Immunology and Cell Biology (2015), 1–9

www.nature.com/icb

REVIEW

The importance of co-stimulation in the orchestration of T helper cell differentiation

Jonathan M Coquet[1], Lisa Rausch[1] and Jannie Borst[2]

Upon their activation, CD4 T cells can differentiate into distinct T helper cell subsets with specialised functions. Different T helper cell subsets produce specific cytokines that mediate beneficial and sometimes detrimental effects, depending on the infection or disease setting. CD4 T-cell priming relies on signals delivered by the T-cell antigen receptor, co-stimulatory receptors and cytokine receptors on the CD4 T-cell surface. Cytokine receptors are well known to deliver instructive signals that direct T helper cell differentiation. However, it is less appreciated that co-stimulatory receptors also exert potent modulatory effects on this process. In this review, we outline the contribution of co-stimulatory and co-inhibitory receptors to the process of T helper cell differentiation, focusing on those pathways for which the underlying mechanisms are best known. Herein, we depict the physiological context of T-cell priming and emphasise the impact of cell–cell communication on directing T helper cell differentiation.
Immunology and Cell Biology advance online publication, 21 April 2015; doi:10.1038/icb.2015.45

T HELPER CELLS—REGULATORS OF IMMUNITY

Mature CD4 and CD8 T cells express T-cell antigen receptors (TCR) that bind to peptides presented in the context of MHC class II or class I molecules. While activated CD8 T cells differentiate into cytotoxic T cells (CTL) that kill infected cells, CD4 T cells differentiate into diverse types of helper cells that regulate the function of T cells, B cells, natural killer cells, antigen presenting cells (APC), phagocytes and non-immune cells. During T-cell development in the thymus, the CD4 T-cell lineage separates from the CD8 T-cell lineage when $CD4^+$ $CD8^+$ thymocytes contact cortical thymic epithelial cells expressing MHC class II.[1] Signals through the TCR then induce the expression of the transcriptional regulators GATA3 and ThPOK, which together solidify the CD4 T-cell lineage.[2] In the thymic medulla, $CD4^+$ thymocytes with an autoreactive TCR are either instructed to die by apoptosis, or acquire Foxp3 expression and develop into natural regulatory T cells.[3] $CD4^+$ thymocytes that have a TCR specific for non-self-antigens become naive conventional CD4 T cells. Both natural regulatory T cells and naive conventional CD4 T cells emigrate from the thymus to peripheral lymphoid organs where natural regulatory T cells maintain tolerance to self-antigens, whereas conventional CD4 T cells respond to invading pathogens.

Naive CD4 T cells that are activated in secondary lymphoid organs differentiate into different T helper cell subsets with specialised functions. Classically, T helper cell subsets are discerned based on their ability to secrete distinct cytokines.[4] Th1 cells produce interferon-γ (IFN-γ) and are important in immunity to viruses and cancer, Th2 cells secrete interleukin- (IL-) 4, IL-5 and IL-13 and promote immunity to large extracellular pathogens, Th17 cells produce IL-17, which induces the release of antimicrobial peptides at mucosal surfaces and has a prominent role in antifungal immunity,[5] and Tfh cells produce IL-4 and IL-21 that regulate B-cell responses.[6] In addition, IL-9-producing Th9 cells and IL-22-producing Th22 cells have recently been described and are implicated in worm expulsion and wound healing respectively.[7] Upon their activation, naive CD4 T cells can also gain Foxp3 expression and differentiate into inducible Treg (iTreg) that suppress overactive immune responses.[8]

T helper cell subsets also differentially express certain chemokine receptors, allowing them to localise to specific niches to exert their function.[9] Chemotactic signals may also help to bring specific cell types together in specialised priming niches.[10,11] Although differential expression is not exclusive, Th1 cells typically express CCR5 and CXCR3, Th2 cells CCR3 and CCR4,[12] Th17 cells CCR6[13] and Tfh cells CXCR5.[14,15]

The differentiation of naive CD4 T cells into specialised T helper cell subsets is a complex process requiring cell proliferation, structural and epigenetic alterations in the genome and gene transcription mediated by sets of core- and inducible transcription factors.[4,16] Certain nuclear proteins called 'master regulators' coordinate the transcriptional programs that are pivotal for T helper cell subset differentiation. Among them, T-bet,[17] GATA3,[18] RORγt,[19] Bcl-6[20] and Foxp3[21] are critical for Th1, Th2, Th17, Tfh and iTreg-cell differentiation, respectively.

In this review, we discuss the contribution of co-stimulatory receptors to T helper cell differentiation, paying particular attention to the physiological context in which T helper cell differentiation takes place. We highlight the expression of co-stimulatory receptors and

[1]Department of Microbiology, Tumor and Cell Biology (MTC), Karolinska Institute, Stockholm, Sweden and [2]Division of Immunology, The Netherlands Cancer Institute, Amsterdam, The Netherlands
Correspondence: Dr J Coquet, Microbiology, Tumor and Cell Biology, Karolinska Institute, Nobels Väg 16, Stockholm 171 77, Sweden.
E-mail: jonathan.coquet@ki.se
Received 26 January 2015; revised 1 March 2015; accepted 18 March 2015; accepted article preview online 24 March 2015

ligands in specific cellular niches and focus on co-stimulatory receptor/ligand systems for which the contribution to T helper differentiation has been substantiated by insight into the underlying molecular mechanism.

CD4 T-CELL PRIMING AND MASTER REGULATORS OF T HELPER CELL DIFFERENTIATION

CD4 T-cell priming and associated effector differentiation is dictated by contact with specialised APC, in particular dendritic cells (DC). Migratory DC that come from the site of infection may initiate naive CD4 T-cell priming. However, it generally takes 12–24 h before tissue-resident DC infiltrate tissue-draining lymph nodes and prior to that,[22] soluble antigens may already be delivered to lymph node-resident DC or B cells through the conduit system.[23] Antigen may also be transferred to lymph node-resident APC from migratory DC.[24] Hence, multiple types of APC can be involved in T-cell priming, and these may act in consecutive fashion.

Studies using two-photon laser scanning microscopy have helped to discern T-cell priming into three phases; an initial phase wherein T cells make transient, yet meaningful contacts with many DC, a second more prolonged phase wherein stable contacts between DC and T cells facilitate T-cell division, and a third phase in which T cells regain motility and may cluster with DC/APC to receive further signals or exit the node.[25,26] The timing and duration of these phases are not set in stone and depend on the quality and quantity of antigen.

The expression pattern of 'master regulators' gives an insight into the complex process of CD4 T-cell priming. For instance, T-bet is expressed at least initially at a similar level in both Th1 and Th2 cells but is subsequently lost from Th2 cells.[27,28] RORγt and Foxp3 can be co-expressed initially upon T-cell activation and act antagonistically.[29] In general, the expression of one of these factors must be silenced for iTreg or Th17 cell differentiation to occur. Likewise the BCL-6 protein that is critical for Tfh-cell differentiation, can also be upregulated early in Tfh or Th1 cells.[30,31] Thus, the initial priming event is generally not deterministic and appears to make many cell fates possible. Specific T helper cell functions must thus be consolidated in subsequent steps of T-cell priming, potentially during contact with other APC types.

T HELPER CELL DIFFERENTIATION RELIES ON INSTRUCTIVE SIGNALS FROM CYTOKINES

If the initial activation of naive CD4 T cells makes many cell fates possible, then more precise instructive signals must be required to focus and consolidate certain fates over others. Interleukins such as IL-1β, IL-4, IL-6 and IL-12, and other cytokines including IFN-γ and TGF-β provide such instructive signals. These cytokines stimulate their receptors on the CD4 T-cell surface and activate downstream Janus kinases and Signal transducer and activator of transcription (STAT) molecules. There are six different STAT molecules that upon phosphorylation by Janus kinases dimerise and translocate to the nucleus. There, the specific cytokine receptors and STATs involved provide unique signals that activate specific T helper cell differentiation pathways.[32] They do this by inducing or enhancing the expression of the aforementioned master regulators of transcription. Subsequently, STATs consolidate T helper cell differentiation by regulating the transcription of other genes and directing epigenetic modifications.[33] In this way, IL-12 and IFN-γ direct Th1 differentiation, IL-4 directs Th2-cell differentiation and IL-1 and IL-6 direct Th17-cell differentiation.[4] TGF-β does not activate STAT molecules but rather directs iTreg-cell differentiation via the Smad transcription factor pathway.[32,34]

T HELPER CELL DIFFERENTIATION RELIES ON INSTRUCTIVE SIGNALS FROM DC AND OTHER APC

DC and other professional APC typically produce the cytokines that instruct T helper cell differentiation and activated CD4 T cells can make contacts with many such APC during priming. A number of factors influence the ability of DC to make distinct T helper cell-skewing cytokines. The first factor is the nature of the pathogen. DC activation by different stimuli results in the induction of distinct cytokines. For instance, stimulation of the pattern recognition receptor (PRR) Toll-like receptor (TLR)-4 by lipopolysaccharide stimulates DC to produce IL-12, giving rise to Th1-cell responses,[35] whereas the activation of the C-type lectin receptor Dectin-1 by fungal carbohydrates induces the production of IL-23, IL-1β and IL-6, thereby promoting Th17-cell responses.[36,37] The Th2-cell differentiation process is initiated differently, as the recently primed CD4 T cell rather than the APC produces the IL-4 that is required for this process. It has been proposed that pathogens such as house dust mite favour Th2-priming via IL-10 and IL-33 production by DC,[38] although it is not definitively proven that these cytokines provide instructive signals for Th2-cell commitment.

The second factor that influences the direction of T helper cell differentiation is the nature of the DC (or other APC) that mediates CD4 T-cell priming. DC are derived from a common dendritic cell precursor and can be divided into classical type I DCs (cDC1), cDC2 and plasmacytoid DC.[39] Batf3-dependent cDC1 induce Th1-cell differentiation, whereas IRF-4-dependent cDC2 can prime Th2-cell differentiation[38,40] and mucosal Th17-cell differentiation.[41] $CD103^+$ $CD11c^+$ DC in the mesenteric lymph nodes are also known to promote the conversion of naive T cells into $Foxp3^+$ iTreg via TGF-β and retinoic acid,[42,43] although whether these are cDC1 or cDC2 is not yet clear.

It is noteworthy that in certain anatomical locations, non-classical APC may also prime T helper cell responses in a specific manner. For example, both epidermal Langerhans cells and a subset of intestinal lamina propria cells resembling macrophages/monocytes can induce Th17-cell differentiation.[44,45] Furthermore, monocyte-derived cells can promote T helper cell differentiation under highly inflammatory conditions.[46] Hence, CD4 T-cell priming can occur in the context of different APC subsets that may direct the effector differentiation of CD4 T cells via the secretion of specific cytokines.

T HELPER CELL DIFFERENTIATION – WHERE CO-STIMULATION FITS IN

In vitro assays that make use of agonistic anti-CD3 and anti-CD28 antibodies together with 'instructive' cytokines facilitate T helper cell differentiation effectively, but it is important to realise that they do so in the absence of many pathways typically active *in vivo*. In the classical view, cytokines direct T helper differentiation, while co-stimulatory signals support TCR-induced CD4 T-cell activation, division and expansion. However, the exact nature of the interaction between TCR and MHC class II/peptide complexes can reportedly impact on T helper cell differentiation[11,47] and co-stimulatory receptors have been found to have prominent and specific roles in T helper cell differentiation in physiological settings.

Co-stimulatory and co-inhibitory receptors not only regulate the magnitude of the T-cell response,[48] but also its quality, as we will highlight here. These receptors fall into two families; the Immunoglobulin (Ig) superfamily including CD28, inducible T-cell costimulator (ICOS), programmed cell death protein 1 (PD-1), and signalling lymphocytic activation molecule (SLAM) family members, and the tumour necrosis factor (TNF) receptor superfamily that contains >20

receptors with membrane-bound homotrimeric TNF-like ligands.[48] We here describe the signalling mechanisms of key co-stimulatory receptors, to set the stage for outlining their contribution to T helper differentiation in the following sections.

CD28 is the prototypical co-stimulatory receptor, which upon binding to its ligands CD80 or CD86, signals in concert with the TCR/CD3 complex to allow naive T cells to pass the threshold for activation.[48,49] CD28 signalling amplifies the tyrosine kinase cascade downstream of the TCR/CD3 complex. This leads to the activation of several classical signal transduction pathways, including MAP kinases and c-Jun N-terminal kinases that activate activator protein 1 (AP-1) transcription factor complexes, protein kinase C that activates nuclear factor kappa B (NF-κB), Phospholipase C gamma that activates nuclear factor of activated T cells (NFAT), and the phosphatidylinositol 3-kinase pathway that activates Protein kinase B/Akt. Other members of the Ig-like superfamily also regulate tyrosine kinase activation downstream of the TCR, either in a positive way via tyrosine kinases, or in a negative way via tyrosine phosphatases (CTLA-4, PD-1).[48] TNF receptor superfamily members do not activate tyrosine kinase signalling. They associate with TNF receptor-associated factor (TRAF) signalling adaptors that via ubiquitin signalling activate NF-κB and also link to c-Jun N-terminal kinase and MAP kinase pathways.[48] Thus, signals through co-stimulatory and co-inhibitory receptors help to amplify or dampen responses to pathogens.

Naive CD4 T cells receive co-stimulatory inputs in various ways. PRR signalling initiates the synthesis of co-stimulatory TNF ligands like CD70 by DC and upregulates expression of CD80 and CD86. However, cognate interactions between naive CD4 T cells and DC in the first priming phase can also facilitate expression of co-stimulatory molecules by DC. This is particularly important for lymph node-resident DC that have received antigenic peptides, but were not directly exposed to the pathogen and hence received limited stimulation through PRRs.[26] When CD4 T cells recognise peptide/MHC class II complexes on otherwise resting DC, CD40 ligand is upregulated on the T cell and interacts with CD40 on the DC. This in turn upregulates CD80 and CD86 expression[50] and induces CD70 expression on the DC.[51,52] The communication of CD4 T cells with DC through CD40: CD40 ligand interactions, alone or in conjunction with signals from PRRs are said to 'license' the DC for T-cell priming. The initial PRR-mediated activation and/or CD4-mediated licensing of DC allows the co-stimulatory receptors CD28 and CD27 to promote CD4 T-cell responses in the context of TCR signals.[52,53] Subsequently, CD8 T cells profit from the same co-stimulatory ligands on the DC and from CD4 T-cell help.[54] Notably, signalling through co-stimulatory TNF receptors can also tune the T-cell response subsequent to initial T-cell activation.

CO-STIMULATION AND iTREG-CELL DIFFERENTIATION

At steady state, DC inhibit responses to autoantigens by promoting the activity of thymus-derived natural regulatory T cells. During an immune response to a pathogen, conventional CD4 T cells may differentiate into Foxp3-expressing iTreg, which restrain immune responses to avoid pathological side-effects. Co-stimulation via CD28 and CD27, which are already expressed on naive T cells, counteract iTreg-cell induction and function.[55,56] While fully licensed DC are capable of inducing all T helper cell populations, DC that lack CD40 or CD80 most potently induce iTreg[55] and poorly direct differentiation of other T helper cell populations.

There is evidence that iTreg-cell formation is favoured under these circumstances because DC lacking CD40 or CD80/CD86 fail to induce certain metabolic changes in the cognate T cell.[55,57] Naive CD4 T cells acquire energy from oxidative phosphorylation, but upon optimal activation the cell prepares for rapid clonal expansion by switching to aerobic glycolysis.[57] The ligation of CD28 by CD80 or CD86 on licensed DC strongly promotes activation of Akt and the downstream mammalian target of rapamycin (mTOR) that acts as a metabolic sensor and governs aerobic glycolysis. The activation of mTOR strongly promotes Th1-, Th2- and Th17-cell differentiation. However, in the absence of mTOR activation, iTreg-cell formation is favoured.[58,59] Hence, iTreg have a different type of metabolism as compared with other T helper cell subsets.

In agreement with the described function of CD28, the co-inhibitory receptor PD-1 that can displace CD28 from the immunological synapse and directly impairs TCR- and CD28-driven signals[48] enhances iTreg-cell development.[60] Thus, cognate interaction of naive CD4 T cells with sub-optimally activated DC can convert naïve CD4 T cells into iTreg due to a lack of CD40 signalling into APC and lack of CD28 and CD27 signalling into T cells. Induced Treg, like other T helper cell subsets likely receive co-stimulatory signals although it remains unclear to which extent these contribute directly to iTreg expansion and survival. Paracrine IL-2 is known to be critical for iTreg-cell expansion and survival, and this may in part negate the requirement for co-stimulatory inputs.[61]

Although licensed DC most strongly oppose the development of iTreg, evidence also indicates that the level of DC activation differentially affects the induction of Th1 and Th2 cells. One facet of DC licensing through CD40 is the upregulation of ICAM,[50,62] which facilitates prolonged interactions between DC and CD4 T cells that may favour Th1-cell differentiation.[63] This is in agreement with an early study suggesting that CD40 signals do not favour Th2-cell differentiation.[62] However, it is important to note that in the complete absence of CD40, Th2-cell responses are impaired.[64] Intriguingly, the PD-1 ligand PDL2 is expressed by Th2-inducing IRF-4-dependent DC and may promote IL-5 and IL-4 production from already differentiated Th2 cells.[40]

Taken together, current data argue that DC licensing steers T helper cell differentiation away from the iTreg-cell fate by effects on cell metabolism and disfavours Th2-cell differentiation.

CO-STIMULATION AND T HELPER CELL DIFFERENTIATION

Two major consequences of the initial priming event in T cells are: (1) the *de novo* expression or upregulation of co-stimulatory and co-inhibitory receptors and ligands, and (2) the altered expression of chemokines and chemokine receptors. Co-stimulatory receptors may directly affect transcription at certain cytokine gene loci or may orchestrate contact of CD4 T cells with specialised APC. The documented impact of various Ig-like and TNF receptor superfamily members on T helper cell differentiation and function is summarised in Supplementary Table 1. We discuss here the most salient examples.

Direct effects of co-stimulation on cytokine gene transcription

T helper cell differentiation and function are co-ordinated by specific transcription factors that recognise elements in gene promoters and enhancers and thereby regulate gene expression. Many co-stimulatory receptors overlap in their ability to activate transcription factors such as AP-1, NFAT and NF-κB,[48] which makes their contribution to T helper cell differentiation difficult to predict. However, some of these receptors have been shown to precisely regulate the expression of critical genes that influence T helper cell fate decisions. These are summarised in Figure 1.

The CD27:CD70 pathway directly affects T helper cell differentiation by regulating gene transcription in activated CD4 T cells. CD27 is already expressed on naive CD4 T cells and is further upregulated after

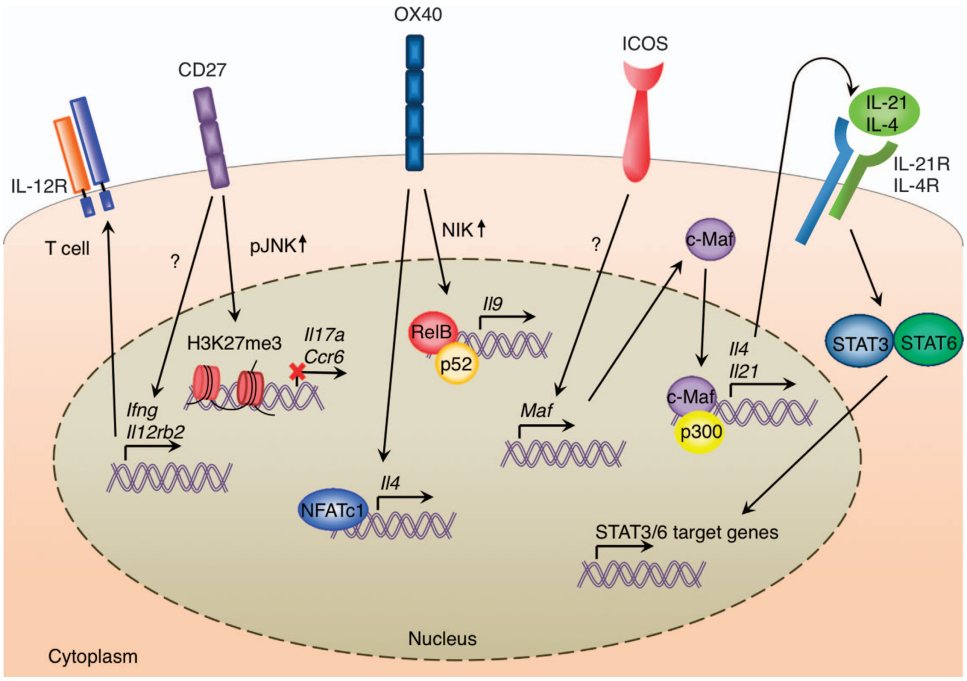

Figure 1 The direct effects of co-stimulatory receptors on gene expression. The figure depicts direct mechanisms by which co-stimulatory receptors can regulate gene transcription and thereby influence T helper cell differentiation and function.

T-cell activation.[65] Its ligand CD70 is expressed on DC that are activated by PRR, licensed by CD4 T cells or both.[51] In human CD4 T cells, CD27 signalling was shown to promote Th1-cell differentiation,[56,66] possibly by the induction of IL-12 Rβ2 expression. In mouse CD4 T cells, CD27 also drives Th1-cell differentiation, by a pathway that was proven to be IL-12 independent,[53,67] but is furthermore uncharacterised. The Th1-cell bias installed by CD27 signalling is highlighted by the phenotype of CD70 transgenic mice that have increased numbers of IFN-γ producing CD4 (and CD8 T cells) and a higher expression of IFN-γ on a per cell basis.[68,69]

Strikingly, in mouse CD4 T cells, CD27 signalling was found to counteract Th17-cell differentiation. It impeded the transcription of the *Il17a* and *Ccr6* genes in differentiated Th17 cells *in vitro* and *in vivo*, despite normal induction of RORγt and other molecular hallmarks of the Th17-cell subset.[68] In CD27-stimulated Th17 cells, c-Jun N-terminal kinase activity and expression of AP-1 binding partners Crem, Atf3 and Batf3 was increased, suggesting that CD27 signalling influenced AP-1 activity, which in turn influenced gene transcription in Th17 cells. Importantly, AP-1 is a critical regulator of Th17-cell differentiation[70] and inhibitors of AP-1 have since been proposed to regulate gene transcription in Th17 cells.[16] CD27 signalling furthermore impeded IL-17 expression by epigenetic effects on the *Il17a* gene,[68] highlighting the specific effects that CD27 can have on T helper cell differentiation.

Co-stimulation through CD28 supports the new synthesis of TNF receptor family member OX40 by activated T cells.[71,72] OX40 has been the focus of Th2 immunity for some time, as it potently induces *Il4* gene transcription, even in the presence of IL-12[73,74] and since OX40L transgenic mice are strongly biased to developing Th2 responses.[75] In the absence of an obvious DC-derived cytokine that polarises CD4 T cells towards the Th2-cell fate, it has been tempting to hypothesise that signalling through OX40 can have an instructive role in Th2-cell differentiation. OX40 is thought to induce NFATc1 translocation to the nucleus where it induces *Il4* gene transcription.[76] However, it is unknown whether OX40 collaborates with other receptors and whether it activates other genes besides the *Il4* gene.

Recently, OX40 was also shown to induce expression of the Th9-associated cytokine IL-9 by rapidly inducing translocation of Rel-b to the *Il9* promoter.[77] This proceeded via the activation of TRAF6 and the non-canonical NF-κB pathway. OX40 signals alone could not induce IL-9 production, but in combination with TGF-β and IL-4, OX40 signalling induced IL-9 expression within the first 24 h of activation.[77] This finding showcases that co-stimulatory receptors can affect T helper cell differentiation by rapidly and specifically altering gene transcription in co-operation with instructive signals provided by cytokines.

Another receptor that has been directly implicated in T helper cell differentiation is ICOS. ICOS signalling into CD4 T cells upregulates the transcription factor c-Maf, which regulates T helper cell differentiation.[78] Early evidence implicated ICOS in Th2-cell differentiation.[78–80] However, Tfh cells also highly express ICOS and are known to benefit from c-Maf activity,[81] suggesting a role for ICOS signalling in Tfh-cell differentiation as well. Accordingly, mice deficient in ICOS or c-Maf have poor Th2- and Tfh-cell responses, whereas mice overexpressing c-Maf are severely impaired in Th1-cell differentiation.[82] The role of c-Maf in Th17-cell differentiation is more complicated. Early after CD4 T-cell activation, c-Maf can repress the expression of Th17 cell-associated cytokines/chemokines and enhance the expression of the suppressive molecules CTLA-4 and IL-10.[16,83] However, later in the Th17-cell response, ICOS and c-Maf promote Th17-cell maintenance.[84] The impact of the ICOS/c-Maf pathway on Th2-, Tfh- and Th17-cell differentiation is attributed to promotion of IL-4 and IL-21 transcription[79,84] and the subsequent induction of STAT3 and STAT6.[85,86] However, c-Maf likely also has a broader role in gene regulation by binding to and regulating DNA modifiers such as CREB-binding protein and p300.[87] This interaction presumably

allows c-Maf to control transcription at many genetic loci in differentiating T helper cells.

Hence, co-stimulatory receptors activate broad transcriptional regulators such as NF-κB, AP-1, NFAT and c-Maf, which can have critical modulatory effects on instructive signals received through cytokine receptors and severely bias T helper cell subset differentiation.

Co-stimulation consolidates T helper cell programs by orchestrating cell contacts

Seminal studies from Sallusto *et al.*[12] identified distinct chemokine receptor expression patterns among distinct T helper cell subsets. Although these have been proposed to allow T helper cells to gain access to sites they would otherwise not enter,[9] there is evidence that they also help to consolidate T helper cell differentiation. Chemokine gradients present on the fibroblastic reticular network of the lymph nodes[88,89] allow activated T cells expressing various co-stimulatory receptors to relocate from the T-cell zone to other niches in the node. In these new niches, antigen presentation and the cytokine milieu may favour a certain T helper cell fate over another. In support of this, dermal- as opposed to epidermal-derived DC, which can differentially direct T helper cell subset specification[44] have been shown to form distinct clusters in skin-draining lymph nodes[90] and T cells in the third stage of priming are known to form tight clusters around DC.[26] Figure 2 summarises the crosstalk between co-stimulatory and chemotactic signals in the process of T helper cell differentiation.

The CXCR3:CXCL9/CXCL10 and CXCR5:CXCL13 chemokine axes, in particular, are closely linked with co-stimulatory pathways and have an important role in T helper cell differentiation. Chemotactic signals mediated by CXCR3 and its ligands CXCL9/CXCL10 are important for Th1-cell differentiation. It was observed that primed CD4 T cells that had gained CXCR3 expression relocated from the T-cell zone to subcapsular CXCL9-rich areas in the lymph node where

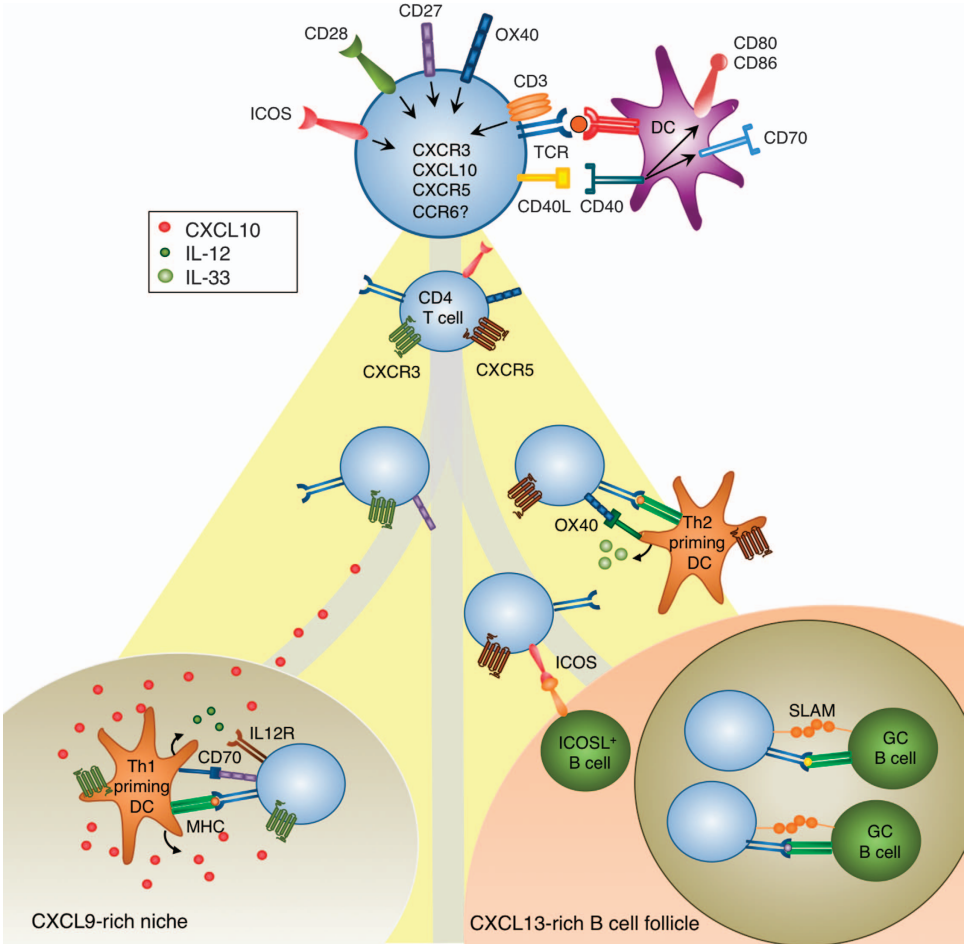

Figure 2 Co-stimulatory receptors and chemotactic signals orchestrate T helper cell differentiation. The figure depicts that co-stimulation induces the expression of chemotactic signals by activated CD4 T cells and thereby coordinates cell contacts with specialised APC. The initial priming of naive T cells by licensed DC leads to the upregulation of other co-stimulatory receptors/ligands, which in turn induces the expression of chemokines and chemokine receptors. Following the initial priming, activated CD4 T cells migrate on the fibroblastic reticular network of lymphoid organs in response to chemotactic signals left there by migrating T cells and DC. Chemokine gradients present in the lymphoid organ attract activated CD4 T cells to niches where the cytokine milieu and APC bias T helper cell differentiation further. CXCR3-expressing CD4 T cells may relocate from the T-cell zone to subcapsular CXCL9-rich areas in the lymph node and contact CXCL10$^+$ CD70-expressing DC, resulting in Th1-cell differentiation. CXCR5 expression allows activated T cells to migrate to the CXCL13-rich area of the B-cell follicle where they may encounter Th2-promoting DC, or enter the follicle and differentiate into Tfh cells. Inputs through the TCR, co-stimulatory and cytokine receptors help to further bias T helper cell differentiation in each of these lymph node niches.

they encountered CXCL10$^+$ DC, that promoted Th1-cell priming.[10] In the genetic absence of CXCR3, Th1-cell priming was impaired.[10]

Activity in the CXCR3:CXCL9/CXCL10 axis is closely linked to co-stimulation through CD27 on T cells. First, CD27 and CXCR3 are co-ordinately expressed on activated CD4 T-cell populations.[91] Intriguingly, CD27 co-stimulation can rapidly induce CXCL10 production in CD8 T cells[92] and this is also possibly the case in CD4 T cells. Upregulation of CXCL10 by T cells may attract CXCR3-expressing DC, which correspond to Th1-priming CD8$^+$ DC in secondary lymphoid organs and to migratory CD103$^+$ DC from the lung (www.immgen.org). Thus the CD27/CD70 pathway and the CXCR3/CXCL10 axis may collaborate to potentiate Th1-cell differentiation.

The differentiation of Tfh and Th2 cells also involves multiple priming steps and relocation of primed CD4 T cells in the lymph node under the guidance of the CXCR5:CXCL13 axis.[6,74,93] CD28, ICOS, CD40 and OX40 are all important for CXCR5 expression on activated CD4 T cells,[72,94–96] allowing the T cells to migrate towards the CXCL13-rich area of the B-cell follicle. At the border between the T- and B-cell zone, activated CD4 T cells are likely not yet committed. Depending on the signals they receive and the APC they contact, they may differentiate into either Th2 or Tfh cells. Their final fate may depend on cross talk with lymph node-infiltrating DC and B cells at this site. DC can be attracted to the T–B cell border by lymphotoxin-expressing B cells and can facilitate further Th2-cell priming.[93] These DC may potentiate full effector Th2-cell differentiation by expressing IL-33[38] or OX40L.[74,97] Subsequent to priming at the T–B cell border, CD4 T cells may exit the node to act as Th2 effector cells in distant tissues such as the lungs or the gut.

Alternatively, CXCR5$^+$ CD4 T cells at the T–B cell border may contact ICOSL-expressing B cells, which facilitates their movement into the follicle. ICOS signalling into the CD4 T cell further increases CXCR5 expression[95] and promotes cell motility through the phosphatidylinositol 3-kinase pathway. This step is independent of cognate interactions with follicular B cells[98] and demonstrates that in activated T cells, co-stimulatory receptors may promote effects independently of TCR triggering. Once these CD4 T cells enter the follicle, differentiation to the Tfh-cell fate is consolidated by cognate interactions with antigen-specific B cells that express receptors of the SLAM family.[99] SLAM receptors typically undergo homotypic interactions (that is SLAM on T cells binds to SLAM on B cells) in the germinal centre and allow for optimal Tfh-cell differentiation, B-cell survival and antibody class switching. The adaptor molecule SAP, which is activated downstream of many SLAM family members is important for IL-4 and IL-21 production by Tfh cells,[100] whereas other SLAM family members such as CD84 facilitate stable cell–cell contact.[101] Hence, in the germinal centre, SLAM family members provide critical inputs for cytokine production by Tfh cells.

A recent study has hypothesised that Th17-cell differentiation can also proceed in dedicated gut niches.[11] Whether this is reliant on CCR6 expression on Th17 cells and what co-stimulatory receptors may trigger CCR6 expression is worth exploration. Thus, co-stimulation induces the expression of chemotactic molecules that promote contacts with other cells, migration within the lymph node and the eventual consolidation of functionally distinct T helper cell subsets.

COMING TO A CONSENSUS ON THE ROLE OF CO-STIMULATORY AND CO-INHIBITORY RECEPTORS IN T HELPER CELL DIFFERENTIATION

Co-stimulatory and co-inhibitory receptors are not typically appreciated to regulate T helper cell differentiation. These cell surface receptor/ligand interactions do not instruct the development of T helper cell subsets in the same way that cytokine receptors, STATs and their downstream master regulators of transcription do. However, two points are important when considering their role in effector T-cell differentiation. The first is that seemingly 'instructive' signals that dictate the function of the master regulator cannot produce an effect without the co-operation of other regulators of gene function. The direct impact of one master regulator RORγt on gene transcription, for example, is in fact quite small when compared to the gene-amplifying or suppressing function of other transcriptional regulators such as NFAT, AP-1, c-Maf, BATF and IRF4.[16] These factors co-operate with one another in complex modules rather than in isolation and many are targets of co-stimulatory receptor activation. Second, the guidance cues that co-stimulatory receptors provide ensure that activated CD4 T cells continue their differentiation in niches where their fate becomes more probabilistic and less stochastic. Hence, although signals through co-stimulatory receptors are not always easy to connect to specific cellular fates, they have crucial regulatory roles in physiological conditions and are potential therapeutic targets.

TARGETING CO-STIMULATORY AND CO-INHIBITORY RECEPTOR FUNCTION IN THE CLINIC

Recently, antibodies that block the co-inhibitory function of PD-1 and CTLA-4 have provided a breakthrough in cancer immunotherapy,[102] as they can markedly promote CD8 T-cell responses to late stage, metastatic cancers. Conversely, agonist antibodies that target co-stimulatory receptors may also boost CD8 T-cell responses to tumours. Based on the experience with TGN1412, an antibody targeting CD28,[103] which induced a cytokine storm and multi-organ failure in six patients, there is a fear that deliberate activation of co-stimulatory receptors is dangerous. However, this case has little predictive value, as TGN1412 was a superagonist antibody, enabling T-cell activation independently of the TCR. A therapeutic agonist antibody to a co-stimulatory receptor should support a TCR-initiated response and not initiate a T-cell response on its own. Such antibodies are currently very actively pursued for cancer immunotherapy and agonist antibodies to CD27, 4-1BB and OX40 are currently in clinical trials.

It is appealing to consider the reverse approach: namely, the agonism of co-inhibitory receptors and blocking of co-stimulatory receptors in autoimmunity and chronic inflammatory diseases, where CD4 T cells have a major role. Steering T helper cell responses in the desired direction (that is, towards iTreg cells and away from pathogenic T helper cell populations in autoimmune patients) by targeting the activity of these molecules is plausible. One recent trial in which OX40L was blocked in patients with mild allergic asthma reported considerable reductions in total IgE and sputum eosinophilia despite little improvement in airway resistance.[104] Soluble CTLA-4 Ig administration also considerably enhanced quality of life in patients with rheumatoid arthritis.[105] These examples encourage the manipulation of co-stimulatory and co-inhibitory pathways in the treatment of inflammatory diseases. The main issues are that many cell types may express these receptors and there is the possibility of unacceptable side-effects.

In humans, rare genetic deficiencies have been reported that indicate the importance of co-stimulatory and co-inhibitory receptors

for normal T- and B-cell function. Patients with genetic loss of CD27, OX40, CD40/CD40 ligand or ICOS suffer from persistent infections and/or virus-induced malignancies, which can have lethal consequences.[106–110] It is important to note that therapeutic intervention with a receptor-targeting antibody is transient, in contrast to the permanent genetic loss of receptor function. Indeed, loss of tolerance to 'self' antigens and autoimmune side-effects do occur in cancer patients as a result of CTLA-4 and/or PD-1 inhibition, but these for the most part have been transient, while tumour regression can be permanent.[111]

OUTLOOK

Discoveries in animal models and *in vitro* work on human cells have led to the successful use of antibodies targeting co-stimulatory and co-inhibitory molecules in patients with cancer. In particular, recombinant mice have provided fundamental insights into the function of co-stimulatory receptors and, in general, have accurately reflected the role of these molecules in humans. We must now endeavour to more vigorously test and translate our preclinical findings to benefit patients with other disorders. Human *in vitro* T helper cell differentiation assays need to be better defined and standardised across the world, especially for Th2 and iTreg cells. The effects of agonist/blocking antibodies and recombinant molecules targeting co-stimulatory and co-inhibitory molecules must be tested in *in vitro* assays and in early phase clinical trials. More thorough diagnostic analysis of T helper cells in human inflammatory diseases, not just from patient blood but also from tissues and lymph nodes must also be conducted.

There is also room to improve on current preclinical models. For instance, humanised mouse models may help to uncover toxicities associated with some therapies before they are tested in humans.[112] Conditional and inducible deletion of co-stimulatory receptors can more precisely define the function of co-stimulatory molecules in specific cell populations. Furthermore, improved models of complex inflammatory and autoimmune diseases such as asthma, arthritis and inflammatory bowel disease are required to uncover the underlying aetiology of these disorders. These models will need to better replicate the genetic and environmental diversity that influences inflammation and autoimmunity in humans.

Finally, a greater understanding of the pathways activated and the outcomes of co-stimulatory and co-inhibitory receptor ligation in T helper cells is required. This involves clearly defining the intracellular cascades and effects on gene transcription triggered by these molecules. Such analyses should be undertaken in human cells whenever possible and we should work towards being able to predict the outcome of co-stimulatory and co-inhibitory signalling events. A clearer understanding of the molecular events will ultimately enhance the efficacy and safety of therapies that target these molecules.

ACKNOWLEDGEMENTS

Jonathan Coquet is supported by a grant from the Swedish Research Council (VR/2015-18/521-2014-2858).

1 Germain RN. T-cell development and the CD4-CD8 lineage decision. *Nat Rev Immunol* 2002; **2**: 309–322.
2 He X, Park K, Kappes DJ. The role of ThPOK in control of CD4/CD8 lineage commitment. *Annu Rev Immunol* 2010; **28**: 295–320.
3 Josefowicz SZ, Rudensky A. Control of regulatory T cell lineage commitment and maintenance. *Immunity* 2009; **30**: 616–625.
4 Wilson CB, Rowell E, Sekimata M. Epigenetic control of T-helper-cell differentiation. *Nat Rev Immunol* 2009; **9**: 91–105.
5 Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. *Annu Rev Immunol* 2009; **27**: 485–517.
6 Ma CS, Deenick EK, Batten M, Tangye SG. The origins, function, and regulation of T follicular helper cells. *J Exp Med* 2012; **209**: 1241–1253.
7 Zhu J, Paul WE. Heterogeneity and plasticity of T helper cells. *Cell Res* 2010; **20**: 4–12.
8 Zhou L, Chong MM, Littman DR. Plasticity of CD4+ T cell lineage differentiation. *Immunity* 2009; **30**: 646–655.
9 Reboldi A, Coisne C, Baumjohann D, Benvenuto F, Bottinelli D, Lira S *et al.* C-C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. *Nat Immunol* 2009; **10**: 514–523.
10 Groom JR, Richmond J, Murooka TT, Sorensen EW, Sung JH, Bankert K *et al.* CXCR3 chemokine receptor-ligand interactions in the lymph node optimize CD4+ T helper 1 cell differentiation. *Immunity* 2012; **37**: 1091–1103.
11 Yang Y, Torchinsky MB, Gobert M, Xiong H, Xu M, Linehan JL *et al.* Focused specificity of intestinal TH17 cells towards commensal bacterial antigens. *Nature* 2014; **510**: 152–156.
12 Sallusto F, Lenig D, Mackay CR, Lanzavecchia A. Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. *J Exp Med* 1998; **187**: 875–883.
13 Annunziato F, Cosmi L, Santarlasci V, Maggi L, Liotta F, Mazzinghi B *et al.* Phenotypic and functional features of human Th17 cells. *J Exp Med* 2007; **204**: 1849–1861.
14 Schaerli P, Willimann K, Lang AB, Lipp M, Loetscher P, Moser B. CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. *J Exp Med* 2000; **192**: 1553–1562.
15 Breitfeld D, Ohl L, Kremmer E, Ellwart J, Sallusto F, Lipp M *et al.* Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. *J Exp Med* 2000; **192**: 1545–1552.
16 Ciofani M, Madar A, Galan C, Sellars M, Mace K, Pauli F *et al.* A validated regulatory network for Th17 cell specification. *Cell* 2012; **151**: 289–303.
17 Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 2000; **100**: 655–669.
18 Zheng W, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* 1997; **89**: 587–596.
19 Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ *et al.* The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* 2006; **126**: 1121–1133.
20 Johnston RJ, Poholek AC, DiToro D, Yusuf I, Eto D, Barnett B *et al.* Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. *Science* 2009; **325**: 1006–1010.
21 Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc Natl Acad Sci USA* 2010; **107**: 12204–12209.
22 Langenkamp A, Messi M, Lanzavecchia A, Sallusto F. Kinetics of dendritic cell activation: impact on priming of TH1, TH2 and nonpolarized T cells. *Nat Immunol* 2000; **1**: 311–316.
23 Sixt M, Kanazawa N, Selg M, Samson T, Roos G, Reinhardt DP *et al.* The conduit system transports soluble antigens from the afferent lymph to resident dendritic cells in the T cell area of the lymph node. *Immunity* 2005; **22**: 19–29.
24 Allan RS, Waithman J, Bedoui S, Jones CM, Villadangos JA, Zhan Y *et al.* Migratory dendritic cells transfer antigen to a lymph node-resident dendritic cell population for efficient CTL priming. *Immunity* 2006; **25**: 153–162.
25 Mempel TR, Henrickson SE, Von Andrian UH. T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. *Nature* 2004; **427**: 154–159.
26 Bousso P. T-cell activation by dendritic cells in the lymph node: lessons from the movies. *Nat Rev Immunol* 2008; **8**: 675–684.
27 Ylikoski E, Lund R, Kylaniemi M, Filen S, Kilpelainen M, Savolainen J *et al.* IL-12 up-regulates T-bet independently of IFN-gamma in human CD4+ T cells. *Eur J Immunol* 2005; **35**: 3297–3306.
28 Fang M, Xie H, Dougan SK, Ploegh H, van Oudenaarden A. Stochastic cytokine expression induces mixed T helper cell States. *PLoS Biol* 2013; **11**: e1001618.
29 Zhou L, Lopes JE, Chong MM, Ivanov II, Min R, Victora GD *et al.* TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. *Nature* 2008; **453**: 236–240.
30 Choi YS, Yang JA, Yusuf I, Johnston RJ, Greenbaum J, Peters B *et al.* Bcl6 expressing follicular helper CD4 T cells are fate committed early and have the capacity to form memory. *J Immunol* 2013; **190**: 4014–4026.
31 Nakayamada S, Kanno Y, Takahashi H, Jankovic D, Lu KT, Johnson TA *et al.* Early Th1 cell differentiation is marked by a Tfh cell-like transition. *Immunity* 2011; **35**: 919–931.
32 O'Shea JJ, Lahesmaa R, Vahedi G, Laurence A, Kanno Y. Genomic views of STAT function in CD4+ T helper cell differentiation. *Nat Rev Immunol* 2011; **11**: 239–250.
33 Wei G, Wei L, Zhu J, Zang C, Hu-Li J, Yao Z *et al.* Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4+ T cells. *Immunity* 2009; **30**: 155–167.
34 Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature* 2003; **425**: 577–584.
35 Re F, Strominger JL. Toll-like receptor 2 (TLR2) and TLR4 differentially activate human dendritic cells. *J Biol Chem* 2001; **276**: 37692–37699.
36 LeibundGut-Landmann S, Gross O, Robinson MJ, Osorio F, Slack EC, Tsoni SV *et al.* Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. *Nat Immunol* 2007; **8**: 630–638.

37 Zenaro E, Donini M, Dusi S. Induction of Th1/Th17 immune response by Mycobacterium tuberculosis: role of dectin-1, Mannose Receptor, and DC-SIGN. *J Leukoc Biol* 2009; **86**: 1393–1401.
38 Williams JW, Tjota MY, Clay BS, Vander Lugt B, Bandukwala HS, Hrusch CL *et al.* Transcription factor IRF4 drives dendritic cells to promote Th2 differentiation. *Nat Commun* 2013; **4**: 2990.
39 Guilliams M, Ginhoux F, Jakubzick C, Naik SH, Onai N, Schraml BU *et al.* Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat Rev Immunol* 2014; **14**: 571–578.
40 Gao Y, Nish SA, Jiang R, Hou L, Licona-Limon P, Weinstein JS *et al.* Control of T helper 2 responses by transcription factor IRF4-dependent dendritic cells. *Immunity* 2013; **39**: 722–732.
41 Persson EK, Uronen-Hansson H, Semmrich M, Rivollier A, Hagerbrand K, Marsal J *et al.* IRF4 transcription-factor-dependent CD103(+)CD11b(+) dendritic cells drive mucosal T helper 17 cell differentiation. *Immunity* 2013; **38**: 958–969.
42 Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y *et al.* A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med* 2007; **204**: 1757–1764.
43 Denning TL, Wang YC, Patel SR, Williams IR, Pulendran B. Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. *Nat Immunol* 2007; **8**: 1086–1094.
44 Igyarto BZ, Haley K, Ortner D, Bobr A, Gerami-Nejad M, Edelson BT *et al.* Skin-resident murine dendritic cell subsets promote distinct and opposing antigen-specific T helper cell responses. *Immunity* 2011; **35**: 260–272.
45 Atarashi K, Nishimura J, Shima T, Umesaki Y, Yamamoto M, Onoue M *et al.* ATP drives lamina propria T(H)17 cell differentiation. *Nature* 2008; **455**: 808–812.
46 Plantinga M, Guilliams M, Vanheerswynghels M, Deswarte K, Branco-Madeira F, Toussaint W *et al.* Conventional and monocyte-derived CD11b(+) dendritic cells initiate and maintain T helper 2 cell-mediated immunity to house dust mite allergen. *Immunity* 2013; **38**: 322–335.
47 Tubo NJ, Jenkins MK. TCR signal quantity and quality in CD4 T cell differentiation. *Trends Immunol* 2014; **35**: 591–596.
48 Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat Rev Immunol* 2013; **13**: 227–242.
49 Acuto O, Michel F. CD28-mediated co-stimulation: a quantitative support for TCR signalling. *Nat Rev Immunol* 2003; **3**: 939–951.
50 Cella M, Scheidegger D, Palmer-Lehmann K, Lane P, Lanzavecchia A, Alber G. Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. *J Exp Med* 1996; **184**: 747–752.
51 Tesselaar K, Xiao Y, Arens R, van Schijndel GM, Schuurhuis DH, Mebius RE *et al.* Expression of the murine CD27 ligand CD70 *in vitro* and *in vivo*. *J Immunol* 2003; **170**: 33–40.
52 Keller AM, Groothuis TA, Veraar EA, Marsman M, Maillette de Buy Wenniger L, Janssen H *et al.* Costimulatory ligand CD70 is delivered to the immunological synapse by shared intracellular trafficking with MHC class II molecules. *Proc Natl Acad Sci USA* 2007; **104**: 5989–5994.
53 Xiao Y, Peperzak V, Keller AM, Borst J. CD27 instructs CD4+ T cells to provide help for the memory CD8+ T cell response after protein immunization. *J Immunol* 2008; **181**: 1071–1082.
54 Feau S, Garcia Z, Arens R, Yagita H, Borst J, Schoenberger SP. The CD4(+) T-cell help signal is transmitted from APC to CD8(+) T-cells via CD27-CD70 interactions. *Nature Commun* 2012; **3**: 948.
55 Wang L, Pino-Lagos K, de Vries VC, Guleria I, Sayegh MH, Noelle RJ. Programmed death 1 ligand signaling regulates the generation of adaptive Foxp3+CD4+ regulatory T cells. *Proc Natl Acad Sci USA* 2008; **105**: 9331–9336.
56 Pen JJ, De Keersmaecker B, Maenhout SK, Van Nuffel AM, Heirman C, Corthals J *et al.* Modulation of regulatory T cell function by monocyte-derived dendritic cells matured through electroporation with mRNA encoding CD40 ligand, constitutively active TLR4, and CD70. *J Immunol* 2013; **191**: 1976–1983.
57 MacIver NJ, Michalek RD, Rathmell JC. Metabolic regulation of T lymphocytes. *Annu Rev Immunol* 2013; **31**: 259–283.
58 Battaglia M, Stabilini A, Migliavacca B, Horejs-Hoeck J, Kaupper T, Roncarolo MG. Rapamycin promotes expansion of functional CD4+CD25+FOXP3+ regulatory T cells of both healthy subjects and type 1 diabetic patients. *J Immunol* 2006; **177**: 8338–8347.
59 Delgoffe GM, Pollizzi KN, Waickman AT, Heikamp E, Meyers DJ, Horton MR *et al.* The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. *Nat Immunol* 2011; **12**: 295–303.
60 Francisco LM, Salinas VH, Brown KE, Vanguri VK, Freeman GJ, Kuchroo VK *et al.* PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. *J Exp Med* 2009; **206**: 3015–3029.
61 Chen Q, Kim YC, Laurence A, Punkosdy GA, Shevach EM. IL-2 controls the stability of Foxp3 expression in TGF-beta-induced Foxp3+ T cells *in vivo*. *J Immunol* 2011; **186**: 6329–6337.
62 Ruedl C, Bachmann MF, Kopf M. The antigen dose determines T helper subset development by regulation of CD40 ligand. *Eur J Immunol* 2000; **30**: 2056–2064.
63 van Panhuys N, Klauschen F, Germain RN. T-cell-receptor-dependent signal intensity dominantly controls CD4(+) T cell polarization in vivo. *Immunity* 2014; **41**: 63–74.
64 MacDonald AS, Straw AD, Dalton NM, Pearce EJ. Cutting edge: Th2 response induction by dendritic cells: a role for CD40. *J Immunol* 2002; **168**: 537–540.
65 Hintzen RQ, de Jong R, Lens SM, Brouwer M, Baars P, van Lier RA. Regulation of CD27 expression on subsets of mature T-lymphocytes. *J Immunol* 1993; **151**: 2426–2435.
66 van Oosterwijk MF, Juwana H, Arens R, Tesselaar K, van Oers MH, Eldering E *et al.* CD27-CD70 interactions sensitise naive CD4$^+$ T cells for IL-12-induced Th1 cell development. *Int Immunol* 2007; **19**: 713–718.
67 Soares H, Waechter H, Glaichenhaus N, Mougneau E, Yagita H, Mizenina O *et al.* A subset of dendritic cells induces CD4$^+$ T cells to produce IFN-gamma by an IL-12-independent but CD70-dependent mechanism *in vivo*. *J Exp Med* 2007; **204**: 1095–1106.
68 Coquet JM, Middendorp S, van der Horst G, Kind J, Veraar EA, Xiao Y *et al.* The CD27 and CD70 costimulatory pathway inhibits effector function of T helper 17 cells and attenuates associated autoimmunity. *Immunity* 2013; **38**: 53–65.
69 Arens R, Tesselaar K, Baars PA, van Schijndel GM, Hendriks J, Pals ST *et al.* Constitutive CD27/CD70 interaction induces expansion of effector-type T cells and results in IFNgamma-mediated B cell depletion. *Immunity* 2001; **15**: 801–812.
70 Li P, Spolski R, Liao W, Wang L, Murphy TL, Murphy KM *et al.* BATF-JUN is critical for IRF4-mediated transcription in T cells. *Nature* 2012; **490**: 543–546.
71 Pollok KE, Kim YJ, Zhou Z, Hurtado J, Kim KK, Pickard RT *et al.* Inducible T cell antigen 4-1BB. Analysis of expression and function. *J Immunol* 1993; **150**: 771–781.
72 Walker LS, Gulbranson-Judge A, Flynn S, Brocker T, Raykundalia C, Goodall M *et al.* Compromised OX40 function in CD28-deficient mice is linked with failure to develop CXC chemokine receptor 5-positive CD4 cells and germinal centers. *J Exp Med* 1999; **190**: 1115–1122.
73 Ohshima Y, Yang LP, Uchiyama T, Tanaka Y, Baum P, Sergerie M *et al.* OX40 costimulation enhances interleukin-4 (IL-4) expression at priming and promotes the differentiation of naive human CD4(+) T cells into high IL-4-producing effectors. *Blood* 1998; **92**: 3338–3345.
74 Flynn S, Toellner KM, Raykundalia C, Goodall M, Lane P. 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* 1998; **188**: 297–304.
75 Ishii N, Ndhlovu LC, Murata K, Sato T, Kamanaka M, Sugamura K. OX40 (CD134) and OX40 ligand interaction plays an adjuvant role during *in vivo* Th2 responses. *Eur J Immunol* 2003; **33**: 2372–2381.
76 So T, Song J, Sugie K, Altman A, Croft M. Signals from OX40 regulate nuclear factor of activated T cells c1 and T cell helper 2 lineage commitment. *Proc Natl Acad Sci USA* 2006; **103**: 3740–3745.
77 Xiao X, Balasubramanian S, Liu W, Chu X, Wang H, Taparowsky EJ *et al.* OX40 signaling favors the induction of T(H)9 cells and airway inflammation. *Nat Immunol* 2012; **13**: 981–990.
78 Nurieva RI, Duong J, Kishikawa H, Dianzani U, Rojo JM, Ho I *et al.* Transcriptional regulation of th2 differentiation by inducible costimulator. *Immunity* 2003; **18**: 801–811.
79 Dong C, Juedes AE, Temann UA, Shresta S, Allison JP, Ruddle NH *et al.* ICOS co-stimulatory receptor is essential for T-cell activation and function. *Nature* 2001; **409**: 97–101.
80 Tafuri A, Shahinian A, Bladt F, Yoshinaga SK, Jordana M, Wakeham A *et al.* ICOS is essential for effective T-helper-cell responses. *Nature* 2001; **409**: 105–109.
81 Kroenke MA, Eto D, Locci M, Cho M, Davidson T, Haddad EK *et al.* Bcl6 and Maf cooperate to instruct human follicular helper CD4 T cell differentiation. *J Immunol* 2012; **188**: 3734–3744.
82 Ho IC, Lo D, Glimcher LH. c-maf promotes T helper cell type 2 (Th2) and attenuates Th1 differentiation by both interleukin 4-dependent and -independent mechanisms. *J Exp Med* 1998; **188**: 1859–1866.
83 Xu J, Yang Y, Qiu G, Lal G, Wu Z, Levy DE *et al.* c-Maf regulates IL-10 expression during Th17 polarization. *J Immunol* 2009; **182**: 6226–6236.
84 Bauquet AT, Jin H, Paterson AM, Mitsdoerffer M, Ho IC, Sharpe AH *et al.* 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* 2009; **10**: 167–175.
85 Stritesky GL, Muthukrishnan R, Sehra S, Goswami R, Pham D, Travers J *et al.* The transcription factor STAT3 is required for T helper 2 cell development. *Immunity* 2011; **34**: 39–49.
86 Yang Y, Ochando J, Yopp A, Bromberg JS, Ding Y. IL-6 plays a unique role in initiating c-Maf expression during early stage of CD4 T cell activation. *J Immunol* 2005; **174**: 2720–2729.
87 Chen Q, Dowhan DH, Liang D, Moore DD, Overbeek PA. CREB-binding protein/p300 co-activation of crystallin gene expression. *J Biol Chem* 2002; **277**: 24081–24089.
88 Bajenoff M, Egen JG, Koo LY, Laugier JP, Brau F, Glaichenhaus N *et al.* Stromal cell networks regulate lymphocyte entry, migration, and territoriality in lymph nodes. *Immunity* 2006; **25**: 989–1001.
89 Acton SE, Astarita JL, Malhotra D, Lukacs-Kornek V, Franz B, Hess PR *et al.* Podoplanin-rich stromal networks induce dendritic cell motility via activation of the C-type lectin receptor CLEC-2. *Immunity* 2012; **37**: 276–289.
90 Kissenpfennig A, Henri S, Dubois B, Laplace-Builhe C, Perrin P, Romani N *et al.* Dynamics and function of Langerhans cells *in vivo*: dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells. *Immunity* 2005; **22**: 643–654.
91 Hikono H, Kohlmeier JE, Takamura S, Wittmer ST, Roberts AD, Woodland DL. Activation phenotype, rather than central- or effector-memory phenotype, predicts the recall efficacy of memory CD8+ T cells. *J Exp Med* 2007; **204**: 1625–1636.

92 Peperzak V, Veraar EA, Xiao Y, Babala N, Thiadens K, Brugmans M *et al.* CD8+ T cells produce the chemokine CXCL10 in response to CD27/CD70 costimulation to promote generation of the CD8$^+$ effector T cell pool. *J Immunol* 2013; **191**: 3025–3036.
93 Leon B, Ballesteros-Tato A, Browning JL, Dunn R, Randall TD, Lund FE. Regulation of T(H)2 development by CXCR5+ dendritic cells and lymphotoxin-expressing B cells. *Nat Immunol* 2012; **13**: 681–690.
94 Salek-Ardakani S, Choi YS, Rafii-El-Idrissi Benhnia M, Flynn R, Arens R, Shoenberger S *et al.* B cell-specific expression of B7-2 is required for follicular Th cell function in response to vaccinia virus. *J Immunol* 2011; **186**: 5294–5303.
95 Choi YS, Kageyama R, Eto D, Escobar TC, Johnston RJ, Monticelli L *et al.* ICOS receptor instructs T follicular helper cell versus effector cell differentiation via induction of the transcriptional repressor Bcl6. *Immunity* 2011; **34**: 932–946.
96 Akiba H, Takeda K, Kojima Y, Usui Y, Harada N, Yamazaki T *et al.* The role of ICOS in the CXCR5+ follicular B helper T cell maintenance *in vivo*. *J Immunol* 2005; **175**: 2340–2348.
97 Besnard AG, Togbe D, Guillou N, Erard F, Quesniaux V, Ryffel B. IL-33-activated dendritic cells are critical for allergic airway inflammation. *Eur J Immunol* 2011; **41**: 1675–1686.
98 Xu H, Li X, Liu D, Li J, Zhang X, Chen X *et al.* Follicular T-helper cell recruitment governed by bystander B cells and ICOS-driven motility. *Nature* 2013; **496**: 523–527.
99 King C, Tangye SG, Mackay CR. T follicular helper (TFH) cells in normal and dysregulated immune responses. *Annu Rev Immunol* 2008; **26**: 741–766.
100 Yusuf I, Kageyama R, Monticelli L, Johnston RJ, Ditoro D, Hansen K *et al.* Germinal center T follicular helper cell IL-4 production is dependent on signaling lymphocytic activation molecule receptor (CD150). *J Immunol* 2010; **185**: 190–202.
101 Cannons JL, Qi H, Lu KT, Dutta M, Gomez-Rodriguez J, Cheng J *et al.* Optimal germinal center responses require a multistage T cell:B cell adhesion process involving integrins, SLAM-associated protein, and CD84. *Immunity* 2010; **32**: 253–265.
102 Drake CG, Lipson EJ, Brahmer JR. Breathing new life into immunotherapy: review of melanoma, lung and kidney cancer. *Nat Rev Clin Oncol* 2014; **11**: 24–37.
103 Suntharalingam G, Perry MR, Ward S, Brett SJ, Castello-Cortes A, Brunner MD *et al.* Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *N Engl J Med* 2006; **355**: 1018–1028.
104 Gauvreau GM, Boulet LP, Cockcroft DW, FitzGerald JM, Mayers I, Carlsten C *et al.* OX40L blockade and allergen-induced airway responses in subjects with mild asthma. *Clin Exp Allergy* 2014; **44**: 29–37.
105 Genovese MC, Becker JC, Schiff M, Luggen M, Sherrer Y, Kremer J *et al.* Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition. *N Engl J Med* 2005; **353**: 1114–1123.
106 van Kooten C, Banchereau J. CD40-CD40 ligand. *J Leukoc Biol* 2000; **67**: 2–17.
107 Salzer E, Daschkey S, Choo S, Gombert M, Santos-Valente E, Ginzel S *et al.* Combined immunodeficiency with life-threatening EBV-associated lymphoproliferative disorder in patients lacking functional CD27. *Haematologica* 2013; **98**: 473–478.
108 van Montfrans JM, Hoepelman AI, Otto S, van Gijn M, van de Corput L, de Weger RA *et al.* CD27 deficiency is associated with combined immunodeficiency and persistent symptomatic EBV viremia. *J Allergy Clin Immunol* 2012; **129**: 787–793 e6.
109 Grimbacher B, Hutloff A, Schlesier M, Glocker E, Warnatz K, Drager R *et al.* Homozygous loss of ICOS is associated with adult-onset common variable immunodeficiency. *Nat Immunol* 2003; **4**: 261–268.
110 Byun M, Ma CS, Akcay A, Pedergnana V, Palendira U, Myoung J *et al.* Inherited human OX40 deficiency underlying classic Kaposi sarcoma of childhood. *J Exp Med* 2013; **210**: 1743–1759.
111 Attia P, Phan GQ, Maker AV, Robinson MR, Quezado MM, Yang JC *et al.* Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. *J Clin Oncol* 2005; **23**: 6043–6053.
112 Brady JL, Harrison LC, Goodman DJ, Cowan PJ, Hawthorne WJ, O'Connell PJ *et al.* Preclinical screening for acute toxicity of therapeutic monoclonal antibodies in a hu-SCID model. *Clin Transl Immunol* 2014; **3**: e29.

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