky, L.H. Miller, and M.F. Good. 1997. Complete protective immunity induced in mice by immunization with the 19-kilodalton carboxyl-terminal fragment of the merozoite surface protein-1 (MSP1[19]) of Plasmodium yoelii expressed in Saccharomyces cerevisiae: correlation of protection with antigen-specific antibody titer, but not with effector CD4+ T cells. *J Immunol* 159:3400-3411.

Hondowicz, B.D., K.S. Kim, M.J. Ruterbusch, G.J. Keitany, and M. Pepper. 2018. IL-2 is required for the generation of viral-specific CD4. *Eur J Immunol* 48:80-86.

Hong, S., Z. Zhang, H. Liu, M. Tian, X. Zhu, W. Wang, X. Zhou, F. Zhang, Q. Ge, B. Zhu, H. Tang, Z. Hua, and B. Hou. 2018. B Cells Are the Dominant Antigen-Presenting Cells that Activate Naive CD4. *Immunity* 49:695-708.

Hopp, C.S., and P. Sinnis. 2015. The innate and adaptive response to mosquito saliva and Plasmodium sporozoites in the skin. *Ann N Y Acad Sci* 1342:37-43.

Howland, K.C., L.J. Ausubel, C.A. London, and A.K. Abbas. 2000. The roles of CD28 and CD40 ligand in T cell activation and tolerance. *J Immunol* 164:4465-4470.

Huang, S., W. Hendriks, A. Althage, S. Hemmi, H. Bluethmann, R. Kamijo, J. Vilcek, R.M. Zinkernagel, and M. Aguet. 1993. Immune response in mice that lack the interferon-gamma receptor. *Science* 259:1742-1745.

Huber, M., and M. Lohoff. 2014. IRF4 at the crossroads of effector T-cell fate decision. *Eur J Immunol* 44:1886-1895.

Iezzi, G., D. Scheidegger, and A. Lanzavecchia. 2001. Migration and function of antigen-primed nonpolarized T lymphocytes in vivo. *J Exp Med* 193:987-993.

Ise, W., T. Inoue, J.B. McLachlan, K. Kometani, M. Kubo, T. Okada, and T. Kurosaki. 2014. Memory B cells contribute to rapid Bcl6 expression by memory follicular helper T cells. *Proc Natl Acad Sci U S A* 111:11792-11797.

Itano, A.A., S.J. McSorley, R.L. Reinhardt, B.D. Ehst, E. Ingulli, A.Y. Rudensky, and M.K. Jenkins. 2003. Distinct dendritic cell populations sequentially present antigen to CD4 T cells and stimulate different aspects of cell-mediated immunity. *Immunity* 19:47-57.

Iwata, A., V. Durai, R. Tussiwand, C.G. Briseño, X. Wu, G.E. Grajales-Reyes, T. Egawa, T.L. Murphy, and K.M. Murphy. 2017. Quality of TCR signaling determined by differential affinities of enhancers for the composite BATF-IRF4 transcription factor complex. *Nat Immunol* 18:563-572.

Iyer, S.S., S. Gangadhara, B. Victor, R. Gomez, R. Basu, J.J. Hong, C. Labranche, D.C. Montefiori, F. Villinger, B. Moss, and R.R. Amara. 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.

Jacob, J., G. Kelsoe, K. Rajewsky, and U. Weiss. 1991. Intraclonal generation of antibody mutants in germinal centres. *Nature* 354:389-392.

Johnston, R.J., Y.S. Choi, J.A. Diamond, J.A. Yang, and S. Crotty. 2012. STAT5 is a potent negative regulator of TFH cell differentiation. *J Exp Med* 209:243-250.

Johnston, R.J., A.C. Poholek, D. DiToro, I. Yusuf, D. Eto, B. Barnett, A.L. Dent, J. Craft, and S. Crotty. 2009. Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. *Science* 325:1006-1010.

Josimovic-Alasevic, O., H. Feldmeier, K. Zwingenberger, G. Harms, H. Hahn, M. Shrisuphanunt, and T. Diamantstein. 1988. Interleukin 2 receptor in patients with localized and systemic parasitic diseases. *Clin Exp Immunol* 72:249-254.

Kallies, A., A. Xin, G.T. Belz, and S.L. Nutt. 2009. Blimp-1 transcription factor is required for the differentiation of effector CD8(+) T cells and memory responses. *Immunity* 31:283-295.

Keck, S., M. Schmaler, S. Ganter, L. Wyss, S. Oberle, E.S. Huseby, D. Zehn, and C.G. King. 2014. Antigen affinity and antigen dose exert distinct influences on CD4 T-cell differentiation. *Proc Natl Acad Sci U S A* 111:14852-14857.

Kerfoot, S.M., G. Yaari, J.R. Patel, K.L. Johnson, D.G. Gonzalez, S.H. Kleinstein, and A.M. Haberman. 2011. Germinal center B cell and T follicular helper cell development initiates in the interfollicular zone. *Immunity* 34:947-960.

Kim, C.H., L.S. Rott, I. Clark-Lewis, D.J. Campbell, L. Wu, and E.C. Butcher. 2001. Subspecialization of CXCR5+ T cells: B helper activity is focused in a germinal center-localized subset of CXCR5+ T cells. *J Exp Med* 193:1373-1381.

Kotov, D.I., J.S. Mitchell, T. Pengo, C. Ruedl, S.S. Way, R.A. Langlois, B.T. Fife, and M.K. Jenkins. 2019. TCR Affinity Biases Th Cell Differentiation by Regulating CD25, Eef1e1, and Gbp2. *J Immunol* 202:2535-2545.

Krieger, J.I., S.F. Grammer, H.M. Grey, and R.W. Chesnut. 1985. Antigen presentation by splenic B cells: resting B cells are ineffective, whereas activated B cells are effective accessory cells for T cell responses. *J Immunol* 135:2937-2945.

Krishnamoorthy, V., S. Kannanganat, M. Maienschein-Cline, S.L. Cook, J. Chen, N. Bahroos, E. Sievert, E. Corse, A. Chong, and R. Sciammas. 2017. The IRF4 Gene Regulatory Module Functions as a Read-Write Integrator to Dynamically Coordinate T Helper Cell Fate. *Immunity* 47:481-497.e487.

Krishnamurty, A.T., C.D. Thouvenel, S. Portugal, G.J. Keitany, K.S. Kim, A. Holder, P.D. Crompton, D.J. Rawlings, and M. Pepper. 2016. Somatically Hypermutated Plasmodium-Specific IgM(+) Memory B Cells Are Rapid, Plastic, Early Responders upon Malaria Rechallenge. *Immunity* 45:402-414.

Langhorne, J., F.M. Ndungu, A.M. Sponaas, and K. Marsh. 2008. Immunity to malaria: more questions than answers. *Nat Immunol* 9:725-732.

Langhorne, J., B. Simon-Haarhaus, and S. Meding. 1990. The role of CD4+ T cells in the protective immune response to *Plasmodium chabaudi* in vivo. *Immunol Lett* 25:101-107.

Lanzavecchia, A. 1985. Antigen-specific interaction between T and B cells. *Nature* 314:537-539.

Lee, S.K., R.J. Rigby, D. Zotos, L.M. Tsai, S. Kawamoto, J.L. Marshall, R.R. Ramiscal, T.D. Chan, D. Gatto, R. Brink, D. Yu, S. Fagarasan, D.M. Tarlinton, A.F. Cunningham, and C.G. Vinuesa. 2011. B cell priming for extrafollicular antibody responses requires Bcl-6 expression by T cells. *J Exp Med* 208:1377-1388.

Li, C., I. Corraliza, and J. Langhorne. 1999. A defect in interleukin-10 leads to enhanced malarial disease in Plasmodium chabaudi chabaudi infection in mice. *Infect Immun* 67:4435-4442.

Li, J., E. Lu, T. Yi, and J.G. Cyster. 2016. EBI2 augments Tfh cell fate by promoting interaction with IL-2-quenching dendritic cells. *Nature* 533:110-114.

Linterman, M.A., R.J. Rigby, R. Wong, D. Silva, D. Withers, G. Anderson, N.K. Verma, R. Brink, A. Hutloff, C.C. Goodnow, and C.G. Vinuesa. 2009. Roquin differentiates the specialized functions of duplicated T cell costimulatory receptor genes CD28 and ICOS. *Immunity* 30:228-241.

Liu, D., H. Xu, C. Shih, Z. Wan, X. Ma, W. Ma, D. Luo, and H. Qi. 2015. T-B-cell entanglement and ICOSL-driven feed-forward regulation of germinal centre reaction. *Nature* 517:214-218.

London, C.A., M.P. Lodge, and A.K. Abbas. 2000. Functional responses and costimulator dependence of memory CD4+ T cells. *J Immunol* 164:265-272.

Loughland, J.R., G. Minigo, D.S. Sarovich, M. Field, P.E. Tipping, M. Montes de Oca, K.A. Piera, F.H. Amante, B.E. Barber, M.J. Grigg, T. William, M.F. Good, D.L. Doolan, C.R. Engwerda, N.M. Anstey, J.S. McCarthy, and T. Woodberry. 2017. Plasmacytoid dendritic cells appear inactive during sub-microscopic Plasmodium falciparum blood-stage infection, yet retain their ability to respond to TLR stimulation. *Sci Rep* 7:2596.

Lu, K.T., Y. Kanno, J.L. Cannons, R. Handon, P. Bible, A.G. Elkahloun, S.M. Anderson, L. Wei, H. Sun, J.J. O'Shea, and P.L. Schwartzberg. 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.

Lyke, K.E., A.S. Ishizuka, A.A. Berry, S. Chakravarty, A. DeZure, M.E. Enama, E.R. James, P.F. Billingsley, A. Gunasekera, A. Manoj, M. Li, A.J. Ruben, T. Li, A.G. Eappen, R.E. Stafford, N. Kc, T. Murshedkar, F.H. Mendoza, I.J. Gordon, K.L. Zephir, L.A. Holman, S.H. Plummer, C.S. Hendel, L. Novik, P.J. Costner, J.G. Saunders, N.M. Berkowitz, B.J. Flynn, M.C. Nason, L.S. Garver, M.B. Laurens, C.V. Plowe, T.L. Richie, B.S. Graham, M. Roederer, B.K. Sim, J.E. Ledgerwood, S.L. Hoffman, and R.A. Seder. 2017. Attenuated PfSPZ Vaccine induces strain-transcending T cells and durable protection against heterologous controlled human malaria infection. *Proc Natl Acad Sci U S A* 114:2711-2716.

Macallan, D.C., D. Wallace, Y. Zhang, C. De Lara, A.T. Worth, H. Ghattas, G.E. Griffin, P.C. Beverley, and D.F. Tough. 2004. Rapid turnover of effector-memory CD4(+) T cells in healthy humans. *J Exp Med* 200:255-260.

MacLeod, M.K., A. David, A.S. McKee, F. Crawford, J.W. Kappler, and P. Marrack. 2011. Memory CD4 T cells that express CXCR5 provide accelerated help to B cells. *J Immunol* 186:2889-2896.

MacLeod, M.K., A. McKee, F. Crawford, J. White, J. Kappler, and P. Marrack. 2008. CD4 memory T cells divide poorly in response to antigen because of their cytokine profile. *Proc Natl Acad Sci U S A* 105:14521-14526.

Malleret, B., C. Claser, A.S. Ong, R. Suwanarusk, K. Sriprawat, S.W. Howland, B. Russell, F. Nosten, and L. Rénia. 2011. A rapid and robust tri-color flow cytometry assay for monitoring malaria parasite development. *Sci Rep* 1:118.

Marshall, H.D., A. Chandele, Y.W. Jung, H. Meng, A.C. Poholek, I.A. Parish, R. Rutishauser, W. Cui, S.H. Kleinstein, J. Craft, and S.M. Kaech. 2011. Differential expression of Ly6C and T-bet distinguish effector and memory Th1 CD4(+) cell properties during viral infection. *Immunity* 35:633-646.

Matloubian, M., R.J. Concepcion, and R. Ahmed. 1994. CD4+ T cells are required to sustain CD8+ cytotoxic T-cell responses during chronic viral infection. *J Virol* 68:8056-8063.

McAdam, A.J., T.T. Chang, A.E. Lumelsky, E.A. Greenfield, V.A. Boussiotis, J.S. Duke-Cohan, T. Chernova, N. Malenkovich, C. Jabs, V.K. Kuchroo, V. Ling, M. Collins, A.H. Sharpe, and G.J. Freeman. 2000. Mouse inducible costimulatory molecule (ICOS) expression is enhanced by CD28 costimulation and regulates differentiation of CD4+ T cells. *J Immunol* 165:5035-5040.

McDonald, V., and R. Phillips. 1978. Plasmodium chabaudi in mice. Adoptive transfer of immunity with enriched populations of spleen T and B lymphocytes. *Immunology* 34:821-830.

McHeyzer-Williams, L.J., P.J. Milpied, S.L. Okitsu, and M.G. McHeyzer-Williams. 2015. Class-switched memory B cells remodel BCRs within secondary germinal centers. *Nat Immunol* 16:296-305.

Meredith, M.M., K. Liu, G. Darrasse-Jeze, A.O. Kamphorst, H.A. Schreiber, P. Guermonprez, J. Idoyaga, C. Cheong, K.H. Yao, R.E. Niec, and M.C. Nussenzweig. 2012. Expression of the zinc finger transcription factor zDC (Zbtb46, Btbd4) defines the classical dendritic cell lineage. *J Exp Med* 209:1153-1165.

Miller, J.F., and G.F. Mitchell. 1968. Cell to cell interaction in the immune response. I. Hemolysin-forming cells in neonatally thymectomized mice reconstituted with thymus or thoracic duct lymphocytes. *J Exp Med* 128:801-820.

Mitchell, G.F., and J.F. Miller. 1968. Cell to cell interaction in the immune response. II. The source of hemolysin-forming cells in irradiated mice given bone marrow and thymus or thoracic duct lymphocytes. *J Exp Med* 128:821-837.

Moncunill, G., S.C. De Rosa, A. Ayestaran, A.J. Nhabomba, M. Mpina, K.W. Cohen, C. Jairoce, T. Rutishauser, J.J. Campo, J. Harezlak, H. Sanz, N. Díez-Padrisa, N.A. Williams, D. Morris, J.J. Aponte, C. Valim, C. Daubenberger, C. Dobaño, and M.J. McElrath. 2017. RTS,S/AS01E Malaria Vaccine Induces Memory and Polyfunctional T Cell Responses in a Pediatric African Phase III Trial. *Front Immunol* 8:1008.

Moon, J.J., H.H. Chu, M. Pepper, S.J. McSorley, S.C. Jameson, R.M. Kedl, and M.K. Jenkins. 2007. Naive CD4(+) T cell frequency varies for different epitopes and predicts repertoire diversity and response magnitude. *Immunity* 27:203-213.

Mordmüller, B., G. Surat, H. Lagler, S. Chakravarty, A.S. Ishizuka, A. Lalremruata, M. Gmeiner, J.J. Campo, M. Esen, A.J. Ruben, J. Held, C.L. Calle, J.B. Mengue, T. Gebru, J. Ibáñez, M. Sulyok, E.R. James, P.F. Billingsley, K.C. Natasha, A. Manoj, T. Murshedkar, A. Gunasekera, A.G. Eappen, T. Li, R.E. Stafford, M. Li, P.L. Felgner, R.A. Seder, T.L. Richie, B.K. Sim, S.L. Hoffman, and P.G. Kremsner. 2017. Sterile protection against human malaria by chemoattenuated PfSPZ vaccine. *Nature* 542:445-449.

Morita, R., N. Schmitt, S.E. Bentebibel, R. Ranganathan, L. Bourdery, G. Zurawski, E. Foucat, M. Dullaers, S. Oh, N. Sabzghabaei, E.M. Lavecchio, M. Punaro, V. Pascual, J. Banchereau, and H. Ueno. 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.

Moss, D.K., E.J. Remarque, B.W. Faber, D.R. Cavanagh, D.E. Arnot, A.W. Thomas, and A.A. Holder. 2012. Plasmodium falciparum 19-kilodalton merozoite surface protein 1 (MSP1)-specific antibodies that interfere with parasite growth in vitro can inhibit MSP1 processing, merozoite invasion, and intracellular parasite development. *Infect Immun* 80:1280-1287.

Mueller, I., M.R. Galinski, T. Tsuboi, M. Arevalo-Herrera, W.E. Collins, and C.L. King. 2013. Natural acquisition of immunity to Plasmodium vivax: epidemiological observations and potential targets. *Adv Parasitol* 81:77-131.

Muramatsu, M., K. Kinoshita, S. Fagarasan, S. Yamada, Y. Shinkai, and T. Honjo. 2000. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell* 102:553-563.

Murphy, J.R. 1980. Host defenses in murine malaria: immunological characteristics of a protracted state of immunity to Plasmodium yoelii. *Infect Immun* 27:68-74.

Nelson, R.W., D. Beisang, N.J. Tubo, T. Dileepan, D.L. Wiesner, K. Nielsen, M. Wüthrich, B.S. Klein, D.I. Kotov, J.A. Spanier, B.T. Fife, J.J. Moon, and M.K. Jenkins. 2015. T cell receptor cross-reactivity between similar foreign and self peptides influences naive cell population size and autoimmunity. *Immunity* 42:95-107.

Niikura, M., S. Kamiya, A. Nakane, K. Kita, and F. Kobayashi. 2010. IL-10 plays a crucial role for the protection of experimental cerebral malaria by co-infection with non-lethal malaria parasites. *Int J Parasitol* 40:101-108.

Nossal, G.J., A. Cunningham, G.F. Mitchell, and J.F. Miller. 1968. Cell to cell interaction in the immune response. 3. Chromosomal marker analysis of single antibody-forming cells in reconstituted, irradiated, or thymectomized mice. *J Exp Med* 128:839-853.

Nurieva, R.I., Y. Chung, D. Hwang, X.O. Yang, H.S. Kang, L. Ma, Y.H. Wang, S.S. Watowich, A.M. Jetten, Q. Tian, and C. Dong. 2008. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. *Immunity* 29:138-149.

Nurieva, R.I., Y. Chung, G.J. Martinez, X.O. Yang, S. Tanaka, T.D. Matskevitch, Y.H. Wang, and C. Dong. 2009. Bcl6 mediates the development of T follicular helper cells. *Science* 325:1001-1005.

Obeng-Adjei, N., S. Portugal, T.M. Tran, T.B. Yazew, J. Skinner, S. Li, A. Jain, P.L. Felgner, O.K. Doumbo, K. Kayentao, A. Ongoiba, B. Traore, and P.D. Crompton. 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, K.J., S.E. Mohn, and A.S. Weinmann. 2012. Molecular mechanisms that control the expression and activity of Bcl-6 in TH1 cells to regulate flexibility with a TFH-like gene profile. *Nat Immunol* 13:405-411.

Ogutu, B.R., O.J. Apollo, D. McKinney, W. Okoth, J. Siangla, F. Dubovsky, K. Tucker, J.N. Waitumbi, C. Diggs, J. Wittes, E. Malkin, A. Leach, L.A. Soisson, J.B. Milman, L. Otieno, C.A. Holland, M. Polhemus, S.A. Remich, C.F. Ockenhouse, J. Cohen, W.R. Ballou, S.K. Martin, E. Angov, V.A. Stewart, J.A. Lyon, D.G. Heppner, M.R. Withers, and M.-M.V.W. Group. 2009. Blood stage malaria vaccine eliciting high antigen-specific antibody concentrations confers no protection to young children in Western Kenya. *PLoS One* 4:e4708.

Omer, F.M., J.B. de Souza, and E.M. Riley. 2003. Differential induction of TGF-beta regulates proinflammatory cytokine production and determines the outcome of lethal and nonlethal Plasmodium yoelii infections. *J Immunol* 171:5430-5436.

Omer, F.M., and E.M. Riley. 1998. Transforming growth factor beta production is inversely correlated with severity of murine malaria infection. *J Exp Med* 188:39-48.

Opata, M.M., V.H. Carpio, S.A. Ibitokou, B.E. Dillon, J.M. Obiero, and R. Stephens. 2015. Early effector cells survive the contraction phase in malaria infection and generate both central and effector memory T cells. *J Immunol* 194:5346-5354.

Otterdal, K., A. Berg, A.E. Michelsen, S. Patel, M.G. Tellevik, C.G. Haanshuus, B. Fevang, P. Aukrust, N. Langeland, and T. Ueland. 2018. Soluble markers of neutrophil, T-cell and monocyte activation are associated with disease severity and parasitemia in falciparum malaria. *BMC Infect Dis* 18:670.

Oxenius, A., M.F. Bachmann, R.M. Zinkernagel, and H. Hengartner. 1998. Virus-specific MHC-class II-restricted TCR-transgenic mice: effects on humoral and cellular immune responses after viral infection. *Eur J Immunol* 28:390-400.

Park, S.O., Y.W. Han, A.G. Aleyas, J.A. George, H.A. Yoon, J.H. Lee, H.Y. Kang, S.H. Kang, and S.K. Eo. 2008. Low-dose antigen-experienced CD4+ T cells display reduced clonal expansion but facilitate an effective memory pool in response to secondary exposure. *Immunology* 123:426-437.

Paus, D., T.G. Phan, T.D. Chan, S. Gardam, A. Basten, and R. Brink. 2006. Antigen recognition strength regulates the choice between extrafollicular plasma cell and germinal center B cell differentiation. *J Exp Med* 203:1081-1091.

Pepper, M., J.L. Linehan, A.J. Pagán, T. Zell, T. Dileepan, P.P. Cleary, and M.K. Jenkins. 2010. Different routes of bacterial infection induce long-lived TH1 memory cells and short-lived TH17 cells. *Nat Immunol* 11:83-89.

Pepper, M., A.J. Pagán, B.Z. Igyártó, J.J. Taylor, and M.K. Jenkins. 2011. Opposing signals from the Bcl6 transcription factor and the interleukin-2 receptor generate T helper 1 central and effector memory cells. *Immunity* 35:583-595.

Phan, T.G., D. Paus, T.D. Chan, M.L. Turner, S.L. Nutt, A. Basten, and R. Brink. 2006. High affinity germinal center B cells are actively selected into the plasma cell compartment. *J Exp Med* 203:2419-2424.

Pinzon-Charry, A., T. Woodberry, V. Kienzle, V. McPhun, G. Minigo, D.A. Lampah, E. Kenangalem, C. Engwerda, J.A. López, N.M. Anstey, and M.F. Good. 2013. Apoptosis and dysfunction of blood dendritic cells in patients with falciparum and vivax malaria. *J Exp Med* 210:1635-1646.

Poholek, A.C., K. Hansen, S.G. Hernandez, D. Eto, A. Chandele, J.S. Weinstein, X. Dong, J.M. Odegard, S.M. Kaech, A.L. Dent, S. Crotty, and J. Craft. 2010. In vivo regulation of Bcl6 and T follicular helper cell development. *J Immunol* 185:313-326.

Prudêncio, M., A. Rodriguez, and M.M. Mota. 2006. The silent path to thousands of merozoites: the Plasmodium liver stage. *Nat Rev Microbiol* 4:849-856.

Qi, H., J.L. Cannons, F. Klauschen, P.L. Schwartzberg, and R.N. Germain. 2008. SAP-controlled T-B cell interactions underlie germinal centre formation. *Nature* 455:764-769.

Reinhardt, R.L., A. Khoruts, R. Merica, T. Zell, and M.K. Jenkins. 2001. Visualizing the generation of memory CD4 T cells in the whole body. *Nature* 410:101-105.

Richard, K., S.K. Pierce, and W. Song. 2008. The agonists of TLR4 and 9 are sufficient to activate memory B cells to differentiate into plasma cells in vitro but not in vivo. *J Immunol* 181:1746-1752.

Ridderstad, A., and D.M. Tarlinton. 1998. Kinetics of establishing the memory B cell population as revealed by CD38 expression. *J Immunol* 160:4688-4695.

Riley, E.M., S.J. Allen, J.G. Wheeler, M.J. Blackman, S. Bennett, B. Takacs, H.J. Schönfeld, A.A. Holder, and B.M. Greenwood. 1992. Naturally acquired cellular and humoral immune responses to the major merozoite surface antigen (PfMSP1) of Plasmodium falciparum are associated with reduced malaria morbidity. *Parasite Immunol* 14:321-337.

Roberts, D.W., R.G. Rank, W.P. Weidanz, and J.F. Finerty. 1977. Prevention of recrudescent malaria in nude mice by thymic grafting or by treatment with hyperimmune serum. *Infect Immun* 16:821-826.

Roestenberg, M., M. McCall, J. Hopman, J. Wiersma, A.J. Luty, G.J. van Gemert, M. van de Vegte-Bolmer, B. van Schaijk, K. Teelen, T. Arens, L. Spaarman, Q. de Mast, W. Roeffen, G. Snounou, L. Rénia, A. van der Ven, C.C. Hermsen, and R. Sauerwein. 2009. Protection against a malaria challenge by sporozoite inoculation. *N Engl J Med* 361:468-477.

Rogers, P.R., C. Dubey, and S.L. Swain. 2000. Qualitative changes accompany memory T cell generation: faster, more effective responses at lower doses of antigen. *J Immunol* 164:2338-2346.

Rolf, J., S.E. Bell, D. Kovesdi, M.L. Janas, D.R. Soond, L.M. Webb, S. Santinelli, T. Saunders, B. Hebeis, N. Killeen, K. Okkenhaug, and M. Turner. 2010. Phosphoinositide 3-kinase activity in T cells regulates the magnitude of the germinal center reaction. *J Immunol* 185:4042-4052.

Rubtsov, A.V., K. Rubtsova, A. Fischer, R.T. Meehan, J.Z. Gillis, J.W. Kappler, and P. Marrack. 2011. Toll-like receptor 7 (TLR7)-driven accumulation of a novel CD11c$^+$ B-cell population is important for the development of autoimmunity. *Blood* 118:1305-1315.

Ryg-Cornejo, V., L.J. Ioannidis, A. Ly, C.Y. Chiu, J. Tellier, D.L. Hill, S.P. Preston, M. Pellegrini, D. Yu, S.L. Nutt, A. Kallies, and D.S. Hansen. 2016. Severe Malaria Infections Impair Germinal Center Responses by Inhibiting T Follicular Helper Cell Differentiation. *Cell Rep* 14:68-81.

Salek-Ardakani, S., Y.S. Choi, M. Rafii-El-Idrissi Benhnia, R. Flynn, R. Arens, S. Shoenberger, S. Crotty, and M. Croft. 2011. B cell-specific expression of B7-2 is required for follicular Th cell function in response to vaccinia virus. *J Immunol* 186:5294-5303.

Sallusto, F., D. Lenig, R. Förster, M. Lipp, and A. Lanzavecchia. 1999. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 401:708-712.

Sanni, L.A., W. Jarra, C. Li, and J. Langhorne. 2004. Cerebral edema and cerebral hemorrhages in interleukin-10-deficient mice infected with Plasmodium chabaudi. *Infect Immun* 72:3054-3058.

Schaerli, P., K. Willimann, A.B. Lang, M. Lipp, P. Loetscher, and B. Moser. 2000. CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. *J Exp Med* 192:1553-1562.

Schwickert, T.A., G.D. Victora, D.R. Fooksman, A.O. Kamphorst, M.R. Mugnier, A.D. Gitlin, M.L. Dustin, and M.C. Nussenzweig. 2011. A dynamic T cell-limited checkpoint regulates affinity-dependent B cell entry into the germinal center. *J Exp Med* 208:1243-1252.

Seder, R.A., L.J. Chang, M.E. Enama, K.L. Zephir, U.N. Sarwar, I.J. Gordon, L.A. Holman, E.R. James, P.F. Billingsley, A. Gunasekera, A. Richman, S. Chakravarty, A. Manoj, S. Velmurugan, M. Li, A.J. Ruben, T. Li, A.G. Eappen, R.E. Stafford, S.H. Plummer, C.S. Hendel, L. Novik, P.J. Costner, F.H. Mendoza, J.G. Saunders, M.C. Nason, J.H. Richardson, J. Murphy, S.A. Davidson, T.L. Richie, M. Sedegah, A. Sutamihardja, G.A. Fahle, K.E. Lyke, M.B. Laurens, M. Roederer, K. Tewari, J.E. Epstein, B.K. Sim, J.E. Ledgerwood, B.S. Graham, S.L. Hoffman, and V.S. Team. 2013. Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. *Science* 341:1359-1365.

Shimoda, M., T. Li, J.P. Pihkala, and P.A. Koni. 2006. Role of MHC class II on memory B cells in post-germinal center B cell homeostasis and memory response. *J Immunol* 176:2122-2133.

Shulman, Z., A.D. Gitlin, J.S. Weinstein, B. Lainez, E. Esplugues, R.A. Flavell, J.E. Craft, and M.C. Nussenzweig. 2014. Dynamic signaling by T follicular helper cells during germinal center B cell selection. *Science* 345:1058-1062.

Silva, A.L., M.V. Lacerda, R.T. Fujiwara, L.L. Bueno, and E.M. Braga. 2013. Plasmodium vivax infection induces expansion of activated naïve/memory T cells and differentiation into a central memory profile. *Microbes Infect* 15:837-843.

Stephens, R., and J. Langhorne. 2010. Effector memory Th1 CD4 T cells are maintained in a mouse model of chronic malaria. *PLoS Pathog* 6:e1001208.

Stuehr, D.J., and M.A. Marletta. 1987. Induction of nitrite/nitrate synthesis in murine macrophages by BCG infection, lymphokines, or interferon-gamma. *J Immunol* 139:518-525.

Su, Z., and M.M. Stevenson. 2000. Central role of endogenous gamma interferon in protective immunity against blood-stage Plasmodium chabaudi AS infection. *Infect Immun* 68:4399-4406.

Sullivan, R.T., C.C. Kim, M.F. Fontana, M.E. Feeney, P. Jagannathan, M.J. Boyle, C.J. Drakeley, I. Ssewanyana, F. Nankya, H. Mayanja-Kizza, G. Dorsey, and B. Greenhouse. 2015. FCRL5 Delineates Functionally Impaired Memory B Cells Associated with Plasmodium falciparum Exposure. *PLoS Pathog* 11:e1004894.

Takahashi, Y., H. Ohta, and T. Takemori. 2001. Fas is required for clonal selection in germinal centers and the subsequent establishment of the memory B cell repertoire. *Immunity* 14:181-192.

Taub, D.D., and G.W. Cox. 1995. Murine Th1 and Th2 cell clones differentially regulate macrophage nitric oxide production. *J Leukoc Biol* 58:80-89.

The RTS, S.C.T.P. 2012. A phase 3 trial of RTS,S/AS01 malaria vaccine in African infants. *N Engl J Med* 367:2284-2295.

Tubo, N.J., A.J. Pagán, J.J. Taylor, R.W. Nelson, J.L. Linehan, J.M. Ertelt, E.S. Huseby, S.S. Way, and M.K. Jenkins. 2013. Single naive CD4+ T cells from a diverse repertoire produce different effector cell types during infection. *Cell* 153:785-796.

Ueffing, K., H. Abberger, A.M. Westendorf, K. Matuschewski, J. Buer, and W. Hansen. 2017. Conventional CD11c. *Front Immunol* 8:1333.

Urban, B.C., D. Cordery, M.J. Shafi, P.C. Bull, C.I. Newbold, T.N. Williams, and K. Marsh. 2006. The frequency of BDCA3-positive dendritic cells is increased in the peripheral circulation of Kenyan children with severe malaria. *Infect Immun* 74:6700-6706.

Urban, B.C., D.J. Ferguson, A. Pain, N. Willcox, M. Plebanski, J.M. Austyn, and D.J. Roberts. 1999. Plasmodium falciparum-infected erythrocytes modulate the maturation of dendritic cells. *Nature* 400:73-77.

Urban, B.C., T.T. Hien, N.P. Day, N.H. Phu, R. Roberts, E. Pongponratn, M. Jones, N.T. Mai, D. Bethell, G.D. Turner, D. Ferguson, N.J. White, and D.J. Roberts. 2005. Fatal Plasmodium falciparum malaria causes specific patterns of splenic architectural disorganization. *Infect Immun* 73:1986-1994.

Van den Eertwegh, A.J., R.J. Noelle, M. Roy, D.M. Shepherd, A. Aruffo, J.A. Ledbetter, W.J. Boersma, and E. Claassen. 1993. In vivo CD40-gp39 interactions are essential for thymus-dependent humoral immunity. I. In vivo expression of CD40 ligand, cytokines, and antibody production delineates sites of cognate T-B cell interactions. *J Exp Med* 178:1555-1565.

Velu, V., G. Mylvaganam, S. Gangadhara, J. Hong, S. Iyer, S. Gumber, C. Ibegbu, F. Villinger, and R. Amara. 2016. Induction of Th1-Biased T Follicular Helper (Tfh) Cells in Lymphoid Tissues during Chronic Simian Immunodeficiency Virus Infection Defines Functionally Distinct Germinal Center Tfh Cells. *J Immunol* 197:1832-1842.

Victora, G.D., T.A. Schwickert, D.R. Fooksman, A.O. Kamphorst, M. Meyer-Hermann, M.L. Dustin, and M.C. Nussenzweig. 2010. Germinal center dynamics revealed by multiphoton microscopy with a photoactivatable fluorescent reporter. *Cell* 143:592-605.

Villegas-Mendez, A., T.N. Shaw, C.A. Inkson, P. Strangward, J.B. de Souza, and K.N. Couper. 2016. Parasite-Specific CD4+ IFN-γ+ IL-10+ T Cells Distribute within Both Lymphoid and Nonlymphoid Compartments and Are Controlled Systemically by Interleukin-27 and ICOS during Blood-Stage Malaria Infection. *Infect Immun* 84:34-46.

Vinetz, J.M., S. Kumar, M.F. Good, B.J. Fowlkes, J.A. Berzofsky, and L.H. Miller. 1990. Adoptive transfer of CD8+ T cells from immune animals does not transfer immunity to blood stage Plasmodium yoelii malaria. *J Immunol* 144:1069-1074.

Voisine, C., B. Mastelic, A.M. Sponaas, and J. Langhorne. 2010. Classical CD11c+ dendritic cells, not plasmacytoid dendritic cells, induce T cell responses to Plasmodium chabaudi malaria. *Int J Parasitol* 40:711-719.

Watanabe, M., C. Fujihara, A.J. Radtke, Y.J. Chiang, S. Bhatia, R.N. Germain, and R.J. Hodes. 2017. Co-stimulatory function in primary germinal center responses: CD40 and B7 are required on distinct antigen-presenting cells. *J Exp Med* 214:2795-2810.

Weber, J.P., F. Fuhrmann, and A. Hutloff. 2012. T-follicular helper cells survive as long-term memory cells. *Eur J Immunol* 42:1981-1988.

Weinbaum, F.I., C.B. Evans, and R.E. Tigelaar. 1976. Immunity to Plasmodium Berghei yoelii in mice. I. The course of infection in T cell and B cell deficient mice. *J Immunol* 117:1999-2005.

Weiss, G.E., P.D. Crompton, S. Li, L.A. Walsh, S. Moir, B. Traore, K. Kayentao, A. Ongoiba, O.K. Doumbo, and S.K. Pierce. 2009. Atypical memory B cells are greatly expanded in individuals living in a malaria-endemic area. *J Immunol* 183:2176-2182.

Weng, N.P., B.L. Levine, C.H. June, and R.J. Hodes. 1995. Human naïve and memory T lymphocytes differ in telomeric length and replicative potential. *Proc Natl Acad Sci U S A* 92:11091-11094.

Whitmire, J.K., N. Benning, and J.L. Whitton. 2006. Precursor frequency, nonlinear proliferation, and functional maturation of virus-specific CD4+ T cells. *J Immunol* 176:3028-3036.

WHO. 2018. World Malaria Report 2018. In World Health Organization, Geneva.

Wikenheiser, D.J., S.L. Brown, J. Lee, and J.S. Stumhofer. 2018. NK1.1 Expression Defines a Population of CD4. *Front Immunol* 9:2277.

Williams, M.A., E.V. Ravkov, and M.J. Bevan. 2008. Rapid culling of the CD4+ T cell repertoire in the transition from effector to memory. *Immunity* 28:533-545.

Woodberry, T., G. Minigo, K.A. Piera, F.H. Amante, A. Pinzon-Charry, M.F. Good, J.A. Lopez, C.R. Engwerda, J.S. McCarthy, and N.M. Anstey. 2012. Low-level Plasmodium falciparum blood-stage infection causes dendritic cell apoptosis and dysfunction in healthy volunteers. *J Infect Dis* 206:333-340.

Wu, T., H.M. Shin, E.A. Moseman, Y. Ji, B. Huang, C. Harly, J.M. Sen, L.J. Berg, L. Gattinoni, D.B. McGavern, and P.L. Schwartzberg. 2015. TCF1 Is Required for the T Follicular Helper Cell Response to Viral Infection. *Cell Rep* 12:2099-2110.

Wykes, M.N., X.Q. Liu, L. Beattie, D.I. Stanisic, K.J. Stacey, M.J. Smyth, R. Thomas, and M.F. Good. 2007. Plasmodium strain determines dendritic cell function essential for survival from malaria. *PLoS Pathog* 3:e96.

Wykes, M.N., Y.H. Zhou, X.Q. Liu, and M.F. Good. 2005. Plasmodium yoelii can ablate vaccine-induced long-term protection in mice. *J Immunol* 175:2510-2516.

Xu, H., X. Li, D. Liu, J. Li, X. Zhang, X. Chen, S. Hou, L. Peng, C. Xu, W. Liu, L. Zhang, and H. Qi. 2013. Follicular T-helper cell recruitment governed by bystander B cells and ICOS-driven motility. *Nature* 496:523-527.

Yu, D., S. Rao, L.M. Tsai, S.K. Lee, Y. He, E.L. Sutcliffe, M. Srivastava, M. Linterman, L. Zheng, N. Simpson, J.I. Ellyard, I.A. Parish, C.S. Ma, Q.J. Li, C.R. Parish, C.R. Mackay, and C.G. Vinuesa. 2009. The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. *Immunity* 31:457-468.

Zander, R.A., J.J. Guthmiller, A.C. Graham, R.L. Pope, B.E. Burke, D.J. Carr, and N.S. Butler. 2016. Type I Interferons Induce T Regulatory 1 Responses and Restrict Humoral Immunity during Experimental Malaria. *PLoS Pathog* 12:e1005945.

Zander, R.A., R. Vijay, A.D. Pack, J.J. Guthmiller, A.C. Graham, S.E. Lindner, A.M. Vaughan, S.H.I. Kappe, and N.S. Butler. 2017. Th1-like Plasmodium-Specific Memory CD4. *Cell Rep* 21:1839-1852.

Zuccarino-Catania, G.V., S. Sadanand, F.J. Weisel, M.M. Tomayko, H. Meng, S.H. Kleinstein, K.L. Good-Jacobson, and M.J. Shlomchik. 2014. CD80 and PD-L2 define functionally distinct memory B cell subsets that are independent of antibody isotype. *Nat Immunol* 15:631-637.