OPINION

# Interplay between the $T_H17$ and $T_{Reg}$ cell lineages: a (co-)evolutionary perspective

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Abstract | The origins of the adaptive immune system and the basis for its unique association with vertebrate species have been a source of considerable speculation. In light of recent advances in our understanding of the developmental and functional links between the induced regulatory T cell and T helper 17 cell lineages, and their specialized relationship to the gut, we speculate that the co-evolution of these adaptive immune pathways might have given primitive vertebrates a means to benefit from the diversification of their commensal microbiota.

The differentiation of distinct functional subsets of effector or regulatory $CD4^+$ T cells from naive precursors of a fixed, single antigenic specificity is a fundamental strategy by which the adaptive immune system orchestrates protective or tolerogenic responses to microorganisms. This developmental flexibility has evolved to allow the innate immune system to instruct $CD4^+$ T cell development based on the properties of different classes of microorganisms, mainly through cytokine cues. This ensures appropriate coordination between the innate and adaptive immune responses. Given that the effector T cells responsible for protective adaptive immunity can be long-lived, and that the cost of an inappropriate recall response could be inadequate host protection from infection or autoimmunity, the immune system has developed robust regulatory constraints on these effector cells.

Nowhere is this more evident than for T helper 17 ($T_H17$) cells, which are effector $CD4^+$ T cells that have evolved close ties to induced regulatory T ($T_{Reg}$) cells[1]. $T_H17$ cells are characterized by their expression of the pro-inflammatory cytokines interleukin-17A (IL-17A), IL-17F and IL-22 and reside mainly at barrier surfaces, particularly the mucosae of the gut, where they function to protect the host from microorganisms that invade through the epithelium[2–5]. The induced $T_{Reg}$ cells, which develop post-thymically, are characterized by expression of the transcription factor forkhead box P3 (FOXP3) and are also found mainly in the intestinal mucosae, where they function to restrain excessive effector T cell responses that might damage host tissues[6]. The discovery that the differentiation of both $T_H17$ cells and induced $T_{Reg}$ cells requires transforming growth factor-β (TGFβ) provided the first evidence that these subsets might be developmentally linked[2,4,7]. More recently, direct interactions between the transcriptional programmes that specify each of these lineages have been described[8], as has a developmental plasticity that seems to allow induced $T_{Reg}$ cells to acquire the functions of $T_H17$ cells when encountering antigen in an inflammatory milieu[9,10]. The overlapping developmental features of the pro-inflammatory $T_H17$ cell and anti-inflammatory induced $T_{Reg}$ cell lineages, and the preferential localization of both cell types at mucosal surfaces where they are in constant contact with a diverse and abundant microbiota, raise the interesting possibility that the evolutionary forces that originally drove the emergence and functional features of these two lineages might also be linked. Here, we highlight recent advances that have extended our understanding of the dynamic interplay between the $T_H17$ cell and induced $T_{Reg}$ cell lineages, and speculate why these lineages might have co-evolved.

## Adaptive immunity: a benefit from bugs?

The simplest of multi-celled organisms have evolved mechanisms for defence against microbial threats. The first tenets of an innate immune system in the form of phagocytosis and antimicrobial peptides are present in the earliest of invertebrates[11]. As protochordates and urochordates (the evolutionary forerunners of vertebrates) began to evolve, the ancestors of many of the cytokine families and the complement system began to appear, providing primitive intercellular pathways for coordinating host defence that have been retained in diverse species[12–14]. Vertebrate evolution coincided with the acquisition of new strategies to generate an almost limitless diversity of anticipatory immune receptors and the capacity for specific immune memory — which are the foundations of adaptive immunity. B and T cells are the core of the adaptive immune system and are present in all jawed vertebrates (gnathostomes). Interestingly, B- and T-like cells have recently been identified in a jawless vertebrate (agnathan), the lamprey[15], as has a novel recombination system to generate highly diverse antigen receptors on these immune cells that uses a recognition strategy distinct from that of the recombination activating protein (RAG1 and RAG2)-based B and T cell receptors of jawed vertebrates[16]. Although it is still debated exactly how the evolutionary emergence of an adaptive immune system in vertebrates — jawless or jawed — conferred a selective advantage[17], the convergent evolution of two distinct mechanisms for achieving highly diverse, anticipatory immune receptors indicates that there is a superior fitness value imparted by adaptive immune recognition[16,18].

Although the evolutionary pressures driving the emergence of new immune strategies have typically been viewed in the context of host defence, recent insights into the relationship between vertebrates and the microbial world offer an alternative view on the evolutionary advantages associated with the development of adaptive immunity: that adaptive immunity evolved to 'encourage', rather than 'discourage', microbial colonization of vertebrate hosts. Although commensal organisms exist in all metazoans[19], studies of the diversity of the resident microorganisms have

shown that there is far greater complexity of microorganisms in (and on) vertebrate compared with invertebrate hosts[20]. Particularly in the gut, which is a highly attractive habitat for microorganisms owing to its role in the ingestion and processing of nutrients, the number and diversity of microbial species, especially bacteria, that take up permanent residence in vertebrates are remarkable[21]; this feature does not seem to be shared by invertebrates. It is known, for example, that vertebrate species as divergent as mice and zebrafish share six divisions of intestinal bacteria, which colonize the gastrointestinal tract soon after birth[22,23].

Perhaps not surprisingly, the largest concentration of B and T cells in vertebrates is also found in the gut-associated lymphoid tissue (GALT), where they reinforce epithelial barrier functions that sequester — but do not eliminate — the commensal microorganisms in the intestinal lumen. It should be noted that although our focus here is on conventional αβ T cells, there are other components of the GALT that contribute to the mutualistic relationship that has evolved between vertebrates and their commensal flora. Several other immune cell types, both innate and adaptive, reside in the intestinal tract where they can be recruited by the recognition of microbial products to produce IL-17 and IL-22, similarly to $T_H17$ cells. In this way, natural killer cells and lymphoid tissue inducer cells contribute to microbial surveillance[24], as do adaptive cells with more primitive direct antigen recognition systems, including γδ T cells[25]. Although precise functions for each of these cell types in the gut are incompletely understood, it is presumed that each can contribute to rapid protective responses that allow time for the development of conventional adaptive immune responses, such as $T_H17$ cell differentiation. These cells might have bridged an evolutionary gap before the emergence of the more fully developed adaptive immune systems of which $T_H17$ cells and induced $T_{Reg}$ cells are a part.

So, how might a strategy to encourage a diverse, resident microbial flora have favoured vertebrate survival and how might the emergence of adaptive immunity have facilitated this? It could simply reflect a shared benefit derived from more efficient resource management. Although the early microbial residents of the digestive tracts of complex organisms were probably unicellular parasites that depleted their host's resources — and provoked host defences to clear them — it is now apparent that the nutrient contribution of the gut flora to vertebrate hosts substantially outweighs the nutrient cost[26]. By providing greater and more flexible access to bacterial metabolic and enzymatic pathways with which to derive nutrients from a broader range of dietary components, such as complex carbohydrates[27,28], a permanent, diverse intestinal flora might have provided a tremendous evolutionary advantage. This advantage could be increased by relegating to the more mutationally (and evolutionarily) flexible microorganisms the task of evolving non-nutrient metabolism that might benefit the host, such as vitamin synthesis. By supporting a highly adaptable, metabolically diverse 'permanent' microbiota, early vertebrates could have rapidly expanded their potential nutrient sources, enabling them to adapt more efficiently to a diversity of environmental niches and increasing their chance of survival during periods of food shortage[20].

However, implicit in any strategy to intentionally harbour a large diversity of microorganisms is the risk of infection; by selecting for resident microorganisms that are mainly innocuous, the survival benefit to the host becomes acceptable. Indeed, by encouraging a microbial ecology that is dominated by more benign residents, a further benefit is derived by the establishment of a 'commensal buffer' against more invasive, and therefore more pathogenic, microorganisms. Here, the limitations of an innate immune system could have been problematic. Without the ability to distinguish or remember the 'good' bacteria from the 'bad' bacteria, organisms that relied only on an innate immune system probably could not risk supporting a diverse range of microorganisms. By extension of this reasoning, it is possible that the evolutionary emergence of an adaptive immune system — based on the development of novel genetic mechanisms for generating diverse, anticipatory antigen recognition receptors on clonal immune populations with long-lived memory — was selected for as a means to recognize, and remember, both beneficial and detrimental members of the enteric microbiota and to foster maintenance of the beneficial microorganisms at the expense of the harmful microorganisms.

Accordingly, it is probable that the adaptive immune system has been largely responsible for refining the co-evolutionary interactions between vertebrate hosts and their microbiota such that commensal bacterial species in vertebrate hosts far outnumber pathogenic bacterial species. To accomplish this, the emerging adaptive immune system required mechanisms to temper innate immune responses that are geared only for the clearance of microorganisms. Here, the emergence of a broad repertoire of immune cells that could suppress, as well as promote, innate inflammatory mechanisms depending on the 'threat level' of the microorganism that was recognized became invaluable. We propose that the linked evolution of the induced $T_{Reg}$ and $T_H17$ cell lineages enabled this balanced regulation of pre-existing innate immune mechanisms. Furthermore, the use of the same core cytokine signalling programme — namely TGFβ — by both lineages might have offered an economy and efficiency of response that allowed the immune suppressive $T_{Reg}$ cell pathway to yield to the immune amplifying $T_H17$ cell pathway as required.

## TGFβ: duality and adaptive immunity

Despite their different functional properties, induced $T_{Reg}$ cells and $T_H17$ cells share a requirement for TGFβ to develop from antigen-naive T cells[29] (FIG. 1). During homeostasis, the development of induced $T_{Reg}$ cells seems to be favoured in the TGFβ-rich GALT, promoted by the cofactor *all-trans* retinoic acid (*at*-RA), a metabolite of dietary vitamin A that is produced by dendritic cells resident in the intestinal mucosa and its draining lymph nodes[30–33]. When dendritic cells are activated by microorganisms that induce production of the pro-inflammatory cytokine IL-6, the TGFβ-induced differentiation of naive T cells is diverted away from the induced $T_{Reg}$ cell pathway and towards the $T_H17$ cell pathway[2,4,7]. $T_H17$ cells are characterized by production of the cytokines IL-17A and IL-17F, which are potent activators of neutrophilic inflammation, and they can also produce IL-22, which acts on the epithelium at barrier surfaces to promote the elimination of infiltrating microorganisms[34]. Thus, the balance between *at*-RA and IL-6 instructs the lineage specification of anti- or pro-inflammatory T cells activated in a TGFβ-rich microenvironment, such as the gut, providing an elegant mechanism to determine whether the adaptive immune system will accommodate or eliminate microorganisms in the gut.

Similar to the forerunners of nearly all currently known cytokine family members, homologues of TGFβ pre-dated the divergence of the vertebrate lineage[35] and therefore the emergence of adaptive immunity. TGFβ was already present when adaptive immunity first evolved, as were structural homologues of IL-1, IL-2, IL-6 and IL-17 (REFS 14,36–38) — all of which are present in many invertebrates and which have become involved in the induced $T_{Reg}$–$T_H17$ cell developmental dichotomy in vertebrates. Given the premise that the evolution of adaptive immunity fuelled the accommodation of a diverse commensal

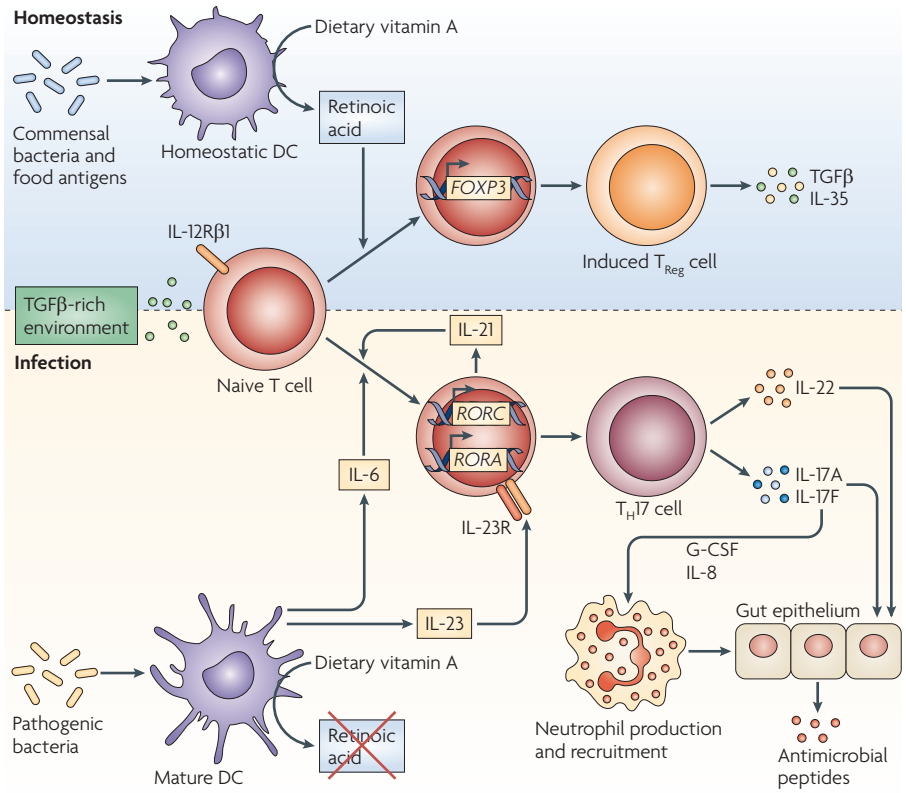


Figure 1 | **A common requirement for transforming growth factor-β in the induced regulatory T cell and T helper 17 cell lineages.** Dendritic cells (DCs) that are conditioned in environments rich in transforming growth factor-β (TGFβ), such as the intestines, present microorganism-derived antigens to induce the differentiation of naive $CD4^+$ T cells to either induced regulatory T ($T_{Reg}$) cells or T helper 17 ($T_H17$) cells, depending on the dominance of retinoic acid or interleukin-6 (IL-6), respectively. During homeostasis, DCs loaded with antigens from commensal bacteria or food produce retinoic acid derived from dietary vitamin A, which favours the upregulation of forkhead box P3 (*FOXP3*) expression and the differentiation of induced $T_{Reg}$ cells. Under conditions of microbial breach of the intestinal epithelial cell barrier, pro-inflammatory stimuli activate DCs, which mature and produce IL-6 and stop retinoic acid production, thereby inducing $T_H17$ cell differentiation. IL-6 and IL-21 (induced by IL-6) suppress *FOXP3* expression and upregulate expression of the retinoic acid-related orphan receptor genes *RORC* and *RORA* (which encode RORγt and RORα, respectively), leading to expression of the inducible component of the IL-23 receptor (IL-23R) and further $T_H17$ cell development. IL-17A and IL-17F produced by $T_H17$ cells increase production of neutrophils in the bone marrow and their recruitment to the gut through intermediary cytokines (such as granulocyte colony-stimulating factor (G-CSF) and IL-8, respectively) and can also act to induce epithelial cell production of antimicrobial peptides. IL-22 produced by $T_H17$ cells promotes enhanced epithelial barrier function and the production of antimicrobial peptides. Collectively, therefore, the $T_H17$ cell response acts both to clear invasive microorganisms and to restore barrier function.

microbiota in the gut, the co-optation of TGFβ for the regulation of these new immune functions makes evolutionary sense. Indeed, this ancient cytokine is involved in a large diversity of regulatory processes[39]. The important role of TGFβ in regulating tissue repair and innate immune responses seems to have pre-dated jawed vertebrate evolution[40], and the prevalence of TGFβ in the gut and its importance in the maintenance and repair of the intestinal epithelium raise the possibility that TGFβ was appropriated by emerging adaptive immune cells as an extension of its function as an intercellular signalling molecule that orchestrates the response of the epithelial barrier to injury.

A common and often confounding feature of TGFβ is its paradoxical ability to have opposing effects in the same pathway, as shown by its ability to both induce and suppress inflammation in different settings[41,42]. This dualistic nature of TGFβ is well suited to control alternative pathways of T cell differentiation in the induced $T_{Reg}$ and $T_H17$ cell lineages. Recently, it was shown that in the carp and goldfish (jawed vertebrates), TGFβ is expressed in skin, thymus, spleen and kidney and has a role in lymphocyte development and the inflammatory response[43,44]. Although the expression and function of TGFβ were not examined in intestinal tissues in these fish, these findings nevertheless indicate that TGFβ might have retained similar functions in adaptive immunity since its emergence at the dawn of vertebrate evolution ~500 million years ago. Accordingly, TGFβ could well have been essential early in the evolutionary transition from innate to adaptive immunity, lending to this new immune strategy its ancient ability to promote different outcomes depending on the homeostatic or inflammatory context.

## Duelling transcription factors

The overlapping functions of TGFβ in $T_H17$ versus induced $T_{Reg}$ cell development are reflected in the transcription factor networks that control their lineage divergence (FIG. 2). Although several transcription factors, including interferon regulatory factor 4 (IRF4), the aryl hydrocarbon receptor (AHR), runt-related transcription factor 1 (RUNX1) and basic leucine zipper transcription factor, ATF-like (BATF)[45–49], have important functions in $T_H17$ cell specification, the retinoic acid-related orphan receptors RORγt and to a lesser extent RORα seem to have a central role[49–51]. Induced $T_{Reg}$ cells are also directed and defined by a lineage-specifying transcription factor, FOXP3 (REF. 52). Surprisingly, however, antigen-activated naive T cells exposed to TGFβ, in the absence or presence of IL-6, are induced to co-express FOXP3 and RORγt early in differentiation, with the expression of RORγt being inhibited as induced $T_{Reg}$ cell differentiation progresses[53,54], or the expression of FOXP3 being inhibited as $T_H17$ cell differentiation proceeds[53,55]. Therefore, RORγt and FOXP3, which are both upregulated downstream of TGFβ signalling in naive T cells, engage in an antagonistic competition for dominance early in T cell differentiation, such that factors that tip the balance in favour of one or the other transcription factor predispose to the development of $T_H17$ cells or induced $T_{Reg}$ cells[55].

Recent discoveries have provided a mechanistic basis for this competition. Both RORα and RORγt have been identified as binding partners of FOXP3 (REFS 55–57), and binding of FOXP3 inhibits the transcriptional activity of RORα and RORγt. FOXP3 binds the ROR factors through a motif encoded by the second exon of the *FOXP3* gene, which interacts with a carboxy-terminal, ligand-dependent transactivation domain of RORα and RORγt[56]. Accordingly, splice variants of FOXP3 that lack the exon 2-encoded domain cannot repress ROR activity[55,56]. Therefore, FOXP3 can directly repress the $T_H17$ cell-promoting transcription factors, RORγt and RORα, and the specific domains that mediate this interaction have been defined.

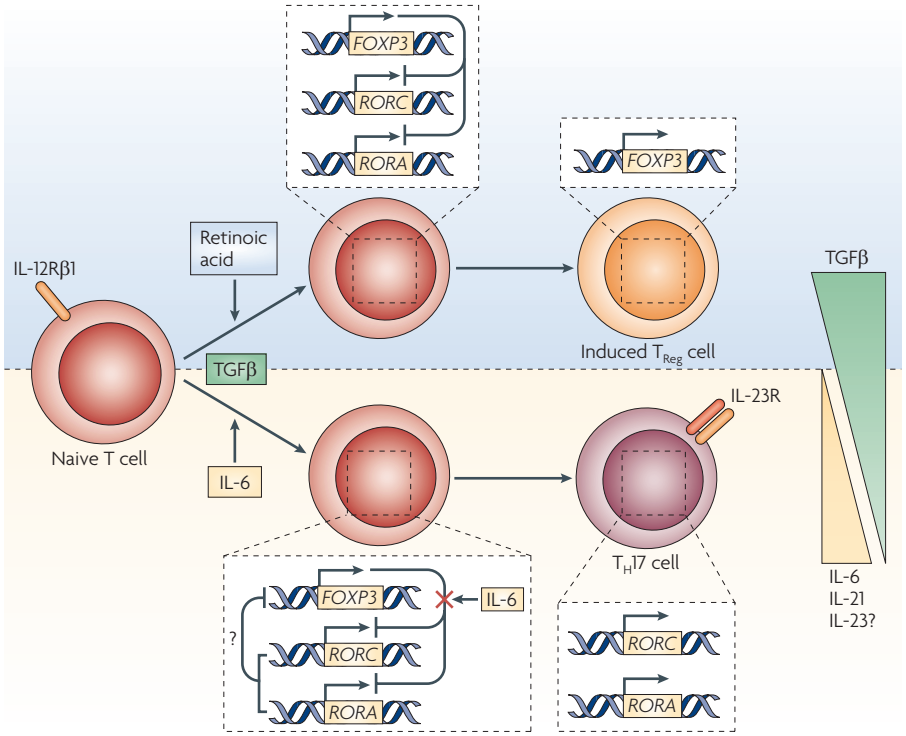


Figure 2 | **Competitive antagonism between FOXP3 and ROR family members dictates induced regulatory T cell versus T helper 17 cell development.** Naive CD4⁺ T cells activated by antigen in the presence of transforming growth factor-β (TGFβ) are induced to express the retinoic acid-related orphan receptor genes *RORC* and *RORA*, as well as forkhead box P3 (*FOXP3*), which encode RORγt, RORα and FOXP3, respectively. These cells can differentiate into either T helper 17 ($T_H17$) cells or induced regulatory T ($T_{Reg}$) cells depending on the dominance of interleukin-6 (IL-6) or retinoic acid, respectively. In the presence of retinoic acid, and absence of IL-6, FOXP3 can bind, and inhibit the activity of, ROR family factors, thereby promoting induced $T_{Reg}$ cell specification. IL-6, and other signal transducer and activator of transcription 3-activating $T_H17$ cell-inducing cytokines (such as IL-21 and IL-23) seem to promote $T_H17$ cell development at least in part by antagonizing the FOXP3-mediated inhibition of ROR family members through an undefined mechanism. ROR factors might also inhibit FOXP3 expression through direct or indirect mechanisms. IL-12Rβ1, interleukin-12 receptor β1.

Importantly, the repression of RORγt-mediated effects by FOXP3 depends on the local concentration of TGFβ[53,55]. At high concentrations of TGFβ, the function of RORγt is repressed through increased expression of FOXP3. At low concentrations of TGFβ, signals initiated by IL-6 through signal transducer and activator of transcription 3 (STAT3) — and sustained by other STAT3-activating cytokines that are important in $T_H17$ cell differentiation, such as IL-21 and IL-23 — override FOXP3-mediated repression of RORγt, leading to the progressive transcriptional dominance of RORγt, silencing of FOXP3 and commitment to the $T_H17$ cell lineage[55]. Accordingly, $T_H17$ cell-promoting cytokines can act through STAT3-dependent pathways to reverse the FOXP3-mediated repression of RORγt and RORα. In keeping with its pleiotropic nature, TGFβ can therefore induce fundamentally different outcomes from the same cellular target through differential effects of dose and co-signalling context.

## FOXP3: instability affords flexibility?

Surprisingly, and contrary to the previously held belief that induced $T_{Reg}$ cells are a stable phenotype, several reports now indicate that mature induced $T_{Reg}$ cells can re-express RORγt and, under certain conditions, can be converted to IL-17-expressing effector cells. However, although subsets of T cells that co-express FOXP3 and RORγt have been identified *in vivo* and *ex vivo*[53,55,58–64], at least some of these cells retain a regulatory phenotype[64]. FOXP3⁺RORγt⁺ T cells identified in the intestines of mice produced substantially less IL-17 than did FOXP3⁻ RORγt⁺ T cells[55], which indicates that FOXP3 directly or indirectly suppresses the ability of RORγt to induce IL-17 expression or that these FOXP3⁺RORγt⁺ T cells might be 'transitional' cells during early $T_H17$ cell development. Nevertheless, dendritic cells activated through dectin 1 (also known as CLEC7A), a pattern recognition receptor that binds β-glucans found in the cell walls of fungi and some bacteria, converted induced $T_{Reg}$ cells into IL-17-producing cells that maintained the expression of FOXP3, which shows that the expression of FOXP3 and IL-17 might not be mutually exclusive and that induced $T_{Reg}$ cells could give rise to 'hybrid' pro-inflammatory effectors in the context of an infectious challenge[61]. In another study, it was shown that mature induced $T_{Reg}$ cells could downregulate expression of FOXP3 and express IL-17 if stimulated with IL-6, indicating that induced $T_{Reg}$ cells can be converted to $T_H17$-like cells late in their development[53]. The downregulation of FOXP3 expression in this study was STAT3 dependent and RORγt and RORα independent, which indicates that ROR factors do not directly repress expression of FOXP3.

Interestingly, IL-6 might not be unique in its ability to induce IL-17 expression by FOXP3⁺ precursors. In studies of human and mouse cells, IL-1 signalling was shown to convert FOXP3⁺ cells to IL-17-producing progeny, although in some cases these IL-17⁺ cells retained expression of FOXP3 (REFS 59,63,65) and in other cases they did not[58,60]. Clearly, then, recent studies establish the possibility that FOXP3⁺ cells in humans and mice can give rise to cells with features of $T_H17$ cells, although controversy exists regarding the conditions under which these IL-17⁺ cells maintain expression of FOXP3 and/or regulatory function, or whether they invariably give rise to pro-inflammatory progeny that have lost immunosuppressive function.

In this regard, an important caveat to these studies concerns the level and isoform of FOXP3 that is expressed, in addition to the fate of the hybrid cells identified. Robust expression of FOXP3 is unequivocally associated with a suppressive cell phenotype, whereas attenuated expression of FOXP3 results in a less effective suppressive programme[52]. It might therefore be possible for a FOXP3⁺IL-17⁺ T cell to express FOXP3 at a sufficiently low level that the RORγt-driven inflammatory profile would be dominant. Alternatively, it is possible that FOXP3⁺IL-17⁺ T cells are a transitional phase in the development of FOXP3⁻IL-17⁺ T cells from induced $T_{Reg}$ cell precursors. Finally, as it is clear that not all splice variants of FOXP3 have comparable effects on the functions of ROR factors, it is possible that the FOXP3⁺IL-17⁺ cells identified in at least some of these studies express isoforms of FOXP3 that do not inhibit ROR functions. Thus, it might be the case that only splice variants of FOXP3 that lack repressive

activity for ROR factors can be stably co-expressed with RORγt and RORα. Although additional studies will be required to resolve these issues, mounting evidence favours the probability that in certain inflammatory settings, FOXP3$^+$ induced $T_{Reg}$ cells can give rise to $T_H17$-type effector cells, whether they co-express isoforms of FOXP3 or not.

Importantly, ROR family members are not the only transcription factor partners with which FOXP3 interacts in T cells. FOXP3 can interact with, and inhibit, the transcription factor RUNX1 (REF. 66), which is expressed during $T_H17$ cell polarization and associates with RORγt at a *cis*-regulatory element upstream of the *IL17A* gene[49]. Given its ability to interact with both FOXP3 and RORγt, it is conceivable that differential binding of RUNX1 to FOXP3 or RORγt might modulate the repressive effects of FOXP3 on RORγt function during the early phase of $T_H17$ cell differentiation when FOXP3 and RORγt are co-expressed. FOXP3 can also bind IRF4, which is required for both $T_H2$ and $T_H17$ cell development. In IRF4-deficient animals, RORγt expression by T cells is decreased whereas FOXP3 expression is increased[45,67], which indicates that IRF4 might promote $T_H17$ cell development by regulating interactions between FOXP3 and RORγt. As more examples of interactions between FOXP3 and transcription factors that control effector T cell fate decisions are discovered[67], it would seem that FOXP3 has a pivotal role in several transcription factor networks that determine the developmental fate of a naive T cell and, once its phenotype is established, its stability. In this regard, it is of note that no clear examples of the diversion of established effector T cells to a FOXP3$^+$ induced $T_{Reg}$ cell phenotype have been characterized. Therefore, whereas FOXP3$^+$ $T_{Reg}$ cells might be recruited to participate in effector responses under threat from infectious agents, the reverse does not seem to be the case: established effector T cells, including $T_H17$ cells, seemingly cannot give rise to FOXP3$^+$ regulatory T cells. This unidirectional developmental plasticity, which allows $T_{Reg}$ cells to convert to effector cells but not the converse, might have evolved as a fail-safe mechanism that reflects the greater immediate threat posed to the host by microorganisms that have breached epithelial barriers, such that the task of silencing effector responses once the immediate threat has subsided is relegated to other immunosuppressive mechanisms, such as the expression by effector T cells of the suppressive cytokine IL-10 (REF. 68).

## Epigenetic influences

Implicit in the divergent development of induced $T_{Reg}$ cells and $T_H17$ cells driven by TGFβ is the differentiation-induced activation or silencing of specific genes, which is associated with their epigenetic modification. As antigen-activated naive T cells differentiate, the epigenetic alterations that are acquired in their chromatin guide and reinforce lineage specification by imparting specific and heritable gene expression programmes on their progeny. Covalent modifications of chromatin components, such as the methylation of histones or of DNA itself, control the accessibility of crucial *cis*-regulatory regions in gene loci to transcriptional activators and repressors. Distinct chromatin marks that are indicative of transcriptionally active or repressed regions of the genome have been identified[69], and these have begun to be studied at cytokine and transcription factor loci in the context of effector and regulatory T cell development[70,71].

It was recently found that, in mice, genes encoding central, lineage-associated T cell transcription factors, such as those that encode the $T_H1$ and $T_H2$ cell-specifying factors — T-bet (*Tbx21*) and GATA3 (*Gata3*), respectively — have both activating (trimethylated histone 3 lysine 4 (H3K4me3)) and repressive (H3K27me3) histone modifications in all of the other effector T cell subsets that have been examined. These bivalent modifications seem to poise the *Tbx21* and *Gata3* genes for induction even after lineage-specific polarization in non-$T_H1$ or -$T_H2$ cells that do not normally express them[70]. This has provided a mechanistic basis for the recently observed flexibility of the $T_H17$ and induced $T_{Reg}$ cell lineages (FIG. 3). The incomplete silencing of the *Tbx21* and *Gata3* genes in alternative T cell lineages is consistent with the recently reported lineage plasticity of mature $T_H17$ cells, which enables $T_H17$ cell precursors to give rise to $T_H1$ or $T_H2$ cell progeny under re-polarizing cytokine conditions[72,73]. Similarly, bivalent marks identified in the promoters of the *Tbx21* and *Gata3* genes in induced $T_{Reg}$ cells are consistent with the observation that these cells might also 'convert' to $T_H1$- and $T_H2$-like cells under re-polarizing cytokine conditions[70]. Furthermore, the gene encoding RORγt (*Rorc*) is associated with bivalent histone modifications in mature $T_{Reg}$ cells and is therefore poised for re-expression, which might underlie the co-expression of FOXP3 and RORγt or IL-17 in the studies discussed above, as well as the ability of induced $T_{Reg}$ cells to convert to $T_H17$ cells. Notably, however, as in $T_H1$ and $T_H2$ cells, no activating (H3K4me3) histone marks were identified in association with the *Foxp3* gene in $T_H17$ cells, which indicates that the reciprocal conversion of mature $T_H17$ cells to induced $T_{Reg}$ cells is unlikely.

Interestingly, FOXP3 cooperates with chromatin modifying enzymes such as histone acetyltransferases and histone deacetylases[74], thereby regulating both repressive and activating epigenetic changes in target genes. Not only can FOXP3 recruit chromatin remodelling factors, but FOXP3 itself can be post-translationally modified by phosphorylation and acetylation, which increases chromatin binding and the transcriptional regulation of target genes. TGFβ signalling seems to increase the binding of acetylated FOXP3 to certain genes and therefore its transcriptional activity, whereas concordant TGFβ and IL-6 signalling inhibits the binding of FOXP3 to chromatin and therefore the transcriptional response[75]. Taken together with the ability of FOXP3 to directly bind numerous transcription factors involved in effector $T_H$ cell development, these results indicate that FOXP3 behaves dominantly, suppressing all but a regulatory developmental programme, with only its down-modulation — or perhaps alternative splicing to functionally distinct isoforms — allowing for the conversion of regulatory cells to effector cells.

## Concluding remarks

If an important selective advantage afforded to primordial vertebrates newly endowed with an adaptive immune system was the means to shape an increasingly diverse intestinal microbiota to serve, rather than threaten, its host, then the co-evolution of induced $T_{Reg}$ cell and $T_H17$ cell lineages in the gut might well have provided the basis for, and been essential to, the development of adaptive immunity. Although it will be difficult to establish proof of this theory ~500 million years after the event, many aspects of the interplay between the induced $T_{Reg}$ and $T_H17$ cell developmental pathways, and the cytokine and transcription factor networks that guide their development, make this increasingly plausible. FOXP3$^+$ induced $T_{Reg}$ cells and RORγt$^+$ $T_H17$ cells are highly enriched in the gut and its associated lymphoid tissues, and the balance between regulatory and effector T cell repertoires is shaped by on-going interactions with an abundant and diverse resident microbiota found mainly in vertebrates, where the commensal microorganisms are separated from $T_{Reg}$ and $T_H17$ cells by an epithelial barrier only one cell thick. These observations support the notion that the evolving T cell arm of the adaptive immune system usurped the primitive TGFβ

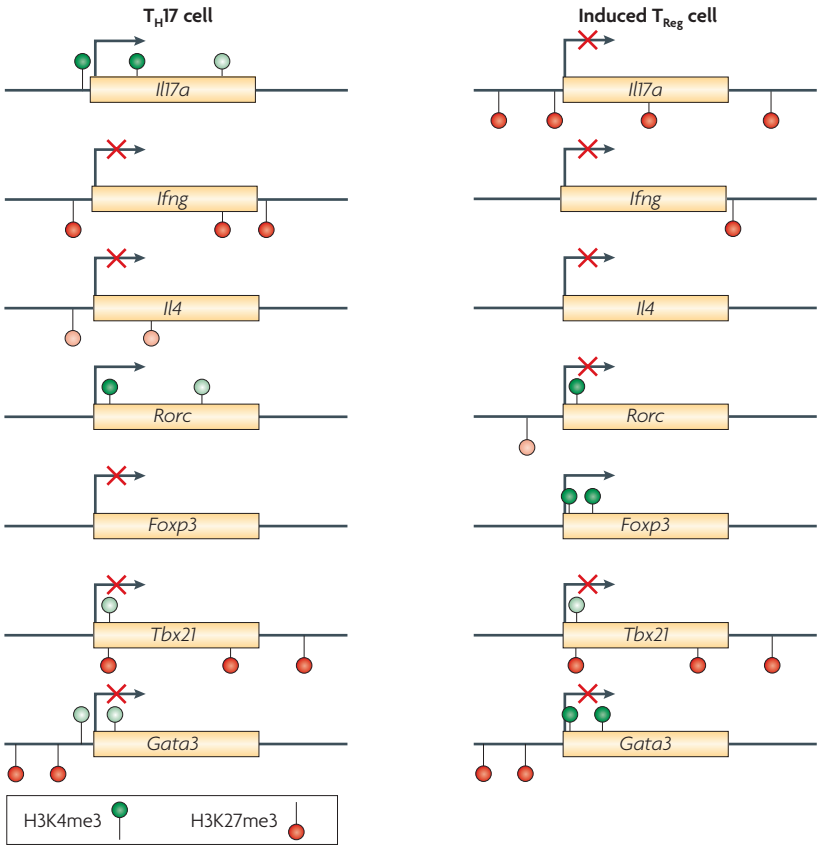


Figure 3 | **Plasticity of induced regulatory T ($T_{Reg}$) cells and T helper 17 ($T_H17$) cells indicated by epigenetic modifications of lineage-specifying transcription factors and cytokines.** Trimethylation of histone 3 lysine 4 (H3K4me3; green circles) is indicative of transcriptionally active genes, whereas H3K27me3 (red circles) is associated with gene silencing. Lighter shading indicates weaker histone modification. Bivalent (active and repressive) histone modifications of the genes encoding retinoic acid-related orphan receptor-γt (*Rorc*), T-bet (*Tbx21*) and GATA3 (*Gata3*) indicate that CD4$^+$ effector or regulatory T cells controlled by these key transcription factors have the ability to switch phenotypes[70]. *Foxp3*, forkhead box P3; *Ifng*, interferon-γ; *Il*, interleukin.

cytokine system, which was already in use for maintenance of the intestinal epithelium, as a means to foster diversification of the commensal flora while protecting against the recurring barrier insults that are intrinsic to gut tissue.

In this regard, it is notable that the role of TGFβ in adaptive immune responses in the mucosae is not unique to the development of the induced $T_{Reg}$ cell and $T_H17$ cell pathways. TGFβ has long been recognized as the main switch factor for secretory IgA[76], which is the predominant immunoglobulin type produced in the intestines. IgA has an important role in both containing the commensal flora inside the gut lumen and shaping the species composition of the commensal microbiota that reside there. Remarkably, FOXP3$^+$ $T_{Reg}$ cell precursors that downregulate FOXP3 expression and acquire features of T follicular helper cells have recently been identified as helpers for IgA production in the Peyer's patches of the gut[77]. In addition to providing evidence of further plasticity in the induced $T_{Reg}$ cell developmental programme, this study identifies additional links between adaptive immune networks that cope with the commensal flora, and extends the hypothesis that the evolutionary benefits derived from immune support for a diverse commensal microbiota might have been central to the emergence of an adaptive immune system — all revolving around the common, ancient cytokine TGFβ.

Similar to TGFβ, the FOX and ROR families of transcription factors that are central to induced $T_{Reg}$ cell and $T_H17$ cell development are also ancient and pre-date the appearance of vertebrates and adaptive immunity. Homologues of most members of both families have been identified in insects, and direct FOXP3 and RORγ orthologues have been identified in bony fish. Therefore, it would seem that both the cytokine and transcription factor networks that gave rise to the complementary, but antagonistic, developmental programmes that ultimately produced the linked induced $T_{Reg}$ cell and $T_H17$ cell lineages were already in place in the first jawed vertebrates. Although $T_{Reg}$ cells have not yet been identified in lower vertebrates, lymphocytes with suppressive activity have been described[78] and we think it is probable that as further phylogenetic studies are forthcoming, the co-evolution of the induced $T_{Reg}$ cell and $T_H17$ cell pathways will become a central theme in the emergence of adaptive immunity.

Indeed, we postulate that the linked induced $T_{Reg}$ cell and $T_H17$ cell lineages are the most ancient of the CD4$^+$ T cell differentiation pathways, providing the developmental template for the $T_H1$ and $T_H2$ cell lineages that followed. In support of this contention, orthologues of IFNγ and IL-4 — the hallmark cytokines of $T_H1$ and $T_H2$ cells, respectively — seem to have appeared after $T_H17$ cell cytokines on the evolutionary stage. Therefore, whereas IFNγ and IL-4 orthologues have not been identified in jawless vertebrates, IL-17 is not only present in agnathans[16,79] but has recently been found to be expressed by activated lamprey lymphocytes, suggesting a distinct similarity to human and mouse $T_H17$ cells[15]. There is also evidence that ancestral flatworms (platyhelminths), which provoke and are cleared by $T_H2$ cell responses, evolved following the divergence of the jawed vertebrates and began to colonize the guts of their hosts after the emergence of $T_H17$ cell effectors[80]. In addition, the ability of $T_H17$ cells to be converted to $T_H1$ or $T_H2$ cell phenotypes, but not the converse, supports the hierarchical position of the $T_H17$ cell lineage evolutionarily[72,73]. Finally, based on the recently appreciated plastic nature of both induced $T_{Reg}$ cells and $T_H17$ cells, we suggest that mechanisms to alter the functional phenotype based on changing environmental cues might be a remnant of the early evolving adaptive immune system that have been conserved over time owing to their efficiency and economy.

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**DATABASES**

**UniProtKB:** http://www.uniprot.org

AHR | BATF | FOXP3 | GATA3 | IL-6 | IL-17A | IL-17F | IL-22 | IRF4 | RAG1 | RAG2 | RORα | RORγt | RUNX1 | STAT3 | T-bet | TGFβ

**FURTHER INFORMATION**

**Casey T. Weaver's homepage:** http://www.microbio.uab.edu/faculty/weaver/index.html

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