CD4+ T cells respond to infections and cancer by using T cell receptors (TCRs) to recognize foreign peptides bound to major histocompatibility complex class II (MHCII) molecules displayed on antigen-presenting cells. Each nascent T cell in the thymus generates a unique TCR by gene segment recombination and then just as Goldilocks chose her porridge, these cells are selected according to TCR affinity for self-peptide:MHCII ligands. Thymocytes with TCRs that have no affinity for self-peptide:MHCII ligands die due to a lack of TCR signaling (neglect). In contrast, thymocytes with TCRs that have moderate affinity for a self-peptide:MHCII ligand receive weak TCR signals that cause the cells to become conventional CD4+ T cells lacking the transcription factor Foxp3. These cells seed the secondary lymphoid organs, forming a vast and diverse repertoire. During an infection, a few cells from this repertoire will by chance express TCRs that recognize MHCII-bound microbial peptides with high affinity. These cells will become activated, proliferate, and differentiate into microbe-killing effector cells. Finally, those thymocytes with TCRs with the highest affinity for self-peptide:MHCII ligands are deleted or become Foxp3+ regulatory T cells that do not produce tissue-damaging cytokines and instead suppress T cells that might. We have recently shown that the amount of self-peptide:MHCII presentation in the thymus determines the size and regulatory T cell composition of self-peptide:MHCII-specific CD4+ T cell repertoires (Malhotra et al., 2016).

For thymically-induced tolerance (central tolerance) to be effective, all MHCII-bound self-peptides that are displayed in secondary lymphoid organs (and thus could activate conventional CD4+ T cells) must also be displayed by thymic antigen-presenting cells. This occurs in large part because many proteins are taken up or expressed by antigen-presenting cells in both locations. However, some proteins have highly tissue-specific functions and therefore restricted patterns of expression. Autoimmune regulator (Aire) drives promiscuous expression of many of these proteins in medullary thymic epithelial cells. Thymic dendritic cells efferocytose epithelial cells and present peptides from the tissue-restricted proteins on MHCII molecules, allowing for tolerance of T cells with high-affinity TCRs for these ligands. The organ-specific autoimmunity that develops in Aire-deficient individuals highlights the importance of this self-tolerance mechanism (Anderson et al., 2002; Mathis and Benoist, 2009).

Several fundamental questions emerge from the observation that the absence of tissue-restricted protein expression in the thymuses of Aire-deficient individuals leads to organ-specific autoimmunity. Are self-reactive thymocytes that encounter MHCII-bound peptides from peripheral tissue-restricted proteins primarily deleted or are they channeled into the regulatory T cell lineage? How does the strength of self-peptide:MHCII recognition influence the balance between the regulatory and conventional T cell fates?

Kieback et al. (2016) and Malchow et al. (2016) tackled these challenging questions by analyzing the repertoires of CD4+ T cells specific for MHCII-bound peptides from tissue-restricted proteins. These complementary studies demonstrate that thymocytes with strong recognition of self-peptide:MHCII ligands had a tendency to become regulatory T cells. Thymocytes with weaker self-peptide:MHCII recognition became conventional CD4+ T cells. Importantly, when the relevant self-protein was ablated from the thymus, many thymocytes that expressed TCRs that normally promoted regulatory T cell induction were instead shunted into the conventional T cell pool and caused pathology (Figure 1). Consistent with a theme emerging from other studies, clonal deletion played a surprisingly small role in tolerance to tissue-restricted proteins (Legoux et al., 2015; Malhotra et al., 2016; Yu et al., 2015).

Both studies assessed how self-peptide:MHCII encounter in the thymus shapes the T cell repertoire by manipulating expression of self-peptides. Kieback et al. studied CD4+ T cells that recognize an MHCII-bound peptide from myelin oligodendrocyte glycoprotein (MOGp) in normal or MOG-deficient mice. Malchow et al. examined mice with or without expression of Aire. Crucially, both studies measured the T cell repertoire by comparing TCR sequences from regulatory and conventional T cells.

Kieback et al. addressed the question of whether regulatory T cells generally have TCRs with higher affinity for a self-peptide:MHCII ligand than conventional T cells with the same specificity. Following immunization with MOGp, mice develop an inflammatory demyelinating disease of the central nervous system. The authors determined TCR sequences from MOGp:MHCII-specific regulatory and conventional T cells that were isolated from central nervous systems of diseased mice. They expressed these TCRs in cell lines and found that cells that expressed regulatory T cell-derived TCRs detected lower amounts of MOGp:MHCII than cells expressing conventional T cell-derived TCRs. The authors also found that in agreement with
The highly self-reactive cells then move into the periphery where they can become activated, proliferate, and to enter the Tconv cell pool (bottom, center). The decision between clonal deletion and Treg cell induction likely depends on the frequency of self-peptide 1:MHCII ligand encounter in the thymus. Repeated high-affinity interactions might promote clonal deletion, whereas fewer such encounters might instead induce Treg cell generation. Lower-affinity interactions with self-peptide 2:MHCII would allow entry into the conventional T cell (Tconv) pool. Treg cell induction following thymic encounter of self-peptide 1:MHCII prevents autoimmune disease in the periphery (top, right). The absence of self-peptide 1:MHCII from the thymus (as might occur in Aire−/− mice due to a lack of promiscuous expression of tissue-restricted proteins) allows developing T cells to undergo lower-affinity interactions with other ligands such as self-peptide 2:MHCII (bottom, left), and to enter the Tconv cell pool (bottom, center). These highly self-reactive cells then move into the periphery where they can become activated, proliferate, and cause inflammation in response to self-peptide 1:MHCII encounter (bottom, right).

Figure 1. High Affinity Interactions between Developing T Cells and Self-Peptide:MHCII Ligands in the Thymus Prevent Autoimmunity by Channeling These Cells to the Regulatory T Cell Lineage

Self-peptides are displayed by major histocompatibility complex molecules (MHC) on the surface of thymic antigen-presenting cells. Thymocyte T cell receptors (TCRs) might have high affinity for one self-peptide:MHCII complex (self-peptide 1:MHCII) and lower affinity for another (self-peptide 2:MHCII). Developing T cells that undergo high-affinity interactions with self-peptide 1:MHCII (top, left) are channeled to the regulatory T cell (Treg) lineage or deleted (top, center). The decision between clonal deletion and Treg cell induction likely depends on the frequency of self-peptide 1:MHCII ligand encounter in the thymus. Repeated high-affinity interactions might promote clonal deletion, whereas fewer such encounters might instead induce Treg cell generation. Lower-affinity interactions with self-peptide 2:MHCII would allow entry into the conventional T cell (Tconv) pool. Treg cell induction following thymic encounter of self-peptide 1:MHCII prevents autoimmune disease in the periphery (top, right). The absence of self-peptide 1:MHCII from the thymus (as might occur in Aire−/− mice due to a lack of promiscuous expression of tissue-restricted proteins) allows developing T cells to undergo lower-affinity interactions with other ligands such as self-peptide 2:MHCII (bottom, left), and to enter the Tconv cell pool (bottom, center). These highly self-reactive cells then move into the periphery where they can become activated, proliferate, and cause inflammation in response to self-peptide 1:MHCII encounter (bottom, right).

previous studies, conventional T cells that were engineered to express MOGp:MHCII-specific TCRs from regulatory T cells caused more severe disease than conventional T cells engineered to express MOGp:MHCII-specific TCRs from other conventional T cells (Hsieh et al., 2004). Conversely, regulatory T cells that were engineered to express MOGp:MHCII-specific TCRs from other regulatory T cells suppressed disease better than regulatory T cells engineered to express MOGp:MHCII-specific TCRs from conventional T cells. Thus, thymocytes with strong recognition of self-peptide:MHCII become regulatory T cells, thereby preventing autoimmunity.

Kieback and colleagues hypothesized that TCR signaling in response to MOGp:MHCII encounter instructs thymic regulatory T differentiation. Indeed, MOG-expressing mice contained more MOGp:MHCII-specific thymic regulatory T cells than MOG-deficient mice. MOGp:MHCII-specific regulatory T cell generation, however, was not completely eliminated in mice lacking MOG. Thus, some MOGp:MHCII-reactive regulatory T cells must be selected on MHCII-bound peptides from proteins other than MOG. These proteins might contain peptides that when bound to MHCII display similar outward pointing amino acids as MOGp, and thus look like MOGp:MHCII complexes to some TCRs (Birnbaum et al., 2014; Nelson et al., 2015).

Malchow et al. sought to understand how Aire-dependent self-peptide:MHCII encounter alters self-reactive CD4+ T cell repertoires. One possibility was that a thymocytes that experiences a high-affinity interaction between its TCR and an Aire-dependent self-peptide:MHCII ligand would be deleted. Another possibility was that such a thymocyte would become a regulatory T cell. If so, then certain TCRs from the regulatory T cell pool of Aire-expressing mice should be missing in Aire-deficient mice. Indeed, Malchow et al. found that ~3% of TCRs from regulatory T cells from normal mice were underrepresented in Aire-deficient mice, including some of the most prevalent TCRs. Malchow et al. also analyzed the TCRs that were overrepresented among conventional T cells in Aire-deficient mice. Were these TCRs that were normally deleted in wild-type mice? No. Remarkably, 74% of these TCRs were expressed by regulatory T cells in secondary lymphoid organs of normal mice. This deviation was also observed for TCRs that were identified from conventional T cells that infiltrated the prostates of Aire-deficient mice. Thus, similar to the results of Kieback et al., these data suggest that in the absence of proper self-peptide:MHCII encounter in the thymus, cells that should become regulatory T cells instead deviate to the conventional T cell pool from which they might cause autoimmunity. Their data also suggest that Aire-mediated clonal deletion negatively regulates a modest ~10% of self-reactive CD4+ T cells, not too far from the 30% that we recently reported (Malhotra et al., 2016). It is prudent, however, not to underestimate clonal deletion as nearly 700,000 thymocytes are clonally deleted per hour in the murine thymus (Stritesky et al., 2013), and these studies could not evaluate thymocytes that were deleted after recognition of self-peptide:MHCII ligands with similar TCR contact residues that were derived from non-Aire-regulated proteins.

These studies suggest that a key function of thymic self-peptide:MHCII encounter is to channel highly self-reactive thymocytes to the regulatory T cell lineage, thereby protecting us from autoimmunity. However, the molecular mechanisms that translate these high-affinity interactions into thymocyte reprogramming remain to be elucidated. Furthermore, an alluring possibility that emerges from these studies is that by virtue of this potent self-reactivity, expression of regulatory T cell-derived TCRs in conventional T cells might yield an alternative therapeutic approach for combating cancers for which self-tolerance limits clearance. Thus, while regulatory T cell development...
in the thymus prevents autoimmunity, it also helpfully tags our best source of highly self-reactive TCRs, which we might one day tap into to avert an entirely different kind of crisis.

REFERENCES


Making Ends Meet: Myeloid Cells Catalyze Blood Vessel Repair in the Brain

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Hemorrhagic stroke, primarily caused by rupture of blood vessels in the brain, is a leading cause of death and disability in adults. In this issue of *Immunity*, Liu et al. (2016) demonstrate that repair of cerebrovascular ruptures can be directly mediated by myeloid cells.

Two types of myeloid cells can be found in the central nervous system (CNS): microglia and infiltrating monocyte-derived macrophages. These two populations were long considered redundant because of their similar morphology, identical molecular markers, and analogous behavior under pathological conditions. Only recently has development of genetic tools that allow differential labeling and ablation of either of these two cell types, along with advances in high-throughput epigenetic, transcriptome, and proteome analysis techniques, enabled demonstration of differential functions of microglia and CNS macrophages (Shechter et al., 2009; Biber et al., 2016; Prinz and Priller, 2014). The microglia, which populate healthy CNS parenchyma in embryogenesis, are currently viewed as auxiliary cells, involved in brain development and multiple aspects of homeostasis, which nevertheless often fail to support the brain under pathological conditions; instead, they assume a pro-inflammatory phenotype, harmful to the delicate, non-regenerating CNS tissue (Crotti and Ransohoff, 2016). On the other hand, monocyte-derived macrophages reside at the borders of the CNS (choroid plexus, meninges, and perivascular spaces) and migrate to the parenchyma only in case of disease (Shechter et al., 2013). In models of acute CNS trauma (e.g., hemorrhagic or ischemic stroke and spinal cord injury), infiltrating macrophages have been shown to use multiple mechanisms to limit the damage and promote neuronal survival and regeneration (Figure 1; Prinz and Priller, 2014; Shechter et al., 2009; Wattananit et al., 2016).

A paper by Liu et al. (2016) in this issue of *Immunity* describes an intriguing role of the CNS-resident myeloid cells. Using a model of laser-induced cerebrovascular injury in zebrafish and in vivo time-lapse imaging, the authors discovered direct involvement of myeloid cells in the process of repair of ruptured blood vessels in the brain. Taking advantage of various fluorescently labeled strains and using pharmacological interventions, the authors visualized the process and studied its molecular basis in great detail. In brief, after a localized injury to the cerebral vasculature, a myeloid cell home to the gap created between the cut ends of the ruptured capillary (attracted by the extracellular ATP leaking from the injured endothelium), extends filopodia or lamellipodia, “grabs” the loose tips of the vessel (via interaction of adhesion molecules, including CDH5 and PECAM1), and uses mechanical traction to “pull” them toward each other (a process dependent on PI3K, F-actin, and myosin-II and regulated by Rac1), leading to endothelial wall fusion. Interestingly, the authors reported that ligation of the vascular ends also occurred