agreement with these observations, the authors also demonstrated that skin inflammation and IL-17 expression in R258W mutant mice were decreased in vivo when animals were treated with IL1β antibodies. Interestingly, IL17 antibodies also improved skin pathology, therefore arguing for an important IL-1β-IL-17 axis in disease progression, at least in cutaneous tissues.

At the cellular level, both groups isolated bone marrow-derived macrophages (BMDMs; Meng et al. [2009]) or bone marrow-derived dendritic cells (BMDCs; Brydges et al. [2009]) from mutant mice and explored their IL-1β-dependent response after exposure to TLR ligands in the presence or absence of ATP. Both studies showed that myeloid cells from mutant mice did not secrete mature IL-1β spontaneously, but displayed a considerably higher sensitivity to TLR ligands than WT cells and, importantly, did not require exogenous addition of ATP for optimal activation (Figure 1). This latter finding is important, because it supports the notion that *NLRP3* mutations associated with disease susceptibility are gain-of-function mutations, and that the mutated NLRP3 protein is constitutively active and therefore does not require detection of upstream stimuli. However, neither study addressed the critical question of whether TLR-dependent accumulation of pro-IL1β transcripts is also required in vivo to ignite IL-1β secretion in *Nlrp3* mutant mice. Indeed, this mode of activation can be envisioned for tissues such as the skin, which might come in contact with microbes after wounding. It does not explain, however, why *Nlrp3* mutant mice did not develop inflammation in the intestine and the lung, which are constantly exposed to microbes. This observation suggests that microbial tolerization mechanisms may efficiently suppress inflammasome activation in mucosal surfaces in contact with microbes in homeostatic conditions.

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The Fate of Human Treg Cells

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In this issue of *Immunity*, Miyara et al. (2009) demonstrate that FoxP3$^+$ cells in human peripheral blood are heterogeneous in function, and CD45RA expression defines their different stages of differentiation.

CD4$^+$CD25^{++}FoxP3$^+$ Treg cells are pivotal in controlling undesired self and non-self immune responses. Importantly, FoxP3$^+$ Treg cells may not be a functionally homogenous population. Various human Treg cell subtypes within peripheral CD4$^+$FoxP3$^+$ T cells have been identified and can be subgrouped based on the expression of CD45RA (Seddiki et al., 2006; Valmori et al., 2005), HLA-DR (Baecher-Allan et al., 2006), and ICOS (Ito et al., 2008). Furthermore, FoxP3 can be expressed in T cells without conferring regulatory properties. Until now, the dynamic differentiation and fate of the various FoxP3-expressing human peripheral CD4$^+$ T cells remained to be clarified. Miyara et al. (2009) in this issue of *Immunity* show that human CD4$^+$FoxP3$^+$ T cells comprise three distinct subpopulations with a precise phenotype and fate: CD25^{++} CD45RA$^+$ (FOXP3^{lo}) resting Treg cells (rTreg cells), CD25^{+++}CD45RA$^-$(FOXP3^{hi}) activated Treg cells (aTreg cells), which represent different stages of Treg cell differentiation and are both suppressive in vitro, and CD25^{++}CD45RA$^-$(FOXP3^{lo}) cytokine secreting T cells, which lack suppressive activity.

Positively selected T cells express CD45RO in the thymus, convert to CD45RA expression at the time of peripheral migration, and switch back to CD45RO after antigen (Ag) recognition. A small percentage of Ag-experienced T cells revert from CD45RO$^+$ to CD45RA$^+$ as they undergo differentiation into terminal effector cells. Initially, it was thought that, in adult peripheral blood, CD4$^+$CD25^{+++} Treg cells reside predominantly within the activated memory CD45RA$^-$ cell subset and within the naive CD45RA$^+$ cell subset in the cord blood. Subsequently, the presence of CD45RA$^+$ Treg cells, in addition to CD45RA$^-$ Treg cells, was appreciated in the periphery of adults (Seddiki et al., 2006; Valmori et al., 2005). Fritzching et al. (2006)

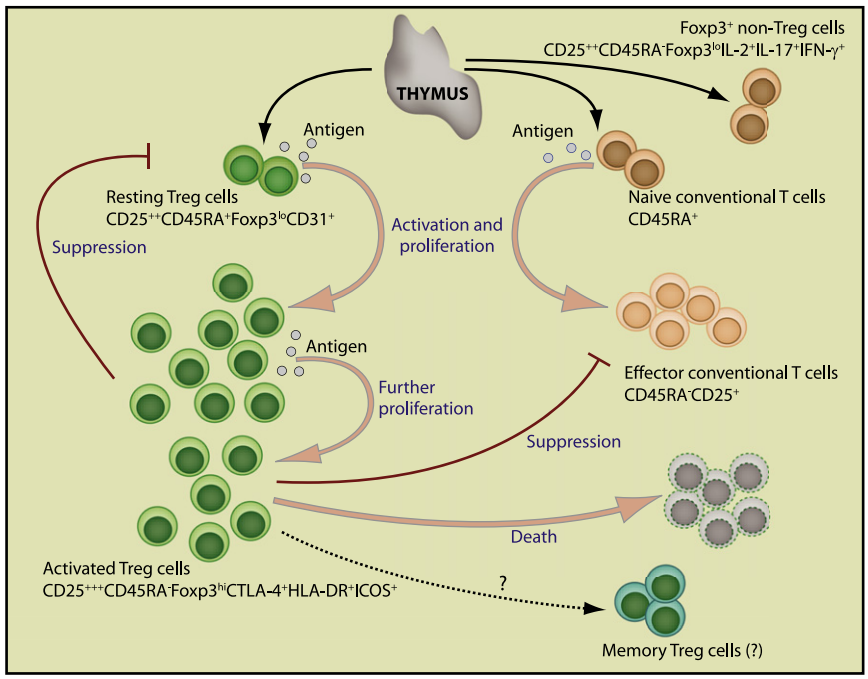

Figure 1. FoxP3$^+$ Treg Cell Fate

Human Treg cells depart from the thymus as resting Treg (rTreg) cells expressing CD31, CD45RA, intermediate amounts of CD25, and low amounts of FOXP3. Upon peripheral Ag encounter, rTreg cells proliferate, downregulate CD45RA, upregulate CD25, FOXP3, CTLA-4, HLA-DR, and ICOS expression and become activated Treg (aTreg) cells that suppress proliferation of conventional T cells (Tconv). aTreg cells are short lived and rapidly die after activation and suppression. Highly proliferating aTreg cells suppress also proliferation of rTreg cells generating a negative feedback loop that stops rTreg cell conversion into aTreg cells and consequent Treg cell exhaustion. It remains to be defined whether: (1) there is a life-long thymic output of CD45RA$^+$ rTreg cells or they are self-maintained; (2) a portion of aTreg cells survive and become memory long-living aTreg cells; and (3) aTreg cells make use of different suppressive mechanisms to inhibit proliferation of Tconv and rTreg cells.

demonstrated that CD45RA$^+$ Treg cells are resistant to CD95L-mediated apoptosis whereas CD45RA$^-$ Treg cells rapidly die after activation. In line with this finding, only CD45RA$^+$ Treg cells gave rise to homogenous regulatory T cell lines upon in vitro expansion (Hoffmann et al., 2009). Although their relationship remained unclear, CD45RA$^+$ and CD45RA$^-$ Treg cells were reported to have comparable function. This was quite surprising, because, in principle, naive Ag-inexperienced CD45RA$^+$ T cells and memory Ag-primed CD45RA$^-$ T cells should exhibit different functions. In the present study, Miyara et al. (2009) confirm both the phenotypical characterization of human peripheral Treg cells based on CD25 and CD45RA expression (Fritzsching et al., 2006; Haas et al., 2007; Seddiki et al., 2006; Valmori et al., 2005) and the apopotosis susceptibility of CD45RA$^-$ Treg cells (Fritzsching et al., 2006). In addition, in line with previous studies (Seddiki et al., 2006; Valmori et al., 2005), they also demonstrate that the ability of CD45RA$^+$ rTreg cells and CD45RA$^-$ aTreg cells to suppress conventional T cells is comparable. However, the gene expression patterns of these two Treg cell subsets are distinct, suggesting different suppressive mechanisms. aTreg cells are, for instance, more active in IL-10 transcription but less active in TGF-β, as compared to rTreg cells. Furthermore, in the present study, it is shown that aTreg cells can inhibit proliferation of rTreg cells.

The key finding of Miyara et al. (2009) is the demonstration that CD45RA$^+$ rTreg cells and CD45RA$^-$ aTreg cells represent a distinct differentiation status of the same T cells. This is proven by an elegant experiment in which clonotypes within the CD45RA$^+$ rTreg cells and CD45RA$^-$ aTreg cells were tracked in vivo 18 months apart in the same individual. A clonotype initially detected only in the rTreg cells was found 18 months later to be dominant in the aTreg cells. We can therefore hypothesize that: (1) the thymus generates CD45RA$^+$ rTreg cells, which are either constantly thymically generated or self-maintained (still to be determined); (2) CD45RA$^+$ rTreg cells, once activated through their TCR, rapidly proliferate and become CD45RA$^-$ aTreg cells that can suppress conventional T cells and die (whether a portion of aTreg cells survive and become a memory long-living Treg cells is still to be clarified); (3) actively dividing aTreg cells can also suppress proliferation of rTreg cells, generating a negative feedback loop that stops rTreg cell conversion into aTreg cells and consequent Treg cell exhaustion (Figure 1). This model, supported by the data presented by Miyara et al. (2009), sheds light on the in vivo cell fate and dynamic differentiation of different human FoxP3$^+$ Treg cell subsets. However, a number of key questions remain to be addressed. Do rTreg cells and aTreg cells use different mechanisms of suppression? Miyara et al. (2009) show that both Treg cell types suppress T cell proliferation in vitro, but the mechanism(s) underlying this suppression is not elucidated and the role of cytokines is not addressed. Do rTreg and aTreg cells reside in different tissues? All the experiments by Miyara et al. (2009) were performed with peripheral blood and the presence of these different Treg cell types in the spleen, lymph nodes, or tissues has not been demonstrated. Finally, what is the relationship between the two Treg cell subsets here described and the inducible Treg cells reported by others?

The fact that activated Treg cells described by Miyara et al. (2009) are superimposable to the previously described CD25^{+++} HLA-DR$^+$ (Baecher-Allan et al., 2006) and the ICOS$^+$ FoxP3$^+$ (Ito et al., 2008) Treg cells, both in term of phenotype and function, remains, in our opinion, uncertain. The HLA-DR$^+$-activated Treg cells were shown to induce early contact dependent in vitro suppression (Baecher-Allan et al., 2006), whereas the ICOS$^+$-activated Treg cells use IL-10 to suppress DC function and TGF-β to suppress T cells (Ito et al., 2008).

Initial studies reported reciprocally regulated and mutually exclusive differentiation programs for Treg cells and Th17 cells. However, the relationship between Treg cells and Th17 cells might be more complex than anticipated, involving plasticity of the respective differentiation programs mediated by epigenetic mechanisms. The

data in Miyara et al. (2009) confirm the recent demonstration that cells coexpressing the master regulator genes for Treg cells (i.e., FoxP3) and for Th17 cells (i.e., the retinoic acid-related orphan receptor gamma t, RORγt) exist (Ayyoub et al., 2009; Voo et al., 2009) and they are members of the CD45RA$^-$ aTreg cell subset. The interesting finding relies on the observation that CD45RA$^-$ aTreg cells express, together with RORγt, high amounts of the Aryl hydrocarbon receptor-repressor (AHR-R), which inhibits Th17 cell differentiation. These new data demonstrate the plasticity of CD4$^+$ T cells whose fate is defined by finely tuned genetic and epigenetic states of critical regulators that skew them toward opposite T cell lineages.

Of particular interest is the wide variety of human autoimmune diseases in which a defect in the Treg cell function has been demonstrated, raising the interesting possibility that this may be a common denominator causing uncontrolled immune responses to self Ags. However, presently there is no consensus on the functional status of Treg cells in human autoimmune diseases. The dynamic change of circulating CD45RA$^+$ rTreg cells into CD45RA$^-$ aTreg cells here described (Miyara et al., 2009) might be of help to better interpret previously conflicting results. Haas et al. (2007) demonstrated that peripheral CD45RA$^+$ rTreg cells are reduced in multiple sclerosis (MS) patients and this might account for the diminished suppressive Treg cell potency observed in MS individuals. Miyara et al. (2009) find that patients with active sarcoidosis, a granulomatous disease of unknown origin, have expanded aTreg cells whereas patients with active systemic lupus erythematosus (SLE), a prototype of autoimmune disease, have increased rTreg cells and FoxP3$^+$ non-Treg cells, as compared to age-matched controls. Overall, these results prove that the field remains complex and needs further exploration.

Finally, the present work (Miyara et al., 2009) challenges three of the widely accepted notions in the Treg cell field, providing important technical advances. First, human Treg cells are not anergic in vitro but, upon TCR-mediated activation, proliferate and the majority die. This finding implies that the thymidine uptake method commonly used for monitoring the in vitro suppressive ability of Treg cells may not be ideal and results may be confounded by the fact that aTreg cells suppress rTreg cells and that aTreg cells die. Second, CD127, which is used as a surface marker to isolate bona fide human Treg cells by flow cytometry, is not a specific marker. CD4$^+$CD25$^+$CD127^{lo} T cells indeed include also FoxP3$^+$ cells that produce IL-17, high amounts of IL-2 and IFN-γ, and do not suppress. CD25 and CD45RA markers should therefore be used to avoid isolation of FoxP3$^+$ non-Treg cells. Finally, cord blood CD4$^+$CD25$^+$ T cells are not all FoxP3$^+$ as previously reported (Fritzsching et al., 2006) and, in addition, RA$^-$FOXP3^{hi} aTreg cells are also present in cord blood. These data prove that natural Treg cells are constantly activated by endogenous self and maternal Ag even in utero and raises some question on the cord blood Treg cell "naiveness."

Overall, the present study sheds light on the tangled and controversial world of human Treg cells but also brings an additional layer of complexity in the phenotypical and functional characterization of these cells.

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