production needed to maintain long term cell survival. Hence, O'Sullivan et al. have not only uncovered an interesting observation for the burgeoning field of immunometabolism but also raised stimulating questions for biochemists to think about regulation of metabolic pathways. Specifically, biochemists will have to incorporate futile metabolic pathways in trying to understand how nutrients fulfill the metabolic demands of cells.

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Regulatory T Cells: Exosomes Deliver Tolerance

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T regulatory (Treg) cells enforce peripheral tolerance through regulation of diverse immune responses in a context-specific manner. Okoye et al. show one way that Treg cells suppress Th1 cell responses is through nonautonomous gene silencing mediated by microRNA-containing exosomes.

T regulatory (Treg) cells employ a diverse set of mechanisms to enforce peripheral tolerance, reflecting both the complexity and plasticity of immune responses. Mechanisms of suppression include production of immunomodulatory cytokines (e.g., interleukin-10 [IL-10], transforming growth factor-β [TGF-β], IL-35), the expression of inhibitory receptors (cytotoxic T lymphocyte antigen 4 [CTLA-4]), cytokine sinks (e.g., IL-2 receptor α chain), direct cytotoxic killing (granzymes, perforin), and several others (Shevach, 2009). These mechanisms have been validated by the identification of human and murine genetic defects that disable individual pathways, leading to immune dysregulation and autoimmunity. More recently, context-specific inhibition has emerged as a strategy to finely tune the regulation of specific T helper cell responses. Treg cells appropriate partial or "aborted" forms of the transcriptional programs of respective target T helper (Th) cell types by expressing their master transcription factors and coopting their function (Chaudhry et al., 2009; Zheng et al., 2009). For example, in the case of a Th1 cell response, Treg cells upregulate the expression of T-bet, which in turn induces the expression of some Th1-cell-related genes such as CX3CR1 but not others, enabling Treg cells to migrate to sites of Th1-cell-mediated inflammation while restraining their differentiation into Th1 cells (Koch et al., 2012).

In this issue of *Immunity*, Okoye et al. (2014) add to this list another mechanism of suppression, that of nonautonomous gene silencing mediated by miRNA-containing exosomes. Exosomes are 40–100 nM vesicles that are generated by the inward invagination of endosomal membranes to generate intraluminal vesicles in multivesicular bodies (Raposo and Stoorvogel, 2013). The latter are trafficked to the cell membrane by a Rab family GTPase-dependent mechanism where the exosomes are released. Exosome formation may proceed by a mechanism involving the endosomal sorting complex for transport, a set of conserved proteins involved in lysosomal and exosomal trafficking, or by an alternative mechanism involving lipid raft segregation in a ceramide-dependent manner. The capacity of exosomes to transfer miRNA and mRNA has been verified in many cell types (Robbins and Morelli, 2014). Okoye et al. (2014) extended this concept to Treg cells by first identifying them as prolific producers of exosomes, whose release was hypoxia sensitive and required Rab27a and Rab27b GTPases and ceramide. Importantly, Treg cell exosomes were laden with miRNA, the profile of which was distinct from those of Th1 and Th2 cells. The authors directly demonstrate that Treg cell exosomes

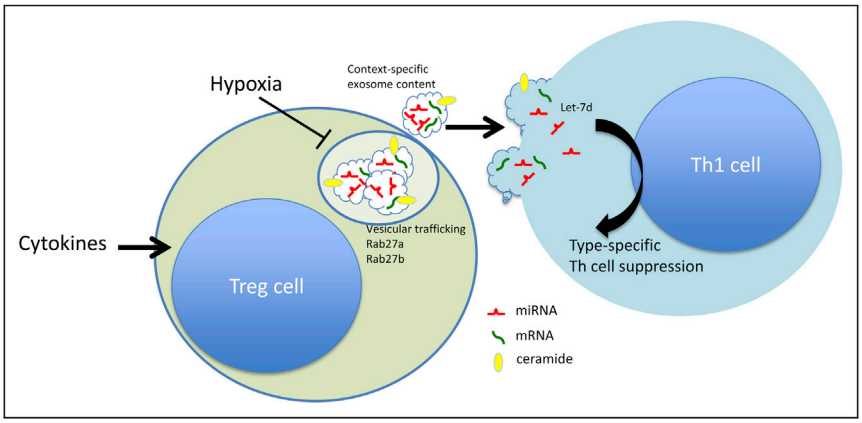

Figure 1. Treg Cell Exosomes Suppress Th1 Cell Responses
Cytokines and metabolic factors regulate Treg cell exosome release. Exosome generation and export depends upon ceramide and Rab GTPases (Rab27a, Rab27b), respectively. Delivery of exosomes containing the miRNA Let-7d to Th1 effector cells results in suppression of proliferation and cytokine secretion.

transferred a specific set of miRNA to conventional T cells, including miR-155, Let-7b, and Let-7d, both in vitro and in vivo. Compromised transfer of Treg cell exosomal miRNAs to conventional T cells, either because of failed miRNA formation (Treg cell Dicer deficiency) or exosome release (Treg cell Rab27a- and Rab27b-deficient Treg cells), abrogated the capacity of Treg cells to prevent disease in a lymphopenia-induced model of colitis.

Okoye et al. (2014) went on to demonstrate a specific role for exosomes in regulating Th1 cell responses (Figure 1). Purified exosomes from WT but not Dicer-deficient Treg cells added to in vitro Th1 cell cultures suppressed cell proliferation and IFN-γ production. Of the three mature exosomal Treg cell miRNAs that were identified in conventional T cells, Let-7d was specifically associated with the control of Th1 cell responses both in vitro and in vivo. Treg cells transfected with a Let-7d inhibitor were compromised in their capacity to suppress Th1 cell proliferation and interferon-γ (IFN-γ) production in vitro and suppress colonic inflammation and IFN-γ expression by conventional T cells in the lymphopenia colitis model.

Employment by Treg cells of miRNA-mediated nonautonomous gene silencing as a suppressive mechanism offers several advantages. It redirects the transcriptional circuitry and cellular function of recipient conventional T cells in favor of a tolerogenic profile. As such, it is a particularly effective mediator of ''infectious tolerance,'' where the effects may range from the transient to the long lasting. Although the studies of Okoye et al. (2014) were focused on the inhibition of Th1 cells by Treg cell exosome miRNA, this mechanism is well suited to context-dependent regulation of other Th cell responses. Specificity for a particular Th cell response may be tailored by the precise combination of miRNA delivered by Treg cell exosomes. Sensitivity of exosome release to hypoxia adds a further layer of control that may fine-tune exosome release in different regions of the gastrointestinal tract.

In addition to miRNA, exosomes also deliver other noncoding RNA, mRNA, proteins, and lipids that have been implicated in immune regulation (Robbins and Morelli, 2014). Okoye et al. (2014) demonstrate a wide range of transcripts enriched in Treg cell exosomes as compared to the parent cells, including those encoding chemokines, interleukins, collagen and matrix proteins, ephrins, and others. The role of Treg cell exosomal mRNA and proteins in modulating target cells remains unknown but seems likely to play a role in their immunomodulatory effects.

There are some caveats to the studies of Okoye et al. (2014), principle among which is the lack of clarity surrounding the differential contribution of nonautonomous gene silencing to Treg-cell-mediated regulation as compared to other well-established suppressive mechanisms. Most of the in vivo observations on the role of this pathway in peripheral tolerance were gleaned from studies using the lymphopenia-colitis model, which suffers from the limitations of an immunodeficient host and a lymphopenic environment. Additionally, Treg-cell-specific ablation of RNaseIII enzymes involved in miRNA maturation, including Dicer and Drosha, results in rapidly fatal autoimmunity, whereas Rab27a and Rab27b double-deficient mice lacking in Treg cell exosome release suffer a relatively mild inflammatory phenotype (Chong et al., 2008; Liston et al., 2008). These observations, although arguing for a dominant cell-intrinsic role for miRNA in controlling Treg cell functions, also hint at a more focused role for exosomal delivery of miRNA and other molecules in peripheral tolerance such as context-specific Th cell regulation and maintenance of mucosal tolerance. Studies employing Treg-cell-lineage-specific genetic approaches that target the exosomal pathway in other disease models and rescue experiments of Foxp3-deficient mice with exosome-sufficient or -deficient Treg cells may further clarify the role of this pathway in peripheral tolerance. Its differential role in natural (thymic) versus induced Treg (iTreg)-cell-mediated tolerance is also relevant, given the importance of the latter for mucosal tolerance.

Although the work of Okoye et al. (2014) centered on the regulation of T effector cell responses, it is easy to envision how Treg cell exosomes might impact the regulatory compartment. In an inflammatory environment, sentinel Treg cells might ''educate'' newly recruited Treg cells. For example, exosome-mediated transfer of miRNA-155 would decrease SOCS1 expression and increase STAT5 activation, based on data from Treg-cell-specific ablation of miRNA-155 (Lu et al., 2009). The result would favor Treg cell homeostasis and stability. Similarly, enhancement of TGF-β signaling pathways in conventional T cells might increase iTreg cell production. Overall, the results of Okoye et al. (2014) foretell a number of immunoregulatory effects offered by Treg-cell-mediated exosomal delivery of miRNA and other agents that will surely be the subject of future investigations.

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Bipotent or Oligopotent? A Macrophage and DC Progenitor Revisited

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Macrophage and dendritic cell (DC) progenitors (MDPs) produce macrophages and DCs but not other hematopoietic lineages. In this issue of *Immunity*, Sathe et al. (2014) show that isolated MDP populations hardly contain such bipotent progenitors at clonal levels, arguing against the existence of MDPs.

Macrophages and dendritic cells (DCs) scavenge dying cells and pathogens by phagocytosis and endocytosis, thereby contributing to tissue homeostasis. Because these cells share similar cell surface makers and functions, their developmental origin and relationship have been subject to debate. Recent studies indicated that some macrophages in adult tissues such as brain, liver, and epidermis are derived from embryonic precursors before birth, whereas other macrophages in the intestine, heart, lung, and dermis are of monocyte origin. Under inflammatory conditions, monocytes are converted to DCs (monocyte-derived DCs) and tissue-resident macrophages. Monocytes and DCs are derived from hematopoietic stem cells (HSCs) in the bone marrow (BM) through intermediate progenitors. By sequentially losing the breadth of their differentiation potential, multipotent progenitors eventually become progenitors committed to specific hematopoietic lineages. Macrophage and DC progenitors (MDPs), which give rise to monocytes-macrophages and DCs but not to other hematopoietic lineages, were proposed to exist in mouse BM (Fogg et al., 2006). MDPs are distinguished from granulocyte macrophage progenitors (GMPs) by their expression of the chemokine receptor CX_3CR1 (Fogg et al., 2006). In addition, common DC progenitors (CDPs) are strictly committed to resident conventional DCs (cDCs) and plasmacytoid DCs (pDCs) (Naik et al., 2007; Onai et al., 2007, 2013). Based on their developmental potential and lineage commitment, GMPs were thought to develop into MDPs by losing their granulocyte potential and further into CDPs and common monocyte progenitors (cMoPs) (Hettinger et al., 2013). Supporting the MDP-to-CDP axis, MDPs injected into BM develop into c-kit$^{int/lo}$ CDP-like cells, although the developmental potential of these CDP-like cells was not evaluated (Liu et al., 2009). Therefore, MDPs have been generally accepted as the branch point of DC versus monocyte-macrophage development (Figure 1).

In this issue of *Immunity*, Sathe et al. (2014) describe the developmental relationship of resident DCs and macrophages. Lymphoid-tissue-resident DCs consist of cDCs and pDCs, and in mice the cDCs are further divided into CD8α^+ Clec9A$^+$ and CD8α^-Clec9A$^-$ subpopulations. Sathe et al. (2014) isolated MDPs based on the original definition, lin$^-$c-kit^{hi} sca-1$^-$CD16/32^{hi}CX_3CR1^+ (Fogg et al., 2006) and lin$^-$M-CSFR$^+$ (Waskow et al., 2008). Adoptive transfer experiments confirmed that the MDPs gave rise to monocytes-macrophages, the resident cDC subpopulations CD8α^+Clec9A$^+$ and CD8α^-Clec9A$^-$, and pDCs in the spleen. However, in contrast to previous findings (Fogg et al., 2006; Hettinger et al., 2013), these MDPs also generated significant amounts of Ly6G$^+$ granulocytes, which was confirmed by colony-forming assays. Of the colonies produced from individual MDPs, 20%–40% were granulocyte colonies, indicating that the MDPs contained substantial amounts of granulocyte-producing clones. Why MDPs showed little