Dendritic cells and B cells in effector T cells decisions

Promotion of antibody induction in lymphoid tissue or gut homing

Samuel Alsén

Department of Microbiology and Immunology
Institute of Biomedicine
Sahlgrenska Academy, University of Gothenburg

UNIVERSITY OF GOTHENBURG

Gothenburg 2018

Cover illustration: Cross-section of a mouse spleen showing T cell zone in blue, B cell follicle in red and germinal centers in green and magenta.

Image courtesy of Frank Liang

Dendritic cells and B cells in effector T cells decisions – promotion of antibody induction in lymphoid tissue or gut homing

Samuel.alsen@gu.se

ISBN 978-91-629-0492-0 (PRINT)
ISBN 978-91-629-0493-7 (PDF)

Printed in Gothenburg, Sweden 2018
Printed by BrandFactory

If "Plan A" doesn´t work...

...Don´t stress, the alphabet has 25 more letters

Dendritic cells and B cells in effector T cells decisions

Promotion of antibody induction in lymphoid tissue or gut homing

Samuel Alsén

Department of Microbiology and Immunology, Institute of Biomedicine
Sahlgrenska Academy, University of Gothenburg
Gothenburg, Sweden

Abstract

$CD4^+$ T cells are principal cells of the adaptive immune system, equipped with the ability to boost innate immune cells and aid B cells in the germinal centers. Every T cell clone carries a variable and unique T cell receptor that recognizes a protein-derived peptide presented on MHC molecules by antigen presenting cells (APC) in secondary lymphoid organs. Dendritic cells (DC) are the most prominent APC due to their unmatched ability to internalize foreign protein antigens, degrade them into peptides, load peptides onto MHC molecules and migrate to lymph nodes and present the antigen to T cells. Recognition of its cognate antigen leads to activation and proliferation of T cells while additional co-stimulatory signals dictate the differentiation and fate of T cells. Although T effector cell differentiation can be induced during the first encounter with an APC, the differentiation of B cell supporting T follicular helper (Tfh) cells require continuous antigen presentation by B cells. These two differentiation pathways of T cells occur in parallel and are guided by reciprocal antagonistic transcription factors. Herein, we have studied how APCs, with a focus on DCs and B cells, influence T cell differentiation.

By adoptively transferring $CD4^+$ T cells with a known antigen specificity into recipient transgenic mice in which DCs can be depleted we show Tfh differentiation in the absence of DCs as long as a sufficient amount of antigen is administered together with the adjuvant. However, depletion of DCs lead to a loss of Th1 effector T cells that had downstream consequences on B cells by preventing class-switching into the Th1-associated antibody isotype. Excluding the altered class-switch, germinal center B cells showed normal affinity maturation and memory formation. This shows that Tfh cells generated in the absence of DCs are fully functional and that DCs therefore do not provide unique accessory signals required for Tfh differentiation.

T cells that differentiate to develop into Tfh cells become programmed to do so already during the primary encounter with an APC. To fulfill the Tfh differentiation program pre-Tfh cells must then interact with antigen presenting B cells to fully adopt Tfh functionality. This step-wise process has been extensively studied but it still remains unclear precisely how B cells enforce the Tfh program. In the second and third study, we exploited mixed bone marrow chimeras to generate mice in which B cells cannot present antigens to T cell thus terminating the Tfh program at the stage of T-B interactions.

In these studies, we reveal a role of B cells in regulating T cell expression of IL-4, its receptor IL4Rα and a H2-Q2, a gene previously not described in T cell biology. We also show that B cells affect the output of T effector cells from the lymph node. In lymph, we identify T cells that exhibit phenotypic characteristics of Tfh cells, show a history of IL-4 secretion and are dependent on cognate B cell interactions. Some of these migratory ex-Tfh cells show gut tropism and can be tracked to small intestinal lamina propria. This suggests that Tfh cells not selected for germinal center entry can convert into tissue-tropic effector T cells.

Keywords: T cells, T follicular helper cells, dendritic cells, B cells, germinal center, small intestinal lamina propria, differentiation, adaptive immunity

ISBN 978-91-629-0492-0 (PRINT)
ISBN 978-91-629-0493-7 (PDF)

Populärvetenskaplig sammanfattning

Trots att människokroppen konstant exponeras för virus och bakterier insjuknar vi sällan, tack vare vårt effektiva immunförsvar. Immunförsvaret består av en mängd olika specialiserade celler som delas upp i två grenar; det medfödda och det adaptiva immunförsvaret. Vid en infektion är det medfödda immunförsvaret först att aktiveras. Cellerna i det medfödda immunförsvaret känner igen strukturer på virus och bakterier som de inte kan förändra eftersom dessa är viktiga för dess överlevnad och funktion. Vissa celler inom det medfödda immunförsvaret har även förmågan att äta upp bakterier och virusinfekterade celler. Dessa celler kallas antigenpresenterande celler då de kan bryta ned proteiner från bakterier och virus till korta peptider som sedan visas upp på cellmembranet för att aktivera antigen-specifka T-celler. Det finns flera olika antigenpresenterande celler men dendritiska celler har visats vara bäst på att ta upp och presentera antigen till T-celler.

T-celler tillsammans med B-celler utgör det adaptiva immunförsvaret. Under deras utveckling så klipps och klistras olika DNA-fragment ihop i de gener som kodar för cellernas antigen-igenkännande receptorer. Denna utveckling ger att varje T-cell och B-cell en receptor med unik specificitet. Då T- och B-celler finns i överflöd så leder denna unika igenkänning på cellnivå till att kroppens T- och B cells som helhet har ett väldigt brett register för igenkännande. Då nya T- och B-celler med olika specificeter hela tiden bildas för att sedan väljas och anpassas efter typen av infektion, tillhör de det adaptiva immunförsvaret.

När en T-cell möter och interagerar med en antigenpresenterande cell som presenterar den peptidsekvens som T-cellens receptor känner igen så börjar T-cellen att dela på sig och därmed bilda ett stort antal celler. Detta sker i lymfknutan och förutom celldelningen så börjar T-cellerna i lymfknutan att specialisera sig för att bli ännu bättre på att bekämpa infektionen. Antingen så lämnar de lymfknutan och beger sig till platsen för infektionshärden för att lokalt bekämpa den eller så stannar T-cellen i lymfknuten för att hjälpa B-cellerna att producera antikroppar. Antikroppar är små lösliga proteiner som främst finns i blod och slemhinnor. Antikroppar kan bland annat neutralisera gifter som utsöndras från bakterier eller förhindra virus från att infektera celler. Precis som T-cellernas receptor har antikropparna en unik specifictet. Om B-cellernas receptorer som utgörs av cellmembransbundna antikroppar känner igen ett protein har de möjlighet att ta upp proteinet och presentera peptider för T-cellerna. På så sätt kan B-celler agera som en antigenpresenterande cell för T-celler. B-celler som visar upp antigen för T-celler kan sedan med hjälp av T-

celln att förbättra de antikropparna som de producerar och på så sätt förstärka immunförsvarets effekt.

Denna avhandling är baser på mina studier om hur olika antigenpresenterade celler, främst dendritiska celler och B-celler, vägleder T-cellernas beslut att antingen stanna i lymfknutan eller att bege sig till infektionshärden. Arbetet är utfört i olika musmodeller där vi genom genetiska manipulationer kan eliminera vissa celler eller där B-celler inte längre har förmågan att presentera antigen till T-celler. Vi fann att dendritiska celler har en unik förmåga att inducera typ-1-immunsvar men att de inte är umbärliga för att hjälpa B-celler. Vi visar även visat att B-celler även kan påverkar vilka T-celler som beger sig till infektionshärden. Dessa studier bidrar en bättre förståelse för hur B-celler och dendrtitiska celler påverkar T-cellernas funktioner. Kunskap om hur man kan styra T-celler till infektionshärden eller uppmuntra dessa att stanna kvar i lymfknutan för att hjälpa B-cellernas antikroppsproduktion är av stort värde för utvecklig av nya vaccin.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

Paper I:

T Follicular Helper, but Not Th1, Cell Differentiation in the Absence of Conventional Dendritic cells.
Dahlgren M. W, Gustafsson-Hedberg T, Livingston M, Cucak H, **Alsén S**, Yrlid U and Johansson-Lindbom B.

*The Journal of Immunology, 2015 June 1; 194(11); 5187-5199**

Paper II:

IL-4 secretion following a Th1 skewing immunization is restricted to T follicular helper cells that also downregulate IL4Rα.
Alsén S, Wenzel A. U, Dahlgren M.W, Erlandsson E, Gustafsson-Hedberg T, Bryder D, Johansson-Lindbom B and Yrlid U.

Manuscript

Paper III:

B cells regulate lymph node exit of tissue tropic T helper cells following immunization.
Alsén S, Cervin J, Cucak H, Livingston M, Johansson-Lindbom B and Yrlid U.

Manuscript

**Reprints were made with permission of the publisher*

Content

Frequently used abbreviations

AID	Activation-induced cytidine deaminase
APC	Antigen presenting cell
Bcl6	B cell lymphoma 6
BCR	B cell receptor
Blimp-1	B lymphocyte-induced maturation protein-1
CCR	C-C chemokine receptor
CXCR	Chemokine (C-X-C motif) receptor
CSR	Class switch recombination
DC	Dendritic cell
DTx	Diphtheria toxin
DZ	Dark zone
GC	Germinal center
IL	Interleukin
Ig	Immunoglobulin
i.p.	Intraperitoneal
LZ	Light zone
MHC	Major histocompatibility complex
MLN	Mesenteric lymph node
OVA	Ovalbumin
PC	Plasma cell
PD-1	Programmed death-1
pDC	Plasmacytoid dendritic cell
p.i.	Post-immunization / post-infection
STAT	Signal transducer and activator of transcription

The cells and anatomical organization of the immune system

The immune system is composed of numerous different cell types, each providing a highly specialized function. The survival of an organism is directly dependent on its immune system and its capacity to provide immunity. The immune system is compartmentalized into separate sites where immune cells are generated, mature, become activated and where they perform their function. In this section I will briefly introduce the principal cells of the immune system, lymphoid organs and peripheral effector sites.

Cells of the immune system

The immune system is generally divided into the innate and the adaptive immune system. The innate, or in-born, immune system is the first line defense against invading pathogens acting instantly upon pathogen encounter. The cells of the innate immune system are activated either by effector molecules provided by other cells or directly by invariant pattern recognition receptors (PRRs). The PRRs recognize evolutionary conserved elements with limited variability called pathogen-associated molecular patterns (PAMPs). These structural components are essential for survival of the microbe. A family of receptors called Toll-like receptors (TLRs) are the most studied PRRs. TLRs are capable of recognizing bacterial structures like lipopolysaccharide and lipoteichoic acid, but other TLRs also sense double-stranded RNA (dsRNA) found in viruses, thus allowing the innate immune system to identify the type of invading pathogen (1).

Some innate immune cells, called granulocytes (neutrophils, eosinophils, basophils and mast cells), contain intracellular granules loaded with pro-inflammatory molecules and effector molecules capable of killing pathogens (2). In response to activation signals, granulocytes rapidly degranulate and initiate the immune response by causing inflammation of the tissue. Phagocytes are cells capable of ingesting and killing whole bacteria targeted for phagocytosis by the complement system and antibodies or following recognition of PAMPs (3). Phagocytes include neutrophils and mast cells of the above-mentioned granulocytes as well as macrophages and dendritic cells (DCs). Natural killer (NK) cells are specialized in killing virus infected cells by secretion of pore forming molecules and proteases which in combination lyse the target cell. Typically, NK cells target cells that fail to express MHC

class 1 molecules (4), or by binding of CD16 (FcRγIII) to the Fc-region of an antibody bound to the target cell which initiate killing through antibody-dependent cellular cytotoxicity (ADCC) (4, 5).

Whereas the innate immune system recognizes conserved elements present on the surface or cytoplasm of microbes, the adaptive immune is highly specific to non-conserved, or variable, elements. The cells of the adaptive immune system have several important functions for overall immunity. B cells produce antibodies that can neutralize toxins, activate complement or target cells for phagocytosis and ADCC. T cells, given their name due to their thymus dependent maturation, are subcategorized into $CD4^+$ T cells and $CD8^+$ T cells. The $CD4^+$ T cells are called T helper cells and play an essential role in promoting immune responses by producing cytokines that acts upon surrounding cells. $CD8^+$ T cells, or cytotoxic T lymphocyte, induce lysis in virus-infected cells or altered self (i.e. cancer) in an antigen dependent manner. Additional cells of the adaptive immune system are NKT cells, mucosal associated invariant $CD8^+$ T cells and γδ T cells that recognizes conserved structure like lipids and bacterial metabolites, giving them innate-like properties in terms of antigen recognition.

The adaptive immune system has several distinct functions that are not shared by the innate immune system. First, cells of the adaptive immune system are able to proliferate in response to antigen recognition, thus amplifying the sheer number of reactive cells. Second, by recombination of the germline DNA encoding their receptors they are capable of generating an almost limitless number of receptors, all with unique specificity. In the case of B cells these receptors can be mutated in activated cells to further increase specificity, or affinity (6, 7). Lastly, antigen experienced cells are capable of surviving for extended periods of time. These cells potently and rapidly respond upon re-exposure to their cognate antigen (8, 9), thus providing immunological memory which is the basis of vaccines.

The link between the innate and adaptive immune system is facilitated by cells that are capable of taking up foreign protein antigens, degrade them into peptides and present them on major histocompatibility complexes (MHC) for T cell recognition. Cells capable of performing this critical function, called antigen presenting cells (APCs), are found throughout the body scanning the tissue for foreign antigen. Cells with antigen presenting function include DCs (conventional and plasmacytoid), B cells, macrophages and monocytes. The conventional DCs (cDCs) are considered to be the most proficient APC in terms of antigen uptake, presentation and activation of T cells.

The key cells for induction of antibody mediated immune responses, $CD4^+$ T cells, DCs and B cells, are central to this thesis and will be covered in full in the coming chapters.

Primary lymphoid organs

The immune system can be compartmentalized into three anatomical distinct regions. The primary lymphoid organs constitute the first of these sites and are responsible for the generation and development of immune cells.

The bone marrow
The immune cells outlined in the previous section are all of hematopoietic origin and generated in the bone marrow (BM). All cells derived from a shared stem cell precursor that through activation of transcription factors gradually guiding their differentiation into separate lineages (Figure 1). The first distinct separation arising from the shared BM stem cells is the differentiation into either a common myeloid precursor or a common lymphoid precursor. With the exception of NK cells, innate immune cells develop from the common myeloid precursor through several intermediary steps and, conversely, NK cells, B cells and T cells are derived from the common lymphoid progenitor. Some of the cells complete their maturation in peripheral tissues, T cells however, exit the BM as progenitor thymocytes and require further development in the thymus to become mature T cells.

Thymus
Thymus is the primary lymphoid organ responsible for development and selection of T cells. The important role of the thymus in generating T cells was discovered by surgical removal of the thymus. Athymic mice lack T cells and fail to reject skin and tumor engraftments (10). The course of T cell development is guided by sequential interactions between different thymic epithelial cells, DCs and the maturing T cells to ensure generation of functional and non-self-reactive T cells. The process of T cell development in thymus will be described in greater detail in the T cell section.

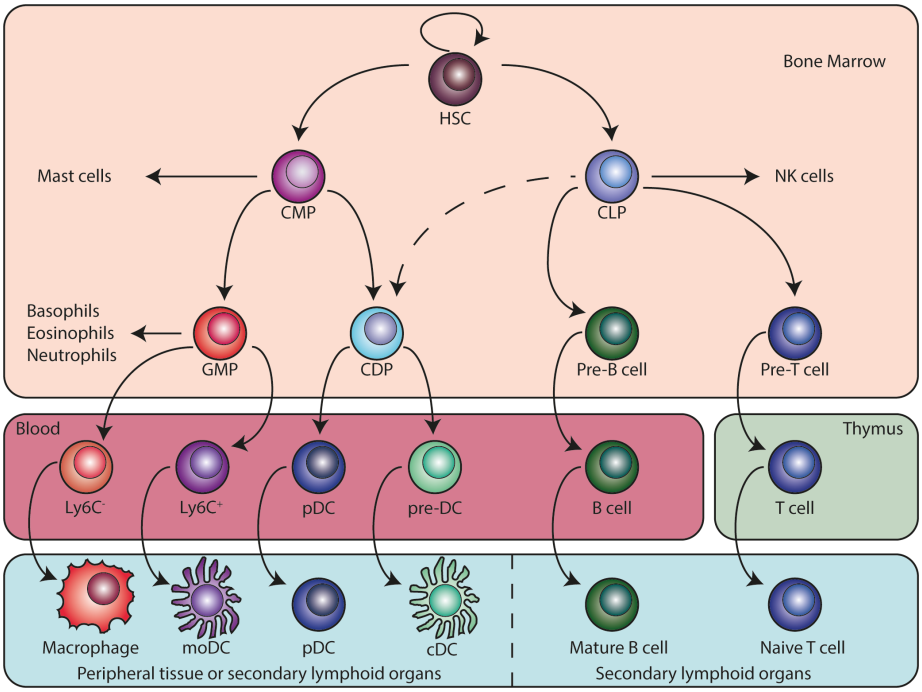

Figure 1. Overview of haematopoiesis. *HSC: Haematopoietic stem cell, CMP: Common myeloid progenitor, CLP: Common lymphoid progenitor, GMP: Granulocyte / Macrophage progenitor, CDP: Common DC precursor*

Secondary lymphoid organ

The secondary lymphoid organs (SLOs) are strategically situated to monitor pathogenic infiltration at all tissues throughout the body. SLOs are connected to blood vessels and the lymphatic system. The lymphatic system connects the tissue to SLOs via afferent lymphatics through which free antigen as well as antigen bearing cells are transported into the SLOs. Lymphocytes exiting SLOs migrate through efferent lymphatics and via ducts emptied back into the blood stream. This allows lymphocytes to circulate through SLOs in search of APCs that present antigens from peripheral tissues. Thus, SLOs serve as a focal point facilitating the encounter between APCs and T cells and therefore function as inductive sites for the adaptive immune system.

The spleen

The spleen is not connected to afferent lymphatics, instead it is dedicated to scan for antigens in the blood stream, degrade aging erythrocytes and iron recycling (11). The spleen is anatomically divided into two large

compartments, the red pulp and the white pulp which are encapsulated by the marginal zone. In the red pulp the blood is filtered from old erythrocytes and iron is recycled. This is also the site for lymphocyte entry and a reservoir for antibody producing plasma cells (PCs) as well as monocytes (11, 12). The marginal zone is rich in DCs and macrophages proficient in acquiring blood borne antigens. A population of B cells (marginal zone B cells), that are important for early immune responses by recognition of conserved microbial elements also resides within the marginal zone (13).

Closest to the central arteriole in the white pulp lies the periarteriolar sheath, forming an area that is populated by T cells and DCs. Situated in-between the periarteriolar sheath and the marginal zone follicles containing B cells can be found. The distinct separation between T and B cells areas is maintained by the secretion of chemokines from fibroblastic reticular cells in the T cell zone and follicular dendritic cells (FDCs) in the B cell follicle (11). In addition to the acquisition of antigens via macrophages and DCs in the marginal zone, a conduit system that runs throughout the white pulp allows for transport of smaller sized antigens that can be sampled by DCs (14). In contrast to lymph nodes (LNs), the spleen does not have efferent lymphatics. Instead lymphocyte egress from the spleen directly into the blood stream, most likely by initial transit from the white pulp into the red pulp via bridging channels in the marginal zone (15).

Mesenteric lymph nodes

The mesenteric lymph nodes (MLNs) are the second line of SLOs (Figure 2) and drain the intestine and efferent lymphatics from Peyer´s patches. Small molecular antigens that enter the MLN via the afferent lymphatics is distributed by a conduit network similar to that seen in spleen (16). Antigen-bearing DCs arrive to the MLN via afferent lymphatics and then enter at the subcapsular sinus. The DCs then translocate into the cortex where B cells reside and then proceed to venture further into the paracortex, the T cell zone of the MLN. T and B cells as well as LN resident DCs enter LNs via high endothelial venules (HEVs). Lymphocytes are able to transmigrate into the MLN via HEVs by binding of CD62L, LFA-1 and CCR7 to PNAd, ICAM-1 and CCL-21, respectively, present on the HEV endothelium (17-21). MLNs and other gut-associated SLOs also express the mucosal vascular addressin MAdCAM-1 that bind to the gut-homing associated integrin α4β7 (22, 23). Furthermore, MAdCAM-1 acts as a ligand for CD62L if properly glycosylated, thereby adding additional means of entry for lymphocytes (24, 25).

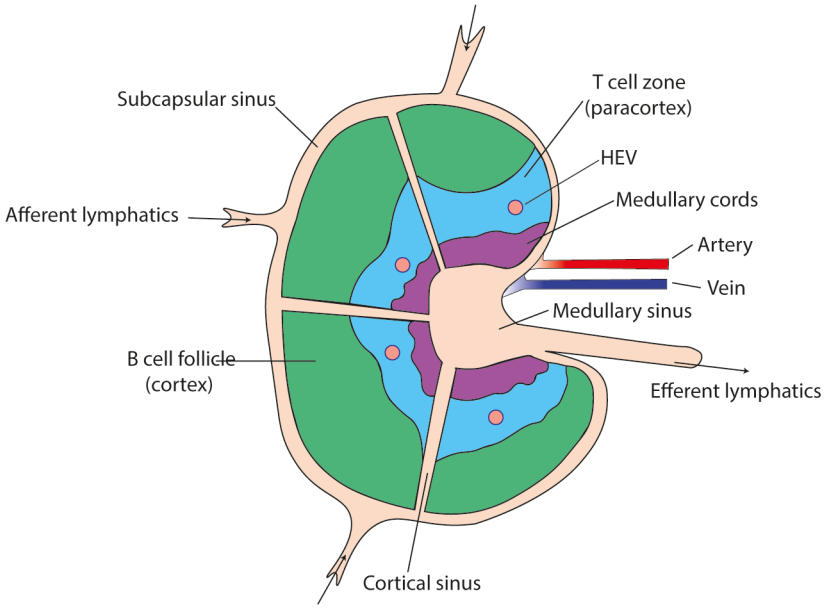

Figure 2. The anatomical organization of a lymph node

The mucosal immune system

Mucosal surfaces cover all internal body surfaces and are constantly exposed to both pathogens and commensal bacteria. Maintenance of its integrity is therefore essential for the survival of the organism. In addition to physical and

chemical barriers, it requires a finely tuned immune landscape able to combat invading pathogenic microorganisms yet adequately handle harmless food-antigens and commensal bacteria. Herein, I will focus on the mucosal immune system and its associated structures.

Peyers´ Patches

Interspersed along the intestine, just underneath the epithelium, lies several highly organized clusters of lymphoid follicles called Peyer´s patches (PP) that serves as the primary induction sites for immune responses in the gut. Due to the anatomical localization, PPs lack afferent lymphatics and the efferent lymphatics connect to MLNs rather than emptying into the blood stream. The epithelial layer of the PP that separates the underlying subepithelial dome (SED) and the gut lumen is called follicle-associated epithelium (FAE). Lodged in the FAE are microfold (M) cells that can transport luminal antigens and microbes into the SED (26, 27). In the SED, DCs acquire antigens and migrate from the SED to the neighboring T cell zone to initiate T cell responses (28, 29). In contrast to other SLOs, entry of lymphocytes into PPs via HEV do not rely on CD62L expression as PP-associated HEVs lack expression of PNAd (17).

Isolated lymphoid follicles & cryptopatches

Cryptopatches (CP) and isolated lymphoid follicles (ILFs) are small solitary follicle like structures encircled by DCs that are present directly underneath the epithelium containing M cells in the small intestine (30). The CPs consists of lineage negative lymphoid cells expressing the stem cell factor c-kit (31) whereas the ILFs contain mostly B cells (32). Whether these structures can, similar to other SLOs, function as inductive sites for conventional T cells is still not known.

Small intestinal lamina propria

The small intestine is comprised of a monolayer of epithelial cells forming crypts and villi lined with mucus on the luminal side. Together the epithelial cells and the mucus constitute both a physical and a chemical barrier against the gut microbiota. The underlying tissue, separated by a thin layer of extracellular matrix, is the small intestinal lamina propria (SI LP). The SI LP serves as one of the main effector sites of mucosal immune responses. LP DCs are able to acquire gut luminal antigens by transepithelial sampling (33, 34), or acquire antigens via M cells that actively transport antigens across the epithelium (35). The LP do not function as an inductive site and therefore antigen bearing DCs migrate to the MLN via the lymphatic system in an CCR7-dependent manner in order to encounter naïve T cells (36, 37). Efficient homing to the SI LP of lymphocytes require expression of $\alpha 4\beta 7$ integrin that

binds to the addressin MAdCAM-1 present on post-capillary venules in the SI LP (22, 23). Furthermore, expression of the chemokine receptor CCR9 can help facilitate gut-homing and transmigration in response to CCL25 produced in the SI LP and presented on the apical surface of the endothelium (38-40). However, the importance of CCR9 expression for gut-homing is not uniform for all cell types as CCR9 deficient $CD4^+$ T cells are not as disadvantaged in their gut-homing as their $CD8^+$ counterparts (41, 42).

Dendritic cells

Discovered in the early 1970´s by Steinman and Cohn, DCs were given their name due to their morphological shape of dendrites extending from the cellular body (43-45). The findings that DCs express PRR and are able to induce T cell proliferation *in vitro* identified DCs as a link between the innate and adaptive immune system (46, 47). This role has been further solidified over the years and DCs are now considered to be the supreme APCs. This is attributed to their unique ability to migrate from the peripheral tissue into the SLO via lymphatic vessels in combination with their unparalleled potency to induce responses in naïve T cells (48-50).

Dendritic cell subsets

DCs are commonly divided into four categories based on their distinct function and tissue localization, namely conventional DCs (cDCs), plasmacytoid DCs (pDCs), Langerhans cells and monocyte-derived DCs (moDCs) (51). The term "DCs" often refers to cDCs as they are the most common of the four. Most frequently identified by expression of CD11c and MHCII, cDCs are present in SLOs as well as at effector sites. Immature cDCs exhibit phagocytic activity, abundant expression of PRRs and intermediate expression of MHCII and CD11c (52). In response to activation by PRR triggering, cDCs mature, halt their phagocytic activity and upregulate MHCII and co-stimulatory molecules to increase the capacity to activate T cells (52). Furthermore, cDCs at effector sites upregulate CCR7 and migrate to SLOs via the lymphatic system in a CCR7 dependent manner (36, 37). cDC are subcategorized into subsets based on their expression of transcription factors, surface markers and associated differential functionality. Murine migratory cDCs expressing CD103 but not CD11b have the ability to present extracellular antigens to $CD8^+$ T cells in what is called cross-presentation, whereas migratory $CD11b^+$ cDCs are more proficient in inducing $CD4^+$ T cell responses (53, 54). In comparison, LN resident cross-presenting cDCs do not express CD103, rather they are commonly distinguished by CD8α expression (55, 56). Similarly to migrating $CD103^+$ cDCs, $CD8\alpha^+$ cDCs are less proficient inducers of $CD4^+$ T cell than the LN resident $CD11b^+$ cDCs (56, 57). A study by Watchmaker et al characterized human cDC subsets and found that SI-LP $SIRP\alpha^+ CD103^+$ cDCs and blood $CD1c^+$ cDCs correlated to murine SI LP $CD11b^+CD103^+$ cDCs (58). Moreover, they found that $CD11b^-CD103^+$ cDCs in murine SI LP corresponded to human SI LP $SIRP\alpha^- CD103^+$ and blood $CD141^+$ cDCs (58). Due to the discrepancy in surface marker expression between species, the two

major subsets of cDCs have now been termed cDC1 ($CD141^+/CD103^+$) and cDC2 ($SIRP\alpha^+/CD11b^+$). While all cDCs share expression of the transcription factor Zbtb46 (59, 60), lineage defining transcription factors promoting cDC1 and cDC2 development have been identified. Initially, the transcription factor Batf3 was found to be critical for cDC1 development (61). However, $Batf3^{-/-}$ mice was shown to develop cDC1s during infection via a Batf3-independent pathway (62) calling into question whether all cDC1s depend on Batf3 for their development. In addition to Batf3, the transcription IRF8 have now been shown to be critical for development of both LN resident and SI LP cDC1s (63, 64). Regarding cDC2s, no single transcription factor have been identified to be absolutely critical for their development although mice deficient in either Notch2 or IRF4 show dramatically decreased numbers in both peripheral tissue and LN resident cDC2s (65, 66).

Preferentially present in spleen, SLOs, liver and bone marrow, pDCs are morphologically spherical and unable to migrate via the lymphatic system (67, 68). In experiments using targeted antigen delivery to pDCs via CD303 and DCIR have shown that they are able to act as APCs and induce $CD4^+$ T cell responses (69, 70). While certainly able to present antigen and induce T cell responses, their specialization lies in their capacity to produce vast amounts of type I interferons in response to PRR signaling, mainly through TLR7 and TLR9 (71).

Langerhans cells reside in the ectodermal tissue and dermis of the skin. They share the migratory trait of cDCs and are able to present and antigens and also transfer antigens to cDCs. Langerhans cells are of myeloid origin but they renew independently of the BM from tissue resident progenitors (72). Genetic tracing experiments also show that Langerhans cells unlike DC precursors, do not express the C-type lectin receptor 1 (DNGR-1) thus questioning if Langerhans cells truly can be classified as a bona-fide DC subset (73).

The last type of DC is the inflammatory monocyte derived DC (moDC) that stems from the macrophage DC progenitor in the BM. Monocytes constitutively seed into tissues as $Ly6C^{high}$ monocytes and differentiate into two populations, CX_3CR1^+ $CD64^+$ macrophages and moDCs. In the SI LP, these macrophages are situated underneath the epithelium and able to extend their dendrites through the small intestinal epithelium into the gut lumen to sample antigens (74) but do not migrate and inefficiently prime T cells (75). During inflammation, the influx of $Ly6C^+$ monocytes dramatically increases (76, 77) and transition of monocytes to moDCs is marked by high expression of CD11c and MHCII, which in combination with intermediate expression of CX_3CR1 and lack of CD64 distinguish them from CX_3CR1^+ $CD64^+$

macrophages (76, 78). In contrast to CX_3CR1^+ $CD64^+$ macrophages, moDCs are migratory (54, 76, 79) and have been shown to produce IL-6 and promote germinal centre responses in the context of CpG immunization (80).

Antigen uptake and presentation on MHCII

The most unique role of APCs is their ability to internalize extracellular antigens that are subsequently degraded into peptides. The peptides are then loaded onto MHCII molecules for recognition by the T cell receptor of $CD4^+$ T cells, or $CD8^+$ T cells by MHCI for cross-presentation (81). cDCs exploit various cellular processes to acquire antigen and deliver them into antigen-processing compartments (see below). The antigen-processing compartments, typically late endosome or lysosomes with an acidic environment, contain proteolytic enzymes and newly produced MHCII molecules loaded with invariant chain (Ii) that must be proteolytically degraded to disassociate from the MHCII molecule (82-84). When Ii is cleaved, a small peptide fragment called class II-associated invariant chain (CLIP) remains bound to the peptide binding groove of the MHCII molecule. By the aid of the chaperone H2-M, CLIP is substituted to the peptide to be presented and subsequently transported to the cell surface for $CD4^+$ T cell recognition (82).

There are four principal mechanisms by which cDCs and other APCs utilize to attain antigen into antigen-processing compartments for MHCII loading. First, an unspecific process called macropinocytosis enables cDCs to sample their surroundings for antigen by internalizing part of their cell membrane into endosomes. This process is carried out in a constitutive manner in immature cDCs and activation, or maturation, by PRR stimuli leads to a temporary burst of macropinocytosis activity that wanes after a few hours (85, 86).

Second, APCs can internalize antigens via receptor-mediated endocytosis via the complement receptors, Fc receptors or lectin receptor like DEC205 that recognize carbohydrate structures on proteins and pathogens. In the case of B cells, the B cell receptor (BCR) function as an antigen specific endocytic receptor. These receptors help facilitate the uptake of antigen into antigen-processing compartments in both clathrin-dependent and independent manners and, at least for the clathrin-dependent endocytosis, the aptitude of receptor-mediated endocytosis in cDCs persists after activation (87). Due to the efficient uptake of antigen into antigen-processing compartments, receptor-mediated endocytosis has been exploited in several experimental systems by conjugation of antigen to antibodies specific for endocytic receptors (88, 89).

Third, phagocytosis of bacteria and apoptotic cells is probably the most important mechanisms of antigen uptake employed by APCs as it leads to both effector T cell responses but also supports the induction of tolerance against self-derived antigens (90, 91). Similar to receptor-mediated endocytosis, phagocytosis is primarily induced by binding to receptors, some of which are shared between the two mechanisms like Fc and complement receptors. Following internalization of particulate antigens the phagosome fuses with lysosomes to create a phagolysosome that contains enzymes that can kill the pathogen and degrade its proteins for loading on MHCII molecules (90). While cDCs efficiently phagocytose bacteria and apoptotic cells to present antigens, macrophages have a lysosome-dominant endocytic compartment better suited for the killing of pathogens (92).

The final mechanism for MHCII presentation is the presentation of cytosolic and nuclear antigens by autophagy. During autophagy, the autophagosome devour macromolecules and organelles and merges with endosome and lysosome containing the MHCII loading machinery to form the autophagolysosome (93, 94). This process is seemingly important for presentation of self-antigens and is therefore of utmost importance during T cell selection in the thymus (95, 96).

T cell interactions

The purpose of antigen presentation by APCs is to initiate T cell responses. cDCs are considered to be the supreme APCs for this task as they are most proficient in capturing, processing and present antigens both on MHCII and cross-presenting extracellular antigens on MHCI (81, 97, 98). The current view of T cell activation conventionally comprises of three signals needed to fully activate a T cell and guide their differentiation. All of these signals can be provided by cDCs. Signal 1 is the interaction between the TCR and its cognate peptide presented on MHC complexes, which triggers TCR signaling via CD3. However, this signal in the absence of signal 2 consequently lead to an unresponsive state or T cell anergy (99).

Signal 2 is delivered by interactions of co-stimulatory molecules present on the cell surface of APCs and their corresponding ligands on the T cells. Immature cDCs express low levels of co-stimulatory molecules and surface expression is readily upregulated upon cDC maturation by PRR signaling induced by PAMPs like dsRNA, LPS and unmethylated CpG DNA-motifs binding to TLR3, TLR4 and TLR9, respectively (100-102). DCs express several co-stimulatory molecules, the best described are CD80 and CD86 that bind to

CD28 on T cells. In combination with CD3 stimuli these co-stimulatory molecules are sufficient to induce T cell proliferation and IL-2 production *in vitro* (103, 104). The function of CD80 and CD86 is not only of stimulatory nature as T cells during the later stages of T cell activation express another ligand, CTLA-4, that transduces inhibitory signals to downregulate T cell responses. Consistent with the findings that co-stimulatory molecules are upregulated upon maturation it has been shown that T cell priming by immature DCs primarily generates regulatory T cells and not effector T cells (105, 106). Moreover, cDCs can also express co-inhibitory molecules like programmed death-ligand 1 (PD-L1) that consequently leads to reduced T cell proliferation and production of the suppressive cytokine IL-10 (107, 108).

The 3rd and final signal is mediated by soluble factors called cytokines. Just like the cell-surface bound co-stimulatory molecules, the production of cytokines is induced in response to PRR signalling (100-102). In combination with the signals provided by co-stimulatory molecules, cytokines help to shape the outcome of T cell responses by modulating expression of transcription factors. In the next chapter I will in-depth describe the role of cytokines and co-stimulatory molecules in regulating T cell responses.

T cells

T lymphocytes, or T cells, were given their name due their thymus dependent development. T cells represent one of the two cell types that constitute the adaptive immune system. In contrast to the cells of the innate immune system which recognize conserved moieties and structures by PPRs, the reactivity of T cells is determined by their TCR that recognize variable protein derived peptides. The majority of T cells are conventional T cells of either $CD4^+$ or $CD8^+$ lineage, each carrying a unique TCR composed by pairing of a TCRα- and TCRβ-chain. TCRs of conventional $CD8^+$ and $CD4^+$ T cells recognize peptides from degraded proteins presented on MHC class I and II molecule, respectively.

While $CD4^+$ T cells are restricted to interact with a limited number of cells capable of presenting peptides originating from the endosome compartment on MHCII, as discussed in the previous chapter, $CD8^+$ T cells recognizes peptides from the cytosolic compartment presented on MHCI on all nucleated cells. $CD8^+$ T cells are commonly referred to as cytotoxic T lymphocytes (CTLs) due to their cytolytic ("killing") activity in response to antigenic stimuli after initial, antigen dependent, activation by cross-presenting DCs in SLOs. Mechanistically, CTLs induce apoptosis in target cells displaying their cognate peptide-MHCI complex, by secretion of perforin and granzyme B which form a pore in the target cells or via FAS-FASL interactions (109). Their ability to selectively induce apoptosis in cells in a controlled manner makes CTLs instrumental in combating virus infections as well as eradicating tumor cells displaying altered-self peptides.

Another set of lymphocytes that do not recognize peptide antigens, consisting of mucosa-associated invariant T (MAIT) cells, natural-killer T cells and γδ T cells which do not use the TCRα and TCRβ-chains, also develop in the thymus. Unlike conventional T cells these lymphocytes do not respond to peptides presented on MHC molecules but rather recognize bacterial metabolites, glycolipids and lipids presented on MR-1 and CD1d molecules. Based on the scope of this thesis, the following section will focus its discussion on $CD4^+$ T cells, their development, differentiation process and function.

Development and thymic selection

Deriving from the common lymphoid progenitor in the BM, T cell precursors exit the BM and through the blood stream enter the thymus, an organ

specialized for the purpose of T cell development. Once in the thymus, T cell precursors start to proliferate to generate a pool of thymocytes and the process of T cell maturation is initiated. The T cell "rite of passage" begins with rearrangement of the TCRβ locus to successfully generate a unique TCR capable of recognizing antigens. In short, the TCRβ locus consists of 52 different V_β gene segments, 2 D_β gene segments and 13 J_β gene segments. By the aid of two endonuclease enzymes called RAG-1 and RAG-2 two random D_β and J_β gene segments are juxtaposed thus forming a hairpin loop of DNA which is separated from the chromosomal DNA by RAG1/2 (110). In order to complete the ligation of the two D_β and J_β gene segments another set of enzymes is involved. One of the enzymes involved, TdT, randomly removes or inserts nucleotides in the ends of the cleaved D_β and J_β gene segments and once matching ends are generated the two segments are joined together (111). To complete the TCRβ chain rearrangement the newly generated DJ_β segment is paired to one of the 52 different V_β segments by the same mechanisms described above.

When thymocytes successfully have recombined a TCRβ chain capable of pairing with a surrogate TCRα chain to form a pre-TCR they rapidly proliferate and acquire expression of both CD4 and CD8 molecules. The $CD4^+$ $CD8^+$ thymocytes are commonly referred to as "Double-positive thymocytes" and it is during this stage of development that the rearrangement of the TCRα chain locus is initiated. Distinct from the TCRβ locus, the TCRα locus is comprised of approximately 70-80 V_α and 61 J_α gene segments and lack D segments altogether. Mechanistically, V_α and J_α genes are joined together as described above but the lack of D segment and surplus of V_α and J_α segments allows for continuous rearrangement of the TCRα locus, until a TCR capable of weakly recognizing self-peptide:self-MHC is generated.

The recognition of self-peptide:self-MHC complexes is the most critical aspect of the T cell development as this checkpoint serves as a double-edged sword in what is known as T cell selection. First, the TCR must be able to bind self-peptide:self-MHC complexes on cortical thymic epithelial cells (cTECs). This ensures that the T cell is reactive to peptides presented on self-MHC molecules. Failure to do so results in apoptosis of the T cell and this process is known as *positive selection* (112). T cells that successfully pass the positive selection migrate from the cortex into the medulla. Secondly, the TCRs are screened for self-reactivity in the medulla where they encounter medullary thymic epithelial cells (mTECs) and thymic DCs that present a vast array of self-derived peptides. If the TCR binds to any of the numerous self-peptide:self-MHC complexes presented with too high affinity they will undergo apoptosis or gain immunosuppressive properties. This mechanism is known as *negative*

selection and serves as an effective way of purging potentially self-reactive T cells that otherwise could cause autoimmunity (112). At this stage T cells lose expression of either CD4 or CD8 depending on if they recognize MHCII or MHCI molecules, respectively. The T cells have thereby completed the maturation process and enter the blood stream as naïve T cells.

All in all, the enormous number of possible combinations between gene segments and junctional diversity caused by TdT have the potential to give rise to a seemingly infinite number of T cells, each carrying a TCR with unique specificity. However, the safe-keeping mechanisms in play during the multi-stage process of thymic selection renders only a small fraction of the thymocytes to mature into functional naïve T cells.

CD4 T helper cell activation and differentiation

Once $CD4^+$ T cells have fulfilled their thymic selection they relocate from the thymus to SLOs via the blood stream. After entry into SLOs, T cells position themselves in the T cell zone in response to a CCL19/21 gradient by the chemokine receptor CCR7. At this stage, $CD4^+$ T cells are considered to be naïve, meaning that they are antigen inexperienced i.e. have not received the signals needed to become activated. These signals can only fully be delivered by APCs. The activation of $CD4^+$ T cells is often considered to require, and be influenced by, three distinct signals. The first signal, or signal 1, is recognition of the cognate antigen presented on MHCII molecules on APCs by the TCR. Signal 2 is comprised by the interactions of co-stimulatory, or co-inhibitory, molecules present on APCs and their ligands on T cells. Signal 1 and 2 are minimum requirements for activation of T cells and proliferation, while signal 1 in the absence of signal 2 leads to anergy. Finally, signal 3 is a polarizing signal mediated by soluble cytokines which promotes differentiation into distinct subsets, each uniquely specialized in propagating immune responses tailor-fitted to fend off the specific pathogen. Each of these specialized subsets produce a defined set of effector molecules and their differentiation is governed by a master transcription factor (Figure 3).

Effector cells

Initially, $CD4^+$ T cell responses were considered to solely be composed of type 1 T helper (Th1) and type 2 T helper (Th2) cell subsets based on their distinct production of cytokines as described by Mosmann and Coffman (113). Later, additional T cell subsets have been discovered, of which the type 17 helper (Th17) cell is the best described. This makes it evident that $CD4^+$ T cell

differentiation is more complex than a binary choice between Th1 and Th2 subsets.

The differentiation and function of Th1 cells is governed by the transcription factor T-bet, encoded by the gene *Tbx21*, which is induced in response to interferon γ (IFNγ) signalling via signal transducer and activator of transcription 1 (STAT1) (114, 115). In turn, T-bet drives the expression of the IL-12β receptor (115) which allows IL-12 signalling through STAT4 to further support T-bet expression thus establishing a step-wise positive feedback loop underlining the importance of IL-12 and STAT4 signalling in Th1 differentiation (116-119). Additionally, IL-18 and IL-27 have also been reported to be beneficial for Th1 differentiation as Th1 responses are reduced in mice deficient for either cytokine (120-123). Once established, T-bet drives the expression of the Th1 associated effector molecules IFNy and tumor necrosis factor α (TNFα) which are potent inducers of macrophage activation and the cellular defence against viruses and intracellular bacteria.

Th2 cells, on the other hand, are dependent on the transcription factor GATA3 for their differentiation (124, 125) hence GATA3 deficient mice entirely fail to generate Th2 cells (126, 127). Early *in vitro* findings showed that the early IL-4 production is dependent on IL-2 signalling via STAT5 (128, 129) which together with GATA3 have been reported to bind the DNase I hypersensitivity site II in the *Il4* promoter (129, 130), a site which strongly regulates IL-4 expression in Th2 cells. STAT5 signalling also induces expression of the IL4R α chain (131), making T cells responsive to IL-4 signalling through STAT6. This manifests GATA3 expression and the Th2 differentiation program (132-134). Although the IL-4 and STAT6 signaling axis are required for Th2 induction and worm expulsion during *Trichuris muris* infection (135), it has been shown both IL-4 and STAT6 deficient animals can support the GATA3 dependent development of Th2 cells (136-138). This suggests that an IL-4 independent pathway towards Th2 generation also exist.

The effector functions of Th2 cells mediate immunity towards extracellular parasites, like nematodes and helminths, but also promote allergic and asthmatic responses. Functionally, these responses are facilitated by GATA3 reinforced production of the Th2 signature cytokines IL-4, IL-5 and IL-13, which recruit and activate eosinophils to degranulate effector molecules capable of killing parasites (139). Besides acting on eosinophils to expel or kill parasites, the cytokines produced by Th2 cells can also support antibody isotype class-switching into IgG1 and IgE. Cross-linking of IgE bound to high-affinity FcεRI receptors on mast cells induced by antigen binding causes

degranulation releasing histamine and pre-stored TNFα. This will promote inflammation but also augment rapid allergic and asthmatic responses.

The Th17 subset was the third subsets of effector T cells to be described, thereby upending the Th1 vs Th2 paradigm. One of the canonical Th17 cytokines, IL-17A of the IL-17 cytokine family, was early known to enhance immunity towards extracellular pathogens as well as to be associated with autoimmunity but the regulation of IL-17 expression remained unknown. The findings that cells expressing IL-17 could not co-express either IFNγ or IL-4 when primed in the presence of microbial lipopeptides (140) sparked the idea that a third subset of effector T cells might exist. Follow-up studies in autoimmune mouse models showed that ICOS and IL-23 were critical for IL-17 but not IFNγ expression (141-143). In 2005 two groups also showed that IL-17 producing cells develop independently from Th1 and Th2 cells (144, 145). While these studies used IL-23 to polarize IL-17 producing T cells it was shown IL-23 was not necessary *in vivo* for early Th17 differentiation as the proliferative effects of IL-23 was intact in Th17 cells derived from IL-23 deficient mice (146). Later, several studies identified IL-6 and transforming growth factor β (TGFβ) as the cytokines involved in early Th17 differentiation in the absence of IL-23 (147-149).

The master transcriptional regulator of Th17 was identified in 2006 when Ivanov and colleagues observed that the transcription factor RORγt was induced in response to TGFβ and IL-6, promoted Th17 responses when overexpressed and conversely showed defective Th17 responses in RORγt deficient cells (150). While TGFβ signalling inhibits SOCS3, a suppressor of STAT3, to promote Th17 responses (151), IL-6 stimulation leads to phosphorylation and activation of STAT3, which is crucial for Th17 development by regulating expression of RORγt (152, 153). In addition, the activation of STAT3 leads to production of IL-21. This enforces the Th17 phenotype by initiating a self-regulated positive feedback loop via IL21R and STAT3 to sustain Th17 phenotype and function (154-156). The pro-inflammatory cytokines secreted by Th17 cell (IL-17A, IL-17F, IL-22, IL-6 and TNFα) typically leads to release of chemokine involved in recruiting neutrophils, monocytes and production of anti-microbial peptides (157, 158). Finally, it was demonstrated in mice that the presence or absence of segmented filamentous bacteria in the intestinal commensal flora was directly linked to the increased Th17 responses and resistance to *Citrobacter rodentium* infection in the host (159). Conversely, the same model was also used to illustrate the ability of Th17 cells to promote arthritic autoimmunity (160), thus illustrating the whole spectra of Th17 mediated immunity.

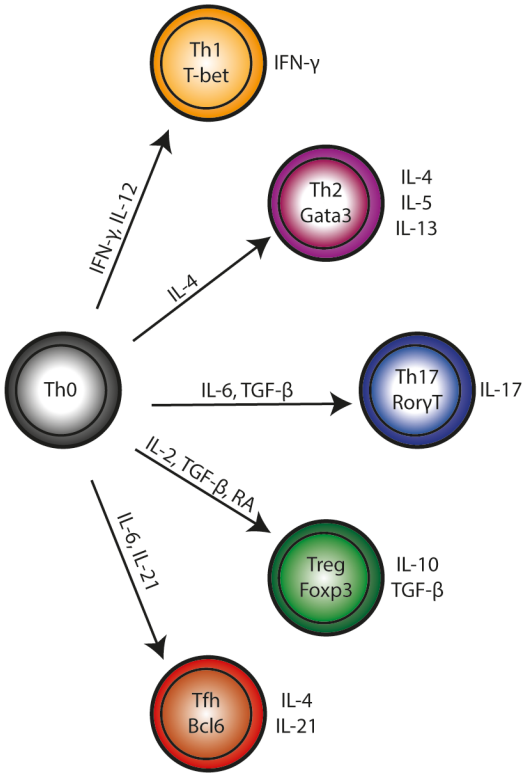

Figure 3. Polarizing signals promoting T cell differentiation into specific subsets with their signature transcription factors and cytokines.

Follicular T helper cells

It had been known for a long time that high quality B cell responses required T cell help. In 1985 Lanzavecchia directly showed that T cells can only solicit B cell help and support proliferation when they interact in an antigen specific and MHCII dependent manner (161). Initial findings identified the chemokine receptor CXCR5, originally known as Burkitt´s lymphoma receptor 1, to be expressed on memory T cells in blood (162). Soon after, when its ligand CXCL13 was shown be produced in B cell follicles (163) and that T cells upregulated CXCR5 in response to activation and responded to chemoattractant cues mediated by CXCL13 (164), the expression of CXCR5 on tonsillar T cells was identified as a bona-fide marker for follicular homing T (Tfh) cells with the ability to provide B cell help (165, 166). Gene expression analysis showed that Tfh cells, identified by CXCR5 and CD57 expression,

were transcriptionally different from Th1 and Th2 cells (167, 168). Later findings showed that Tfh develop independently from other T effector cell subsets (169) which strongly implied that Tfh constituted a novel T cell subset. A year later, a series of seminal papers identified the transcriptional suppressor Bcl6 as the master regulator of the Tfh subset (170-172). These studies solidified that Tfh cells constitute a separate subset by showing that loss of Bcl6 prevented Tfh cell generation while overexpression of Bcl6 forced Tfh subset commitment, and consequentially blocked T effector differentiation.

As Tfh cells continued to be studied in great detail, it became increasingly evident that the development of Tfh is more multifaceted and refined compared to that of effector T cells. In contrast to T effector cell differentiation, which can be described as an event guided by one APC providing a polarizing set of signals during initial T cell activation, the differentiation of Tfh cells is best considered a multi-stage process in which not only the correct ligands and cytokines are needed but also where the timing is of utmost importance. Polarizing signals provided at the wrong time may have detrimental effect on Tfh differentiation. The fact that all T effector cell linages can be generated *in vitro* by following established protocols while to date, no such protocol exists for the successful generation of functional Tfh cells also provides a testament to the complexity of Tfh differentiation. To fully appreciate the intricacy of Tfh cell differentiation, a more in-depth discussion is warranted.

The first stage of the multi-step process is the interaction with an APC that presents the cognate antigen and co-stimulatory ligands and cytokines. This initial interaction leads to an early commitment to Tfh cell or T effector cell differentiation marked by acquisition of Bcl6 or its antagonist Blimp-1 (170-172), respectively. Exactly what signal(s) that determines the choice between Bcl6 and Blimp1 is not fully understood but early findings showed that the affinity of the TCR influenced T cell differentiation as higher TCR affinity correlated with preferential Tfh differentiation (173). However, later findings showed Tfh cells, in contrast to T effector cells, require continuous TCR stimuli and that antigen dose and sustained availability also influence T cell differentiation (174-176). At the moment, TCR:pMHCII dwell-time, the half-life of productive TCR-pMHCII interaction, is generally considered the best TCR-based predictor of cell-fate preference (177).

The first co-stimulatory signal received by the T cells is mediated by CD28 interaction with CD80 or CD86 on the APC which provides necessary co-stimulation and leads to IL-2 production (178). Although IL-2 is important in the early stages of T cell proliferation, IL-2 stimulation and subsequent STAT5 signalling quickly impairs Tfh cell differentiation (179-181). To counteract

these negative signals, T cells committed to Tfh differentiation actively shield themselves from IL-2 signalling by several mechanisms. First, expressing lower levels of CD25 (IL2Rα), the high affinity receptor for IL-2, compared to T effector cells is a hallmark of early Tfh differentiation as demonstrated by Crotty and colleagues (182, 183). Secondly, T cells that commit to Tfh differentiation reposition themselves to the T-B border and interfollicular areas. This occurs in response to chemotactic cues by EBI2 and CXCR5 where Tfh cells interact with DCs expressing high levels of CD25 that do not lead to downstream signalling, thus effectively sequestering IL-2 (184). Finally, Tfh cells are commonly characterized by their high expression of CXCR5 and programmed cell death protein 1 (PD-1) (185). PD-1 signalling efficiently supresses CD28 mediated IL-2 expression (186, 187), thus preventing autocrine IL-2 signalling.

OX40 was one of the first co-stimulatory molecules to be assigned a role in Tfh differentiation. OX40 stimulation was shown to promote IL-4 and CXCR5 expression while supressing IFNγ production induced by IL-12 *in vitro* (188). $CD4^+$ T cell proliferation is also reduced in the absence of OX40 stimuli (189). Indeed, forced expression of OX40L on DCs boosts follicular homing (190) and *in vivo* administration of agonistic OX40L-hIgG1 in $CD40^{-/-}$ mice rescued follicular homing of $CD4^+$ T cells (191). However, early OX40 stimuli during LCMV infection divert T cells from Tfh differentiation to T effector differentiation by upregulating Blimp-1 (192). In addition, humans deficient in OX40 have normal antibody titres (193). These later findings would suggest that OX40 rather plays a context dependent, and perhaps redundant, role in dictating Tfh generation or function.

Perhaps one of the most important Tfh promoting co-stimulatory signal by the APC at this point is ICOSL that binds ICOS on the T cell. ICOS and CD28 belong to the same receptor family but unlike CD28, ICOS signalling does not lead to IL-2 production due to difference in a single amino-acid in the cytoplasmic tail (194, 195). CD28 is also expressed on resting naïve T cells while ICOS is upregulated following TCR signalling under the governance of the transcription factor Foxp1 (196). ICOS signalling promotes Tfh differentiation in a multitude of different manners. For instance, it potently induces expression of the master regulator Bcl6 (182) as well as protecting Bcl6 from ubiquitin-dependent protosomal degradation via PI3K mediated osteopontin translocation (197). Furthermore, ICOS signalling directly blocks the activity of two transcription factors, Klf2 and FOXO1, both acting to supress Tfh cell differentiation (198-200). Moreover, ICOS signalling is important for induction of the canonical Tfh cytokines IL-4 (201-203) and IL-21 (204). While the expression of IL-21 is driven by c-Maf in Tfh cells as well

as Th17 cells (204, 205) it is still not clear how the *Il-4* locus is operated in Tfh cells. Primarily, the transcription factor BATF have been assigned a role in governing IL-4 expression in Tfh cells (206, 207) and more recently, Notch dependent activation of the transcription factor RBP-J was identified as an important regulator of IL-4 expression Tfh cells but not Th2 cells (208).

Recently, the Wnt-signaling induced transcription factor Achaete-scute homologue 2 (Ascl2) was found to potently induce CXCR5 expression when expression was forced *in vitro,* independently of Bcl6 expression (209). Ascl2 overexpression *in vivo* caused an increased proportion of CXCR5 expressing $CD4^+$ T cells at day 2 p.i. but did not lead to increased $CXCR5^+$ $Bcl6^+$ Tfh cell numbers at day 6 p.i. when GCs are established, suggesting that Ascl2 is involved in early Tfh differentiation (209). In addition to Ascl2, Wnt signaling also activates the transcription factors TCF-1 and LEF-1 which have been reported to early Tfh differentiation by regulating expression of Bcl6 and Blimp-1 (210-212) as well as a number of other genes associated with Tfh effector function (211). While conditional deletion of *Tcf7* (encoding TCF-1) and *Lef1* negatively impacts Tfh differentiation, Tfh differentiation in *Tcf7* deficient cells can at least be rescued by Bcl6 overexpression (210).

Several soluble mediators, or cytokines, have been implicated in Tfh differentiation (213). IL-6 is perhaps the best described as it was shown to induce expression of the Tfh associated cytokine IL-21 (169, 214), which then can self-sustain IL-21 expression through IL21R signalling. The necessity of IL-6 or IL-21 for the successful generation of Tfh cells is questionable as several studies have shown that neither cytokine is an absolute requirement for Tfh differentiation and germinal center (GC) formation (214-217). Necessary or not, the effects of IL-6 and IL-21 deficiency are more profound when both cytokines are lacking, suggesting either overlapping or synergistic roles (214, 216).

The last important factor that have been implicated in the first stage of Tfh differentiation is the question of which cells that provide the stimulatory signals discussed above and where this interaction takes place. Under normal circumstances, cDCs have been attributed the role of the initiating APC as antigen presentation restricted to cDCs is sufficient to promote early Tfh differentiation (218). More recent studies have identified LN resident cDC2 (CD4-expressing) (184) as well as migrating cDC2 (219) to be important for Tfh differentiation. Both sentinel and migratory cDC2 localize near the T-B border and the interfollicular area of the LN (184, 219), a niche where long-lasting T-B interactions occur and the Tfh fate appears to be sealed (220). The cDC2s also express higher levels of ICOSL and CD25 than cDC1 where the

latter receptor permits quenching of IL-2 signalling in T cells, (184, 219). At least for the migratory cDC2, the position to this microenvironment is likely guided by higher expression levels of CXCR5, EBI2 in combination with reduced levels of CCR7 (219). Upon activation, T cells poised to become Tfh cells downregulate PSGL1 and CCR7 (185, 217), which anchors T cells in the T cell zone, and upregulate CXCR5 and EBI2 which consequently leads to homing to the T-B border and interfollicular area (164, 184, 185, 221). Although DCs are able to position themselves in the correct microenvironment and provide all the required signals for Tfh differentiation, they are not critical for Tfh differentiation and do not provide any unique signals necessary for Tfh differentiation. This is supported by the findings that Tfh cell develop in mice in which MHCII presentation is restricted to B cells during LCMV infection, albeit at reduced numbers, (222) as well is in mice where cDCs are transiently depleted as long as antigen is not limiting (223).

At this point during Tfh differentiation, Bcl6 is established as the master transcriptional regulator and T cells have positioned themselves in the T-B border and interfollicular area where a cognate interaction with B cells initiates the second phase of Tfh differentiation (220). The relationship between T and B cells is of symbiotic nature as disruption of T-B interactions or depletion of one cell type leads to a reciprocal loss of the other during their concomitant differentiation towards GC cells (170, 185, 224). Even though CD40 expression on non-B cell APCs is sufficient for T cells to migrate to the T-B border (191), CD40-CD40L interactions between T and B cells become crucial at this stage during development as $CD40^{-/-}$ B cells cannot support Tfh differentiation (225). For T cells to migrate further into the B cell follicle ICOS-ICOSL interaction between T and B cells, not necessarily in a cognate manner, have been shown to be of importance (226). This interaction potentially increases the contact duration and promote CD40 expression on B cells thereby creating a positive feedback loop (227).

The signalling lymphocytic activation molecule family (SLAMF), consisting of seven family members, four of which are expressed on $CD4^{+}$ T cells, bind in a homotypic manner to SLAMFs expressed on B cells. This family of molecules have been reported to be important at the stage of T-B interactions as in mice lacking CD84 (SLAMF5) the longevity of T-B interactions was compromised between T and B cells (228). This is also the case in mice lacking SLAM-associated protein (SAP). SAP functions as an adaptor protein to enhance SLAMF signalling, and T cells deficient in SAP show unaltered interaction with DC but fail to form stable conjugates with B cells (229). T cells deficient in SLAM (SLAMF1) also develop into Tfh cells but show a marked reduction in IL-4 production (230). Deletion of Ly108 (SLAMF6) in

$SAP^{-/-}$ mice restores the GC responses in these mice due to inhibitory signals mediated by tyrosine motifs in the cytosolic domain of Ly108, that SAP efficiently quench under normal conditions (231). Interestingly, while deletion of SAP negates Tfh differentiation at the stage of T-B interaction it was recently shown that GC responses were intact in mice with a complete deletion of the SLAM family (232). This suggests that the different members of the SLAM family may both positively and negatively regulate Tfh differentiation while not deliver any critical signals by themselves.

The final phase of Tfh cell development is completed in the GC where Tfh cells, often termed GC Tfh cells at this stage, exert their effector functions. This is done by secreting cytokines, providing cognate co-stimulatory ligands to GC B cells that successfully acquired antigens, thereby selecting for B cells with the highest affinity. While several T cell derived cytokines can influence class-switching in B cells in a tailor-fitted manner to yield appropriate effector functions to the elicited immune response, IL-4 and IL-21 are the two cytokines associated with all GC responses. At first, IL-21 was thought to be critical to support maintained GC Tfh function through autocrine signaling (169). However, later studies contradicts this as both IL-21 and IL-21R deficient animals are capable of generating functional GC Tfh cells and defects are largely confined to the B cell compartment (233, 234). As stated earlier, IL-4 is the canonical cytokine secreted by the Th2 subset and was originally identified as a B cell stimulating cytokine (235). More recent studies using a murine model system in which active secretion of IL-4 can be detected by surface expression of human CD2 (236) revealed that IL-4 secreted in LNs is restricted to Tfh cells (203, 224, 237) and IL-4 / IL-13 co-secreting cells are largely confined to peripheral effector tissues (238). A study using fluorescent intravital imaging showed that prolonged cognate T-B interactions in the GCs sparked intracellular Ca^{2+} release which corresponded with IL-4 and IL-21 production (239). This highlights the importance of continuous cognate interactions between T-B cells to maintain GC Tfh effector functions. Weinstein et al used a dual reporter system for IL-4 and IL-21 production and showed that there is a differential spatial distribution of IL-4 and IL-21 producing cells within the GC (240). IL-21 producing cells locate in closer proximity to the dark zone than their IL-4 producing counterparts, which in turn express higher levels of CD40L. The finding that IL-4 producing GC Tfh cells express higher levels of CD40L is an interesting observation, considering that IL-4 and CD40 signalling in B cells synergistically induce an increased expression of activation-induced deaminase in B cells (241). In addition to IL-4 and IL-21 production, novel studies have revealed GC Tfh cells to be a source of both dopamine and IL-9 (242, 243). Functionally, dopamine signalling in B

cells leads to transportation of ICOSL to the B cell surface (242) and IL-9 was shown to promote generation of B cell memory (243).

Regulatory T cells

The first observation of a $CD4^+$ T cell subset with immunosuppressive functions was made by Sakaguchi et al who identified $CD4^+CD25^+$ T cells as a subset capable of supporting self-tolerance (244). The findings that deficiency of the transcription factor FoxP3 caused fatal immunopathology and loss of $CD4^+CD25^+$ T cells established regulatory T cells (Tregs) as a separate T cell subset (245). Tregs supress target cells by three distinct modes of actions; first, they can mediate suppression by cell-cell interaction by providing the suppressive ligands CTLA-4 (246) and LAG-3 (247). Second, secretion of cytokines, most commonly, IL-10 is strongly associated with immunosuppression (248) and finally, expression of the ectoenzymes CD39 and CD73 can convert pro-inflammatory ATP into adenosine to yield an immunosuppressive niche (249). An early study identified Tregs capable of regulating GC response in human tonsils (250). Based upon this study, a subset of Tregs termed T follicular regulatory cells (Tfr) residing in the GCs and exhibiting the Tfh-associated $CXCR5^+PD\text{-}1^+Bcl\text{-}6^+$ phenotype in addition to FoxP3 expression was identified by three independent groups in 2011 (251-253). Initially, Tfr cells were thought to be reactive against self-antigens as a way to prevent auto-immunity until a recent study demonstrated that Tfr cells with the same reactivity can be generated against an antigen recognized either as self or foreign (254). This suggests that Tfr cells may also regulate the magnitude of non-self GC responses. CTLA-4 mediated suppression has been reported to regulate GC Tfh function (255, 256) but the exact mode of action of Tfr mediated suppression of the GC responses remains to be fully elucidated.

Homing and recruitment

Early work by Eugene Butchers´ lab demonstrated that $CD4^+$ T cells prone to follicular or tissue homing developed in parallel (257) and that the site of priming consequently lead to expression of tissue-specific homing receptor associated with skin or gut trafficking shortly after activation in skin or gut draining LNs, respectively (258) (Figure 4).

Follicular homing

While Tfh cells rely on downregulation of CCR7 and concomitant upregulation of CXCR5 and EBI2 to reach the T-B border and interfollicular area as discussed earlier, GC Tfh cells rely on slightly different mechanisms to

migrate into the follicle and position themselves in the GC. First, in contrast to developing Tfh cells, GC Tfh cells have downregulated EBI2 (259) just like B cells that have received T cell help (260, 261), thus localizing in the GC in a oxysterol independent manner. The GC can be subdivided into two anatomically distinct areas, namely the dark zone (DZ) where B cells proliferate and undergo somatic hypermutation (SHM) and light zone (LZ), in which B cells receive T cell help from Tfh cells. This compartmentalization is governed by the chemoattractants CXCL13 produced by follicular dendritic cells (FDCs) in the LZ (262) and CXCL12, also known as stromal cell-derived factor 1 (SDF-1), produced by CXCL12-expressing reticular cells (CRCs) in the DZ (263, 264). Tfh cells are largely confined to the light zone due to their high expression of CXCR5 attracting them to the CXCL13 rich area of the dark zone. Interestingly, besides being a prime source of CXCL13, FDCs were shown to induce CXCR4 expression on $CD4^+$ T cells when co-cultured *in vitro* and GC Tfh cells showed higher levels of CXCR4 compared to non-GC T cells *in vivo* (265). However, despite their expression of CXCR4, GC Tfh cells are essentially nonresponsive to CXCL12 due to FDC mediated upregulation of RGS13 and RGS16 (266), two proteins capable of regulating G protein receptor signalling. This highlights the crucial role of FDCs in orchestrating T cell positioning within the GC. While the GC is an immobile anatomical structure, GC Tfh cells readily shuttle between existing GCs within a LN (267). This could possibly be a mechanism to increase diversity in potential T cell help although exactly what mechanisms that operate this dynamic inter-GC migration are still not known.

Peripheral homing

The first gut homing-associated marker observed on lymphocytes was the α4β7 integrin, capable of binding MAdCAM-1 present on post-capillary endothelial venules in the small intestine (22, 23). MLNs draining the gut potently imprint α4β7 expression in comparison to skin draining LNs (258) and during the early stages of $CD4^+$ T cell priming in MLNs expression of α4β7 and CXCR5 is mutually exclusive (268). Co-culture experiments with T cells and cDCs showed that cDCs from MLNs and also PPs efficiently induce T cell expression of α4β7 as well as CCR9 (269-271), a chemokine receptor associated with small intestinal homing (272). A study by Iwata et al demonstrated that the vitamin A metabolite retinoic acid (RA) strongly induced both α4β7 and CCR9 expression on T cells activated *in vitro* under non-polarizing conditions (273). Furthermore, the same study showed that cDCs in gut-draining lymphoid organs were capable of metabolizing vitamin A into RA. Consequently vitamin A deficient diet lead to depletion of lamina propria T cells (273). While $CD103^+$ and not $CD103^-$ cDCs in MLN are the only cDC subset capable of generating $\alpha4\beta7^+$ $CCR9^+$ gut-homing $CD8^+$ T cells

(37), this distinction does not fully apply to $CD4^+$ as $CD103^-$ cDCs from MLN can induce α4β7 but not CCR9 expression (274). The expression of CCR9 allows $CD4^+$ T cells to interact with CCL25 produced by epithelial cells in the SI LP (38) but also presented on the endothelial cells in post-capillary venules to support transmigration over the endothelium (39, 40). Lastly, the relative importance of CCR9 contribution to SI LP homing appears more profound among $CD8^+$ T cells as in competitive transfer experiments CCR9-deficient cells were prominently outcompeted by their wild-type counterparts following immunization (41). In contrast CCR9-deficient $CD4^+$ T cells only show a modest reduction in SI LP homing compared to wild type $CD4^+$ T cells (42).

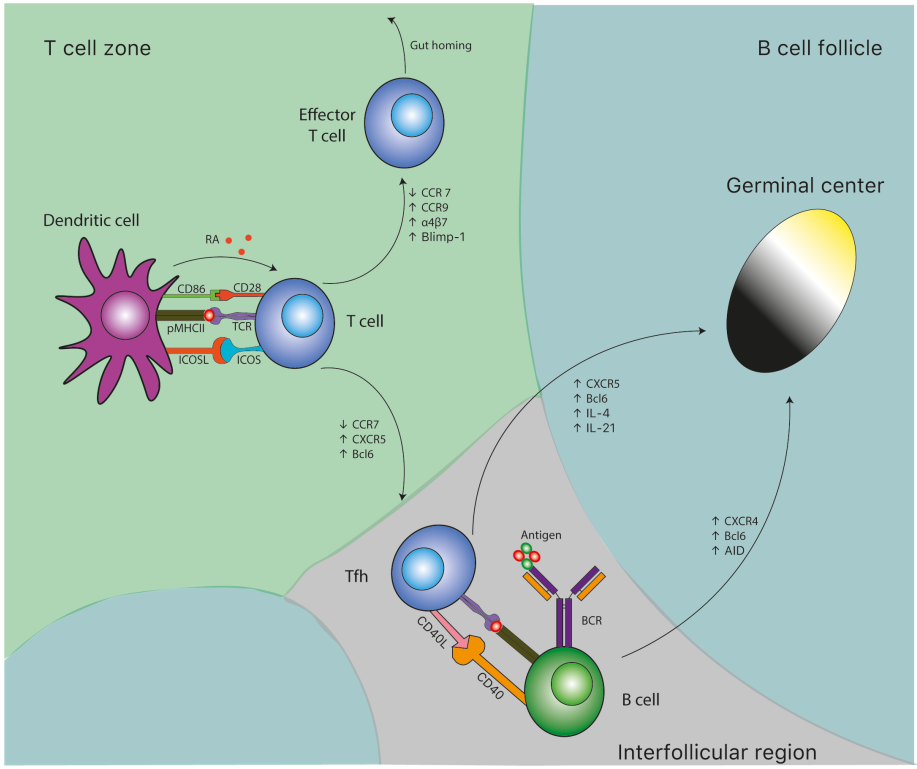

Figure 4. General overview of T cell movement after activation in MLNs

Memory

The findings that the subset defining transcription factors actively repress other subset associated transcription factors, thereby competing for dominance of the transcriptional landscape, introduced the idea that T cells commit to perform one function at a time. However, strong environmental cues can reprogram

effector T cells into other T helper subsets (Reviewed in (275, 276)). This plasticity of T cells to re-differentiate into other T helper subsets is perhaps best conserved in Tfh cells as they resourcefully re-differentiate into effector T cells after transfer into naïve hosts and subsequent challenge in both Th1 and Th2 skewing systems (224, 277-279).

The ability of antigen experienced T cells to survive over extended periods of time, proliferate, re-differentiate and quickly respond upon antigen re-challenge is one of the most important features of the adaptive immune system, namely immunological memory. Traditionally, T cell memory has been divided into two compartments, T central memory cells (Tcm) and T effector memory cells (Tem) based on their differential localization. Tem, defined as $CD44^{+}CD62L^{-}CCR7^{-}$ cells, reside in peripheral tissue, poised to swiftly react to re-infection whereas Tcm, which maintain CCR7 and CD62L expression, are harboured in secondary lymphoid organs and preferentially proliferate to generate more effector cells (280). The generation of Tem cells was shown to be independent of Bcl6 while the generation of Tcm cells requires Bcl6 expression (281), suggesting that Tcm may arise from Tfh cells.

Indeed, studies have identified that memory GC Tfh cells have the capacity to efficiently become GC Tfh cells upon antigen re-challenge while non-CXCR5 expressing T cells poorly reconstitute the GC Tfh pool (277, 278, 282). When Tfh cells enter the resting memory phase the expression of both Bcl6 and ICOS are reduced while the levels of CCR7 and CD62L are upregulated (283). Thus, memory Tfh cells will adopt a phenotype more akin to the classical Tcm phenotype. Over the course of the immune response memory T cells disseminate into non-draining LNs and aptly respond to antigenic stimuli (284). Interestingly, it was recently shown that the memory pool in non-reactive LNs preferentially consists of T cells with lower TCR affinity (285). Moreover, memory cells in the reactive LN were spatially localized to the outer follicle or in close proximity to the subcapsular sinus whereas circulating Tfh cells resided in the T cell zone (285, 286). In summary, T cell memory is maintained by several distinct memory cells that serve different purposes, where Tfh cells are important for re-initiation of the GC response in both previously exposed LNs as well as non-draining LNs.

B cells

The second arm of the adaptive immune system is composed of B cells that can develop into plasma cells capable of producing immunoglobulins (Ig), i.e. antibodies, to confer humoral immunity. Antibodies are heterodimeric glycoproteins consisting of two light chains and two heavy chains paired to each other capable of binding antigens with high specificity. Antibodies mediate a wide variety of effector functions ranging from toxin neutralization to complement activation or opsonization. In this chapter, I will briefly cover the B cells development, how high-affinity antibodies are generated in T-dependent response in the GC and the outcomes of the GC reaction.

Development and maturation

B cells develop in the BM from progenitor (pro)-B cells committed to the B cell lineage. In a manner similar to T cells Pro-B cells undergo a series of error prone random gene rearrangement of the Ig heavy chain locus to generate a uniquely recombined set of V-D-J segments guided by RAG1/2 and TdT enzymes (287, 288). The successful recombination of a V segment to DJ segments yields a functional heavy chain paired with a surrogate light chain, called pre-B-cell receptor, which induces allelic exclusion of the IgH locus to prevent further rearrangements of the heavy chain (289). This subsequently allows for V-J recombination of the light chain locus to be initiated. Once light chain recombination has resulted in a light chain capable of pairing to the IgM isotype heavy chain the developing B cell starts expressing the dimeric immunoglobulin on the cell surface which serves as the BCR. Before leaving the BM, the possible autoreactivity of the BCR is controlled. Self-antigens are presented in the BM and immature B cells carrying a self-reactive BCR are eliminated or undergo receptor editing to replace the light chain (290). Immature B cells then egress from the BM to the spleen where they receive survival signals and complete their early development by differentiating into naïve follicular or marginal zone B cells (291, 292).

T dependent B cell responses

Follicular B cells acquire antigens by the antigen binding regions of the BCR that, in contrast to the TCR, which recognizes MHC-peptide complexes, directly interact with the native form of the antigen. Soluble antigen arriving to the LN can either directly diffuse into the follicle through pores or be

retrieved by subcapsular sinus-lining macrophages which transfers the antigen to FDCs (293-295). B cells acquire the antigen from either of these cells by binding of antigen to the BCR. This leads to B cell activation by inducing intracellular activation signals and internalization of the antigen-bound BCR for degradation in endocytic vesicles. If the antigen contains a protein component this leads to antigen presentation of antigen-derived peptides on MHCII molecules (296). Following activation, B cells migrate to the T-B border in a CCR7-dependent manner (297) aided by EBI2 towards the interfollicular region (260, 298). This interfollicular region-aimed movement occurs in parallel with that of CXCR5 expressing activated Tfh cells which facilitates the encounter of antigen specific T cells and antigen presenting B cells (220).

The phase following initial T-B interactions, referred to as the extrafollicular response, involves clonal expansion and fate commitment to differentiate into extrafollicular plasma cells (PC), IgM^+ memory B cells or proceed onto the GC B cell differentiation pathway. Extrafollicular PCs are short-lived cells with an estimated life span of 3 days (299) that provide early protection by producing low-affinity antibodies (300). The magnitude and clonal selection of the extrafollicular response is determined by the affinity of the reactive BCRs and antigen dose, which corresponds to expansion of PCs and size of the extrafollicular foci (301, 302). T cells also play a role in the differentiation of extrafollicular plasma cells as T cells deficient for Bcl6 (303) or CD40L (304) show significantly impaired extrafollicular responses during T dependent responses. Presumably, T cells provide signals, possibly via IL-21 (305), that regulate Blimp-1 expression to promote PC differentiation of B cells (306).

In response to stimuli from T cells, B cells start to switch isotype expression from IgM and IgD to IgA, IgE or IgG constant regions. This process known as class-switch recombination (CSR) is a means to adequately adapt the antibody mediated effector response to the invading pathogen. This process is initiated by cytokine induced expression of germline transcripts which guides the enzyme activation-induced cytidine deaminase (AID) to switch-regions (307). AID was first identified in cells undergoing CSR *in vitro* and was shown to be able to convert cytosine into uracil by deamination (6, 308). This ultimately leads to single strand breaks and C-G to A-T transition after DNA repair. Switch regions have high density of C-G base pairing, making them a prime target for AID activity. The introduction of multiple strand breaks in the switch region eventually causes double strand break. Such breaks at C_μ and C_γ switch regions allow non-homologous end-joining to replace C_μ with C_γ resulting in isotype switch from IgM to IgG.

Similar to GC Tfh cells, B cells undergoing GC B cell differentiation must suppress Blimp-1 expression and express high levels of Bcl6, which actively represses Blimp-1 expression (309, 310). In addition to repressing Blimp-1 and PC differentiation Bcl6 also suppresses the anti-apoptotic protein Bcl-2. Hence GC B cells are prone to apoptosis and require continuous survival signals from T cells. Expression of Bcl6 in B cells is, at least partially, induced by the Tfh associated cytokines IL-4 and IL-21 (311-313). Consequently, GC responses in mice deficient for both IL-4 and IL-21R are severely impaired (313). However, IL-4 and IL-21 play a dual role in this aspect of B cell survival, as they also stimulate B cell proliferation and survival (233, 235, 314), although neither signal is individually critical for GC B cell survival. A non-redundant signal in GC B cell survival is CD40 stimulation which is provided by CD40L on T cells. Cell cultures of GC B cells stimulated with α-Ig, which rapidly undergo apoptosis due to the pro-apoptotic state induced by Bcl6, can be rescued from apoptosis if agonistic CD40 antibodies are present (315). *In vivo*, the necessity of CD40L-CD40 interactions have been shown to be important during both early GC B development and late GC B cell survival. Mice treated with blocking CD40L antibodies during the first 3 days of an immune response failed to generate functional GCs (316, 317) and treatment at later stages rapidly abolished already formed GCs (316, 318). Furthermore, CD40 signalling in B cells is important for expression of AID (241), the enzyme driving CSR and somatic hypermutation (SHM). Hence absence of CD40 signalling leads to loss of class-switched antibodies in T dependent but not T independent responses (304, 317, 319). At this stage B cells that have been selected to become GC B cells start to downregulate EBI2 (260, 261) and migrate into the follicle in responses to chemotactic cues by CXCL13 produced by FDCs (262) and CRC derived CXCL12 (263, 264). The aggregation of B cells centred around FDCs and CRCs results in the formation of the GC and its polarization into two distinct compartments with differential functions.

Germinal center process

The germinal center (GC) is the location where B cells undergo rapid proliferation and SHM in an attempt to increase the antigen affinity of the BCR. The GC reaction is perhaps best described as a microcosm where evolution takes place at the single cell level by SHM and survival is regulated in a Darwinistic fashion, in which fitness is defined by BCR affinity. These processes take place separately in the two distinctive compartments of the germinal center, namely the dark zone (DZ) and the light zone (LZ). In this section, the processes taking place in these compartments will be dissected (Figure 5).

The dark zone

The positioning of GC B cells into the DZ is dependent on CXCR4 mediated chemotaxis towards the gradient of CXCL12 produced by stromal cells in the DZ (320). In the DZ, GC B cells, called centroblasts at this stage, rapidly proliferate to generate a large pool of centroblasts. Newly generated centroblasts express AID that introduces nucleotide changes in the V region of Ig genes, which contain the antigen binding complementarity determining regions. Each and every progeny cell from the centroblasts acquires a unique set of somatic mutations resulting in a great spread of BCR affinities within the pool. Given that AID is involved in CSR, it begs the question whether CSR also occurs in AID expressing centroblasts in the DZ. While GC B cells can express low amounts of germline transcripts (321), expression levels of germline transcripts peaks early during the immune response. Antigen specific class-switched B cells can be found already day 2 post-immunization which suggests that CSR preferentially occurs prior to GC entry (322, 323). Once SHM is completed centroblasts internalize CXCR4 and start migrating to the LZ.

The light zone

If the DZ is considered to be where the evolution of GC B cells transpires, the LZ is where their fitness is tested. In the LZ antigen can be retained for extended periods of time in immune complexes bound to complement receptor 2 present on the surface of FDCs (324). GC B cells in the LZ, commonly referred to as centrocytes, are able to capture antigen present on the FDCs via the BCR which triggers internalization of the antigen bound BCR. Centrocytes compete for antigen binding of the immune complexes and the capacity of centrocytes to acquire antigen is directly dependent on the affinity of their BCR (325). This competition of antigen binding is indeed "a matter of life and death" as antigen uptake directly correlates to peptide presentation and consequently T cell help from GC Tfh cells that provide survival signals. Due to their Bcl6 mediated pro-apoptotic nature, centrocytes that fail to compete for antigen are deprived of survival signals and undergo apoptosis.

Centrocytes expressing higher affinity BCR that successfully acquired antigen and received T cell help can differentiate into antibody producing PCs, become memory B cells or re-enter the DZ to clonally expand and be subjected to SHM once again. Centrocytes that opt to re-enter the DZ undergo additional rounds of competition between centrocytes that have accumulated even more mutations during SHM thus potentially increasing their BCR affinity even further. Antibodies produced by GC derived PCs can replace antibodies bound to immune complexes thereby increasing the minimum affinity requirements

of centrocytes to acquire antigen (326). This mechanism is suggested to guarantee the generation of antibodies with higher affinity.

The critical role of T cells in GC B cell selection has been elegantly elucidated by Victora et al by using targeted antigen delivery to GC B cells using a αDEC205 antibody conjugated to Ovalbumin (OVA) in mice that received adoptive transfer of $DEC205^{+/+}$ or $DEC205^{-/-}$ B cells (89). This model bypasses affinity based BCR mediated uptake of antigen as $DEC205^{+/+}$ B cells will acquire and present more pMHCII compared to $DEC205^{-/-}$ B cells and vastly outcompete $DEC205^{-/-}$ B cells in receiving T cell help (89). Furthermore, when αDEC205-OVA antibodies are administered to wild-type ($DEC205^{+/+}$) mice causing all B cells to present equal amounts of pMHCII regardless of BCR affinity, there was a profound effect on affinity maturation (89). As discussed earlier, several T cell mediated signals have been reported to be critical for GC maintenance. ICOS-ICOSL interactions was early identified to be important for GC maintenance and IgG class-switch as ICOS signalling induces CD40L and IL-4 expression in T cells (201, 202). However, ICOSL delivered in a bystander fashion is sufficient for normal GC induction with only a modest reduction in affinity selection (227). In addition, administration of agonistic αCD40 antibodies rescues class-switching in $ICOS^{-/-}$ mice (327), indicating that the effects of ICOS-ICOSL is largely T cell dependent. A recent study observed that BCR signalling induced Bcl6 degradation at the protein level (328). However, IL-4 and IL-21 signalling via STAT3 and STAT6, respectively, protected Bcl6 from degradation. This identifies a novel role for IL-4 and IL-21 in the safeguarding of GC B cells. These experiments illustrate how BCR affinity and T cell help in concert ensures cyclic affinity-based selection in the GC.

Germinal center output

The GC reaction results in generation of long-lived antibody producing PCs that can survive for extended periods of time in the absence of antigenic stimuli. It also generates a pool of memory B cells that swiftly respond upon secondary infections and differentiate into PCs or re-enter the GC reaction. The longevity of PCs in combination with the capacity of memory cells to respond to secondary challenges together constitutes the foundation of serological immunity. In contrast to extrafollicular PCs that have few mutations and relatively low affinity, PCs originating from the GC reaction are highly mutated and, due to the selection processes in the GC, secrete antibodies with significantly higher affinity than the antibodies generated by their extrafollicular counterparts. PCs with a history of GC reaction also produce

class-switched antibodies guided by cytokines and therefore have their function tailor-fitted to the needed immune response.

Selection into the PC compartment is directed by Blimp-1 (306, 329). The exact mechanism that promotes Blimp-1 induction is not entirely known. IL-21 has been shown to induce both Blimp-1 and Bcl6 expression in B cells (305), suggesting that IL-21 signalling may act as a rheostat in GC B cell decisions. Indeed, several studies have reported impaired PCs formation, CSR and SHM in the absence of IL-21 signalling (217, 233, 314), coupled to an accelerated generation of memory B cells with lower mutation frequency (233). However, it should be noted that these studies have largely been conducted with IL-21$^{-/-}$ and IL-21R$^{-/-}$ animals, making discrimination between T and B cell intrinsic effects difficult. A study by McGuire et al used adoptive transfer of transgenic IL-21R$^{+/+}$ or IL-21R$^{-/-}$ CD4^{+} T cells into IL-21R sufficient and deficient hosts to dissect this (234). The authors found that IL-21R$^{+/+}$ but not IL-21R$^{-/-}$ T cells could restore both GC B cell numbers and high affinity IgG during secondary responses. Several studies have observed that GC derived PCs have a greater frequency of mutations leading to higher affinity than GC B cells in two separate model systems in which the high affinity mutations are well described, indicating that BCR signaling strength also regulates PC differentiation (301, 330, 331). The mechanisms that dictate memory selection are less well understood. Memory B cells have lower affinity than PCs, suggesting that either strong BCR stimuli prevent memory cell generation by promoting PC differentiation or that memory cells are generated early during the GC reaction before acquiring high affinity mutations (330). Indeed, Weisel et al showed that CD80^{-} PD-L2^{-} memory B cells primarily arise at the early stages of the GC response whereas memory B cells that arise during the later stages of the GC reaction express CD80 and PD-L2 (332). This phenotypic distinction of memory B cells have been shown to be associated with differential functions. Upon rechallenge, CD80^{-} PD-L2^{-} memory B cell preferentially re-enter the GC while CD80^{+} PD-L2^{+} memory B cells predominantly become antibody producing cells (333). GC Tfh-produced IL-9 was recently shown to induce cell cycle arrest in the S-phase followed by acquisition of memory phenotype in GC B cells (243). Conversely, IL-9 blockade prevented memory formation indicating that GC Tfh cells may help govern memory B cell output.

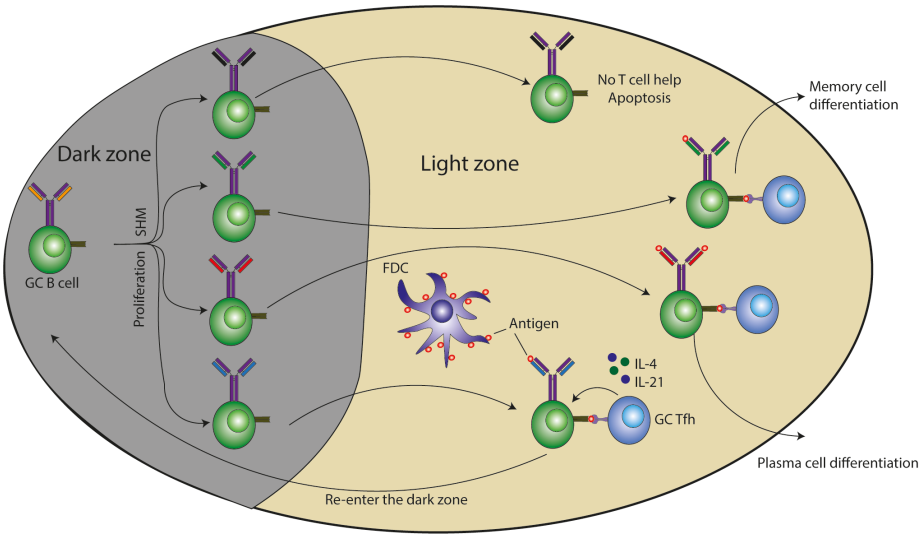

Figure 5. The process and outcomes of the germinal center reaction

Aim

The overall aim of this thesis was to investigate the role of different APCs, with a focus on DCs and B cells, in terms of T effector cell versus Tfh cell differentiation in the context of protein adjuvant immunization.

Specific aims were:

- To determine if cDCs send unique accessory signals required for the differentiation of Tfh cells
- To identify genes in Th cells that are regulated by B cells early following immunization
- To investigate if B cells play a role in the generation and output of peripheral T effector cells

Key methodologies

Mice

The complex nature of the immune system cannot in its entirety be faithfully replicated *in vitro* and is best studied *in vivo*. For this purpose, we have used mice as our model animal. This is due to the rapid reproduction cycle, many commercially available genetic models in addition to their immune system being relatively similar to that seen in humans. However, as mice are inbred over several generations to maintain the genetic identity they lack the diversity at individual level seen in the human population. Throughout this thesis we have used the C57BL6/J strain as our wild type (wt) control mouse and all transgenic and knock-out (KO) mice used are based on the C57BL6/J background. Transgenic mice are mice that carry a DNA insert from another source used as a means to give the cells in the mouse additional properties. Conversely, KO mice have a specific deletion of a gene. In this thesis we have exploited several transgenic and KO mice with specific phenotypes. For instance, the CD11c-DTR mice in Paper I express the diphtheria toxin (DTx) receptor, which is not naturally expressed in mice, under the control of the CD11c promotor (81). This allows for ablation of CD11c expressing cells by administration of DTx. Another mouse strain central to this thesis is the OT-II mice in which $CD4^+$ T cells express a TCR transgene that specifically recognizes an OVA-derived peptide presented on MHCII. The OT-II mice strain is crossed onto a congenic mouse expressing the allelic variant CD45.1 whereas C57BL6/J mice carry the CD45.2 variant. This discrete difference in CD45.1 alleles allows for tracking of $CD45.1^+$ OT-II cells after being adoptively transferred into $CD45.2^+$ congenic hosts. For a summary of mice strains used in this thesis, see **Table 1**.

Table 1 Mouse strains used in this thesis

STRAIN	PHENOTYPE
CD11c-DTR	*Mice expressing diphtheria toxin receptor under control of the CD11c promotor*
$MHCII^{-/-}$	*KO mice deficient for MHCII*
CD11c-DTR x $MHCII^{-/-}$	*MHCII KO mice carrying the CD11c-DTR transgene*
μMT	*KO mice unable to generate B cells*
B1-8^{hi} - GFP	*B cells express the transgenic high affinity anti-NP BCR*
$CX_3CR1^{-/GFP}$	*CX_3CR1 KO mice with transgenic GFP replacing one of the two alleles*
OT-II	*CD4 T cells express a transgenic TCR specific for an OVA-derived peptide. $CD45.1^+$*
$ICOS^{-/-}$ OT-II	*ICOS KO mice with the OT-II transgene*
KN2xOT-II	*OT-II mice in which one IL-4 allele is replaced with hCD2 to track IL-4 secretion*

Bone marrow chimeras

Transplantation of BM into lethally irradiated hosts is a technique that can be used to transfer a phenotype from a donor mouse onto the hematopoietic compartment of the recipient mice. For instance, CD11c-DTR mice administered DTx will succumb after a few days due to loss of non-hematopoietic CD11c expressing cells (81). Hence, in CD11c-DTR BM chimeric mice DTx can repeatedly be administered over extended periods of time without affecting the non-hematopoietic compartment since only the cells of the hematopoietic compartment reconstituted by CD11c-DTR bone marrow express the receptor. Moreover, the transferred BM can be composed of two different backgrounds to generate a hematopoietic compartment with mixed phenotypes. The mixed BM strategy has been exploited in all papers and in particular Paper II and Paper III in which we have reconstituted mice with 4:1 ratio of μMT / C57BL6/J (Control) and μMT / MHCII$^{-/-}$ (MHCII$^{B-/-}$) to generate bone marrow chimeras with a mixed phenotype. In this case, 80% the hematopoietic compartment is of μMT origin which cannot support B cell development, effectively causing all B cells generated to be of either 20% C57BL6/J or MHCII$^{-/-}$ origin rendering B cells either capable of presenting antigens on MHCII molecules or not. This strategy was also employed in Paper I where 4:1 mixed bone marrow chimeras from CD11c-DTR/μMT and MHCII$^{-/-}$ were used to generate mice in which B cells could not present antigens and cDCs were either deficient for MHCII or could be transiently depleted by DTx administration.

Thoracic duct cannulation

For collection of bona fide migrating T cells, we cannulated the thoracic duct and collected the efferent lymph containing thoracic duct lymphocytes (TDL) overnight. The thoracic duct is the largest lymphatic vessel and serves as a focal point for many efferent lymphatics vessels, including all of the intestinal draining LNs. This method has been used to collect migratory APCs in mice that have had their MLNs removed by mesenteric lymphadenectomy (50). In mice with intact MLNs, migratory cDCs do not exit the LN into the efferent lymph and the TDLs largely consists of recirculating T and B lymphocytes.

Flow cytometry & fluorescence activated cell sorting

The identification of immune cells is based on their expression of cell-type associated markers either present on the cell surface or intracellularly. In flow cytometry, target specific antibodies conjugated to fluorochromes that are excited by light, or laser, of a certain wave length and emit light of a higher wavelength upon excitation are used to label markers of interest. Each laser used can excite multiple fluorochromes with distinct emission spectra, allowing analysis of a multitude of targets simultaneously at a rate of over

15 000 cells per second. This combination of speed and possibility to analyze multiple targets at the same time makes flow cytometry the best method to perform multi-parameter characterization and analysis of cells within a diverse population like a lymphoid organ.

By forcing the antibody labelled cell suspension through a vibrating nozzle under high pressure, a stream of small fluid droplets containing a single cell is formed. The stream of droplets subsequently passes through a series of lasers serving as excitation sources for different wavelengths. The emitted light is separated by a series of wavelength specific mirrors and filters into detectors and the emission spectra and signal strength for each antibody labelled target as well as the light scatter caused by the cell is analysed to determine the size and granularity of cells.

An additional feature of flow cytometry is the ability to isolate individual cells or all cells defined by a desired phenotype in what is called fluorescence activated cell sorting (FACS). By applying a high voltage over two metal plates situated on opposing sides of the droplet stream it is possible to sort individual droplets containing cells of interest into tubes or cell culture plates. In this thesis we have sorted OT-II cells based on their phenotype into tubes containing lysis buffer to perform gene expression analysis.

Gene expression

To measure the expression of genes at the mRNA level we performed quantitative real-time PCR on sorted OT-II cells activated both *in vivo* and *in vitro*. After sorting into lysis buffer, we purified mRNA transcriptome by spin column purification for subsequently reverse transcription to generate a cDNA library of the transcriptome. Similar to traditional PCR, target specific primers allows for amplification of gene specific fragments. The amplified dsDNA product allows for the binding of a probe that becomes fluorescent when bound to dsDNA. Hence, the fluorescent signal will increase in a logarithmic manner with every amplification cycle. By comparing the fluorescent signal of the gene of interest with a house keeping gene, a highly and stably expressed gene, the relative levels of mRNA transcripts can be estimated.

Results & Discussion

Paper I – The role of DCs in initiating CD4$^+$ T cell responses

The development of Tfh cells is dependent on several molecular interactions including ICOS-ICOSL engagement and cellular interactions with B cells to form functional GC residing Tfh cells (222, 334). DCs have long been considered to be necessary and sufficient for early Tfh induction as antigen presentation restricted to cDCs in both molecular models and adoptive transfer of antigen loaded cDCs is sufficient to initiate the Tfh differentiation program (218, 335). Early Tfh differentiation is marked by an upregulation of CXCR5 and movement to the T-B border that occurs independently of expression of Bcl6 the bona-fide transcription factor of Tfh cells (277). B cell restricted antigen presentation is sufficient to induce functional Tfh cells during LCMV infection but not protein immunization (222), calling into question whether cDCs deliver unique signals necessary for Tfh differentiation provided in trans or if it is their potency in inducing T cell expansion that make them non-redundant in conventional immunization regimens. To address if cDCs provide critical and non-redundant signals in Tfh development we studied Tfh generation in the absence of cDCs during initial T cell priming. To this end, we adoptively transferred congenic CD45.1$^+$ OT-II cells that carry a transgenic TCR specific for OVA into CD45.2$^+$ CD11c-DTR hosts. The cDC population in CD11c express a transgenic receptor for DTx allowing for transient depletion of cDCs that lasts for approximately 72h (81). After immunization with OVA and the dsRNA analogue polyI:C which stimulates TLR3, this model system allows for analysis of antigen specific CD4$^+$ T cells, at selected time points, primed in the absence or presence of cDCs.

T cell expansion in the absence of cDCs require higher antigen dosing
Given their unparalleled ability to efficiently take up and present antigens, cDCs are considered to be the most potent APC in terms of inducing T cell responses. Ablation of cDCs have previously been reported to severely impair CD4$^+$ T cell priming when antigen is administered at mucosal sites but not when high antigen dose is administered systemically (336). In initial experiments we immunized mice intraperitoneally (i.p.) with 300 μg OVA and 100 μg polyI:C and observed a reduction in expansion of OT-II in cDC depleted mice (Figure 6A) accompanied with failure to downregulate CD62L and decreased expression of CXCR5, suggesting impaired CD4$^+$ T cell activation. To distinguish between the antigen presentation and accessory functions of cDCs, we pursued by administering a 10-fold higher antigen dose

to allow antigen uptake and presentation by other, less proficient, APCs.

In response to non-limiting antigen dose OT-II proliferation, Tfh differentiation and GC B cell generation was rescued, showing that other APCs can support the induction of T cells responses and that cDCs play a non-redundant role in this aspect (Figure 6A and C). This is in line with a previous study that report a correlation between antigen dose, Tfh and GC B cell generation (318), albeit more pronounced in the absence of cDCs. Interestingly, OT-II cells primed in the absence of cDCs showed reduced amount of the Th1-associated cytokine IFNγ (Figure 6B). Intracellular staining and mRNA expression levels for the Th1 and Tfh associated transcription factors T-Bet and Bcl6, respectively, confirmed that the Th1 but not Tfh program was compromised in cDC ablated mice. This indicates that cDCs may be more important for Th1 responses than Tfh differentiation. Functionally, the reduction of Th1 polarization had downstream implications of the B cell response as GC B cells preferentially expressed Tfh associated IgG1 and not Th1 associated IgG2c antibody isotypes in cDC depleted mice. Besides of the bias towards IgG1 isotype switching, GC responses were normal as cDC ablated animals showed normal affinity maturation and memory responses. Collectively, this shows that cDCs are redundant for Tfh cell differentiation but necessary for Th1 cell differentiation and their associated responses.

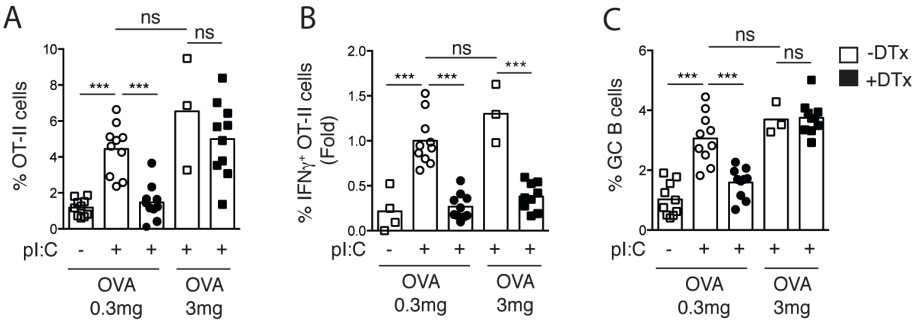

Figure 6. ***High antigen dose by-passes the requirements for cDC in germinal center formation.*** *(A-C) OT-II expansion (A), IFNγ production (B) and induction of GC B cells (C) d14 p.i. following indicated doses of OVA and DTx treatment.*

The issues of antigen dosage and de novo generated cDCs

One of the key questions that arose during this study concerned the antigen dosage. While another study have shown that antigen presentation restricted to B cells can promote Tfh induction, albeit inefficiently, during chronic viral infection (222), it is difficult to evaluate how a chronic infection translates into protein immunization in terms of antigen availability. Certainly, 3 mg of OVA certainly can be considered as an extreme amount of antigen and we sought to address whether the induction of Tfh cells could be initiated by *de novo* generated cDCs that present lingering antigen. To test this, we first assessed when cDCs start to re-emerge in the spleen after DTx depletion and noted that the reconstitution of cDCs is first evident at 86h post depletion. By transferring OT-II cells at various time points in relation to immunization into wild type mice that had received 3 mg we found that OT-II cells need to be present at the time of immunization to be efficiently primed.

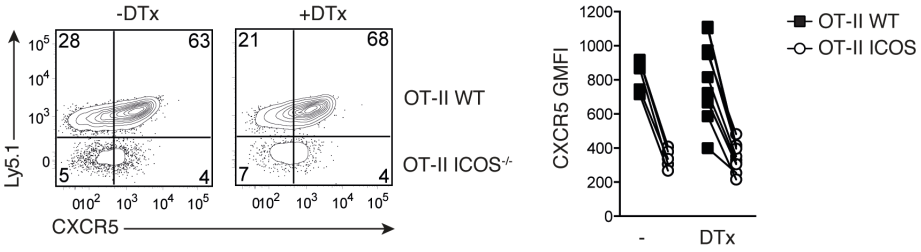

Figure 7. Early Tfh differentiation is dependent on ICOS-ICOSL in cDC depleted animals. *Competetive transfer experiment of wt and ICOS$^{-/-}$ OT-II cells d3 p.i. with or without DTx treatment in B$^{MHCII-/-}$ CD11c-DTR/B6 mice*

Since GC B cells eventually start to express the DTR transgene in CD11c-DTR mice, continuous administration of DTx will deplete GC B cells and consequently terminate the GC reaction (318). To circumvent this effect and further dissect which APCs were responsible for T cell priming in the absence of cDCs, we generated bone marrow chimeras in which cDCs are sensitive to DTx and B cells are resistant but cannot present antigens. During continuous cDC depletion, OT-II cells were still dependent on ICOS-ICOSL interactions to acquire CXCR5 expression during the initial stages of Tfh differentiation and cognate B interactions to fulfil Tfh development (Figure 7). Hence, generation of (OT-II) Tfh still depend on normal cellular and molecular interactions and administration of 3 mg of OVA in CD11c-DTR mice does not generate an antigen deposit that repopulating cDCs can utilize as a means to efficiently prime OT-II cells.

Addressing the role of other APCs in the absence of cDCs

Since the necessity of cDCs could be mitigated when antigen is not limiting we set out address to role of other APCs in terms of T cell priming and Tfh induction proficiency. To approach this, we first identified MHCII expressing cells that express ICOSL as the ability to present antigen and provide ICOS-ICOSL interactions is critical for Tfh induction (182). By this criteria, we observed that primarily Ly6C^{high} monocytes, but also pDCs, gained expression of ICOSL in response to polyI:C. Administration DTx in CD11c-DTR mice have been reported to result in blood neutrophilia (337) as well as deplete marginal zone macrophages (338) indicating that the APC landscape may be influenced in response to DTx. In addition to neutrophils we also observed an increased influx of Ly6C^{high} monocytes suggesting that inflammatory monocytes may function as the primary APC after DTx mediated cDC depletion.

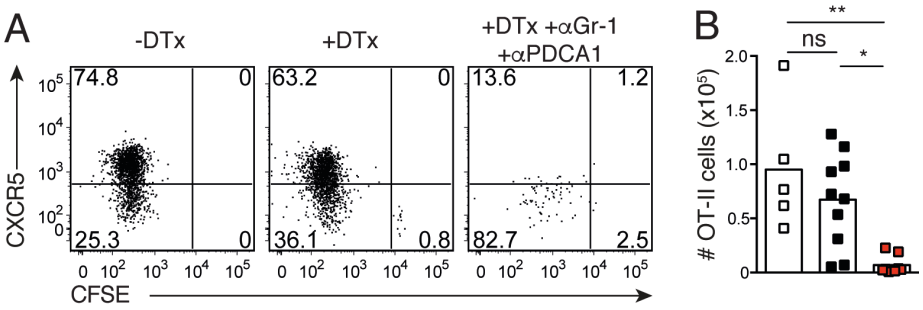

Figure 8. Ly6C^{+} monocytes are complementary with B cells in driving CD4^{+} T cell expansion in the absence of cDCs. *(A) CXCR5 expression and cell division by CFSE labelling d3 p.i. in CD11c-DTR mice with indicated treatment. (B) Total number of OT-II cells and CXCR5 expressing OT-II cells.*

Co-administration of DTx and αGR-1 antibodies in CD11c-DTR mice counteracted the influx of granulocytes and caused a partial reduction in Ly6C^{high} monocytes compared to untreated animals but did not affect T cell priming or GC responses. Furthermore, GC responses remained intact after combined cDC, monocyte and pDC depletion by αPDCA-1 antibodies suggesting a possible redundancy between remaining Ly6C^{high} monocytes and B cells in driving T cell expansion and Tfh differentiation. To delineate the roles of Ly6C^{high} monocytes and B cells in terms of initial T cell priming we treated B$^{MHCII-/-}$ / CD11c-DTR mice with DTx in combination, or without, with αGR-1 and antibodies αPDCA-1. Strikingly, monocytes and pDCs in this system were able to support early T cell activation but in their absence T cells failed to robustly proliferate (Figure 8). These results suggest that in the absence of cDCs, Ly6C^{high} monocytes along with B cells, and possibly pDCs, are capable of inducing T cell responses. Although B cells present more

peptide-MHCII complex when antigen dose is increased (294), B cells as sole APCs are poor inducers of T cell priming and Tfh differentiation in response to protein immunization and even in high peptide dose experiments (222). Thus, we cannot exclude an auxiliary role of residual Ly6C^{high} monocytes after αGR-1 treatment in amplifying initial T cell expansion and supporting Tfh differentiation as they previously have been ascribed to perform this role (80) as long as functional B cells are present.

Conclusions

When high dose of antigen in the immunization is used:

- cDCs are not required for robust T cell priming and Tfh differentiation.
- monocytes and B cells, potentially also pDCs, can support early T cell priming and Tfh differentiation.
- GC development, affinity maturation and memory responses still occur in the absence of cDCs
- Th1 differentiation and associated IgG2c class-switch are drastically impaired in cDC depleted mice

Paper II – Identification of genes regulated by B cells

While cDCs are important, but not critical, for initial Tfh induction antigen presenting B cells is an absolute requirement for their complete differentiation and sustainment (222, 223). Given that the onset of Tfh differentiation marked by expression of CXCR5 and PD-1 can be initiated without the help of B cells (218, 223), the proposed model of Tfh differentiation is a step-wise interaction where the role of B cells is to propagate the Tfh differentiation pathway to generate GC Tfh cells. Although some co-stimulatory molecules provided by B cells like CD40 (225) and ICOSL (226) are known to be crucial for Tfh generation, the full extent of B cell influence on Th differentiation is not completely understood.

Identification of genes in Th selectively regulated by B cells
To address which genes in Th cells that are selectively regulated by B cells we opted for a wide and unbiased approach by MicroArray analysis of total OT-II

cells collected five days post a Th1 skewing immunization from mice in which B cells can present antigen or not. As expected in MHCII$^{B-/-}$ mice, several Tfh associated genes, including *Bcl6*, *c-Maf*, *Il21* and *Cxcr4*, showed a decline in expression that coincided with a loss of the CXCR5^{+} PD-1^{+} Tfh phenotype. Interestingly, we observed an adverse relationship between the expression of *Il4* and the gene encoding for the IL-4 receptor, *Il4ra*, as well as a total loss of the gene *H2-Q2* (Figure 9) which encodes a polymorphic non-conventional MHC class I molecule (339, 340). To address whether the dysregulation of *Il4*, *Il4ra*, and *H2-Q2* seen in MHCII$^{B-/-}$ mice was due to a loss of Tfh cells we sorted CD62L^{-} CXCR5^{-} and CD62L^{-} CXCR5^{+} OT-II cells to be able to compare a bona-fide effector population to the follicular homing population. Indeed, the differential expression seen among total OT-II cells was restricted to CD62L^{-} CXCR5^{+} OT-II cells. Furthermore, surface staining of IL-4Rα and intracellular cytokine staining for IL-4 confirmed that the expression of these genes was restricted to the CD62L^{-} CXCR5^{+} population, suggesting that these genes were in fact related to Tfh cells. Moreover, *in vitro* re-stimulation of CD62L^{-} CXCR5^{+} OT-II cells by agonistic αCD3 antibodies could not revert *Il4ra* or *H2-Q2* expression back to levels seen in control mice, suggesting that these genes are not regulated by TCR signalling but rather by other co-stimulatory signals provided by B cells.

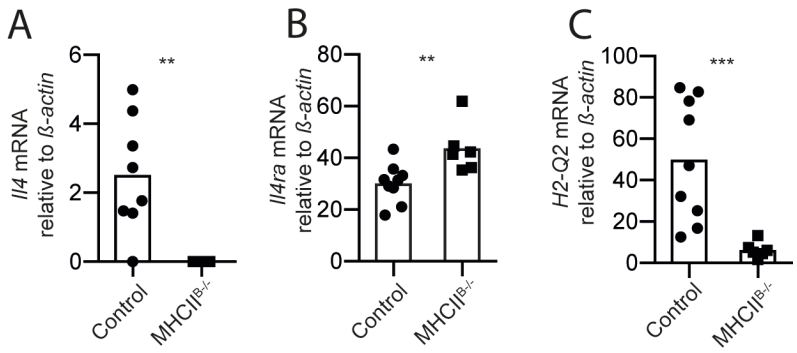

Figure 9. Genes regulated by B cells. *(A-C) OT-II cells were sorted from MLNs of control and MHCII$^{B-/-}$ mice 5 days p.i. with OVA + polyI:C. Gene expression of Il4 (A), Il4ra (B) and H2-Q2 (C) relative to β-actin.*

Il-4 and IL4Rα expression in Tfh cells

While production of IL-4 in Tfh cells have been reported by several studies (230, 237, 240), a concurrent downregulation or role of IL4Rα in Tfh biology have up until this point has not been described. First, we analysed the expression of IL4Rα in CXCR5^{-}, CXCR5^{+} and CXCR5^{+} PD-1^{+} cells in OT-II cells as they developed following immunization. Although the three populations showed a decline in IL4Rα over time surface expression was

noticeably lower in Tfh cells at all time points. Given the close relationship between Th2 and Tfh cells (224, 237, 279), we speculated that the selective down regulation of IL4Rα in IL-4 producing Tfh cells could be a mechanism to circumvent autocrine IL-4 signalling by Tfh cells that otherwise could drive Th2 differentiation in Th1-skewed responses. In order to analyse IL4Rα expression among IL-4 producing cells *in vivo* we transferred KN2xOT-II cells that marks IL-4 secretion by surface expression of the hCD2 transgene (236). KN2xOT-II cells primed under Th1-skewing regimen showed a converse relationship between IL-4 secretion and expression of IL4Rα where active IL-4 secretion was only detectable in Tfh cells (Figure 10). To dissect if this relationship between IL-4 and IL4Rα expression was selective for Th-1 biased immunizations or a general phenomenon we used the Th2 permissive adjuvant cholera toxin (CT) (341, 342). Analogous analysis in CT immunized mice showed similar results as polyI:C immunized mice. In addition, there was no evident difference in IL4Rα expression among IL-4-producing and non-producing Tfh cells. These results show that IL4Rα downregulation and IL-4 production are general features of Tfh cells regardless of adjuvant and IL-4 secretion status.

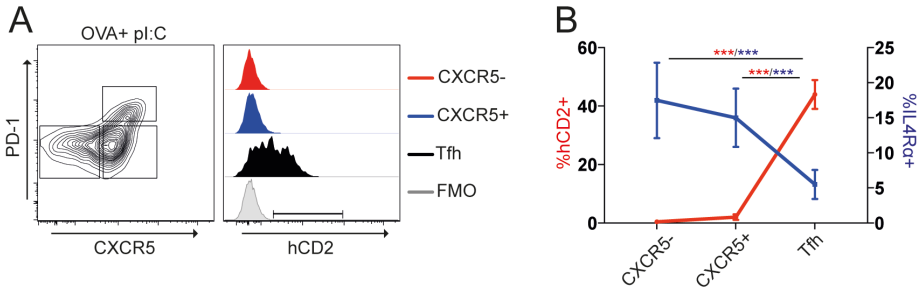

Figure 10. Differential expression of IL-4 and IL4Rα in different T cell populations. *(A-B) KN2xOT-II cells in MLN 5d p.i. with OVA + polyI:C. (A) hCD2 expression in indicated populations. (B) Expression of hCD2 and IL4Rα in different T cell populations as gated in (A).*

To investigate if the downregulation of IL4Rα had any functionality in Tfh cells, we aimed to force expression in OT-II cells by transducing them with a retrovirus encoding IL4Rα. However, this experimental approach was unsuccessful as STAT5 signalling cytokines readily induce IL4Rα expression (131), most likely in response to autocrine IL-2 as a result of αCD28 co-stimulation during *in vitro* activation (343). To counteract the upregulation of IL4Rα, we cultured *in vitro* activated OT-II cells in the presence of a neutralizing αIL-2 antibody to avoid IL-2 induced expression of IL4Rα. We

also adoptively transferred transduced OT-II cells into recipient mice to investigate if *in vivo* rest in the presence of natural survival signals would allow *in vitro* activated cells to downregulate IL4Rα expression. Regardless of stimulation conditions or extended *in vivo* rest, a difference in IL4Rα expression by the control-transduced cells was not discernible compared to cells expressing the IL4Rα construct. Hence, we could not generate a model system in which IL4Rα was differentially expressed during priming and forced to remain expressed during the GC reaction when IL-4 is produced by Tfh cells. Thus, we have not been able to address if the downregulation of IL4Rα serves as mechanism to shield Tfh cells from autocrine signaling or if it's a means to increase IL-4 availability for GC B cells.

Conclusions

- Tfh cells produce IL-4 and downregulate its receptor, IL4Rα in a B cell dependent manner
- The converse relationship between IL-4 secretion and IL4Rα expression in Tfh is not selective for Th1-biased adjuvants
- At the transcript level in T cells, the expression of *H2-Q2* encoding for an unconventional MHCI molecule is regulated by B cells

Paper III – B cells influence effector T cell output

After T cell priming, Tfh cells and T effector cells develop in parallel governed by the transcription factors Bcl6 and Blimp-1 (170), respectively. We have previously described that early during the immune response developing Tfh cells and effector cells can be discriminated based on their exclusionary expression of the follicular homing receptor CXCR5 and the gut-tropic associated integrin α4β7 (268). While the signals that induce expression of CXCR5 in T cells still remain to be elucidated the vitamin A metabolite RA have been shown to induce α4β7 in T cells (270, 273). The expression of α4β7 enables T cells to transmigrate from the blood into the SI LP by binding to MAdCAM-1 present on post-capillary venules along the SI. In a previous study we observed a population of T cells displaying co-expression of CXCR5 and α4β7 (268). This suggested a conflict in homing preference between B cell follicle and gut. How these $CXCR5^+$ $\alpha4\beta7^+$ T cells were generated and their peripheral homing capacity was not known. We speculated that these $CXCR5^+$

α4β7^{+} T cells may, at least partially, constitute former pre-Tfh cells that were not selected for GC entry. By combining thoracic duct cannulation and the OT-II adoptive transfer system in mice in which B cells can or cannot present antigen, we set out to address if B cells play a role in the generation and output of gut homing α4β7^{+} Th cells.

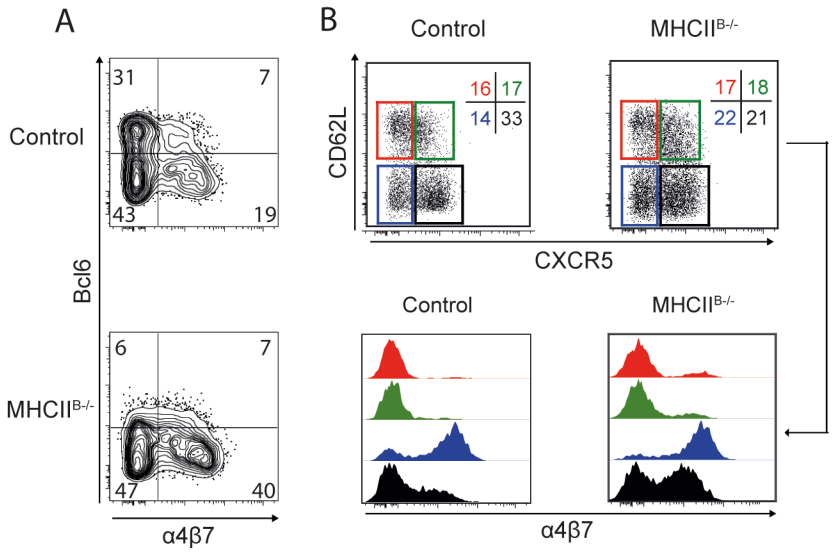

Figure 11. Loss of cognate B cell interaction leads to an increase of effector T cells and a loss of Tfh cells. *(A-B) OT-II cells in MLNs d5 p.i. of control or MHCII$^{B-/-}$ mice that were immunized with OVA + polyI:C. (A) Facs plots showing the relationship between Bcl6 and α4β7. (B) α4β7 expression in T cell populations as determined by differential expression of CD62L and CXCR5.*

Antigen presenting B cells in T cell differentiation

First, we addressed the generation of α4β7^{+} gut-tropic OT-II cells in control and MHCII$^{B-/-}$ and observed an increase of total α4β7^{+} OT-II cells (Figure 11A). This increment in α4β7^{+} was even further emphasized in B cell deficient μMT mice and accompanied by a loss of Tfh cells. By separating the T cells into four populations based on their expression of CD62L and CXCR5 it became clear that the increased expression of α4β7 was primarily concentrated to the CD62L^{-}CXCR5^{+} population that also includes Tfh cells (Figure 11B). This suggests that prevention of Tfh cell development could lead to reprogramming into effector cells. In addition to α4β7 integrin, RA promote expression of the chemokine receptor CCR9 which further enhances the gut-tropism by attracting T cell to CCL25 produced by SI epithelial cells (38). Although there was an evident increase in CCR9 expression among both

CD62L$^-$CXCR5$^-$ and CD62L$^-$CXCR5$^+$ OT-II cells in the MLN, no increase in OT-II cells was observed in the SI LP. Moreover, there was no bias towards CCR9$^+$ OT-II in the SI LP despite their increased generation in MLN.

Characterization of CD4$^+$ T cells that have left the LN

The apparently unaltered SI entry of Th in MHCII$^{B-/-}$ mice could potentially mean that the newly generated effector cells remained in the MLN, possibly by virtue of their CXCR5 expression. Alternatively, transmigration over the endothelium is a rate-limiting event that is saturated in our adoptive transfer model. To faithfully study Th cells that have left the LN we cannulated the thoracic duct and collected TDLs. To our knowledge emigrant CD4$^+$ T cells in TDL have not previously been studied. We therefor assessed the phenotype of recent emigrant T cells at various time-points post immunization in wt mice and used MLN as a reference SLO. Although TDLs contain T cells primed in all lower limbs it became clear that egress from gut draining LNs constitute the major part of TDLs as approximately 80% of all OT-II cells expressed α4β7 day 3 p.i. (Figure 12A). This is in line with a previous study in our group using FTY720 to block LN output showing that during the first 3 days of the immune response LN egress is dominated by α4β7$^+$ cells (268). Moreover, most of the α4β7 expressing TDL OT-II cells co-expressed CCR9, a clear enrichment compared to the MLNs (Figure 12B). At this time point, Tfh cells in the MLN can be identified by high expression of PD-1 or Bcl6 coupled to CXCR5 expression. However, the Tfh population during this time point is confined to the α4β7$^-$ OT-II cell compartment. Indeed, cells exhibiting a Tfh profile day 3 p.i. could not be seen among TDLs, indicating that Tfh cells remain in the MLN at this stage (Figure 12C).

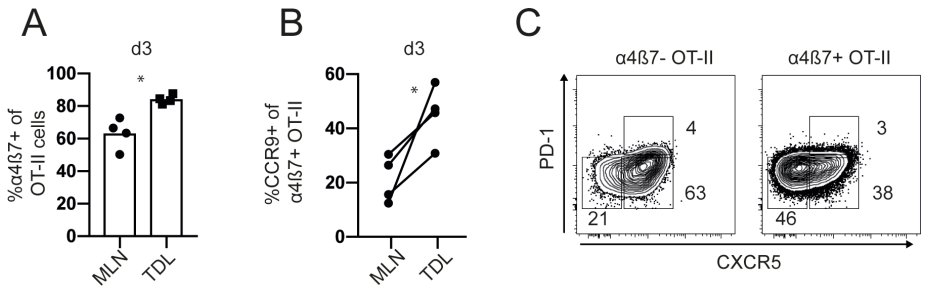

Figure 12. Early LN output consists of effector cells. *(A-C) Phenotypic analysis of OT-II cells in TDL d3 p.i. after OVA + polyI:C. (A-B) Expression of α4β7 (A) and frequency of CCR9$^+$ cells of α4β7$^+$ cells (B). (C) Representative contour plots showing CXCR5 and PD-1 expression in MLN and TDL.*

Identification of ex-Tfh cells migrating in lymph

Next, we sought to address whether the CXCR5$^+$ α4β7$^+$ population evident at later time points (268) was migratory in its nature. At day 5 p.i. the egress of α4β7$^+$ as well as CCR9$^+$ had waned and the OT-II cells in TDL showed a remarkable resemblance to those in MLN in terms of α4β7 and CCR9 expression. Interestingly, we observed migratory populations exhibiting the characteristic CXCR5$^+$ PD-1$^+$ Tfh phenotype, albeit with a slightly lower expression of PD-1 compared to MLN, in both α4β7$^+$ and α4β7$^-$ OT-II cells. This indicates that former Tfh cell (ex-Tfh) do exit LNs early during the immune response. However, ex-Tfh cells among TDLs showed an almost complete loss of Bcl6 expression suggesting that either these cells never fully committed to the Tfh differentiation pathway or that Bcl6 is downregulated before LN egress. A previous study reported that blood CXCR5$^+$ PD-1^{int} T cells corresponded to circulating Tfh-memory cells (344). The generation of these cells was shown to dependent on Bcl6 and ICOS sufficiency but independent of SAP signalling. This suggests that CXCR5$^+$ PD-1^{int} T cells are generated and egress before GC formation. To test whether CXCR5$^+$ PD-1$^+$ OT-II TDL cells were generated independently of GCs we performed thoracic duct cannulation in control and MHCII$^{B-/-}$ mice. Consistent with our initial findings that α4β7$^+$ and CCR9$^+$ gut-tropic effector cells are abundantly generated in the MLN of MHCII$^{B-/-}$ mice, α4β7 and CCR9 expressing OT-II cells are also more prevalent among TDLs of MHCII$^{B-/-}$ mice compared to control (Figure 13A-B). Moreover, CXCR5$^+$ PD-1$^+$ ex-Tfh cells are exclusively found in TDLs from control mice regardless of α4β7 expression or not (Figure 13C). He et al showed that the generation of blood CXCR5$^+$ PD-1^{int} cells was independent of long lasting B cell interactions at later time points (344). This suggests that the presence of migrating ex-Tfh could be dependent on kinetics. Thus, we collected TDLs from control and MHCII$^{B-/-}$ mice at day 8 p.i. when GC are fully established in control animals but absent in MHCII$^{B-/-}$ mice and found that ex-Tfh were absent in TDLs of both groups. This shows that egress of ex-Tfh displaying the bona-fide CXCR5$^+$ PD-1$^+$ Tfh phenotype is an early event and that migratory ex-Tfh cells are generated in a B cell dependent manner.

The reason why these ex-Tfh cells opt to migrate is unclear, one possibility could be that Tfh cells are generated in abundance relative to what is needed to generate an optimal GC response and therefore some Tfh cells are forced to leave by competition. A recent study has demonstrated that Tfh-memory cells with high affinity TCRs remain in the reactive LN after resolution of the GC response whereas low affinity Tfh clones migrate to non-reactive LNs to serve as memory cells (285). Due to our model system of tracking transgenic OT-II we cannot determine if TCR affinity regulates the output of ex-Tfh cells. However, the vast difference in kinetics between the model systems and our finding that a fair share of ex-Tfh express α4β7 and have downregulated CD62L, hallmarks of gut-tropic effector T cells, implies that we are not studying disseminating memory Tfh cells bound for LNs.

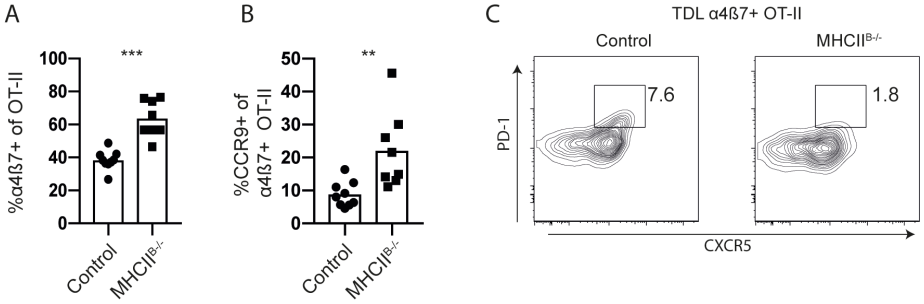

Figure 13. Loss of cognate B cell interaction increases output of effector cells. *(A-C) OT-II cells were adoptively transferred into control or MHCII$^{B-/-}$ mice that were cannulated d4 p.i. Expression of α4β7 (A) and frequency of CCR9^{+} cells of α4β7^{+} cells (B). (C) Identification of α4β7^{+} Tfh cells in TDL of control and MHCII$^{B-/-}$ mice.*

ex-Tfh cells have a history of IL-4 secretion

Since the ex-Tfh population was dependent on cognate B cell interaction for their generation, we wanted to examine whether ex-Tfh cells had participated in the GC reaction before migrating or if they were selected for LN egress at the initial T-B interaction in the interfollicular region. To address this we exploited the KN2xOT-II mouse model in which IL-4 secretion can be monitored for at least 24h by surface expression of hCD2 (236). As seen in paper II, as well as other studies (237, 238), secretion of IL-4 in SLOs is restricted to the Tfh population (though not all Tfh cell produce IL-4). We therefore used hCD2 expression as a retrospective marker of GC history. Tracking of recent emigrant KN2xOT-II cells in TDLs revealed that former IL-4 secreting cells are present among both α4β7^{+} gut-tropic and α4β7^{-} KN2xOT-II cells. Furthermore, within the α4β7^{+} KN2xOT-II compartment in MLN and TDL the surface expression of hCD2 was restricted to the CCR9^{-} cells. Even though CCR9 and hCD2 expression was mutually exclusive in

MLN and TDL hCD2 expression was also clearly evident among CCR9$^+$ KN2xOT-II cells in the SI LP (Figure 14A).

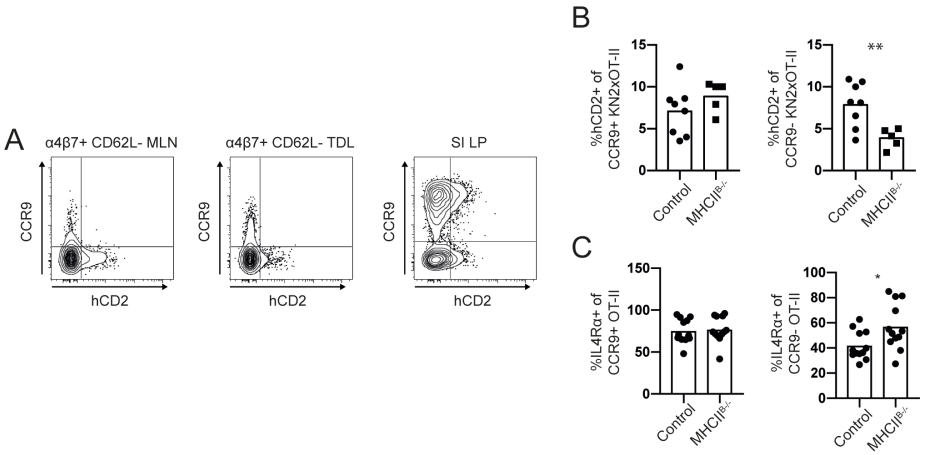

Figure 14. ex-Tfh cells have a history of IL-4 secretion. *(A-C) KN2xOT-II cells were transferred into wildtype (A) or control and MHCII$^{B-/-}$ chimeras (B-C) and analysed for hCD2 and IL4Rα expression day 5 p.i.. (A) Representative flow cytometry plots showing CCR9 versus hCD2 expression on KN2xOT-II cells in MLN, TDL and SI LP. (B-C) Analysis of hCD2 (B) and IL4Rα (C) expression in CCR9$^+$ (left panels) and CCR9$^-$ (right panels) KN2xOT-II cells in SI LP.*

This finding was seemingly confusing and could possibly be explained by either upregulation of CCR9 by hCD2$^+$ cells or that CCR9$^+$ cells start to secrete IL-4 upon arrival into the effector tissue. Other studies using the KN2 model combined with a hCD4 reporter for IL-13 secretion have reported that Th2 effector cells *produce* IL-4 and IL-13 in SLOs but do not *secrete* these cytokines until arrival in the effector tissue (238). Hence, we speculated that hCD2$^+$ CCR9$^+$ KN2xOT-II cells in the SI LP were not ex-Tfh cells but rather an IL-4 producing effector cell generated under Th1-polarizing conditions. In particular, Tfh and IL-4 producing Th2 cells have a close relationship as their development seems to be intertwined although IL-4 competent T cells can be generated in the absence of B cells (224, 279). To distinguish CD4$^+$ T cells that secreted IL-4 in a B cell dependent manner from bona-fide effector cells we adoptively transferred KN2xOT-II cells into control and MHCII$^{B-/-}$ chimeras. Although PD-1 expression was lost at day 5 p.i. in MHCII$^{B-/-}$ chimeras we were able to detect remaining Bcl6 expression identifying cells that once were poised to become Tfh cells. However, only CXCR5$^+$ Bcl6$^+$ cells in control animals showed a history of IL-4 secretion, indicating that pre-Tfh cells do not actively secrete IL-4 before antigen is presented by B cells. When analysing hCD2 expression (IL4 secretion) by KN2xOT-II cells in the SI LP it became clear that the CCR9$^+$ population was unaffected in MHCII$^{B-/-}$ mice whereas

hCD2 expression was drastically reduced among $CCR9^-$ cells (Figure 14B). In line with paper II but not shown in paper III, we also analysed expression of IL4Rα in OT-II cells from SI LP. This analysis revealed that $CCR9^-$ expressed lower levels of IL4Rα compared to $CCR9^+$ cells. This reduced expression was annulled in $MHCII^{B-/-}$ mice (Figure 14C) which further supports that $CCR9^-$ OT-II cells in the SI-LP have interacted with B cells. Collectively, our data show that ex-Tfh cells, by expression of α4β7 and IL4 secretion history, can be traced from the MLN via circulation into the SI LP where they, at least partially, make up the $CCR9^-$ population (Figure 15). If these cells have a particular function in mucosal immune homeostasis, possibly by unique cytokine production or by supporting B cells in isolated lymphoid follicles, merits further research.

Conclusions

- Gut-tropic effector cells are generated at the expense of Tfh cells in the absence of cognate B cell interaction
- Five days p.i. ex-Tfh cells emigrate from the lymph node and some express markers associated with mucosal homing
- Migratory ex-Tfh cells that have interacted with B cells, traced by IL-4 secretion are able to home to the SI LP
- Eight days p.i. ex-Tfh cells in TDL can no longer be identified by CXCR5 and PD-1 expression

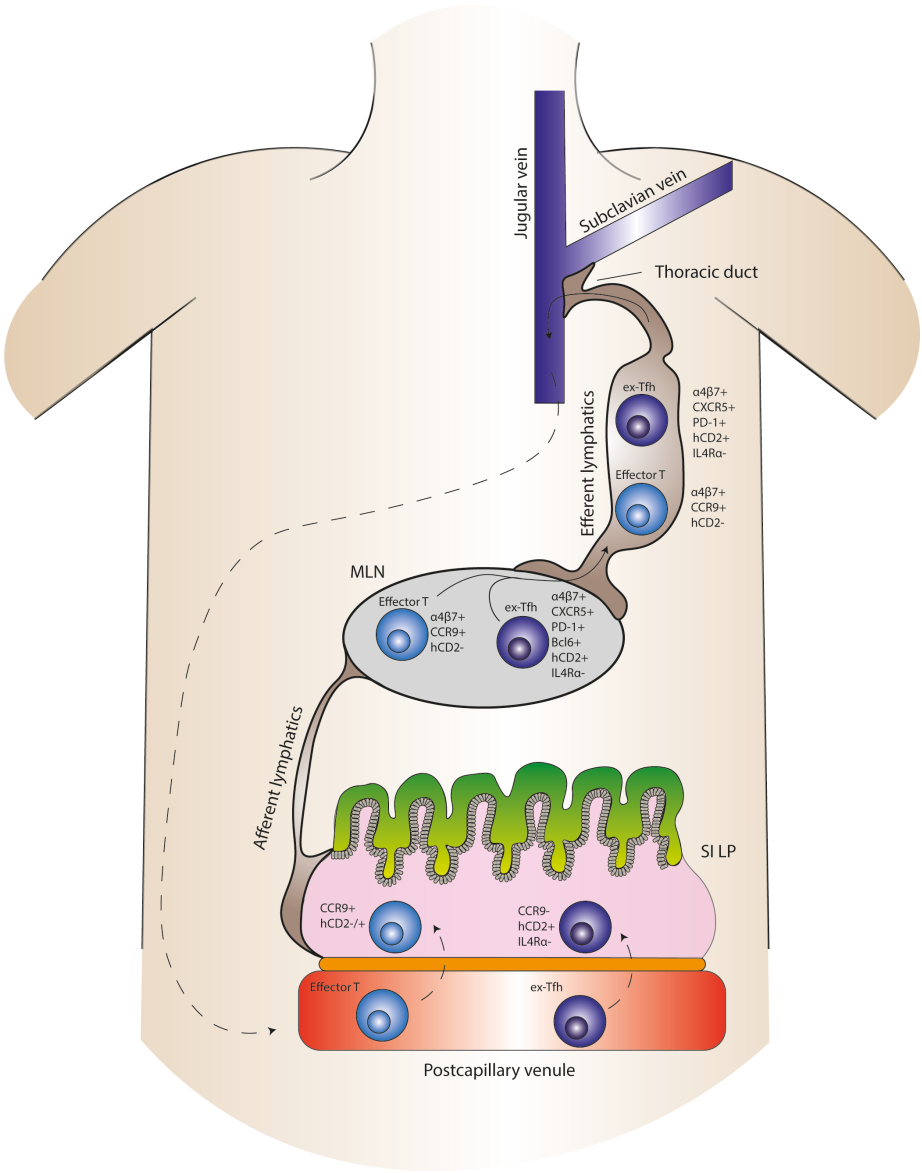

Figure 15. Graphic summary of ex-Tfh and T effector migration

TL:DR: The immune system is complex

Acknowledgement

Finally, the most important and most read part of any thesis, acknowledgements! Throughout the course of my thesis work there have been a lot of people whom, in various ways, have made this journey both enjoyable and memorable. While I´d like to thank all of my co-workers for creating a friendly atmosphere at work, there are some that I´d like to thank in particular.

First and foremost, **Ulf**, my supervisor for taking me on as a PhD student. Your never-ending optimism and enthusiasm for science is contagious which, for a student, is very motivating. I really appreciate that you have been so accessible and taken your time to listen to all of my ideas as well as letting me pursue those ideas experimentally with your guidance. My time as PhD student in your lab have been educational in every aspect thanks to you and your pedagogical ways of explaining things. But…. Ice hockey > Basket

My former supervisor **Bengt**, for sparking my interest in immunology back in Lund. I had no intentions to do a PhD when I started working in your lab, that changed awfully quick. Thanks for spending so much time and energy on me, being fully engaged in my project and introducing me to Ulf. **Madde**, I could not have wished for a better person to teach me how to work in a lab. Always happy and supportive with a patience that knows no boundaries (I know I was a total disaster in the lab when I first started).

My current group members, **Jakob** & **Frank** for bringing so much laughter into the lab every day. **Frank**, thank you so much for setting up all the immunohistochemistry for my project when I´ve been away, I really appreciate it! **Jakob**, my partner in crime and brother in mouse faeces ☺ Without your help with all the animal work my project would look completely different. You have been a great friend and colleague over the years and I´ll really miss working with you every day.

Former group members, **Tobbe** & **Jessica** for taking great care of me when I first started. Previous master students, **Louis** & **Cris**, I´m glad to see that you guys decided to become PhDs students *even though* you had to endure and Jakob and I as your tutors. **Louis**, thanks for taking over the lead on the human project and doing a great job with that part.

The lab ladies **Frida, Madde** and **Annelie E**, for all the candy and good laughs in the lab. I know I´m the worst at tidying up after my late nights in the lab, but hey, you should be glad I´m not DJing the radio ☺.

Maria S for our mutually beneficial snus-for-candy deal, especially when you provide liquorice penguins.

To past and present office mates; **Josefine**, **Jib**, **Sean**, **Astrid**, **Inta**, **Frank** for tolerating my chatting and loud music without too many complaints. **Inta**, thank you so much for all the chocolate that you bring whenever you have been out travelling, Latvian chocolate is niiiiice. **Astrid**, your heaters have a measurable impact on global warming, you should use my hoodie instead ☺

My mental coaches **Lisa** and **Jonas** for all your efforts in making my viral construct work, it was no easy task but we got it done. I´m looking forward to finally get started on the cancer project in your lab.

Patrik, **Filip** and **Marianne** for being helpful and forthcoming in sharing biopsies.

Alex, for sharing your expertise in flow cytometry as well as your help with running qPCRs while I´ve been away.

Karin & **Annelie**, I can´t even remember how many times I´ve come to you guys with no notice and in a hurry to ask for help with mice and other things.

Pia, for the being so helpful and all the work you do at EBM to make our research easier.

To all the great friends from work that I got know over the years; brew master **Johan** & **Lotta** for all things work & after-work related. The pub people from Cancer Centrum, **Mohammed**, **Rebecca** and **Elin** for all the good times we had organizing the pubs.

To my childhood friends up north, **Emmoth, Micke K, Joel, Axel, Sterling, Morin, Erik B**. I know I don´t come to visit you guys as frequently as I should and want to. Whenever we do have the chance to get together it is like I never left, thank you all for your friendship throughout the years.

The people down south whom I got to know during my studies; **Miche**, **Jacke**, **Deas**, **Runesson**, **Teddan, Tim** and **Ässet**. There is never a boring moment with you guys, can´t wait to join you guys for all the festivities during the summer. **Miche**, thanks for letting me sleep at your place numerous times when I had to be in Lund for work. Rock on!

SSK – Robban, **Albin**, **Mats**, **Fejjan** and **Micke J**, the perfect company for reunions and hiking in the wilderness. Remember that time when I almost needed a kidney transplant? Those were the days….

My friends here in Göteborg, **Björn, Madde, Snore, Mats, Krull, Märt, Carlos, Nicke** & **Sofie**. You guys mean the world to me and I´m so glad that I´ve gotten to know you all. I consider myself lucky to have such amazing friends, I really do. **Snore**, **Björn** & **Madde**, you guys have been absolutely fantastic during the final stretch of my PhD, constantly cheering me up when the workload have been almost unbearable at times.

Robert, you are like family to me and I would definitely not be here without you. I´m so thankful for your friendship throughout the years and all the memories we share. True friendship is waking up in the same bed with blood stained sheets or getting fed with Coca-Cola and Djungelvrål when hungover, right?

Cissi for all your support and encouragement, I could not have done this without your help. Thank you for all the good times we shared over the years.

To my **Family**, for your unwavering support. **Mom** & **Dad**, every time I come to visit you it feels like I´m checking into a SPA, please stop helicopter-parenting and start taking care of yourself ☺ **Simon, Isabelle, David & Jennica** for being the best brothers and sisters-in-law one could ask for. I wished I lived closer to all of you because I don´t get to see you as much as I would like to… and **David**, sorry (not sorry) for having my dissertation at the same time as you were supposed to enjoy front-row tickets to Iron Maiden, at least I get to see them perform this summer. Nice!

References

1. Janeway, C. A., Jr., and R. Medzhitov. 2002. Innate immune recognition. *Annu. Rev. Immunol.* 20: 197–216.
2. Chertov, O., D. Yang, O. M. Z. Howard, and J. J. Oppenheim. 2000. Leukocyte granule proteins mobilize innate host defenses and adaptive immune responses. *Immunol. Rev.* 177: 68–78.
3. Underhill, D. M., and A. Ozinsky. 2002. Phagocytosis of Microbes: Complexity in Action. *Annu. Rev. Immunol.* 20: 825–852.
4. Ljunggren, H.-G., and K. Kärre. 1990. In search of the "missing self": MHC molecules and NK cell recognition. *Immunology Today* 11: 237–244.
5. Campbell, K. S., and J. Hasegawa. 2013. Natural killer cell biology: An update and future directions. *Journal of Allergy and Clinical Immunology* 132: 536–544.
6. 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.
7. Weigert, M., R. Perry, D. Kelley, T. Hunkapiller, J. Schilling, and L. Hood. 1980. The joining of V and J gene segments creates antibody diversity. *Nature* 283: 497–499.
8. Seddon, B., P. Tomlinson, and R. Zamoyska. 2003. Interleukin 7 and T cell receptor signals regulate homeostasis of CD4 memory cells. *Nat Immunol* 4: 680–686.
9. Zhang, X., S. Sun, I. Hwang, D. F. Tough, and J. Sprent. 1998. Potent and Selective Stimulation of Memory-Phenotype CD8+ T Cells In Vivo by IL-15. *Immunity* 8: 591–599.
10. Miller, J. F. A. P. 2002. The discovery of thymus function and of thymus-derived lymphocytes. *Immunol. Rev.* 185: 7–14.
11. Mebius, R. E., and G. Kraal. 2005. Structure and function of the spleen. *Nat Rev Immunol* 5: 606–616.
12. Swirski, F. K., M. Nahrendorf, M. Etzrodt, M. Wildgruber, V. Cortez-Retamozo, P. Panizzi, J.-L. Figueiredo, R. H. Kohler, A. Chudnovskiy, P. Waterman, E. Aikawa, T. R. Mempel, P. Libby, R. Weissleder, and M. J. Pittet. 2009. Identification of Splenic Reservoir Monocytes and Their Deployment to Inflammatory Sites. *Science (New York, N.Y.)* 325: 612–616.
13. Cerutti, A., M. Cols, and I. Puga. 2013. Marginal zone B cells: virtues of innate-like antibody-producing lymphocytes. *Nat Rev Immunol* 13: 118–132.
14. Nolte, M. A., J. A. M. Beliën, I. Schadee-Eestermans, W. Jansen, W. W. J. Unger, N. Van Rooijen, G. Kraal, and R. E. Mebius. 2003. A Conduit System Distributes Chemokines and Small Blood-borne Molecules through the Splenic White Pulp. *J Exp Med* 198: 505–512.
15. Mitchell, J. 1973. Lymphocyte circulation in the spleen: Marginal zone bridging channels and their possible role in cell traffic. *Immunology* 24: 93–107.
16. Gretz, J. E., C. C. Norbury, A. O. Anderson, A. E. I. Proudfoot, and S. Shaw. 2000. Lymph-Borne Chemokines and Other Low Molecular Weight Molecules Reach High Endothelial Venules via Specialized Conduits While a Functional Barrier Limits Access to the Lymphocyte Microenvironments in Lymph Node Cortex. *J Exp Med* 192: 1425–1440.
17. Streeter, P. R. 1988. Immunohistologic and functional characterization of a vascular addressin involved in lymphocyte homing into peripheral lymph nodes. *J. Cell Biol.* 107: 1853–1862.
18. Berg, E. L. 1991. The human peripheral lymph node vascular addressin is a ligand for LECAM-1, the peripheral lymph node homing receptor. *J. Cell Biol.* 114: 343–349.
19. Hamann, A., D. Jablonski-Westrich, K. U. Scholz, A. Duijvestijn, E. C. Butcher, and H. G. Thiele. 1988. Regulation of lymphocyte homing. I. Alterations in homing receptor expression and organ-specific high endothelial venule binding of lymphocytes upon activation. *J. Immunol.* 140: 737.
20. Warnock, R. A., S. Askari, E. C. Butcher, and U. H. V. Andrian. 1998. Molecular Mechanisms of Lymphocyte Homing to Peripheral Lymph Nodes. *J Exp Med* 187: 205–216.
21. Campbell, J. J., E. P. Bowman, K. Murphy, K. R. Youngman, M. A. Siani, D. A. Thompson, L. Wu, A. Zlotnik, and E. C. Butcher. 1998. 6-C-kine (SLC), a Lymphocyte Adhesion-triggering Chemokine Expressed by High Endothelium, Is an Agonist for the MIP-3β Receptor CCR7. *J. Cell Biol.* 141: 1053–1059.
22. Berlin, C., R. F. Bargatze, J. J. Campbell, U. H. von Andrian, M. C. Szabo, S. R. Hasslen, R. D. Nelson, E. L. Berg, S. L. Erlandsen, and E. C. Butcher. 1995. α4 integrins mediate lymphocyte attachment and rolling under physiologic flow. *Cell* 80: 413–422.
23. 1993. α4β7 integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. *Cell* 74: 185–195.

24. Streeter, P. R., E. L. Berg, B. T. N. Rouse, R. F. Bargatze, and E. C. Butcher. 1988. A tissue-specific endothelial cell molecule involved in lymphocyte homing. *Nature* 331: 41–46.
25. Berg, E. L., L. M. McEvoy, C. Berlin, R. F. Bargatze, and E. C. Butcher. 1993. L-selectin-mediated lymphocyte rolling on MAdCAM-1. *Nature* 366: 695–698.
26. Neutra, M. R., E. Pringault, and J.-P. Kraehenbuhl. 1996. Antigen sampling across epithelial barriers and induction of mucosal immune responses. *Annu. Rev. Immunol.* 14: 275–300.
27. Neutra, M. R., A. Frey, and J.-P. Kraehenbuhl. 1996. Epithelial M Cells: Gateways for Mucosal Infection and Immunization. *Cell* 86: 345–348.
28. Hopkins, S. A., F. Niedergang, I. E. Corthesy-Theulaz, and J. P. Kraehenbuhl. 2000. A recombinant Salmonella typhimurium vaccine strain is taken up and survives within murine Peyer's patch dendritic cells. *Cell. Microbiol.* 2: 59–68.
29. Kunkel, D., D. Kirchhoff, S.-I. Nishikawa, A. Radbruch, and A. Scheffold. 2003. Visualization of peptide presentation following oral application of antigen in normal and Peyer's patches-deficient mice. *Eur. J. Immunol.* 33: 1292–1301.
30. Hamada, H., T. Hiroi, Y. Nishiyama, H. Takahashi, Y. Masunaga, S. Hachimura, S. Kaminogawa, H. Takahashi-Iwanaga, T. Iwanaga, H. Kiyono, H. Yamamoto, and H. Ishikawa. 2002. Identification of Multiple Isolated Lymphoid Follicles on the Antimesenteric Wall of the Mouse Small Intestine. *The Journal of Immunology* 168: 57–64.
31. Kanamori, Y. 1996. Identification of novel lymphoid tissues in murine intestinal mucosa where clusters of c-kit+ IL-7R+ Thy1+ lympho-hemopoietic progenitors develop. *J Exp Med* 184: 1449–1459.
32. Moghaddami, M., A. Cummins, and G. Mayrhofer. 1998. Lymphocyte-filled villi: Comparison with other lymphoid aggregations in the mucosa of the human small intestine. *Gastroenterology* 115: 1414–1425.
33. Niess, J. H. 2005. CX3CR1-Mediated Dendritic Cell Access to the Intestinal Lumen and Bacterial Clearance. *Science* 307: 254–258.
34. Rescigno, M., M. Urbano, B. Valzasina, M. Francolini, G. Rotta, R. Bonasio, F. Granucci, J.-P. Kraehenbuhl, and P. Ricciardi-Castagnoli. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol* 2: 361 EP –.
35. Jang, M. H., M. N. Kweon, K. Iwatani, M. Yamamoto, K. Terahara, C. Sasakawa, T. Suzuki, T. Nochi, Y. Yokota, P. D. Rennert, T. Hiroi, H. Tamagawa, H. Iijima, J. Kunisawa, Y. Yuki, and H. Kiyono. 2004. Intestinal villous M cells: An antigen entry site in the mucosal epithelium. *Proceedings of the National Academy of Sciences* 101: 6110–6115.
36. Worbs, T., U. Bode, S. Yan, M. W. Hoffmann, G. Hintzen, G. Bernhardt, R. Forster, and O. Pabst. 2006. Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. *J Exp Med* 203: 519–527.
37. Johansson-Lindbom, B., M. Svensson, O. Pabst, C. Palmqvist, G. Marquez, R. Förster, and W. W. Agace. 2005. Functional specialization of gut CD103+ dendritic cells in the regulation of tissue-selective T cell homing. *J Exp Med* 202: 1063–1073.
38. Wurbel, M.-A., J.-M. Philippe, C. Nguyen, G. Victorero, T. Freeman, P. Wooding, A. Miazek, M.-G. Mattei, M. Malissen, B. R. Jordan, B. Malissen, A. Carrier, and P. Naquet. 2000. The chemokine TECK is expressed by thymic and intestinal epithelial cells and attracts double- and single-positive thymocytes expressing the TECK receptor CCR9. *Eur. J. Immunol.* 30: 262–271.
39. Papadakis, K. A., J. Prehn, V. Nelson, L. Cheng, S. W. Binder, P. D. Ponath, D. P. Andrew, and S. R. Targan. 2000. The Role of Thymus-Expressed Chemokine and Its Receptor CCR9 on Lymphocytes in the Regional Specialization of the Mucosal Immune System. *The Journal of Immunology* 165: 5069–5076.
40. Hieshima, K., Y. Kawasaki, H. Hanamoto, T. Nakayama, D. Nagakubo, A. Kanamaru, and O. Yoshie. 2004. CC Chemokine Ligands 25 and 28 Play Essential Roles in Intestinal Extravasation of IgA Antibody-Secreting Cells. *The Journal of Immunology* 173: 3668–3675.
41. Stenstad, H., M. Svensson, H. Cucak, K. Kotarsky, and W. W. Agace. 2007. Differential homing mechanisms regulate regionalized effector CD8αβ(+) T cell accumulation within the small intestine. *Proc Natl Acad Sci U S A* 104: 10122–10127.
42. Stenstad, H. 2006. Gut-associated lymphoid tissue-primed CD4+ T cells display CCR9-dependent and -independent homing to the small intestine. *Blood* 107: 3447–3454.
43. Steinman, R. M., and Z. A. Cohn. 1973. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J Exp Med* 137: 1142–1162.
44. Steinman, R. M. 1974. IDENTIFICATION OF A NOVEL CELL TYPE IN PERIPHERAL LYMPHOID ORGANS OF MICE: II. FUNCTIONAL PROPERTIES IN VITRO. *J Exp Med* 139: 380–397.

45. Steinman, R. M., D. S. Lustig, and Z. A. Cohn. 1974. Identification of a novel cell type in peripheral lymphoid organs of mice. 3. Functional properties in vivo. *J Exp Med* 139: 1431–1445.
46. Nussenzweig, M. C. 1980. Dendritic cells are accessory cells for the development of anti-trinitrophenyl cytotoxic T lymphocytes. *J Exp Med* 152: 1070–1084.
47. Reis e Sousa, C. 2004. Activation of dendritic cells: translating innate into adaptive immunity. *Curr Opin Immunol* 16: 21–25.
48. Itano, A. A., and M. K. Jenkins. 2003. Antigen presentation to naive CD4 T cells in the lymph node. *Nat Immunol* 4: 733–739.
49. Huang, F. P., N. Platt, M. Wykes, J. R. Major, T. J. Powell, C. D. Jenkins, and G. G. MacPherson. 2000. A discrete subpopulation of dendritic cells transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. *J Exp Med* 191: 435–444.
50. Turnbull, E. L., U. Yrlid, C. D. Jenkins, and G. G. MacPherson. 2005. Intestinal dendritic cell subsets: differential effects of systemic TLR4 stimulation on migratory fate and activation in vivo. *J. Immunol.* 174: 1374–1384.
51. Merad, M., P. Sathe, J. Helft, J. Miller, and A. Mortha. 2013. The Dendritic Cell Lineage: Ontogeny and Function of Dendritic Cells and Their Subsets in the Steady State and the Inflamed Setting. *Annu. Rev. Immunol.* 31: 563–604.
52. Banchereau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature* 392: 245–252.
53. Jaensson, E., H. Uronen-Hansson, O. Pabst, B. Eksteen, J. Tian, J. L. Coombes, P.-L. Berg, T. Davidsson, F. Powrie, B. Johansson-Lindbom, and W. W. Agace. 2008. Small intestinal CD103+ dendritic cells display unique functional properties that are conserved between mice and humans. *Journal of Experimental Medicine* 205: 2139–2149.
54. cerovic, V., S. A. Houston, C. L. Scott, A. Aumeunier, U. Yrlid, A. M. Mowat, and S. W. F. Milling. 2013. Intestinal CD103(-) dendritic cells migrate in lymph and prime effector T cells. *mucosal Immunology* 6: 104–113.
55. Haan, den, J. M., S. M. Lehar, and M. J. Bevan. 2000. CD8(+) but not CD8(-) dendritic cells cross-prime cytotoxic T cells in vivo. *J Exp Med* 192: 1685–1696.
56. Pooley, J. L., W. R. Heath, and K. Shortman. 2001. Cutting Edge: Intravenous Soluble Antigen Is Presented to CD4 T Cells by CD8- Dendritic Cells, but Cross-Presented to CD8 T Cells by CD8+ Dendritic Cells. *The Journal of Immunology* 166: 5327–5330.
57. Kamphorst, A. O., P. Guermonprez, D. Dudziak, and M. C. Nussenzweig. 2010. Route of Antigen Uptake Differentially Impacts Presentation by Dendritic Cells and Activated Monocytes. *The Journal of Immunology* 185: 3426–3435.
58. Watchmaker, P. B., K. Lahl, M. Lee, D. Baumjohann, J. Morton, S. J. Kim, R. Zeng, A. Dent, K. M. Ansel, B. Diamond, H. Hadeiba, and E. C. Butcher. 2013. Comparative transcriptional and functional profiling defines conserved programs of intestinal DC differentiation in humans and mice. *Nat Immunol* 15: 98–108.
59. 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.
60. Satpathy, A. T., W. KC, J. C. Albring, B. T. Edelson, N. M. Kretzer, D. Bhattacharya, T. L. Murphy, and K. M. Murphy. 2012. Zbtb46 expression distinguishes classical dendritic cells and their committed progenitors from other immune lineages. *J Exp Med* 209: 1135–1152.
61. Edelson, B. T., W. KC, R. Juang, M. Kohyama, L. A. Benoit, P. A. Klekotka, C. Moon, J. C. Albring, W. Ise, D. G. Michael, D. Bhattacharya, T. S. Stappenbeck, M. J. Holtzman, S.-S. J. Sung, T. L. Murphy, K. Hildner, and K. M. Murphy. 2010. Peripheral CD103+ dendritic cells form a unified subset developmentally related to CD8alpha+ conventional dendritic cells. *Journal of Experimental Medicine* 207: 823–836.
62. Tussiwand, R., W.-L. Lee, T. L. Murphy, M. Mashayekhi, W. KC, J. C. Albring, A. T. Satpathy, J. A. Rotondo, B. T. Edelson, N. M. Kretzer, X. Wu, L. A. Weiss, E. Glasmacher, P. Li, W. Liao, M. Behnke, S. S. K. Lam, C. T. Aurthur, W. J. Leonard, H. Singh, C. L. Stallings, L. D. Sibley, R. D. Schreiber, and K. M. Murphy. 2012. Compensatory dendritic cell development mediated by BATF-IRF interactions. *Nature*.
63. Aliberti, J. 2002. Essential role for ICSBP in the in vivo development of murine CD8alpha + dendritic cells. *Blood* 101: 305–310.
64. Luda, K. M., T. Joeris, E. K. Persson, A. Rivollier, M. Demiri, K. M. Sitnik, L. Pool, J. B. Holm, F. Melo-Gonzalez, L. Richter, B. N. Lambrecht, K. Kristiansen, M. A. Travis, M. Svensson-Frej, K.

Kotarsky, and W. W. Agace. 2016. IRF8 Transcription-Factor-Dependent Classical Dendritic Cells Are Essential for Intestinal T Cell Homeostasis. *Immunity* 44: 860–874.
65. Lewis, K. L., M. L. Caton, M. Bogunovic, M. Greter, L. T. Grajkowska, D. Ng, A. Klinakis, I. F. Charo, S. Jung, J. L. Gommerman, I. I. Ivanov, K. Liu, M. Merad, and B. Reizis. 2011. Notch2 receptor signaling controls functional differentiation of dendritic cells in the spleen and intestine. *Immunity* 35: 780–791.
66. Persson, E. K., H. Uronen-Hansson, M. Semmrich, A. Rivollier, K. Hägerbrand, J. Marsal, S. Gudjonsson, U. Håkansson, B. Reizis, K. Kotarsky, and W. W. Agace. 2013. IRF4 transcription-factor-dependent CD103(+)CD11b(+) dendritic cells drive mucosal T helper 17 cell differentiation. *Immunity* 38: 958–969.
67. Sapoznikov, A., J. A. Fischer, T. Zaft, R. Krauthgamer, A. Dzionek, and S. Jung. 2007. Organ-dependent in vivo priming of naive CD4+,but not CD8+,T cells by plasmacytoid dendritic cells. *J Exp Med*.
68. Yrlid, U., V. Cerovic, S. Milling, C. D. Jenkins, J. Zhang, P. R. Crocker, L. S. Klavinskis, and G. G. MacPherson. 2006. Plasmacytoid dendritic cells do not migrate in intestinal or hepatic lymph. *J. Immunol.* 177: 6115–6121.
69. Dzionek, A., Y. Sohma, J. Nagafune, M. Cella, M. Colonna, F. Facchetti, G. Günther, I. Johnston, A. Lanzavecchia, T. Nagasaka, T. Okada, W. Vermi, G. Winkels, T. Yamamoto, M. Zysk, Y. Yamaguchi, and J. Schmitz. 2001. BDCA-2, a Novel Plasmacytoid Dendritic Cell–specific Type II C-type Lectin, Mediates Antigen Capture and Is a Potent Inhibitor of Interferon α/β Induction. *J Exp Med* 194: 1823–1834.
70. Meyer-Wentrup, F., D. Benitez-Ribas, P. J. Tacken, C. J. A. Punt, C. G. Figdor, I. J. M. de Vries, and G. J. Adema. 2008. Targeting DCIR on human plasmacytoid dendritic cells results in antigen presentation and inhibits IFN- production. *Blood* 111: 4245–4253.
71. Asselin-Paturel, C., A. Boonstra, M. Dalod, I. Durand, N. Yessaad, C. Dezutter-Dambuyant, A. Vicari, A. O'Garra, C. Biron, F. Briere, and G. Trinchieri. 2001. Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology. *Nat Immunol* 2: 1144–1150.
72. Chorro, L., A. Sarde, M. Li, K. J. Woollard, P. Chambon, B. Malissen, A. Kissenpfennig, J. B. Barbaroux, R. Groves, and F. Geissmann. 2009. Langerhans cell (LC) proliferation mediates neonatal development, homeostasis, and inflammation-associated expansion of the epidermal LC network. *J Exp Med* 206: 3089–3100.
73. Schraml, B. U., J. van Blijswijk, S. Zelenay, P. G. Whitney, A. Filby, S. E. Acton, N. C. Rogers, N. Moncaut, J. J. Carvajal, and C. Reis e Sousa. 2013. Genetic Tracing via DNGR-1 Expression History Defines Dendritic Cells as a Hematopoietic Lineage. *Cell* 154: 843–858.
74. Rescigno, M., M. Urbano, B. Valzasina, M. Francolini, G. Rotta, R. Bonasio, F. Granucci, J. P. Kraehenbuhl, and P. Ricciardi-Castagnoli. 2001. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol* 2: 361–367.
75. Schulz, O., E. Jaensson, E. K. Persson, X. Liu, T. Worbs, W. W. Agace, and O. Pabst. 2009. Intestinal CD103+, but not CX3CR1+, antigen sampling cells migrate in lymph and serve classical dendritic cell functions. *Journal of Experimental Medicine* 206: 3101–3114.
76. Bain, C. C., C. L. Scott, H. Uronen-Hansson, S. Gudjonsson, O. Jansson, O. Grip, M. Guilliams, B. Malissen, W. W. Agace, and A. M. Mowat. 2012. mi201289a. *mucosal Immunology* 6: 498–510.
77. Dunay, I. R., R. A. Damatta, B. Fux, R. Presti, S. Greco, M. Colonna, and L. D. Sibley. 2008. Gr1(+) inflammatory monocytes are required for mucosal resistance to the pathogen Toxoplasma gondii. *Immunity* 29: 306–317.
78. Tamoutounour, S., S. Henri, H. Lelouard, B. de Bovis, C. de Haar, C. J. van der Woude, A. M. Woltman, Y. Reyal, D. Bonnet, D. Sichien, C. C. Bain, A. M. Mowat, C. Reis e Sousa, L. F. Poulin, B. Malissen, and M. Guilliams. 2012. CD64 distinguishes macrophages from dendritic cells in the gut and reveals the Th1-inducing role of mesenteric lymph node macrophages during colitis. *Eur. J. Immunol.* 42: 3150–3166.
79. Satpathy, A. T., C. G. Briseño, J. S. Lee, D. Ng, N. A. Manieri, W. KC, X. Wu, S. R. Thomas, W.-L. Lee, M. Turkoz, K. G. McDonald, M. M. Meredith, C. Song, C. J. Guidos, R. D. Newberry, W. Ouyang, T. L. Murphy, T. S. Stappenbeck, J. L. Gommerman, M. C. Nussenzweig, M. Colonna, R. Kopan, and K. M. Murphy. 2013. Notch2-dependent classical dendritic cells orchestrate intestinal immunity to attaching-and-effacing bacterial pathogens. *Nat Immunol* 14: 937–948.
80. Chakarov, S., and N. Fazilleau. 2014. Monocyte-derived dendritic cells promote T follicular helper cell differentiation. *EMBO Mol Med* n/a–n/a.

81. Jung, S., D. Unutmaz, P. Wong, G.-I. Sano, K. De los Santos, T. Sparwasser, S. Wu, S. Vuthoori, K. Ko, F. Zavala, E. G. Pamer, D. R. Littman, and R. A. Lang. 2002. In vivo depletion of CD11c+ dendritic cells abrogates priming of CD8+ T cells by exogenous cell-associated antigens. *Immunity* 17: 211–220.
82. Blum, J. S., P. A. Wearsch, and P. Cresswell. 2013. Pathways of Antigen Processing. *Annu. Rev. Immunol.* 31: 443–473.
83. Roche, P. A., and P. Cresswell. 1990. Invariant chain association with HLA-DR molecules inhibits immunogenic peptide binding. *Nature* 345: 615–618.
84. Neefjes, J. 1999. CIIV, MIIC and other compartments for MHC class II loading. *Eur. J. Immunol.* 29: 1421–1425.
85. Sallusto, F. 1995. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. *J Exp Med* 182: 389–400.
86. West, M. A., R. P. Wallin, S. P. Matthews, H. G. Svensson, R. Zaru, H. G. Ljunggren, A. R. Prescott, and C. Watts. 2004. Enhanced dendritic cell antigen capture via toll-like receptor-induced actin remodeling. *Science* 305: 1153–1157.
87. Garrett, W. S., L.-M. Chen, R. Kroschewski, M. Ebersold, S. Turley, S. Trombetta, J. E. Galán, and I. Mellman. 2000. Developmental Control of Endocytosis in Dendritic Cells by Cdc42. *Cell* 102: 325–334.
88. Bonifaz, L. C., D. P. Bonnyay, A. Charalambous, D. I. Darguste, S. Fujii, H. Soares, M. K. Brimnes, B. Moltedo, T. M. Moran, and R. M. Steinman. 2004. In vivo targeting of antigens to maturing dendritic cells via the DEC-205 receptor improves T cell vaccination. *J Exp Med* 199: 815–824.
89. 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.
90. Stuart, L. M., and R. A. B. Ezekowitz. 2005. Phagocytosis. *Immunity* 22: 539–550.
91. Steinman, R. M., K. Inaba, S. Turley, P. Pierre, and I. Mellman. 1999. Antigen capture, processing, and presentation by dendritic cells: recent cell biological studies. *Hum. Immunol.* 60: 562–567.
92. Mellman, I. 1998. Antigen processing for amateurs and professionals. *Trends in Cell Biology* 8: 231–237.
93. Crotzer, V. L., and J. S. Blum. 2010. Autophagy and adaptive immunity. *Immunology* 451: no–no.
94. Schmid, D., M. Pypaert, and C. Münz. 2007. Antigen-Loading Compartments for Major Histocompatibility Complex Class II Molecules Continuously Receive Input from Autophagosomes. *Immunity* 26: 79–92.
95. Nedjic, J., M. Aichinger, J. Emmerich, N. Mizushima, and L. Klein. 2008. Autophagy in thymic epithelium shapes the T-cell repertoire and is essential for tolerance. *Nature* 455: 396–400.
96. Aichinger, M., C. Wu, J. Nedjic, and L. Klein. 2013. Macroautophagy substrates are loaded onto MHC class II of medullary thymic epithelial cells for central tolerance. *J Exp Med* 210: 287–300.
97. Inaba, K. 1990. Dendritic cells pulsed with protein antigens in vitro can prime antigen- specific, MHC-restricted T cells in situ [published erratum appears in J Exp Med 1990 Oct 1;172(4):1275]. *J Exp Med* 172: 631–640.
98. Mayordomo, J. I., T. Zorina, W. J. Storkus, L. Zitvogel, C. Celluzzi, L. D. Falo, C. J. Melief, S. T. Ildstad, W. Martin Kast, A. B. Deleo, and M. T. Lotze. 1995. Bone marrow-derived dendritic cells pulsed with synthetic tumour peptides elicit protective and therapeutic antitumour immunity. *Nat Med* 1: 1297–1302.
99. Schwartz, R. 1990. A cell culture model for T lymphocyte clonal anergy. *Science* 248: 1349–1356.
100. Longhi, M. P., C. Trumpfheller, J. Idoyaga, M. Caskey, I. Matos, C. Kluger, A. M. Salazar, M. Colonna, and R. M. Steinman. 2009. Dendritic cells require a systemic type I interferon response to mature and induce CD4+ Th1 immunity with poly IC as adjuvant. *Journal of Experimental Medicine* 206: 1589–1602.
101. De Smedt, T. 1996. Regulation of dendritic cell numbers and maturation by lipopolysaccharide in vivo. *J Exp Med* 184: 1413–1424.
102. Shah, J. A., P. A. Darrah, D. R. Ambrozak, T. N. Turon, S. Mendez, J. Kirman, C.-Y. Wu, N. Glaichenhaus, and R. A. Seder. 2003. Dendritic Cells Are Responsible for the Capacity of CpG Oligodeoxynucleotides to Act as an Adjuvant for Protective Vaccine Immunity Against Leishmania majorin Mice. *J Exp Med* 198: 281–291.
103. Jenkins, M. K., P. S. Taylor, S. D. Norton, and K. B. Urdahl. 1991. CD28 delivers a costimulatory signal involved in antigen-specific IL-2 production by human T cells. *J. Immunol.* 147: 2461.
104. Norton, S. D., L. Zuckerman, K. B. Urdahl, R. Shefner, J. Miller, and M. K. Jenkins. 1992. The CD28 ligand, B7, enhances IL-2 production by providing a costimulatory signal to T cells. *J. Immunol.* 149: 1556.

105. Roncarolo, M.-G., M. K. Levings, and C. Traversari. 2001. Differentiation of T Regulatory Cells by Immature Dendritic Cells: Figure 1. *J Exp Med* 193: F5–F10.
106. Jonuleit, H., E. Schmitt, K. Steinbrink, and A. H. Enk. 2001. Dendritic cells as a tool to induce anergic and regulatory T cells. *Trends in Immunology* 22: 394–400.
107. Freeman, G. J., A. J. Long, Y. Iwai, K. Bourque, T. Chernova, H. Nishimura, L. J. Fitz, N. Malenkovich, T. Okazaki, M. C. Byrne, H. F. Horton, L. Fouser, L. Carter, V. Ling, M. R. Bowman, B. M. Carreno, M. Collins, C. R. Wood, and T. Honjo. 2000. Engagement of the Pd-1 Immunoinhibitory Receptor by a Novel B7 Family Member Leads to Negative Regulation of Lymphocyte Activation. *J Exp Med* 192: 1027–1034.
108. Dong, H., G. Zhu, K. Tamada, and L. Chen. 1999. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nat Med* 5: 1365–1369.
109. Russell, J. H., and T. J. Ley. 2002. Lymphocyte-mediated cytotoxicity. *Annu. Rev. Immunol.* 20: 323–370.
110. Willerford, D. M., W. Swat, and F. W. Alt. 1996. Developmental regulation of V(D)J recombination and lymphocyte differentiation. *Current Opinion in Genetics & Development* 6: 603–609.
111. Benedict, C. L., S. Gilfillan, T.-H. Thai, and J. F. Kearney. 2000. Terminal deoxynucleotidyl transferase and repertoire development. *Immunol. Rev.* 175: 150–157.
112. Starr, T. K., S. C. Jameson, and K. A. Hogquist. 2003. Positive and negative selection of T cells. *Annu. Rev. Immunol.* 21: 139–176.
113. Mosmann, T. R., H. Cherwinski, M. W. Bond, M. A. Giedlin, and R. L. Coffman. 1986. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* 136: 2348.
114. Lighvani, A. A., D. M. Frucht, D. Jankovic, H. Yamane, J. Aliberti, B. D. Hissong, B. V. Nguyen, M. Gadina, A. Sher, W. E. Paul, and J. J. O'Shea. 2001. T-bet is rapidly induced by interferon- in lymphoid and myeloid cells. *Proceedings of the National Academy of Sciences* 98: 15137–15142.
115. Afkarian, M., J. R. Sedy, J. Yang, N. G. Jacobson, N. Cereb, S. Y. Yang, T. L. Murphy, and K. M. Murphy. 2002. T-bet is a STAT1-induced regulator of IL-12R expression in naïve CD4+ T cells. *Nat Immunol* 3: 549–557.
116. Thierfelder, W. E., J. M. van Deursen, K. Yamamoto, R. A. Tripp, S. R. Sarawar, R. T. Carson, M. Y. Sangster, D. A. A. Vignali, P. C. Doherty, G. C. Grosveld, and J. N. Ihle. 1996. Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells. *Nature* 382: 171–174.
117. Kaplan, M. H., Y.-L. Sun, T. Hoey, and M. J. Grusby. 1996. Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. *Nature* 382: 174–177.
118. Cai, G., T. Radzanowski, E. N. Villegas, R. Kastelein, and C. A. Hunter. 2000. Identification of STAT4-Dependent and Independent Mechanisms of Resistance to Toxoplasma gondii. *The Journal of Immunology* 165: 2619–2627.
119. Hsieh, C. S., S. E. Macatonia, C. S. Tripp, S. F. Wolf, A. O'Garra, and K. M. Murphy. 1993. Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. *Science* 260: 547–549.
120. Takeda, A., S. Hamano, A. Yamanaka, T. Hanada, T. Ishibashi, T. W. Mak, A. Yoshimura, and H. Yoshida. 2003. Cutting Edge: Role of IL-27/WSX-1 Signaling for Induction of T-Bet Through Activation of STAT1 During Initial Th1 Commitment. *The Journal of Immunology* 170: 4886–4890.
121. Yoshida, H., S. Hamano, G. Senaldi, T. Covey, R. Faggioni, S. Mu, M. Xia, A. C. Wakeham, H. Nishina, J. Potter, C. J. M. Saris, and T. W. Mak. 2001. WSX-1 Is Required for the Initiation of Th1 Responses and Resistance to L. major Infection. *Immunity* 15: 569–578.
122. Takeda, K., H. Tsutsui, T. Yoshimoto, O. Adachi, N. Yoshida, T. Kishimoto, H. Okamura, K. Nakanishi, and S. Akira. 1998. Defective NK Cell Activity and Th1 Response in IL-18–Deficient Mice. *Immunity* 8: 383–390.
123. Yoshimoto, T., K. Takeda, T. Tanaka, K. Ohkusu, S.-I. Kashiwamura, H. Okamura, S. Akira, and K. Nakanishi. 1998. IL-12 Up-Regulates IL-18 Receptor Expression on T Cells, Th1 Cells, and B Cells: Synergism with IL-18 for IFN-γ Production. *J. Immunol.* 161: 3400.
124. Zheng, W.-P., and R. A. Flavell. 1997. The Transcription Factor GATA-3 Is Necessary and Sufficient for Th2 Cytokine Gene Expression in CD4 T Cells. *Cell* 89: 587–596.
125. Zhang, D.-H., L. Cohn, P. Ray, K. Bottomly, and A. Ray. 1997. Transcription Factor GATA-3 Is Differentially Expressed in Murine Th1 and Th2 Cells and Controls Th2-specific Expression of the Interleukin-5 Gene. *Journal of Biological Chemistry* 272: 21597–21603.
126. Zhu, J., B. Min, J. Hu-Li, C. J. Watson, A. Grinberg, Q. Wang, N. Killeen, J. F. Urban, L. Guo, and W. E. Paul. 2004. Conditional deletion of Gata3 shows its essential function in TH1-TH2 responses. *Nat Immunol* 5: 1157–1165.

127. Pai, S.-Y., M. L. Truitt, and I.-C. Ho. 2004. GATA-3 deficiency abrogates the development and maintenance of T helper type 2 cells. *Proceedings of the National Academy of Sciences* 101: 1993–1998.
128. Cote-Sierra, J., G. Foucras, L. Guo, L. Chiodetti, H. A. Young, J. Hu-Li, J. Zhu, and W. E. Paul. 2004. Interleukin 2 plays a central role in Th2 differentiation. *Proceedings of the National Academy of Sciences* 101: 3880–3885.
129. Zhu, J., J. Cote-Sierra, L. Guo, and W. E. Paul. 2003. Stat5 Activation Plays a Critical Role in Th2 Differentiation. *Immunity* 19: 739–748.
130. Tanaka, S., Y. Motomura, Y. Suzuki, R. Yagi, H. Inoue, S. Miyatake, and M. Kubo. 2010. The enhancer HS2 critically regulates GATA-3-mediated Il4 transcription in TH2 cells. *Nat Immunol* 12: 77–85.
131. Liao, W., D. E. Schones, J. Oh, Y. Cui, K. Cui, T.-Y. Roh, K. Zhao, and W. J. Leonard. 2008. Priming for T helper type 2 differentiation by interleukin 2–mediated induction of interleukin 4 receptor α-chain expression. *Nat Immunol* 9: 1288–1296.
132. Kaplan, M. H., U. Schindler, S. T. Smiley, and M. J. Grusby. 1996. Stat6 Is Required for Mediating Responses to IL-4 and for the Development of Th2 Cells. *Immunity* 4: 313–319.
133. Takeda, K., T. Tanaka, W. Shi, M. Matsumoto, M. Minami, S. Kashiwamura, K. Nakanishi, N. Yoshida, T. Kishimoto, and S. Akira. 1996. Essential role of Stat6 in IL-4 signalling. *Nature* 380: 627–630.
134. Shimoda, K., J. van Deursen, M. Y. Sangster, S. R. Sarawar, R. T. Carson, R. A. Tripp, C. Chu, F. W. Quelle, T. Nosaka, D. A. Vignali, Doherty, P. C., G. Grosveld, W. E. Paul, and J. N. Ihle. 1996. Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. *Nature* 380: 630–633.
135. Else, K. J. 1994. Cytokine-mediated regulation of chronic intestinal helminth infection. *J Exp Med* 179: 347–351.
136. van Panhuys, N., S.-C. Tang, M. Prout, M. Camberis, D. Scarlett, J. Roberts, J. Hu-Li, W. E. Paul, and G. Le Gros. 2008. In vivo studies fail to reveal a role for IL-4 or STAT6 signaling in Th2 lymphocyte differentiation. *Proc Natl Acad Sci U S A* 105: 12423–12428.
137. Jankovic, D., M. C. Kullberg, N. Noben-Trauth, P. Caspar, W. E. Paul, and A. Sher. 2000. Single Cell Analysis Reveals That IL-4 Receptor/Stat6 Signaling Is Not Required for the In Vivo or In Vitro Development of CD4+ Lymphocytes with a Th2 Cytokine Profile. *The Journal of Immunology* 164: 3047–3055.
138. Finkelman, F. D., S. C. Morris, T. Orekhova, M. Mori, D. Donaldson, S. L. Reiner, N. L. Reilly, L. Schopf, and J. F. Urban. 2000. Stat6 Regulation of In Vivo IL-4 Responses. *The Journal of Immunology* 164: 2303–2310.
139. Walker, J. A., and A. N. J. McKenzie. 2017. TH2 cell development and function. *Nat Rev Immunol* 18: 121–133.
140. Infante-Duarte, C., H. F. Horton, M. C. Byrne, and T. Kamradt. 2000. Microbial Lipopeptides Induce the Production of IL-17 in Th Cells. *The Journal of Immunology* 165: 6107–6115.
141. Nurieva, R. I., P. Treuting, J. Duong, R. A. Flavell, and C. Dong. 2003. Inducible costimulator is essential for collagen-induced arthritis. *Journal of Clinical Investigation* 111: 701–706.
142. Murphy, C. A., C. L. Langrish, Y. Chen, W. Blumenschein, T. McClanahan, R. A. Kastelein, J. D. Sedgwick, and D. J. Cua. 2003. Divergent Pro- and Antiinflammatory Roles for IL-23 and IL-12 in Joint Autoimmune Inflammation. *J Exp Med* 198: 1951–1957.
143. Cua, D. J., J. Sherlock, Y. Chen, C. A. Murphy, B. Joyce, B. Seymour, L. Lucian, W. To, S. Kwan, T. Churakova, S. Zurawski, M. Wiekowski, S. A. Lira, D. Gorman, R. A. Kastelein, and J. D. Sedgwick. 2003. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* 421: 744–748.
144. Park, H., Z. Li, X. O. Yang, S. H. Chang, R. Nurieva, Y.-H. Wang, Y. Wang, L. Hood, Z. Zhu, Q. Tian, and C. Dong. 2005. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* 6: 1133–1141.
145. Harrington, L. E., R. D. Hatton, P. R. Mangan, H. Turner, T. L. Murphy, K. M. Murphy, and C. T. Weaver. 2005. Interleukin 17–producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 6: 1123–1132.
146. Langrish, C. L., Y. Chen, W. M. Blumenschein, J. Mattson, B. Basham, J. D. Sedgwick, T. McClanahan, R. A. Kastelein, and D. J. Cua. 2005. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med* 201: 233–240.
147. Veldhoen, M., R. J. Hocking, C. J. Atkins, R. M. Locksley, and B. Stockinger. 2006. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* 24: 179–189.

148. Bettelli, E., Y. Carrier, W. Gao, T. Korn, T. B. Strom, M. Oukka, H. L. Weiner, and V. K. Kuchroo. 2006. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 441: 235–238.
149. Mangan, P. R., L. E. Harrington, D. B. O'Quinn, W. S. Helms, D. C. Bullard, C. O. Elson, R. D. Hatton, S. M. Wahl, T. R. Schoeb, and C. T. Weaver. 2006. Transforming growth factor-β induces development of the TH17 lineage. *Nature* 441: 231–234.
150. Ivanov, I. I., B. S. McKenzie, L. Zhou, C. E. Tadokoro, A. Lepelley, J. J. Lafaille, D. J. Cua, and D. R. Littman. 2006. The Orphan Nuclear Receptor RORγt Directs the Differentiation Program of Proinflammatory IL-17+ T Helper Cells. *Cell* 126: 1121–1133.
151. Qin, H., L. Wang, T. Feng, C. O. Elson, S. A. Niyongere, S. J. Lee, S. L. Reynolds, C. T. Weaver, K. Roarty, R. Serra, E. N. Benveniste, and Y. Cong. 2009. TGF- Promotes Th17 Cell Development through Inhibition of SOCS3. *The Journal of Immunology* 183: 97–105.
152. Yang, X. O., A. D. Panopoulos, R. Nurieva, S. H. Chang, D. Wang, S. S. Watowich, and C. Dong. 2007. STAT3 Regulates Cytokine-mediated Generation of Inflammatory Helper T Cells. *Journal of Biological Chemistry* 282: 9358–9363.
153. Laurence, A., C. M. Tato, T. S. Davidson, Y. Kanno, Z. Chen, Z. Yao, R. B. Blank, F. Meylan, R. Siegel, L. Hennighausen, E. M. Shevach, and J. J. O'Shea. 2007. Interleukin-2 Signaling via STAT5 Constrains T Helper 17 Cell Generation. *Immunity* 26: 371–381.
154. Korn, T., E. Bettelli, W. Gao, A. Awasthi, A. Jäger, T. B. Strom, M. Oukka, and V. K. Kuchroo. 2007. IL-21 initiates an alternative pathway to induce proinflammatory TH17 cells. *Nature* 448: 484–487.
155. Zhou, L., I. I. Ivanov, R. Spolski, R. Min, K. Shenderov, T. Egawa, D. E. Levy, W. J. Leonard, and D. R. Littman. 2007. IL-6 programs TH-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol* 8: 967–974.
156. Nurieva, R., X. O. Yang, G. Martinez, Y. Zhang, A. D. Panopoulos, L. Ma, K. Schluns, Q. Tian, S. S. Watowich, A. M. Jetten, and C. Dong. 2007. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. *Nature* 448: 480–483.
157. Zelante, T., R. Iannitti, A. De Luca, and L. Romani. 2011. IL-22 in antifungal immunity. *Eur. J. Immunol.* 41: 270–275.
158. Wolk, K., S. Kunz, E. Witte, M. Friedrich, K. Asadullah, and R. Sabat. 2004. IL-22 Increases the Innate Immunity of Tissues. *Immunity* 21: 241–254.
159. Ivanov, I. I., K. Atarashi, N. Manel, E. L. Brodie, T. Shima, U. Karaoz, D. Wei, K. C. Goldfarb, C. A. Santee, S. V. Lynch, T. Tanoue, A. Imaoka, K. Itoh, K. Takeda, Y. Umesaki, K. Honda, and D. R. Littman. 2009. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 139: 485–498.
160. Wu, H.-J., I. I. Ivanov, J. Darce, K. Hattori, T. Shima, Y. Umesaki, D. R. Littman, C. Benoist, and D. Mathis. 2010. Gut-Residing Segmented Filamentous Bacteria Drive Autoimmune Arthritis via T Helper 17 Cells. *Immunity* 32: 815–827.
161. Lanzavecchia, A. 1985. Antigen-specific interaction between T and B cells. *Nature* 314: 537–539.
162. Forster, R., T. Emrich, E. Kremmer, and M. Lipp. 1994. Expression of the G-protein--coupled receptor BLR1 defines mature, recirculating B cells and a subset of T-helper memory cells. *Blood* 84: 830–840.
163. Gunn, M. D., V. N. Ngo, K. M. Ansel, E. H. Ekland, J. G. Cyster, and L. T. Williams. 1998. A B-cell-homing chemokine made in lymphoid follicles activates Burkitt's lymphoma receptor-1. *Nature* 391: 799–803.
164. Ansel, K. M., L. J. McHeyzer-Williams, V. N. Ngo, M. G. McHeyzer-Williams, and J. G. Cyster. 1999. In vivo-activated CD4 T cells upregulate CXC chemokine receptor 5 and reprogram their response to lymphoid chemokines. *J Exp Med* 190: 1123–1134.
165. 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.
166. 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.
167. Chtanova, T., S. G. Tangye, R. Newton, N. Frank, M. R. Hodge, M. S. Rolph, and C. R. Mackay. 2004. T follicular helper cells express a distinctive transcriptional profile, reflecting their role as non-Th1/Th2 effector cells that provide help for B cells. *The Journal of Immunology* 173: 68–78.
168. Kim, C. H. 2004. Unique gene expression program of human germinal center T helper cells. *Blood* 104: 1952–1960.

169. 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.
170. 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.
171. 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.
172. 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.
173. Fazilleau, N., L. J. McHeyzer-Williams, H. Rosen, and M. G. McHeyzer-Williams. 2009. The function of follicular helper T cells is regulated by the strength of T cell antigen receptor binding. *Nat Immunol* 10: 375–384.
174. Baumjohann, D., T. Okada, and K. M. Ansel. 2011. Cutting Edge: Distinct Waves of BCL6 Expression during T Follicular Helper Cell Development. *The Journal of Immunology* 187: 2089–2092.
175. Benson, R. A., M. K. MacLeod, B. G. Hale, A. Patakas, P. Garside, and J. M. Brewer. 2015. Antigen presentation kinetics control T cell/dendritic cell interactions and follicular helper T cell generation in vivo. *eLife* 4: e06994.
176. Tam, H. H., M. B. Melo, M. Kang, J. M. Pelet, V. M. Ruda, M. H. Foley, J. K. Hu, S. Kumari, J. Crampton, A. D. Baldeon, R. W. Sanders, J. P. Moore, S. Crotty, R. Langer, D. G. Anderson, A. K. Chakraborty, and D. J. Irvine. 2016. Sustained antigen availability during germinal center initiation enhances antibody responses to vaccination. *Proceedings of the National Academy of Sciences* 113: E6639–E6648.
177. 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.
178. Shapiro, V. S., K. E. Truitt, J. B. Imboden, and A. Weiss. 1997. CD28 mediates transcriptional upregulation of the interleukin-2 (IL-2) promoter through a composite element containing the CD28RE and NF-IL-2B AP-1 sites. *Mol. Cell. Biol.* 17: 4051–4058.
179. Nurieva, R. I., A. Podd, Y. Chen, A. M. Alekseev, M. Yu, X. Qi, H. Huang, R. Wen, J. Wang, H. S. Li, S. S. Watowich, H. Qi, C. Dong, and D. Wang. 2012. STAT5 protein negatively regulates T follicular helper (Tfh) cell generation and function. *Journal of Biological Chemistry* 287: 11234–11239.
180. Johnston, R. J., Y. S. Choi, J. A. Diamond, J. A. Yang, and S. Crotty. 2012. STAT5 is a potent negative regulator of T FHcell differentiation. *J Exp Med* 209: 243–250.
181. Ballesteros-Tato, A., B. León, B. A. Graf, A. Moquin, P. S. Adams, F. E. Lund, and T. D. Randall. 2012. Interleukin-2 Inhibits Germinal Center Formation by Limiting T Follicular Helper Cell Differentiation. *Immunity* 36: 847–856.
182. 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.
183. Choi, Y. S., J. A. Yang, and S. Crotty. 2013. Dynamic regulation of Bcl6 in follicular helper CD4 T (Tfh) cells. *Curr Opin Immunol* 25: 366–372.
184. 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.
185. Haynes, N. M., C. D. C. 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-1 HighGerminal Center-Associated Subpopulation. *The Journal of Immunology* 179: 5099–5108.
186. Kamphorst, A. O., A. Wieland, T. Nasti, S. Yang, R. Zhang, D. L. Barber, B. T. Konieczny, C. Z. Daugherty, L. Koenig, K. Yu, G. L. Sica, A. H. Sharpe, G. J. Freeman, B. R. Blazar, L. A. Turka, T. K. Owonikoko, R. N. Pillai, S. S. Ramalingam, K. Araki, and R. Ahmed. 2017. Rescue of exhausted CD8 T cells by PD-1–targeted therapies is CD28-dependent. *Science* 355: 1423–1427.
187. Hui, E., J. Cheung, J. Zhu, X. Su, M. J. Taylor, H. A. Wallweber, D. K. Sasmal, J. Huang, J. M. Kim, I. Mellman, and R. D. Vale. 2017. T cell costimulatory receptor CD28 is a primary target for PD-1–mediated inhibition. *Science* 355: 1428–1433.
188. Flynn, S., K.-M. Toellner, C. Raykundalia, M. Goodall, and P. Lane. 1998. CD4 T Cell Cytokine Differentiation: The B Cell Activation Molecule, OX40 Ligand, Instructs CD4 T Cells to Express Interleukin 4 and Upregulates Expression of the Chemokine Receptor, Blr-1. *J Exp Med* 188: 297–304.

189. Kopf, M., C. Ruedl, N. Schmitz, A. Gallimore, K. Lefrang, B. Ecabert, B. Odermatt, and M. F. Bachmann. 1999. OX40-Deficient Mice Are Defective in Th Cell Proliferation but Are Competent in Generating B Cell and CTL Responses after Virus Infection. *Immunity* 11: 699–708.
190. Brocker, T., A. Gulbranson-Judge, S. Flynn, M. Riedinger, C. Raykundalia, and P. Lane. 1999. CD4 T cell traffic control: *in vivo* evidence that ligation of OX40 on CD4 T cells by OX40-ligand expressed on dendritic cells leads to the accumulation of CD4 T cells in B follicles. *Eur. J. Immunol.* 29: 1610–1616.
191. Fillatreau, S., and D. Gray. 2003. T Cell Accumulation in B Cell Follicles Is Regulated by Dendritic Cells and Is Independent of B Cell Activation. *Journal of Experimental Medicine* 197: 195–206.
192. Boettler, T., Y. S. Choi, S. Salek-Ardakani, Y. Cheng, F. Moeckel, M. Croft, S. Crotty, and M. von Herrath. 2013. Exogenous OX40 Stimulation during Lymphocytic Choriomeningitis Virus Infection Impairs Follicular Th Cell Differentiation and Diverts CD4 T Cells into the Effector Lineage by Upregulating Blimp-1. *The Journal of Immunology* 191: 5026–5035.
193. Byun, M., C. S. Ma, A. Akçay, V. Pedergnana, U. Palendira, J. Myoung, D. T. Avery, Y. Liu, A. Abhyankar, L. Lorenzo, M. Schmidt, H. K. Lim, O. Cassar, M. Migaud, F. Rozenberg, N. Canpolat, G. Aydoğan, B. Fleckenstein, J. Bustamante, C. Picard, A. Gessain, E. Jouanguy, E. Cesarman, M. Olivier, P. Gros, L. Abel, M. Croft, S. G. Tangye, and J.-L. Casanova. 2013. Inherited human OX40 deficiency underlying classic Kaposi sarcoma of childhood. *J Exp Med* 210: 1743–1759.
194. Harada, Y., D. Ohgai, R. Watanabe, K. Okano, O. Koiwai, K. Tanabe, H. Toma, A. Altman, and R. Abe. 2003. A Single Amino Acid Alteration in Cytoplasmic Domain Determines IL-2 Promoter Activation by Ligation of CD28 but Not Inducible Costimulator (ICOS). *J Exp Med* 197: 257–262.
195. Watanabe, R., Y. Harada, K. Takeda, J. Takahashi, K. Ohnuki, S. Ogawa, D. Ohgai, N. Kaibara, O. Koiwai, K. Tanabe, H. Toma, K. Sugamura, and R. Abe. 2006. Grb2 and Gads Exhibit Different Interactions with CD28 and Play Distinct Roles in CD28-Mediated Costimulation. *The Journal of Immunology* 177: 1085–1091.
196. Wang, H., J. Geng, X. Wen, E. Bi, A. V. Kossenkov, A. I. Wolf, J. Tas, Y. S. Choi, H. Takata, T. J. Day, L.-Y. Chang, S. L. Sprout, E. K. Becker, J. Willen, L. Tian, X. Wang, C. Xiao, P. Jiang, S. Crotty, G. D. Victora, L. C. Showe, H. O. Tucker, J. Erikson, and H. Hu. The transcription factor Foxp1 is a critical negative regulator of the differentiation of follicular helper T cells. *Nat Immunol* 15: 667 EP –.
197. Leavenworth, J. W., B. Verbinnen, J. Yin, H. Huang, and H. Cantor. 2014. A p85α-osteopontin axis couples the receptor ICOS to sustained Bcl-6 expression by follicular helper and regulatory T cells. *Nat Immunol* 16: 96–106.
198. Weber, J. P., F. Fuhrmann, R. K. Feist, A. Lahmann, M. S. Al Baz, L.-J. Gentz, D. Vu Van, H. W. Mages, C. Haftmann, R. Riedel, J. R. Grün, W. Schuh, R. A. Kroczek, A. Radbruch, M.-F. Mashreghi, and A. Hutloff. 2015. ICOS maintains the T follicular helper cell phenotype by down-regulating Krüppel-like factor 2. *J Exp Med* 212: 217–233.
199. Lee, J.-Y., C. N. Skon, Y. J. Lee, S. Oh, J. J. Taylor, D. Malhotra, M. K. Jenkins, M. G. Rosenfeld, K. A. Hogquist, and S. C. Jameson. 2015. The Transcription Factor KLF2 Restrains CD4+ T Follicular Helper Cell Differentiation. *Immunity* 42: 252–264.
200. Stone, E. L., M. Pepper, C. D. Katayama, Y. M. Kerdiles, C.-Y. Lai, E. Emslie, Y. C. Lin, E. Yang, A. W. Goldrath, M. O. Li, D. A. Cantrell, and S. M. Hedrick. 2015. ICOS Coreceptor Signaling Inactivates the Transcription Factor FOXO1 to Promote Tfh Cell Differentiation. *Immunity* 42: 239–251.
201. Dong, C., A. E. Juedes, U.-A. Temann, S. Shresta, J. P. Allison, N. H. Ruddle, and R. A. Flavell. 2001. ICOS co-stimulatory receptor is essential for T-cell activation and function. *Nature* 409: 97–101.
202. Tafuri, A., A. Shahinian, F. Bladt, S. K. Yoshinaga, M. Jordana, A. Wakeham, L.-M. Boucher, D. Bouchard, V. S. F. Chan, G. Duncan, B. Odermatt, A. Ho, A. Itie, T. Horan, J. S. Whoriskey, T. Pawson, J. M. Penninger, P. S. Ohashi, and T. W. Mak. ICOS is essential for effective T-helper-cell responses. *Nature* 409: 105 EP –.
203. Reinhardt, R. L., H.-E. Liang, and R. M. Locksley. 2009. Cytokine-secreting follicular T cells shape the antibody repertoire. *Nat Immunol* 10: 385–393.
204. Bauquet, A. T., H. Jin, A. M. Paterson, M. Mitsdoerffer, I.-C. Ho, A. H. Sharpe, and V. K. Kuchroo. 2008. The costimulatory molecule ICOS regulates the expression of c-Maf and IL-21 in the development of follicular T helper cells and TH-17 cells. *Nat Immunol* 10: 167–175.
205. Kroenke, M. A., D. Eto, M. Locci, M. Cho, T. Davidson, E. K. Haddad, and S. Crotty. 2012. Bcl6 and Maf Cooperate To Instruct Human Follicular Helper CD4 T Cell Differentiation. *The Journal of Immunology* 188: 3734–3744.
206. Bao, K., T. Carr, J. Wu, W. Barclay, J. Jin, M. Ciofani, and R. L. Reinhardt. 2016. BATF Modulates the Th2 Locus Control Region and Regulates CD4 +T Cell Fate during Antihelminth Immunity. *The Journal of Immunology* 197: 4371–4381.

207. Sahoo, A., A. Alekseev, K. Tanaka, L. Obertas, B. Lerman, C. Haymaker, K. Clise-Dwyer, J. S. McMurray, and R. Nurieva. 2015. Batf is important for IL-4 expression in T follicular helper cells. *Nature communications* 6: 1.
208. Dell'Aringa, M., and R. L. Reinhardt. 2018. Notch signaling represents an important checkpoint between follicular T-helper and canonical T-helper 2 cell fate. *mucosal Immunology*.
209. Liu, X., X. Chen, B. Zhong, A. Wang, X. Wang, F. Chu, R. I. Nurieva, X. Yan, P. Chen, L. G. van der Flier, H. Nakatsukasa, S. S. Neelapu, W. Chen, H. Clevers, Q. Tian, H. Qi, L. Wei, and C. Dong. 2014. Transcription factor achaete-scute homologue 2 initiates follicular T-helper-cell development. *Nature* 1–21.
210. 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 Reports* 12: 2099–2110.
211. Choi, Y. S., J. A. Gullicksrud, S. Xing, Z. Zeng, Q. Shan, F. Li, P. E. Love, W. Peng, H.-H. Xue, and S. Crotty. 2015. LEF-1 and TCF-1 orchestrate TFH differentiation by regulating differentiation circuits upstream of the transcriptional repressor Bcl6. *Nat Immunol* 16: 980–990.
212. Xu, L., Y. Cao, Z. Xie, Q. Huang, Q. Bai, X. Yang, R. He, Y. Hao, H. Wang, T. Zhao, Z. Fan, A. Qin, J. Ye, X. Zhou, L. Ye, and Y. Wu. The transcription factor TCF-1 initiates the differentiation of TFH cells during acute viral infection. *Nat Immunol* 16: 991 EP –.
213. Read, K. A., M. D. Powell, and K. J. Oestreich. 2016. T follicular helper cell programming by cytokine-mediated events. *Immunology* 149: 253–261.
214. Eto, D., C. Lao, D. DiToro, B. Barnett, T. C. Escobar, R. Kageyama, I. Yusuf, and S. Crotty. 2011. IL-21 and IL-6 Are Critical for Different Aspects of B Cell Immunity and Redundantly Induce Optimal Follicular Helper CD4 T Cell (Tfh) Differentiation. *PLoS ONE* 6: e17739.
215. 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. *The Journal of Immunology* 190: 3049–3053.
216. Karnowski, A., S. Chevrier, G. T. Belz, A. Mount, D. Emslie, K. D'Costa, D. M. Tarlinton, A. Kallies, and L. M. Corcoran. 2012. B and T cells collaborate in antiviral responses via IL-6, IL-21, and transcriptional activator and coactivator, Oct2 and OBF-1. *J Exp Med* 209: 2049–2064.
217. 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. *The Journal of Immunology* 185: 313–326.
218. Goenka, R., L. G. Barnett, J. S. Silver, P. J. O'Neill, C. A. Hunter, M. P. Cancro, and T. M. Laufer. 2011. Cutting Edge: Dendritic Cell-Restricted Antigen Presentation Initiates the Follicular Helper T Cell Program but Cannot Complete Ultimate Effector Differentiation. *The Journal of Immunology* 187: 1091–1095.
219. Krishnaswamy, J. K., U. Gowthaman, B. Zhang, J. Mattsson, L. Szeponik, D. Liu, R. Wu, T. White, S. Calabro, L. Xu, M. A. Collet, M. Yurieva, S. Alsén, P. Fogelstrand, A. Walter, W. R. Heath, S. N. Mueller, U. Yrlid, A. Williams, and S. C. Eisenbarth. 2017. Migratory CD11b +conventional dendritic cells induce T follicular helper cell–dependent antibody responses. *Sci. Immunol.* 2: eaam9169.
220. 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.
221. Hardtke, S. 2005. Balanced expression of CXCR5 and CCR7 on follicular T helper cells determines their transient positioning to lymph node follicles and is essential for efficient B-cell help. *Blood* 106: 1924–1931.
222. Barnett, L. G., H. M. A. 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. 1–11.
223. Dahlgren, M. W., T. Gustafsson, M. Livingston, H. Cucak, S. Alsén, U. Yrlid, and B. Johansson-Lindbom. 2015. T follicular helper but not Th1 cell differentiation in the absence of conventional dendritic cells. *J. Immunol.* Accepted for publication.
224. 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. *Journal of Experimental Medicine* 206: 991–999.
225. 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. *Journal of Experimental Medicine* 214: 2795–2810.

226. 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.
227. Liu, D., H. Xu, C. Shih, Z. Wan, X. Ma, W. Ma, D. Luo, and H. Qi. 2014. T-B-cell entanglement and ICOSL-driven feed-forward regulation of germinal centre reaction. *Nature* 517: 214–218.
228. 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.
229. 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.
230. Yusuf, I., R. Kageyama, L. Monticelli, R. J. Johnston, D. DiToro, K. Hansen, B. Barnett, and S. Crotty. 2010. Germinal Center T Follicular Helper Cell IL-4 Production Is Dependent on Signaling Lymphocytic Activation Molecule Receptor (CD150). *The Journal of Immunology* 185: 190–202.
231. Kageyama, R., J. L. Cannons, F. Zhao, I. Yusuf, C. Lao, M. Locci, P. L. Schwartzberg, and S. Crotty. 2012. The Receptor Ly108 Functions as a SAP Adaptor-Dependent On-Off Switch for T Cell Help to B Cells and NKT Cell Development. *Immunity* 36: 986–1002.
232. Chen, S., C. Cai, Z. Li, G. Liu, Y. Wang, M. Blonska, D. Li, J. Du, X. Lin, M. Yang, and Z. Dong. 2017. Dissection of SAP-dependent and SAP-independent SLAM family signaling in NKT cell development and humoral immunity. *J Exp Med* 214: 475–489.
233. Zotos, D., J. M. Coquet, Y. Zhang, A. Light, K. D'Costa, A. Kallies, L. M. Corcoran, D. I. Godfrey, K.-M. Toellner, M. J. Smyth, S. L. Nutt, and D. M. Tarlinton. 2010. IL-21 regulates germinal center B cell differentiation and proliferation through a B cell–intrinsic mechanism. *J Exp Med* 207: 365–378.
234. McGuire, H. M., A. Vogelzang, J. Warren, C. Loetsch, K. D. Natividad, T. D. Chan, R. Brink, M. Batten, and C. King. 2015. IL-21 and IL-4 Collaborate To Shape T-Dependent Antibody Responses. *The Journal of Immunology* 195: 5123–5135.
235. Howard, M. 1982. Identification of a T cell-derived b cell growth factor distinct from interleukin 2. *J Exp Med* 155: 914–923.
236. Mohrs, K., A. E. Wakil, N. Killeen, R. M. Locksley, and M. Mohrs. 2005. A Two-Step Process for Cytokine Production Revealed by IL-4 Dual-Reporter Mice. *Immunity* 23: 419–429.
237. King, I. L., and M. Mohrs. 2009. IL-4-producing CD4+ T cells in reactive lymph nodes during helminth infection are T follicular helper cells. *Journal of Experimental Medicine* 206: 1001–1007.
238. Liang, H.-E., R. L. Reinhardt, J. K. Bando, B. M. Sullivan, I.-C. Ho, and R. M. Locksley. 2011. Divergent expression patterns of IL-4 and IL-13 define unique functions in allergic immunity. *Nat Immunol* 13: 58–66.
239. 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.
240. Weinstein, J. S., E. I. Herman, B. Lainez, P. Licona-Limón, E. Esplugues, R. Flavell, and J. Craft. 2016. TFH cells progressively differentiate to regulate the germinal center response. *Nat Immunol* 17: 1197–1205.
241. Dedeoglu, F., B. Horwitz, J. Chaudhuri, F. W. Alt, and R. S. Geha. 2004. Induction of activation-induced cytidine deaminase gene expression by IL-4 and CD40 ligation is dependent on STAT6 and NFκB. *Int Immunol* 16: 395–404.
242. Papa, I., D. Saliba, M. Ponzoni, S. Bustamante, P. F. Cañete, P. Gonzalez-Figueroa, H. A. McNamara, S. Valvo, M. Grimbaldeston, R. A. Sweet, H. Vohra, I. A. Cockburn, M. Meyer-Hermann, M. L. Dustin, C. Doglioni, and C. G. Vinuesa. TFH-derived dopamine accelerates productive synapses in germinal centres. *Nature* 547: 318 EP –.
243. Wang, Y., J. Shi, J. Yan, Z. Xiao, X. Hou, P. Lu, S. Hou, T. Mao, W. Liu, Y. Ma, L. Zhang, X. Yang, and H. Qi. Germinal-center development of memory B cells driven by IL-9 from follicular helper T cells. *Nat Immunol* 18: 921 EP –.
244. Sakaguchi, S., N. Sakaguchi, M. Asano, M. Itoh, and M. Toda. 1995. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* 155: 1151.
245. Fontenot, J. D., M. A. Gavin, and A. Y. Rudensky. 2003. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol* 4: 330–336.
246. Wing, K., Y. Onishi, P. Prieto-Martin, T. Yamaguchi, M. Miyara, Z. Fehervari, T. Nomura, and S. Sakaguchi. 2008. CTLA-4 Control over Foxp3+ Regulatory T Cell Function. *Science* 322: 271–275.

247. Liang, B., C. Workman, J. Lee, C. Chew, B. M. Dale, L. Colonna, M. Flores, N. Li, E. Schweighoffer, S. Greenberg, V. Tybulewicz, D. Vignali, and R. Clynes. 2008. Regulatory T Cells Inhibit Dendritic Cells by Lymphocyte Activation Gene-3 Engagement of MHC Class II. *The Journal of Immunology* 180: 5916–5926.
248. Asseman, C., S. Mauze, M. W. Leach, R. L. Coffman, and F. Powrie. 1999. An Essential Role for Interleukin 10 in the Function of Regulatory T Cells That Inhibit Intestinal Inflammation. *J Exp Med* 190: 995–1004.
249. Deaglio, S., K. M. Dwyer, W. Gao, D. Friedman, A. Usheva, A. Erat, J.-F. Chen, K. Enjyoji, J. Linden, M. Oukka, V. K. Kuchroo, T. B. Strom, and S. C. Robson. 2007. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. *J Exp Med* 204: 1257–1265.
250. Wollenberg, I., A. Agua-Doce, A. Hernandez, C. Almeida, V. G. Oliveira, J. Faro, and L. Graca. 2011. Regulation of the Germinal Center Reaction by Foxp3+ Follicular Regulatory T Cells. *The Journal of Immunology* 187: 4553–4560.
251. Chung, Y., S. Tanaka, F. Chu, R. I. Nurieva, G. J. Martinez, S. Rawal, Y.-H. Wang, H. Lim, J. M. Reynolds, X.-H. Zhou, H.-M. Fan, Z.-M. Liu, S. S. Neelapu, and C. Dong. Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions. *Nat Med* 17: 983 EP –.
252. Linterman, M. A., W. Pierson, S. K. Lee, A. Kallies, S. Kawamoto, T. F. Rayner, M. Srivastava, D. P. Divekar, L. Beaton, J. J. Hogan, S. Fagarasan, A. Liston, K. G. C. Smith, and C. G. Vinuesa. Foxp3+ follicular regulatory T cells control the germinal center response. *Nat Med* 17: 975 EP –.
253. Aloulou, M., E. J. Carr, M. Gador, A. Bignon, R. S. Liblau, N. Fazilleau, and M. A. Linterman. 2016. Follicular regulatory T cells can be specific for the immunizing antigen and derive from naive T cells. *Nature communications* 7: 10579.
254. Sage, P. T., A. M. Paterson, S. B. Lovitch, and A. H. Sharpe. 2014. The Coinhibitory Receptor CTLA-4 Controls B Cell Responses by Modulating T Follicular Helper, T Follicular Regulatory, and T Regulatory Cells. *Immunity* 41: 1026–1039.
255. Wing, J. B., W. Ise, T. Kurosaki, and S. Sakaguchi. 2014. Regulatory T Cells Control Antigen-Specific Expansion of Tfh Cell Number and Humoral Immune Responses via the Coreceptor CTLA-4. *Immunity* 41: 1013–1025.
256. Campbell, D. J., C. H. Kim, and E. C. Butcher. 2001. Separable effector T cell populations specialized for B cell help or tissue inflammation. *Nat Immunol* 2: 876–881.
257. Campbell, D. J., and E. C. Butcher. 2002. Rapid Acquisition of Tissue-specific Homing Phenotypes by CD4(+) T Cells Activated in Cutaneous or Mucosal Lymphoid Tissues. *J Exp Med* 195: 135–141.
258. Suan, D., A. Nguyen, I. Moran, K. Bourne, J. R. Hermes, M. Arshi, H. R. Hampton, M. Tomura, Y. Miwa, A. D. Kelleher, W. Kaplan, E. K. Deenick, S. G. Tangye, R. Brink, T. Chtanova, and T. G. Phan. 2015. T follicular helper cells have distinct modes of migration and molecular signatures in naive and memory immune responses. *Immunity* 42: 704–718.
259. Hannedouche, S., J. Zhang, T. Yi, W. Shen, D. Nguyen, J. P. Pereira, D. Guerini, B. U. Baumgarten, S. Roggo, B. Wen, R. Knochenmuss, S. Noël, F. Gessier, L. M. Kelly, M. Vanek, S. Laurent, I. Preuss, C. Miault, I. Christen, R. Karuna, W. Li, D.-I. Koo, T. Suply, C. Schmedt, E. C. Peters, R. Falchetto, A. Katopodis, C. Spanka, M.-O. Roy, M. Detheux, Y. A. Chen, P. G. Schultz, C. Y. Cho, K. Seuwen, J. G. Cyster, and A. W. Sailer. Oxysterols direct immune cell migration via EBI2. *Nature* 475: 524 EP –.
260. Liu, C., X. V. Yang, J. Wu, C. Kuei, N. S. Mani, L. Zhang, J. Yu, S. W. Sutton, N. Qin, H. Banie, L. Karlsson, S. Sun, and T. W. Lovenberg. Oxysterols direct B-cell migration through EBI2. *Nature* 475: 519 EP –.
261. Cyster, J. G., K. M. Ansel, K. Reif, E. H. Ekland, P. L. Hyman, H. L. Tang, S. A. Luther, and V. N. Nigo. 2003. Follicular stromal cells and lymphocyte homing to follicles. *Immunol. Rev.* 176: 181–193.
262. Rodda, L. B., O. Bannard, B. Ludewig, T. Nagasawa, and J. G. Cyster. 2015. Phenotypic and Morphological Properties of Germinal Center Dark Zone Cxcl12-Expressing Reticular Cells. *The Journal of Immunology* 195: 4781–4791.
263. Bannard, O., R. M. Horton, C. D. C. Allen, J. An, T. Nagasawa, and J. G. Cyster. 2013. Germinal Center Centroblasts Transition to a Centrocyte Phenotype According to a Timed Program and Depend on the Dark Zone for Effective Selection. *Immunity* 39: 912–924.
264. Estes, J. D., B. F. Keele, K. Tenner-Racz, P. Racz, M. A. Redd, T. C. Thacker, Y. Jiang, M. J. Lloyd, S. Gartner, and G. F. Burton. 2002. Follicular Dendritic Cell-Mediated Up-Regulation of CXCR4 Expression on CD4 T Cells and HIV Pathogenesis. *The Journal of Immunology* 169: 2313–2322.
265. Estes, J. D., T. C. Thacker, D. L. Hampton, S. A. Kell, B. F. Keele, E. A. Palenske, K. M. Druey, and G. F. Burton. 2004. Follicular Dendritic Cell Regulation of CXCR4-Mediated Germinal Center CD4 T Cell Migration. *The Journal of Immunology* 173: 6169–6178.

266. Shulman, Z., A. D. Gitlin, S. Targ, M. Jankovic, G. Pasqual, M. C. Nussenzweig, and G. D. Victora. 2013. T Follicular Helper Cell Dynamics in Germinal Centers. *Science* 341: 673–677.
267. Cucak, H., U. Yrlid, B. Reizis, U. Kalinke, and B. Johansson-Lindbom. 2009. Type I Interferon Signaling in Dendritic Cells Stimulates the Development of Lymph-Node-Resident T Follicular Helper Cells. *Immunity* 31: 491–501.
268. Johansson-Lindbom, B., M. Svensson, M. A. Wurbel, B. Malissen, G. Marquez, and W. Agace. 2003. Selective Generation of Gut Tropic T Cells in Gut-associated Lymphoid Tissue (GALT): Requirement for GALT Dendritic Cells and Adjuvant. *Journal of Experimental Medicine* 198: 963–969.
269. Mora, J. R., M. R. Bono, N. Manjunath, W. Weninger, L. L. Cavanagh, M. Rosemblatt, and U. H. von Andrian. 2003. Selective imprinting of gut-homing T cells by Peyer's patch dendritic cells. *Nature* 424: 88–93.
270. Stagg, A. J., M. A. Kamm, and S. C. Knight. 2002. Intestinal dendritic cells increase T cell expression of α4β7 integrin. *Eur. J. Immunol.* 32: 1445–1454.
271. Kunkel, E. J., J. J. Campbell, G. Haraldsen, J. Pan, J. Boisvert, A. I. Roberts, E. C. Ebert, M. A. Vierra, S. B. Goodman, M. C. Genovese, A. J. Wardlaw, H. B. Greenberg, C. M. Parker, E. C. Butcher, D. P. Andrew, and W. W. Agace. 2000. Lymphocyte Cc Chemokine Receptor 9 and Epithelial Thymus-Expressed Chemokine (Teck) Expression Distinguish the Small Intestinal Immune Compartment. *J Exp Med* 192: 761–768.
272. Iwata, M., A. Hirakiyama, Y. Eshima, H. Kagechika, C. Kato, and S.-Y. Song. 2004. Retinoic Acid Imprints Gut-Homing Specificity on T Cells. *Immunity* 21: 527–538.
273. Annacker, O., J. L. Coombes, V. Malmstrom, H. H. Uhlig, T. Bourne, B. Johansson-Lindbom, W. W. Agace, C. M. Parker, and F. Powrie. 2005. Essential role for CD103 in the T cell-mediated regulation of experimental colitis. *J Exp Med* 202: 1051–1061.
274. Zhou, L., M. M. W. Chong, and D. R. Littman. 2009. Plasticity of CD4+ T Cell Lineage Differentiation. *Immunity* 30: 646–655.
275. O'Shea, J. J., and W. E. Paul. 2010. Mechanisms Underlying Lineage Commitment and Plasticity of Helper CD4+ T Cells. *Science* 327: 1098–1102.
276. Liu, X., X. Yan, B. Zhong, R. I. Nurieva, A. Wang, X. Wang, N. Martin-Orozco, Y. Wang, S. H. Chang, E. Esplugues, R. A. Flavell, Q. Tian, and C. Dong. 2012. Bcl6 expression specifies the T follicular helper cell program in vivo. *Journal of Experimental Medicine* 209: 1841–52– S1–24.
277. Lüthje, K., A. Kallies, Y. Shimohakamada, G. T. Belz, A. Light, D. M. Tarlinton, and S. L. Nutt. 2012. The development and fate of follicular helper T cells defined by an IL-21 reporter mouse. *Nat Immunol* 13: 491–498.
278. Ballesteros-Tato, A., T. D. Randall, F. E. Lund, R. Spolski, W. J. Leonard, and B. León. 2016. T Follicular Helper Cell Plasticity Shapes Pathogenic T Helper 2 Cell-Mediated Immunity to Inhaled House Dust Mite. *Immunity* 44: 259–273.
279. Sallusto, F., J. Geginat, and A. Lanzavecchia. 2004. Central Memory and Effector Memory T Cell Subsets: Function, Generation, and Maintenance. *Annu. Rev. Immunol.* 22: 745–763.
280. 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.
281. MacLeod, M. K. L., 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. *The Journal of Immunology* 186: 2889–2896.
282. 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.
283. Luther, S. A., K. Serre, A. F. Cunningham, M. Khan, H. Acha-Orbea, I. C. M. MacLennan, and K.-M. Toellner. 2007. Recirculating CD4 memory T cells mount rapid secondary responses without major contributions from follicular CD4 effectors and B cells. *Eur. J. Immunol.* 37: 1476–1484.
284. Asrir, A., M. Aloulou, M. Gador, C. Pérals, and N. Fazilleau. 2017. Interconnected subsets of memory follicular helper T cells have different effector functions. *Nature communications* 8: 847.
285. Suan, D., A. Nguyen, I. Moran, K. Bourne, J. R. Hermes, M. Arshi, H. R. Hampton, M. Tomura, Y. Miwa, A. D. Kelleher, W. Kaplan, E. K. Deenick, S. G. Tangye, R. Brink, T. Chtanova, and T. G. Phan. 2015. T Follicular Helper Cells Have Distinct Modes of Migration and Molecular Signatures in Naive and Memory Immune Responses. *Immunity* 42: 704–718.
286. Schatz, D. G., M. A. Oettinger, and D. Baltimore. 1989. The V(D)J recombination activating gene, RAG-1. *Cell* 59: 1035–1048.
287. Alt, F. W., and D. Baltimore. 1982. Joining of immunoglobulin heavy chain gene segments: implications from a chromosome with evidence of three D-JH fusions. *Proc Natl Acad Sci USA* 79: 4118.

288. Löffert, D., A. Ehlich, W. Müller, and K. Rajewsky. 1996. Surrogate Light Chain Expression Is Required to Establish Immunoglobulin Heavy Chain Allelic Exclusion during Early B Cell Development. *Immunity* 4: 133–144.
289. Gay, D. 1993. Receptor editing: an approach by autoreactive B cells to escape tolerance. *J Exp Med* 177: 999–1008.
290. Schiemann, B. 2001. An Essential Role for BAFF in the Normal Development of B Cells Through a BCMA-Independent Pathway. *Science* 293: 2111–2114.
291. Harless, S. M., V. M. Lentz, A. P. Sah, B. L. Hsu, K. Clise-Dwyer, D. M. Hilbert, C. E. Hayes, and M. P. Cancro. 2001. Competition for BLyS-mediated signaling through Bcmd/BR3 regulates peripheral B lymphocyte numbers. *Current Biology* 11: 1986–1989.
292. Suzuki, K., I. Grigorova, T. G. Phan, L. M. Kelly, and J. G. Cyster. 2009. Visualizing B cell capture of cognate antigen from follicular dendritic cells. *J Exp Med* 206: 1485–1493.
293. Pape, K. A., D. M. Catron, A. A. Itano, and M. K. Jenkins. 2007. The Humoral Immune Response Is Initiated in Lymph Nodes by B Cells that Acquire Soluble Antigen Directly in the Follicles. *Immunity* 26: 491–502.
294. Unanue, E. R., J. C. CEROTTINI, and M. BEDFORD. 1969. Persistence of Antigen on the Surface of Macrophages. *Nature* 222: 1193–1195.
295. Clark, M. R., D. Massenburg, K. Siemasko, P. Hou, and M. Zhang. 2004. B-cell antigen receptor signaling requirements for targeting antigen to the MHC class II presentation pathway. *Curr Opin Immunol* 16: 382–387.
296. Okada, T., M. J. Miller, I. Parker, M. F. Krummel, M. Neighbors, S. B. Hartley, A. O'Garra, M. D. Cahalan, and J. G. Cyster. 2005. Antigen-Engaged B Cells Undergo Chemotaxis toward the T Zone and Form Motile Conjugates with Helper T Cells. *PLoS Biol* 3: e150.
297. Gatto, D., K. Wood, and R. Brink. 2011. EBI2 Operates Independently of but in Cooperation with CXCR5 and CCR7 To Direct B Cell Migration and Organization in Follicles and the Germinal Center. *The Journal of Immunology* 187: 4621–4628.
298. Ho, F., J. E. Lortan, I. C. M. MacLennan, and M. Khan. 1986. Distinct short-lived and long-lived antibody-producing cell populations. *Eur. J. Immunol.* 16: 1297–1301.
299. Shlomchik, M. J., and F. Weisel. 2012. Germinal center selection and the development of memory B and plasma cells. *Immunol. Rev.* 247: 52–63.
300. 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.
301. Chan, T. D., D. Gatto, K. Wood, T. Camidge, A. Basten, and R. Brink. 2009. Antigen Affinity Controls Rapid T-Dependent Antibody Production by Driving the Expansion Rather than the Differentiation or Extrafollicular Migration of Early Plasmablasts. *The Journal of Immunology* 183: 3139–3149.
302. Di Yu, and C. G. Vinuesa. 2009. Multiple checkpoints keep follicular helper T cells under control to prevent autoimmunity. *Nature* 1–6.
303. Cunningham, A. F., K. Serre, E. Mohr, M. Khan, and K.-M. Toellner. 2004. Loss of CD154 impairs the Th2 extrafollicular plasma cell response but not early T cell proliferation and interleukin-4 induction. *Immunology* 113: 187–193.
304. Ozaki, K., R. Spolski, R. Ettinger, H. P. Kim, G. Wang, C. F. Qi, P. Hwu, D. J. Shaffer, S. Akilesh, D. C. Roopenian, H. C. Morse, P. E. Lipsky, and W. J. Leonard. 2004. Regulation of B Cell Differentiation and Plasma Cell Generation by IL-21, a Novel Inducer of Blimp-1 and Bcl-6. *The Journal of Immunology* 173: 5361–5371.
305. Shaffer, A. L., K.-I. Lin, T. C. Kuo, X. Yu, E. M. Hurt, A. Rosenwald, J. M. Giltnane, L. Yang, H. Zhao, K. Calame, and L. M. Staudt. 2002. Blimp-1 Orchestrates Plasma Cell Differentiation by Extinguishing the Mature B Cell Gene Expression Program. *Immunity* 17: 51–62.
306. Stavnezer, J., and C. E. Schrader. 2014. IgH Chain Class Switch Recombination: Mechanism and Regulation. *The Journal of Immunology* 193: 5370–5378.
307. Muramatsu, M., V. S. Sankaranand, S. Anant, M. Sugai, K. Kinoshita, N. O. Davidson, and T. Honjo. 1999. Specific Expression of Activation-induced Cytidine Deaminase (AID), a Novel Member of the RNA-editing Deaminase Family in Germinal Center B Cells. *Journal of Biological Chemistry* 274: 18470–18476.
308. Martins, G., and K. Calame. 2008. Regulation and functions of Blimp-1 in T and B lymphocytes. *Annu. Rev. Immunol.* 26: 133–169.

309. Shaffer, A. L., X. Yu, Y. He, J. Boldrick, E. P. Chan, and L. M. Staudt. 2000. BCL-6 Represses Genes that Function in Lymphocyte Differentiation, Inflammation, and Cell Cycle Control. *Immunity* 13: 199–212.
310. Arguni, E., M. Arima, N. Tsuruoka, A. Sakamoto, M. Hatano, and T. Tokuhisa. 2006. JunD/AP-1 and STAT3 are the major enhancer molecules for high Bcl6 expression in germinal center B cells. *Int Immunol* 18: 1079–1089.
311. Avery, D. T., E. K. Deenick, C. S. Ma, S. Suryani, N. Simpson, G. Y. Chew, T. D. Chan, U. Palendira, J. Bustamante, S. Boisson-Dupuis, S. Choo, K. E. Bleasel, J. Peake, C. King, M. A. French, D. Engelhard, S. Al-Hajjar, S. Al-Muhsen, K. Magdorf, J. Roesler, P. D. Arkwright, P. Hissaria, D. S. Riminton, M. Wong, R. Brink, D. A. Fulcher, J.-L. Casanova, M. C. Cook, and S. G. Tangye. 2010. B cell–intrinsic signaling through IL-21 receptor and STAT3 is required for establishing long-lived antibody responses in humans. *J Exp Med* 207: 155–171.
312. Ozaki, K. 2002. A Critical Role for IL-21 in Regulating Immunoglobulin Production. *Science* 298: 1630–1634.
313. Linterman, M. A., L. Beaton, D. Yu, R. R. Ramiscal, M. Srivastava, J. J. Hogan, N. K. Verma, M. J. Smyth, R. J. Rigby, and C. G. Vinuesa. 2010. IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. *Journal of Experimental Medicine* 207: 353–363.
314. Liu, Y. J., D. E. Joshua, G. T. Williams, C. A. Smith, J. Gordon, and I. C. M. MacLennan. 1989. Mechanism of antigen-driven selection in germinal centres. *Nature* 342: 929–931.
315. Han, S., K. Hathcock, B. Zheng, T. B. Kepler, R. Hodes, and G. Kelsoe. 1995. Cellular interaction in germinal centers. Roles of CD40 ligand and B7-2 in established germinal centers. *J. Immunol.* 155: 556.
316. Foy, T. M. 1993. In vivo CD40-gp39 interactions are essential for thymus-dependent humoral immunity. II. Prolonged suppression of the humoral immune response by an antibody to the ligand for CD40, gp39. *J Exp Med* 178: 1567–1575.
317. 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.
318. Xu, J., T. M. Foy, J. D. Laman, E. A. Elliott, J. J. Dunn, T. J. Waldschmidt, J. Elsemore, R. J. Noelle, and R. A. Flavell. 1994. Mice deficient for the CD40 ligand. *Immunity* 1: 423–431.
319. Allen, C. D. C., K. M. Ansel, C. Low, R. Lesley, H. Tamamura, N. Fujii, and J. G. Cyster. 2004. Germinal center dark and light zone organization is mediated by CXCR4 and CXCR5. *Nat Immunol* 5: 943–952.
320. Liu, Y. J., F. Malisan, O. de Bouteiller, C. Guret, S. Lebecque, J. Banchereau, F. C. Mills, E. E. Max, and H. Martinez-Valdez. 1996. Within Germinal Centers, Isotype Switching of Immunoglobulin Genes Occurs after the Onset of Somatic Mutation. *Immunity* 4: 241–250.
321. Toellner, K. M. 1996. Immunoglobulin switch transcript production in vivo related to the site and time of antigen-specific B cell activation. *J Exp Med* 183: 2303–2312.
322. Pape, K. A., V. Kouskoff, D. Nemazee, H. L. Tang, J. G. Cyster, L. E. Tze, K. L. Hippen, T. W. Behrens, and M. K. Jenkins. 2003. Visualization of the Genesis and Fate of Isotype-switched B Cells during a Primary Immune Response. *J Exp Med* 197: 1677–1687.
323. Mandel, T. E., R. P. Phipps, A. P. Abbot, and J. G. Tew. 1981. Long-term antigen retention by dendritic cells in the popliteal lymph node of immunized mice. *Immunology* 43: 353–362.
324. Yang Shih, T.-A., E. Meffre, M. Roederer, and M. C. Nussenzweig. 2002. Role of BCR affinity in T cell–dependent antibody responses in vivo. *Nat Immunol* 3: 570–575.
325. Zhang, Y., M. Meyer-Hermann, L. A. George, M. T. Figge, M. Khan, M. Goodall, S. P. Young, A. Reynolds, F. Falciani, A. Waisman, C. A. Notley, M. R. Ehrenstein, M. Kosco-Vilbois, and K.-M. Toellner. 2013. Germinal center B cells govern their own fate via antibody feedback. *J Exp Med* 210: 457–464.
326. McAdam, A. J., R. J. Greenwald, M. A. Levin, T. Chernova, N. Malenkovich, V. Ling, G. J. Freeman, and A. H. Sharpe. 2001. ICOS is critical for CD40-mediated antibody class switching. *Nature* 409: 102–105.
327. Chevrier, S., T. Kratina, D. Emslie, D. M. Tarlinton, and L. M. Corcoran. 2017. IL4 and IL21 cooperate to induce the high Bcl6 protein level required for germinal center formation. *Immunol Cell Biol* 95: 925–932.
328. Angelin-Duclos, C., G. Cattoretti, K. I. Lin, and K. Calame. 2000. Commitment of B Lymphocytes to a Plasma Cell Fate Is Associated with Blimp-1 Expression In Vivo. *The Journal of Immunology* 165: 5462–5471.
329. Smith, K. G. C. 1997. The extent of affinity maturation differs between the memory and antibody-forming cell compartments in the primary immune response. *The EMBO Journal* 16: 2996–3006.

330. 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.
331. Weisel, F. J., G. V. Zuccarino-Catania, M. Chikina, and M. J. Shlomchik. 2016. A Temporal Switch in the Germinal Center Determines Differential Output of Memory B and Plasma Cells. *Immunity* 44: 116–130.
332. 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.
333. Choi, Y. S., J. A. Yang, I. Yusuf, R. J. Johnston, J. Greenbaum, B. Peters, and S. Crotty. 2013. Bcl6 expressing follicular helper CD4 T cells are fate committed early and have the capacity to form memory. *The Journal of Immunology* 190: 4014–4026.
334. Baumjohann, D., T. Okada, and K. M. Ansel. 2011. Cutting Edge: Distinct Waves of BCL6 Expression during T Follicular Helper Cell Development. *The Journal of Immunology* 187: 2089–2092.
335. Fahlén-Yrlid, L., T. Gustafsson, J. Westlund, A. Holmberg, A. Strömbeck, M. Blomquist, G. G. MacPherson, J. Holmgren, and U. Yrlid. 2009. CD11c(high)dendritic cells are essential for activation of CD4+ T cells and generation of specific antibodies following mucosal immunization. *J. Immunol.* 183: 5032–5041.
336. Tittel, A. P., C. Heuser, C. Ohliger, C. Llanto, S. Yona, G. J. Hämmerling, D. R. Engel, N. Garbi, and C. Kurts. 2012. Functionally relevant neutrophilia in CD11c diphtheria toxin receptor transgenic mice. *Nat. Methods* 9: 385–390.
337. Probst, H. C., K. Tschannen, B. Odermatt, R. Schwendener, R. M. Zinkernagel, and Van Den Broek M. 2005. Histological analysis of CD11c-DTR/GFP mice after in vivo depletion of dendritic cells. *Clin Exp Immunol* 141: 398–404.
338. Cullen, M. K. 1993. Identification of a recombinogenic major histocompatibility complex Q gene with diverse alleles. *J Exp Med* 177: 1803–1807.
339. Guidry, P. A., and I. Stroynowski. 2005. The Murine Family of Gut-Restricted Class Ib MHC Includes Alternatively Spliced Isoforms of the Proposed HLA-G Homolog, "Blastocyst MHC." *The Journal of Immunology* 175: 5248–5259.
340. Blázquez, A. B., and M. C. Berin. 2008. Gastrointestinal dendritic cells promote Th2 skewing via OX40L. *J. Immunol.* 180: 4441–4450.
341. Xu-Amano, J., R. J. Jackson, K. Fujihashi, H. Kiyono, H. F. Staats, and J. R. McGhee. 1994. Helper Th1 and Th2 cell responses following mucosal or systemic immunization with cholera toxin. *Vaccine* 12: 903–911.
342. Carter, L. L., L. A. Fouser, J. Jussif, L. Fitz, B. Deng, C. R. Wood, M. Collins, T. Honjo, Honjo, G. J. Freeman, and B. M. Carreno. 2009. PD-1:PD-L inhibitory pathway affects both CD4+ and CD8+ T cells and is overcome by IL-2. *Eur. J. Immunol.* 32: 634–643.
343. He, J., L. M. Tsai, Y. A. Leong, X. Hu, C. S. Ma, N. Chevalier, X. Sun, K. Vandenberg, S. Rockman, Y. Ding, L. Zhu, W. Wei, C. Wang, A. Karnowski, G. T. Belz, J. R. Ghali, M. C. Cook, D. S. Riminton, A. Veillette, P. L. Schwartzberg, F. Mackay, R. Brink, S. G. Tangye, C. G. Vinuesa, C. R. Mackay, Z. Li, and Di Yu. 2013. Circulating Precursor CCR7loPD-1hi CXCR5+ CD4+ T Cells Indicate Tfh Cell Activity and Promote Antibody Responses upon Antigen Reexposure. *Immunity* 39: 770–781.