VIEWPOINT

The functional plasticity of T cell subsets

Jeffrey A. Bluestone, Charles R. Mackay, John J. O'Shea and Brigitta Stockinger

Abstract | In 1986, Robert Coffman and Timothy Mossman first described the division of CD4$^+$ T cells into functional subsets, termed T helper 1 (T_H1) and T_H2, based on cytokine production, and in doing so unwittingly opened a Pandora's box of complexity and controversy. Although the mechanisms that regulate T_H1 and T_H2 cells are now well known, recent descriptions of other CD4$^+$ T cell subsets — such as regulatory T cells, T follicular helper cells, T_H17, T_H22 and most recently T_H9 and T_H22 cells — have questioned how we think of T cell subsets and what commitment to a functional T cell subset means. Here, *Nature Reviews Immunology* asks four leaders in the field their thoughts on the functional plasticity of T cell subsets.

Are T cell subsets flexible?

Brigitta Stockinger. Although past studies that were largely carried out *in vitro* emphasized terminal commitment of effector T cells, it has now become clear that flexibility in T cell commitment is probably not the exception, but rather the norm.

The decision to commit to a specific T cell phenotype will depend on the integration of numerous signals received by an effector T cell, and it makes sense that some activated T cells might then be pushed towards a terminal effector programme and eventually death by apoptosis. However, activated T cells that are retained as memory cells may indeed preserve flexibility to alter their cytokine programme according to the stimuli received. It remains to be shown whether there are preferential directions for plasticity or whether effector T cells can change in any direction from every starting point.

To date, there is good evidence that T helper 17 (T_H17) cells tend to convert to T_H1 cells but not vice versa[1,2] and that T_H2 cells can change into an interleukin-9 (IL-9)-producing T cell type but may not easily become T_H1 cells[3]. It is of course entirely possible that not all conditions and factors that drive the various T cell phenotypes have been identified, but it is equally likely that there are some T cell programmes that are more related than others. For instance, although T cells producing IL-9 do not produce IL-4 they are nevertheless associated with T_H2-type responses such as helminth-specific immune responses or allergy.

Jeffrey A. Bluestone. It is increasingly clear that certain subsets of T cells defined by their function and by the expression of a particular transcription factor are not necessarily stable. We and other groups recently reported that some forkhead box P3 (FOXP3)$^+$ T regulatory (T_{Reg}) cells lose FOXP3 expression and take on an effector memory T cell phenotype[4–6], producing interferon-γ (IFNγ), and in some instances have the potential to cause rapid pancreatic cell destruction and immune-mediated diabetes[5]. Moreover, in humans a subset of activated T_{Reg} cells with low FOXP3 expression levels that have lost their suppressor function and produce more IL-17 as a population percentage than any other CD4$^+$ T cell fraction analysed has been described[7]. Importantly, the proportion of this FOXP3^{low} T_{Reg} cell population is increased in the blood during active systemic lupus erythematosus. Thus, T_{Reg} cells that have lost their suppressor function and are potentially pathogenic have been described in healthy mice and in patients with autoimmune disease and may have a role in autoimmunity.

Charles R. Mackay. The definition of effector T cell subsets has been somewhat arbitrary but usually relates to the cytokines that they produce, the transcription factors expressed and in some cases the chemoattractant receptors expressed. T cell subsets defined by the expression of CD4 or CD8, or by the presence of αβ and γδ T cell receptors (TCRs), are inflexible and are determined during ontogeny in the thymus, whereas the turning on or off of transcription factors in peripheral T cells and the gene expression programmes driven by these transcription factors, for example those encoding cytokines and chemokine receptors, are much more flexible. The degree of flexibility between subsets should become clearer as we gain a better understanding of the molecular nature of all the transcription factors that determine T cell subset fate.

There are now several clear examples that show that T cells can be flexible. However, it is unlikely that a given subset can transform to any and every other subset. Perhaps the T cell subset that has the greatest potential for flexibility with respect to origin is T follicular helper (T_{FH}) cells, the T effector cell subset that provides help to B cells and supports antibody class switching in germinal centres. T_{FH} cells can develop independently of other effector T cell subsets[8] but almost certainly also derive from T cell types such as T_H1, T_H2 or T_H17 cells. T_{FH} cells are mostly defined by their follicular localization, which is dictated by the expression of CXC-chemokine receptor 5 (CXCR5). Thus, flexibility in this instance might simply be a change in the homing behaviour of T_H1, T_H2 or T_H17 cells through the expression of CXCR5. T_H1, T_H2 and T_H17 cell subsets normally express chemoattractant receptors such as CC-chemokine receptor 5 (CCR5), chemoattractant-receptor homologous molecule expressed by T_H2 cells (CRTH2; also known as GPR44) and CCR6 that facilitate homing to tissues and not follicles. Recent studies clearly support a model in which T_H2 cells can transform into CXCR5$^+$ T_{FH} cells.

Three studies used IL-4 reporter mice[9–11] and showed that, during helminth infection, most IL-4-expressing $CD4^+$ T cells also expressed the T_{FH} cell markers CXCR5, programmed cell death protein 1 (PD1), inducible T cell co-stimulator (ICOS), B cell lymphoma 6 (BCL-6) and IL-21 and localized to the B cell follicles. However, they also expressed GATA3, which is the main transcription factor for T_H2 cell differentiation, indicative perhaps of their pedigree. These IL-4-expressing T_{FH} cells seemed to have developed directly from T_H2-type cells after their transfer into naive mice[9]. Sceptics might argue that these T_{FH} cells are simply T_H2 cells; however, mice deficient in IL-4 or GATA3 still make good antibody responses. One possibility is that a T_H2-derived T_{FH} cell produces IL-4 but loses features of peripheral T_H2 cells such as IL-5 production or CRTH2 expression.

Another example of T_{FH} cell flexibility is the ability of $FOXP3^+CD4^+$ T cells to differentiate into T_{FH} cells in mouse Peyer's patches (although apparently not in other lymphoid tissues) and promote IgA production[12]. T cell subsets can be flexible, but they show clear preferences in this flexibility.

John J. O'Shea. The answer is simple — an unequivocal yes — but the question is not. There are now abundant examples of flexibility in terms of cytokine production[5,13,14] but perhaps the best example is IL-10. Initially viewed as a T_H2-type cytokine, it is now recognized that T_H1, T_H17 and T_{Reg} cells all make IL-10. T cells that express FOXP3 and the T_H17 cell-associated transcription factor RORγt have been described, as have $FOXP3^+$ T_{Reg} cells that become IL-17 producers, express the T_H1 cell-associated transcription factor T-bet (also known as TBX21) or make IFNγ[15–17]. *In vivo*, $IL\text{-}17^+IFN\gamma^+$ T cells have been described and T_H17 cells have been shown to develop into T_H1 cells[18]. T_{FH} cells can express FOXP3, and IL-4-producing T_H2 cells can become T_{FH} cells. T_{FH} cells produce IL-21, but so do T_H17 cells. In addition, T_H17 cells produce IL-22, but cells that make IL-22 and not IL-17 have now been identified[19,20].

Many of the classical studies that led to our current notion of T cell lineages have relied heavily on extensive *in vitro* manipulation; it is less well established whether T cells generated *in vivo* follow the same rules. Moreover, human effector T cells are often more flexible in terms of cytokine production than their mouse counterparts. These data beg the question: why should we think that cytokine production by T cell subsets is not flexible?

Although the distinction between lineages and subsets may seem pedantic, the term lineage implies stability of phenotype, whereas the term subset does not. The concept of lineage commitment arises from developmental biology and has strong biological and molecular underpinnings. The terms lineage commitment and cell fate determination refer to the programming of a cell to follow a specified path, often resulting in terminal differentiation, and immediately imply limited flexibility. So, is it more accurate to characterize T cells that selectively produce certain cytokines as subsets or lineages? Is the distinction useful or is it just semantics? If T_H cells are just subsets, then it is not a big deal — sometimes they make cytokines and sometimes they do not; there is no requirement that a subset necessarily behaves like a terminally differentiated cell. However, the issue of flexibility in immune responses is clearly not just semantics for those interested in manipulating immune responses in a therapeutically useful manner. If the goal is to understand the molecular basis of specification, it is important to know when T cells are committed to a fate and when they are just making cytokines, and what the determining factors are for the difference. Moreover, when the terms are not used appropriately we can become prisoners of our own semantics; clearly it is timely to revisit how we think of T_H cell subsets and what commitment means. Fortunately, more genetic tools are becoming available to carefully map the fates of immune cells *in vivo* in the setting of homeostasis and infection.

Q *What are the environmental (extrinsic) factors that allow for plasticity?*

J.J.O'S. It is again useful to consider the precedents from developmental biology. Lineage commitment indicates that the developmental fate of a cell and its progeny is restricted. External signals drive lineage commitment by acting through transcription factors. For instance, gradients of the morphogen activin, a transforming growth factor-β (TGFβ)-related cytokine, promote mesoderm development by turning on the transcription factor T-box protein brachyury. Other lineage-specifying transcription factors such as homeobox, forkhead and signal transducer and activator of transcription (STAT) family members

Glossary

γδ T cells
T cells that express the γδ T cell receptor. These T cells are present in the skin, vagina and intestinal epithelium as intraepithelial lymphocytes. Although the exact function of these T cells is unknown, it has been suggested that mucosal γδ T cells are involved in innate immune responses.

Asymmetrical cell division
A type of division that produces two daughter cells with different properties. This is in contrast to normal cell division, which give rise to equivalent daughter cells. Notably, stem cells can divide asymmetrically to give rise to two distinct daughter cells: one copy of themselves and one cell programmed to differentiate into another cell type.

Class switching
The switch from expressing IgM to expressing other isotypes such as IgG, IgA or IgE that some B cells make after recognizing their cognate antigen. The decision of which isotype is generated is strongly influenced by the specific cytokine milieu and other cells such as T helper cells.

Germinal centre
A highly specialized and dynamic microenvironment that gives rise to secondary B cell follicles during an immune response. It is the main site of B cell maturation, leading to the generation of memory B cells and plasma cells, which produce high-affinity antibody.

Lymphoid-tissue inducer cell
A cell that is present in developing lymph nodes, Peyer's patches and nasopharynx-associated lymphoid tissue (NALT) and is required for the development of these lymphoid organs.

Lymphopenic mice
Mice that have lost both B and T cells, for example severe combined immunodeficiency mice or recombination activation gene-deficient mice, which lack an enzyme required for the generation of T and B cell receptors, or a loss of T cells only, as seen in *nu/nu* mice, which lack a thymus. T cell lymphopenia can be induced in mice by thymectomy on day three of life.

MicroRNAs
Small RNA molecules that regulate the expression of genes by binding to the 3′-untranslated regions of specific mRNAs.

Non-obese diabetic (NOD) mice
NOD mice spontaneously develop type 1 diabetes mellitus as a result of autoreactive T cell-mediated destruction of pancreatic β-islet cells.

Peyer's patches
Collections of lymphoid tissue located in the mucosa of the small intestine, with an outer epithelium layer consisting of specialized epithelial cells called M cells.

Systemic lupus erythematosus
(SLE). An autoimmune disease in which autoantibodies that are specific for DNA, RNA or proteins associated with nucleic acids form immune complexes that damage small blood vessels, particularly in the kidney. Patients with SLE generally have abnormal B and T cell function.

T follicular helper (T_{FH}) cell
A $CD4^+$ T cell that provides help to B cells in follicles and germinal centres. The T_{FH} cell signature includes the expression of CXCR5, ICOS, CD40 ligand and IL-21, factors that mediate T_{FH} cell homing to follicles and B cell help.

also induce cell differentiation in response to other signals. The changes associated with lineage commitment are hereditable, and various epigenetic modifications ensure that this is the case; even without continued external stimulation, cell identity can remain stable owing to these modifications[21,22]. In addition, cell polarity is another important factor that influences the lineage commitment of daughter cells.

Viewed from this perspective, a legitimate argument can be made that differentiation of T_H cells looks like lineage commitment. Cytokines provide morphogen-like signals that induce the expression of lineage-specifying transcription factors, which belong to the same families that drive development in model organisms (for example T-bet, GATA3, H2.0-like homeobox (HLX) and STATs). Recent evidence points to an instructive role of cytokines in lineage determination, above and beyond their effects on cell survival[23]. Lineage-defining cytokine genes also have the expected permissive and repressive epigenetic marks[17].

However, lineage commitment does not exclude reversibility — it does not necessarily imply terminal differentiation. Classically, lineage commitment is divided into two phases: specification and determination. Specified lineage commitment denotes that cells differentiate autonomously under neutral conditions but that this commitment can be reversed. By contrast, a tissue that is determined is irreversibly committed. Determination is followed by differentiation, which is shown by biochemical, structural, functional and histological changes. So it is probably more precise to think of lineage commitment in all T cells as not being 'determined' (as in segmentation or organogenesis). Rather, subsets of cytokine-producing T cells seem to have undergone a process more like specification, meaning that although differentiation occurs, opportunities for alternative fates persist. Consistent with this idea is the observation that epigenetic marks of genes encoding the master regulators T-bet (T_H1 cells) and GATA3 (T_H2 cells) are not uniformly repressed in opposing lineages; such lineages have bivalent epigenetic marks, indicating that both permissive and repressive epigenetic marks are present[17]. Furthermore, the epigenetic modifications of genes that contribute to aspects of T_H cells beyond the lineage-defining cytokine genes (for example, genes encoding other cytokines and chemokine receptors) do not necessarily conform to a simple view of a T_H cell phenotype. The good news is that advances in the understanding of epigenetic regulation along with new technologies allow these changes to be measured across the genome. Another factor that has clearly been documented to affect the stability of T_H cells is microRNAs (miRNAs). For instance, T_{Reg} cells deficient for the miRNA processing enzymes Dicer and Drosha (also known as ribonuclease 3) are unstable, downregulate FOXP3 expression and may become effector T cells[24]. Thus, miRNAs seem to be important in preserving cellular phenotype, and emerging discoveries that relate to the interplay between chromatin modifications, miRNA and large non-coding RNAs should be watched closely.

C.R.M. The cellular and molecular signals associated with germinal centre reactions seem to be necessary for T_{FH} cell development. In a recent study, $CD4^+$ T cells resembling classic T_H2 cells (that is, $CXCR5^-PD1^-IL\text{-}4^+$ T cells) were shown to transform into T_{FH} cells in recipient mice, but only when they had a normal B cell system and could form germinal centres[19]. ICOS–ICOS ligand and CD40–CD40 ligand interactions were also shown to be important for T_{FH} cell development[9,11], regardless of whether the cells developed from T_H2-like cells or from activated (unpolarized) T cells. In addition, IL-6 and IL-21 are two extrinsic factors that promote T cell differentiation to the T_{FH} cell phenotype[8,25] or that mediate upregulation of BCL-6 (an important transcription factor for T_{FH} cell development) expression in T cells (see below).

J.A.B. The microenvironment seems to instruct the outcome of functional change of differentiated T cells. For example, under normal conditions, we have found that T cells that once expressed FOXP3 (which we have termed ex-FOXP3 cells) produced IFNγ in the spleen, liver and peripheral lymph nodes but produced IL-17A in gut-associated lymphoid tissues[6]. *In vitro* studies have shown that IL-6, TGFβ and other unidentified dendritic cell-secreted, and possibly epithelial cell-secreted, factors can modulate T_{Reg} cell function and FOXP3 expression[5,26,27]. There is also growing evidence that other inflammatory cytokines, retinoic acid, Toll-like receptor (TLR) ligands and perhaps lymphopenia can alter T_{Reg} cell stability and function.

In fact, low levels of subset-promoting cytokines may also control plasticity. IL-2-induced STAT5 activation is required for T_{Reg} cell development and survival[28,29]. The T_{Reg} cell-specific determining region in an intron of *Foxp3* contains a STAT5-binding site[30] and T_{Reg} cells with low IL-2Rα (also known as CD25) expression levels are unstable and lose FOXP3 expression when transferred to lymphopenic mice or in an IL-2-deficient autoimmune setting[4,6]. In non-obese diabetic (NOD) mice, intra-islet T_{Reg} cells express decreased levels of IL-2Rα[31], and a subset of these cells loses FOXP3 expression[6]. Treatment with a low dose of recombinant IL-2 restores IL-2Rα expression and increases T_{Reg} cell numbers in the pancreas to provide protection from diabetes[31]. Determining the hierarchy of factors that promote stability or plasticity will be crucial for therapeutic interventions.

B.S. It is likely that plasticity is determined by the sum of interactions with antigen-presenting cells and stromal cells in the environment of initial activation. It was shown in cell culture experiments that T cells develop different cell fates as a consequence of random encounters with antigen-presenting cells and cytokines that last for different time periods[32]. This was determined for effector and memory T cell fates but may similarly apply to the degree of plasticity in adopting distinct effector T cell profiles. However, it is difficult to follow such events on the single-cell level *in vivo* and one can only hope that future advances in intravital microscopy techniques will facilitate these kinds of analyses[33].

Cytokines are important factors that drive $CD4^+$ effector T cell differentiation, and paradigms for T_H1, T_H2 and T_H17 cell differentiation in response to distinct cytokines have been established. However, it is clear that effector T cell subsets generated *in vitro* are not exposed to the full range of mediators that may influence differentiation *in vivo*. *In vitro* conditions lack multiple factors secreted by other cells types present in the physiological environment, such as stromal cells, and the *in vitro* use of antibodies to the TCR or high doses of peptide and antigen-presenting cells artificially enhances T cell activation. It is therefore difficult to be sure to what degree $CD4^+$ effector T cell subsets that are generated *in vitro* bear the hallmarks of those that arise *in vivo*.

Q *What regulates lineage commitment versus flexibility?*

J.A.B. This question raises other fundamental questions: what is a T cell lineage versus a T cell subset versus a T cell differentiation state? Is it the cytokines that are produced, the transcription factors that are expressed and/or the origin of the cell type (thymus versus periphery)? The notion of T cell lineages

started with the concepts of B cells versus T cells, $CD4^+$ T cells versus $CD8^+$ T cells and αβ T cells versus γδ T cells. $CD4^+$ T cells do not turn into $CD8^+$ T cells (or vice versa) and αβ T cells have not been shown to change into γδ T cells. By contrast, the defined T cell subsets are different.

The issue of what criteria to use to define distinct T cell lineages seems to have become increasingly complicated. In fact, recently, cell populations with restricted cytokine profiles have been designated as belonging to unique lineages, such as T_H9 cells[3] and T_H22 cells[19]. Classically, the three criteria that have been used to define T_H cell subsets are their cytokine profiles, the transcription factors that regulate cytokine gene expression and, most recently, epigenetic modifications that affect cytokine gene loci.

Although it is convenient to link T cell subsets to the expression of specific transcription factors, the definitions of T cell subsets formed on the basis of these genetic regulatory elements are blurry, especially in humans. For example, a fraction of T_{Reg} cells transferred to lymphopenic mice lose FOXP3 expression and change differentiation states[6,12] as a consequence of cell division[4]. In addition, FOXP3 is expressed transiently by most activated human T cells, GATA3 is expressed in thymocytes, T-bet is expressed by T cells and dendritic cells, and RORγt is crucial for the development of thymocytes and lymphoid-tissue inducer cells. Therefore, it will be essential that the field comes to an agreement on what constitutes a T cell lineage versus a T cell differentiation state. The definition must include stability, a set of definable molecular and functional markers and a distinct developmental pathway.

CpG islands, histone methylation and acetylation, and miRNA status are major determinants for stable *FOXP3* gene expression[6,24,30]. Most ex-T_{Reg} cells have T_{Reg} cell-specific determining region CpG methylation patterns of non-T_{Reg} cells (that is, methylated CpG islands) and some ex-T_{Reg} cells have methylation patterns that are transitional between non-T_{Reg} and T_{Reg} cells[6]. We argue that global epigenetic changes can 'lock' differentiated T cells into certain functional and phenotypic subsets but that under certain immune insults these T cells retain some plasticity[29]. Recent studies by Reiner and colleagues[34] showing asymmetrical cell division can influence T cell subset stability. So, extrinsic factors and the microenvironment probably determine stability versus flexibility during population expansion so that the daughter chromatin might keep or change its epigenetic status[5,35].

B.S. Quantitative differences in the expression of numerous external factors can influence T cell lineage commitment. As an example, T_H17 cells are influenced by numerous cytokines as well as environmental signals through activation of the transcription factor aryl hydrocarbon receptor (AHR). IL-6 and TGFβ are necessary and sufficient to drive initial T_H17 cell differentiation, but other cytokines such as IL-1β and autocrine IL-21 enhance this step. Furthermore, there is an absolute requirement for IL-23 for the maintenance and function of T_H17 cells *in vivo*[36]; however, this cytokine is dispensable for *in vitro* differentiation[37]. In addition, activation of AHR is essential to drive the expression of IL-22 in T_H17 cells[38]. A particularly poignant reminder of the potential artefacts encountered with *in vitro* systems is the demonstration that T_H17 cell polarization works better in some culture media than others, partly owing to the differential presence of natural AHR ligands[39]. Thus, it is possible that T_H17 cells exposed to all these factors are more committed to their programme than those that encounter only a few of these influences.

In terms of flexibility, in the case of T_H17 cells it is interesting that they express receptors for both IL-12 and IL-23 and are thus susceptible to the action of IL-12, which positively regulates T-bet expression[40], potentially allowing for T_H17 to T_H1 cell plasticity. This would be in line with data from Wei *et al.*[17] who showed that some transcription factors such as T-bet display bivalent epigenetic modifications that may allow rapid transition between repressive and active states. Thus, lineage commitment versus flexibility is influenced by numerous signals and extrinsic influences that shape the molecular programme of a T cell. Findings from *in vitro* conditions will always be just a snapshot of the potential *in vivo* influences that a T cell experiences.

C.R.M. We and others showed recently that the transcriptional repressor BCL-6 directs T_{FH} cell lineage differentiation[41]. It does this by turning on an extensive gene repressor programme that inhibits key transcriptional regulators of other T_H cell lineages, including T-bet, RORγt and GATA3 (REFS 41,42). Our studies suggest that BCL-6 can reprogramme T_H1, T_H2 and T_H17 cells to a T_{FH} cell phenotype, although whether this is reversible is uncertain. BCL-6 also repressed the transcription of a large number of miRNAs, many of which negatively regulate, or are predicted to regulate, key T_{FH} cell-associated molecules, such as CXCR5, IL-21 and ICOS[41]. T_{FH} cells are distinguished from non-T_{FH} effector T cells only through a quantitative increase in the expression of molecules such as CXCR5, CXCR4, PD1, ICOS and IL-21, and so repression of miRNA may be essential for differentiation to the T_{FH} cell phenotype. Repression (or not) of these miRNAs would allow for flexibility in the expression of cytokines and chemokine receptors that are associated with T effector cells. In addition, we found that overexpression of BCL-6 induced the T_{FH} cell phenotype under non-polarizing conditions and also in T cells already polarized to T_H1, T_H2 and T_H17 cell phenotypes. Our feeling is that the induction of BCL-6, through whatever means, may lead to the transformation of non-T_{FH} effector T cells into T_{FH} cells, with the consequent changes in cytokine and chemokine receptor expression.

J.J.O'S. Various extrinsic factors contribute to the plasticity of developing T_H cells, including the cytokine milieu. Microbial pathogens and commensal bacteria also clearly influence the T cell environment. Retinoic acid is another classic differentiating factor that has effects on multiple tissues; recent work has identified a role for retinoic acid in inhibiting the production of inflammatory cytokines and enhancing FOXP3 expression[43]. We also know that environmental factors that activate AHR influence T cell cytokine production. Understanding how these extrinsic signals link to genetic and epigenetic regulation is obviously an important challenge.

Trying to define factors that influence lineage commitment versus plasticity is not new and not unique to T cells; arguments regarding determinism and flexibility have existed in developmental biology since the nineteenth century. Perhaps the most striking example of plasticity is the generation of inducible pluripotent stem cells by expression of a limited number of transcription factors[44,45]. There are other examples of 'reprogramming' that do not require de-differentiation to stem cells[46]; for example, transfection of master regulators such as myoblast determination protein into terminally differentiated cells can reprogramme them into muscle cells. Clearly, these are extreme and artificial circumstances; however, as we come to better understand the extrinsic signals that drive expression of transcription factors, we might be surprised by the plasticity that we see. As discussed earlier, bivalent epigenetic marks on the genes encoding T-bet and GATA3 suggest that,

with the appropriate extrinsic signals (that is, the right cytokines), reprogramming T cells might be easier than we thought. Equally, we now know that epigenetic modifications are dynamic. For example, although trimethylation of histone 3 lysine 27 (H3K27) is associated with repression of gene expression, H3K27 demethylases, such as jumonji d3 (JMJD3; also known as KDM6B) and UTX (also known as KDM6A), can remove repressive marks. Consequently, induction of these demethylases provides another mechanism for plasticity. Understanding the signals that induce these proteins and how they are recruited to some genes and not others will surely provide new insights into how phenotypes can be altered.

Q *Why do we need so many functional subsets?*

J.J.O'S. This is the easiest question to answer — we are surrounded by numerous different pathogens and commensal organisms and a changing environment. T cells traverse throughout the body in multiple niches to do their job. And they need to do this without causing damage to the host. Flexibility makes a lot of sense; if new haematopoietic stem cells are continually generated, one might think that there is little need for flexibility as new cells would be generated to deal with new circumstances. However, as the thymus involutes in the adult and *de novo* responses become limited with increasing age, flexibility of memory and effector T cell responses seems desirable.

C.R.M. First, the immune system needs to respond to diverse pathogens and tumour cells, all of which are dealt with in different ways, involving different leukocyte types and different cytokines. Immunoglobulin production is an important layer of immunity and requires dedicated T_{FH} cells to migrate to follicles and regulate B cell selection, tolerance and immunoglobulin isotype switching. The products of the germinal centre reaction (that is, plasma cells, which produce high-affinity antibody) can be long-lived, so control over this response must be stringent to avoid long-term autoimmunity. Second, functional subsets need to operate in numerous locations, and so subsets that operate in lymphoid tissues will be phenotypically distinct from those that function, for instance, at epithelial surfaces. IL-4-secreting T cells in follicles are probably functionally distinct from IL-4-secreting T cells in peripheral tissues[11]. T_{FH} cells express CXCR5 and high levels of ICOS, which facilitate follicular homing and T cell–B cell interactions, whereas peripheral T_H2 cells regulate other immune responses, such as eosinophil recruitment, through IL-5 production.

Additional $CD4^+$ effector T cell subsets probably exist. For instance, T_{FH} cells can be subdivided into IL-4-, IFNγ- and possibly IL-17-producing cells, and I would argue that such T_{FH} cells are distinct from peripheral T_H1, T_H2 or T_H17 cells because of their high expression of the T_{FH} cell signature molecules ICOS, CXCR5 and IL-21. Another potential $CD4^+$ T_H cell subset and possible relative of T_{FH} cells is non-polarized $CD4^+$ T cells that produce IL-21, that are localized in peripheral tissues and that express chemokine receptors other than CXCR5. IL-21 produced by such cells probably serves as a helper cytokine for $CD8^+$ T cells to facilitate antiviral responses[47].

J.A.B. T_{Reg} cells suppress rather than contribute to immune activation. This is undesirable in cases in which rapid immune responses are required, such as during viral or bacterial infections. Plasticity allows for flexibility, which is key when the immune system is attempting to combat an array of infectious agents. By providing optimal 'early' plasticity, individual T cells with crucial T cell receptor specificities can differentiate and adapt as needed to the environment. Specifically, the ability to locally disable T_{Reg} cells in an inflamed tissue, even transiently, may help to promote immunity. Even more speculatively, one might imagine that ex-T_{Reg} cells may have a crucial role in promoting early immunity in the infectious disease setting. As T_{Reg} cells have a self-reactive repertoire, cells that have lost FOXP3 expression and turned on potentially pathogenic cytokines in the local environment may participate early as an innate type of cell to produce IFNγ, granzymes or other cytokines in response to recognition of self in a potent co-stimulatory environment to promote immunity before the adaptive T cell response is initiated. Thus, ex-T_{Reg} cells might help to allow an immune response or even act as a mechanism of early defence.

B.S. An obvious answer is that we need multiple effector pathways to deal with highly variable pathogen threats. In addition, adaptation of effector T cells to particular circumstances may be necessary in order to avoid side effects of immune reactions or to allow initiation of repair programmes following an immune response initiated to deal with a pathogen threat. This would necessitate changes in cytokine secretion patterns. Thus, the switch from IL-4 to IL-9 production may be linked with TGFβ and tissue remodelling, a switch from IL-17 to IFNγ production during autoimmune inflammation may indicate amelioration rather than exacerbation of disease, and IL-10 production by T_H1 cells in infections may indicate a counterbalance to the pro-inflammatory response.

Many immunologists (let alone non-immunologists) are turned off by the proliferation of T cell subsets with illogical names jumping from T_H1 and T_H2 to T_H9 or T_H17. Now that plasticity in effector profiles seems to be accepted, rather than conjecture, the old classifications as T_H1 cells and so on have lost their finality. For instance, IL-9 is still regarded as a T_H2-type cytokine in all textbooks, despite the fact that it is now clear that it is never co-expressed with IL-4, IL-5 or IL-13 and its expression does not even correlate with GATA3 expression levels in polarized IL-9-producing T cells[3]. IL-17 is not obligatorily co-expressed with IL-22, and the fact that some cells may make only IL-22 does not necessarily qualify them as T_H22 cells.

Nevertheless, it is difficult to prove that a given effector T cell that expresses a particular cytokine programme can turn on a different programme. Despite the fact that such claims were made from the beginning of the description of T_H17 cells, they were not assessed in a rigorous precursor–product manner until fairly recently[1,2,48,49]. However, at present we do not know whether plasticity is limitless in any direction. Until this has become clear, it will be difficult to replace the current unsatisfactory nomenclature with something more logical.

Jeffrey A. Bluestone is at the University of California, San Francisco Diabetes Center and Department of Medicine, University of California, San Francisco 513 Parnassus Avenue, Box 0540–HSW 1114, San Francisco, California 94143, USA.
e-mail: jbluest@diabetes.ucsf.edu

Charles R. Mackay is at Monash University, Faculty of Medicine, Nursing and Health Services, Clayton, Victoria, VIC 3800, Australia, and the Garvan Institute for Medical Research and St Vincent's Clinical School, University of New South Wales, Sydney, New South Wales, NSW 2010, Australia.
e-mail: charles.mackay@med.monash.edu.au

John J. O'Shea is at the Molecular Immunology and Inflammation Branch, National Institutes of Arthritis and Musculoskeletal and Skin Diseases, US National Insitutes of Health, Bethesda, Maryland 20892, USA.
e-mail: osheaj@arb.niams.nih.gov

Brigitta Stockinger is at the Division of Molecular Immunology, Medical Research Council National Institute for Medical Research, Mill Hill, London, NW7 1AA, UK.
e-mail: bstocki@nimr.mrc.ac.uk

doi: 10.1038/nri2654
Published online 7 October 2009

1. Bending, D. *et al.* Highly purified Th17 cells from BDC2.5NOD mice convert into Th1-like cells in NOD/SCID recipient mice. *J. Clin. Invest.* **119**, 565–572 (2009).
2. Lee, Y. K. *et al.* Late developmental plasticity in the T helper 17 lineage. *Immunity* **30**, 92–107 (2009).
3. Veldhoen, M. *et al.* Transforming growth factor-β 'reprograms' the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. *Nature Immunol.* **9**, 1341–1346 (2008).
4. Komatsu, N. *et al.* Heterogeneity of natural Foxp3$^+$ T cells: a committed regulatory T-cell lineage and an uncommitted minor population retaining plasticity. *Proc. Natl Acad. Sci. USA* **106**, 1903–1908 (2009).
5. Zhou, X., Bailey-Bucktrout, S., Jeker, L. T. & Bluestone, J. A. Plasticity of CD4$^+$ FoxP3$^+$ T cells. *Curr. Opin. Immunol.* **21**, 281–285 (2009).
6. Zhou, X. *et al.* Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells *in vivo*. *Nature Immunol.* **10**, 1000–1007 (2009).
7. Miyara, M. *et al.* Functional delineation and differentiation dynamics of human CD4$^+$ T cells expressing the FoxP3 transcription factor. *Immunity* **30**, 899–911 (2009).
8. Nurieva, R. I. *et al.* 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 (2008).
9. Zaretsky, A. G. *et al.* T follicular helper cells differentiate from Th2 cells in response to helminth antigens. *J. Exp. Med.* **206**, 991–999 (2009).
10. King, I. L. & Mohrs, M. IL-4-producing CD4$^+$ T cells in reactive lymph nodes during helminth infection are T follicular helper cells. *J. Exp. Med.* **206**, 1001–1007 (2009).
11. Reinhardt, R. L., Liang, H. E. & Locksley, R. M. Cytokine-secreting follicular T cells shape the antibody repertoire. *Nature Immunol.* **10**, 385–393 (2009).
12. Tsuji, M. *et al.* Preferential generation of follicular B helper T cells from Foxp3$^+$ T cells in gut Peyer's patches. *Science* **323**, 1488–1492 (2009).
13. Lee, Y. K., Mukasa, R., Hatton, R. D. & Weaver, C. T. Developmental plasticity of Th17 and Treg cells. *Curr. Opin. Immunol.* **21**, 274–280 (2009).
14. Zhou, L., Chong, M. M. & Littman, D. R. Plasticity of CD4$^+$ T cell lineage differentiation. *Immunity* **30**, 646–655 (2009).
15. Xu, L., Kitani, A., Fuss, I. & Strober, W. Cutting edge: regulatory T cells induce CD4$^+$CD25$^-$Foxp3$^-$ T cells or are self-induced to become Th17 cells in the absence of exogenous TGF-β. *J. Immunol.* **178**, 6725–6729 (2007).
16. Koch, M. A. *et al.* The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation. *Nature Immunol.* **10**, 595–602 (2009).
17. Wei, G. *et al.* Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4$^+$ T cells. *Immunity* **30**, 155–167 (2009).
18. Shi, G. *et al.* Phenotype switching by inflammation-inducing polarized Th17 cells, but not by Th1 cells. *J. Immunol.* **181**, 7205–7213 (2008).
19. Duhen, T. *et al.* Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. *Nature Immunol.* **10**, 857–863 (2009).
20. Trifari, S. *et al.* Identification of a human helper T cell population that has abundant production of interleukin 22 and is distinct from T_H-17, T_H1 and T_H2 cells. *Nature Immunol.* **10**, 864–871 (2009).
21. Bernstein, B. E., Meissner, A. & Lander, E. S. The mammalian epigenome. *Cell* **128**, 669–681 (2007).
22. Ng, R. K. & Gurdon, J. B. Epigenetic inheritance of cell differentiation status. *Cell Cycle* **7**, 1173–1177 (2008).
23. Rieger, M. A. *et al.* Haematopoietic cytokines can instruct lineage choice. *Science* **325**, 217–218 (2009).
24. Zhou, X. *et al.* Selective miRNA disruption in T reg cells leads to uncontrolled autoimmunity. *J. Exp. Med.* **205**, 1983–1991 (2008).
25. Vogelzang, A. *et al.* A fundamental role for interleukin-21 in the generation of T follicular helper cells. *Immunity* **29**, 127–137 (2008).
26. Pasare, C. & Medzhitov, R. Toll pathway-dependent blockade of CD4$^+$CD25$^+$ T cell-mediated suppression by dendritic cells. *Science* **299**, 1033–1036 (2003).
27. Yang, X. O. *et al.* Molecular antagonism and plasticity of regulatory and inflammatory T cell programs. *Immunity* **29**, 44–56 (2008).
28. Burchill, M. A. *et al.* IL-2 receptor β-dependent STAT5 activation is required for the development of Foxp3$^+$ regulatory T cells. *J. Immunol.* **178**, 280–290 (2007).
29. Wei, L., Laurence, A. & O'Shea, J. J. New insights into the roles of Stat5a/b and Stat3 in T cell development and differentiation. *Semin. Cell Dev. Biol.* **19**, 394–400 (2008).
30. Floess, S. *et al.* Epigenetic control of the foxp3 locus in regulatory T cells. *PLoS Biol.* **5**, e38 (2007).
31. Tang, Q. *et al.* Central role of defective interleukin-2 production in the triggering of islet autoimmune destruction. *Immunity* **28**, 687–697 (2008).
32. Gett, A. V., Sallusto, F., Lanzavecchia, A. & Geginat, J. T cell fitness determined by signal strength. *Nature Immunol.* **4**, 355–360 (2003).
33. Sumen, C., Mempel, T. R., Mazo, I. B. & von Andrian, U. H. Intravital microscopy: visualizing immunity in context. *Immunity* **21**, 315–329 (2004).
34. Reiner, S. L. Decision making during the conception and career of CD4$^+$ T cells. *Nature Rev. Immunol.* **9**, 81–82 (2009).
35. Locksley, R. M. Nine lives: plasticity among T helper cell subsets. *J. Exp. Med.* **206**, 1643–1646 (2009).
36. McGeachy, M. J. *et al.* The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells *in vivo*. *Nature Immunol.* **10**, 314–324 (2009).
37. Veldhoen, M. *et al.* TGFβ in the context of an inflammatory cytokine milieu supports *de novo* differentiation of IL-17-producing T cells. *Immunity* **24**, 179–189 (2006).
38. Veldhoen, M. *et al.* The aryl hydrocarbon receptor links T_H17-cell-mediated autoimmunity to environmental toxins. *Nature* **453**, 106–109 (2008).
39. Veldhoen, M. *et al.* Natural agonists for aryl hydrocarbon receptor in culture medium are essential for optimal differentiation of Th17 T cells. *J. Exp. Med.* **206**, 43–49 (2009).
40. Takemoto, N. *et al.* Cutting Edge: IL-12 inversely regulates T-bet and eomesodermin expression during pathogen-induced CD8$^+$ T cell differentiation. *J. Immunol.* **177**, 7515–7519 (2006).
41. Yu, D. *et al.* The transcriptional repressor Bcl-6 directs T follicular helper lineage commitment. *Immunity* 22 Jul 2009 (doi:10.1016/j.immuni.2009.07.002).
42. Kusam, S., Toney, L. M., Sato, H. & Dent, A. L. Inhibition of Th2 differentiation and GATA-3 expression by BCL-6. *J. Immunol.* **170**, 2435–2441 (2003).
43. Curotto de Lafaille, M. A. & Lafaille, J. J. Natural and adaptive Foxp3$^+$ regulatory T cells: more of the same or a division of labor? *Immunity* **30**, 626–635 (2009).
44. Yamanaka, S. Pluripotency and nuclear reprogramming. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **363**, 2079–2087 (2008).
45. Gurdon, J. B. & Melton, D. A. Nuclear reprogramming in cells. *Science* **322**, 1811–1815 (2008).
46. Zhou, Q. & Melton, D. A. Extreme makeover: converting one cell into another. *Cell Stem Cell* **3**, 382–388 (2008).
47. Johnson, L. D. & Jameson, S. C. Immunology. A chronic need for IL-21. *Science* **324**, 1525–1526 (2009).
48. Lexberg, M. H. *et al.* Th memory for interleukin-17 expression is stable *in vivo*. *Eur. J. Immunol.* **38**, 2654–2664 (2008).
49. Annunziato, F. *et al.* Phenotypic and functional features of human Th17 cells. *J. Exp. Med.* **204**, 1849–1861 (2007).

Acknowledgements

J.B. would like to thank S. Bailey-Bucktrout, L. Jeker and X. Zhou for their hard work and thoughtful comments, M. Anderson, R. Locksley and A. Abbas for critical reading of this piece and the US National Institute of Health, Juvenile Diabetes Research Foundation, and University of California, San Francisco Diabetes Center for financial support.

C.R.M.'s views in this article are derived from work and discussions with D. Yu, C. Vinuesa and C. King. Charles Mackay is supported by the National Health and Medical Research Council of Australia.

J.J.O'S. would like to thank A. Laurence, L. Wei, V. Sartorelli, M. Gadina, R. Siegel and Y.e Belkaid for their lively discussion of this issue; however, I also wish to apologize to the members of my laboratory for taking so much time in lab meetings and journal clubs for endlessly debating this topic.

DATABASES

UniProtKB: http://www.uniprot.org
AHR | BCL-6 | brachyury | CCR5 | CCR6 | CRTH2 | CXCR5 | Dicer | Drosha | FOXP3 | GATA3 | HLX | ICOS | IFNγ | IL-1β | IL-2 | IL-2Rα | IL-4 | IL-5 | IL-6 | IL-9 | IL-17 | IL-21 | IL-22 | jumonji d3 | PD1 | RORγt | T-bet | UTX

FURTHER INFORMATION

Jeffery A. Bluestone's homepage: http://www.diabetes.ucsf.edu/members/jeffrey-bluestone
John J. O'Shea's homepage: http://www.niams.nih.gov/Research/Ongoing_Research/Branch_Lab/report_individual.asp?id=564&Branch_LabID=48&email=osheajo@mail.nih.gov&includefile=LCBS
Brigitta Stockinger's homepage: http://www.nimr.mrc.ac.uk/molimm/stockinger/
CD4$^+$ T cell diversity: http://www.nature.com/nri/posters/cd4t-celldiversity/index.html

ALL LINKS ARE ACTIVE IN THE ONLINE PDF