a LTβR-dependent fashion. Moreover, stimulated mVSMCs form a fibroblastic reticular cell network-like structure that is typical for SLOs (Gräbner et al., 2009). However, the LTo cell function is not confined to fibroblastic cells because lymph node formation, for example, requires LTβR signaling in vascular endothelial cells (Onder et al., 2013). Hu et al. (2015) solved not only the question concerning the nature of the organizer cell for ATLO formation, but also found an elegant way around the confounding factor that altered immune responsiveness in globally LTβR-deficient mice led to accelerated atherosclerosis. In the core dataset of this study, conditional *Ltbr* gene ablation specifically in VSMC has been achieved through SM22a promoter-driven Cre recombinase expression. Thorough characterization of SLOs of conditionally LTβR-deficient animals on the apolipoprotein E-deficient background revealed the absence of general immune system alterations. Importantly, selective ablation of the LTβR revealed that VSMCs limit atherosclerotic disease progression in aging apolipoprotein E-deficient mice, while initial lesion development was not affected. Hence, these findings suggest that the senescent immune system selectively employs the LTβR on VSMCs to foster ATLO formation and thereby locally restrains the atherosclerotic process. However, it needs to be clarified in future studies how T cell education within ATLOs impinges on the chronic atherosclerotic lesion development. It is possible that ATLO-dependent local induction of regulatory T cells fosters generation of anti-inflammatory macrophages and antagonizes foam cell formation (Figure 1).

The findings of Hu et al. (2015) provide not only novel insight into core processes during atherogenesis, but also teach a new lesson on the role of TLOs in the progression of local inflammation. In fact, it has been a matter of debate whether TLOs enforce or attenuate chronic inflammation or autoimmune disease. One major obstacle in resolving this issue has been the genetic link between SLO development and TLO formation. Hence, uncoupling of TLO formation from SLO development through VSMC-specific genetic *Ltbr*-ablation clearly points toward a novel and important immune regulatory function of TLOs. Identification of specific markers for LTo cells in TLOs of other tissues would open new options for genetic manipulation of these cells in vivo and to revisit their role in chronic inflammatory reactions.

In sum, TLOs in the wall of atherosclerotic arteries might guide us to new breakthroughs in getting control on chronic inflammation.

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DC-SIGN: The Strange Case of Dr. Jekyll and Mr. Hyde

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http://dx.doi.org/10.1016/j.immuni.2015.05.021

In this issue of *Immunity*, Conde et al. (2015) showed that a costimulatory blockade favors the accumulation of CD209a$^+$ macrophages which, upon interaction with fucosylated tissue ligands, promotes the expansion of CD4$^+$Foxp3$^+$ Treg cell number.

The control of peripheral tolerance to self-antigens is one of the mechanisms that define immune homeostasis. An effective peripheral tolerance ensures that autoreactive T cells that have escaped negative selection during thymic education are kept under control to avoid autoimmunity. In addition, excess of tolerance is avoided to allow the immune system to respond with proper capacity against pathogens. Dysregulation of this fine equilibrium leads to important repercussions on both sides of the balance: the development of autoimmunity when tolerance is poor and the facilitation of tumor

immune escape when tolerance is excessive. The central elements in the control of peripheral tolerance are the suppressor T cells, generically referred to as regulatory T (Treg) cells, which use different inhibitory modules to achieve their inhibitory function. Now classical experiments have demonstrated that although mice depleted of Treg cells spontaneously develop autoimmunity and chronic inflammation, they are better prepared to reject incipient tumors. In contrast, in vivo enrichment with Treg cells allows mice to accept allogeneic transplantations (Sakaguchi et al., 2008). Thus, Treg cells have emerged as a component of the immune system with tremendous potential in the treatment of autoimmunity and cancer and in transplantation medicine. Still, our understanding of the endogenous mechanisms regulating the generation and maintenance of Treg cells remains poor.

Several antigen-presenting cell types have been associated with the differentiation of naive T cells into Treg cells, mostly in the context of immaturity or partial maturation. In this setting, co-inhibitory molecules and suppressing cytokines, such as transforming growth factor β (TGF-β) and interleukin-10 (IL-10), provide negative signaling to the T cells that promotes anergy and immunosuppression. Among the antigen-presenting cells able to trigger the differentiation of Treg cells are tumor-infiltrating myeloid-derived suppressor cells and macrophages and dendritic cells (DCs) that are subverted by pathogens to produce IL-10 (Walsh and Mills, 2013). In both tumor-infiltrating myeloid-derived suppressor cells and pathogen-modulated DCs and macrophages, the microenvironment plays a critical role in determining the tolerogenic transcriptional profile that triggers the expression of inhibitory co-stimulatory molecules and the key cytokine IL-10. Tolerogenic profiles in antigen-presenting cells are instructed through the pattern-recognition receptor DC-SIGN that senses the microenvironment (García-Vallejo and van Kooyk, 2013).

In humans, CD209, also known as DC-SIGN (DC-specific intercellular adhesion molecule-3 grabbing non-integrin), has long been considered a DC marker because of its expression on immature DCs in peripheral tissue and mature DCs in lymphoid tissues, although not on follicular DCs, plasmacytoid DCs, or CD1a$^+$ Langerhans cells (Geijtenbeek et al., 2000). However, CD209 has also been described on different types of macrophage-like subpopulations, such as microglia, tumor-infiltrating "M2" macrophages, myeloid-derived suppressor cells, and CD14$^+$ dermal, decidual, and intestinal macrophages. As a type 2 C-type lectin receptor, CD209 is equipped with a carbohydrate recognition domain (CRD) that mediates the recognition of fucose (Le^a, Le^b, Le^X, Le^Y, and sulfo-Le^a) and high-mannose glycans in a Ca^{2+}-dependent manner (Feinberg et al., 2001). These carbohydrate structures can be found in multiple pathogens (e.g., HIV, Dengue virus, Lassa virus, Ebola virus, *M. tuberculosis*, *C. albicans*, *S. mansoni*, and *H. pylori*, among others), but also on human glycoproteins, such as ICAM-2, ICAM-3, Mac-1, carcinoembriogenic antigen, butyrophilin, milk bile-salt stimulated lipase, myelin-oligodendrocyte glycoprotein, and semen clusterin (García-Vallejo and van Kooyk, 2013). Most importantly, interaction of DC-SIGN with some of its endogenous and pathogenic ligands, and in the context of simultaneous triggering of Toll-like receptor 4 (TLR4), has been shown to elicit a synergistic increase in the expression and secretion of IL-10, setting on anti-inflammatory circuits characterized by decreased T cell proliferation and the generation of anergic or Treg cells. Now, Conde et al. report in this issue of *Immunity* (Conde et al., 2015) that one of the eight genetic paralogs of DC-SIGN in mice, CD209a (also reported in literature as SIGNR5, gene ID: 170786) is expressed on a CSF1-induced subset of suppressive macrophages characterized as CD11b$^+$CSF1R$^+$Ly6C^{lo}Ly6G$^-$CD169$^+$ cells. These cells are generated in allogeneic grafts from CD11b$^+$CSF1R$^+$Ly6C^{hi}Ly6G$^-$CD169$^-$ cells by the action of allograft-produced CSF1. CD209a on CD11b$^+$CSF1R$^+$Ly6C^{lo}Ly6G$^-$CD169$^+$ cells engages with fucosylated glycans in the allogeneic graft and, in the context of TLR4 signaling, trigger the expression and secretion of IL-10 (Figure 1) which, in turns, mediates the differentiation of Treg cells that are crucial for the survival of the graft (Conde et al., 2015). Elegant experiments using organs from FucT-IV and FucT-VII double-deficient mice demonstrate that the absence of α1,3-fucosylated glycans in the allogeneic graft prevents the triggering of CD209a-dependent IL-10 production. As expected, *Cd209a*$^{-/-}$ mice failed to accept the graft due to the lack of the necessary IL-10 to build the peripheral tolerance against the allogeneic heart.

The mouse model reported by Conde et al. (2015) closely resembles the findings observed in vitro on human CD209 (Figure 1) and suggests that the mouse CD209a might be the most approximate functional homolog of human CD209 (García-Vallejo and van Kooyk, 2013). Yet, several structural differences of mouse CD209a versus human CD209 might posit a warning with regards to this assumption, because mouse CD209a has a considerably lower affinity for the ligand, slightly different specificity, and a shorter stem region, and is unable to internalize (García-Vallejo and van Kooyk, 2013). In addition, the contribution of other fucose-specific C-type lectins, such as mouse MGL-1 (CD301a), which has also been described to trigger macrophage-dependent anti-inflammatory circuits in an IL-10-dependent fashion, is worth exploring. Yet, the protective role of the IL-10-producing CD11b$^+$CSF1R$^+$Ly6C^{lo}Ly6G$^-$CD169$^+$ cells in preventing allograft rejection is indisputable and paves the way to new therapeutic avenues in transplantation. Interestingly, DC-SIGN$^+$ cells have been described to infiltrate acute rejecting kidney human allografts, correlating with poor prognosis (Zuidwijk et al., 2012). Although the phenotype of the DC-SIGN$^+$ cells in this report was certainly more pro-inflammatory, it would be extremely interesting to investigate whether tissue-specific differences in glycosylation might explain a lack of DC-SIGN ligands in the kidney that could aggravate the deficit of peripheral tolerance to the transplanted organ.

CD209a is not a specific marker of tolerogenic macrophages as it could be interpreted from the report of Conde et al. (2015). Previous research has demonstrated the presence of CD209a in a subpopulation of DCs arising from monocytes in vivo under the influence of TLR4 signaling (Figure 1). Such DCs lacked the expression of monocyte markers, but have high expression of TLR4 and CD14, acquired the probing morphology of DCs, localized to the T cell areas, and showed powerful antigen-capturing, as well as a highly effective capacity to present antigens in MHC-I and -II (Cheong

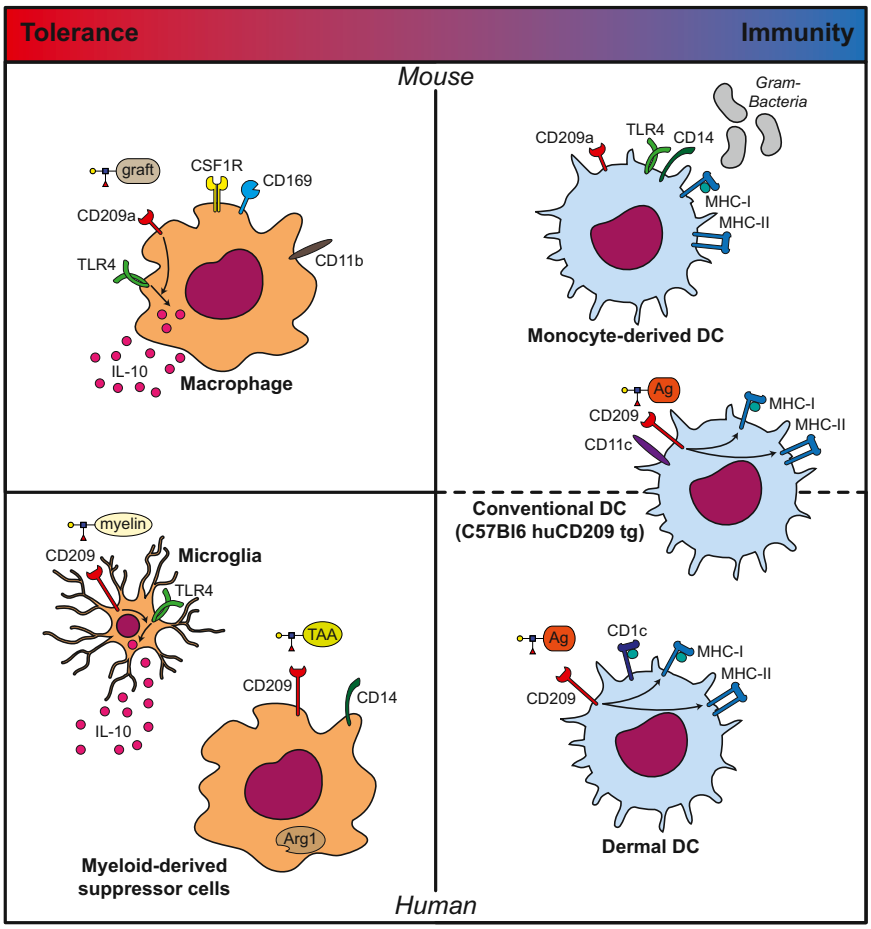

Figure 1. The Multifaceted Function of DC-SIGN

The interaction of CD209a on $CD11b^+CSF1R^+Ly6C^{lo}Ly6G^-CD169^+$ with fucosylated glycans on graft glycoproteins together with TLR4 triggering leads to the activation of a tolerogenic phenotype dominated by IL-10 secretion. The human equivalent could be represented by microglia or myeloid-derived suppressor cells. CD209 expressed on microglia interacts with fucosylated glycans on myelin oligodendrocyte glycoprotein resulting in a synergistic upregulation of the TLR4-dependent IL-10 production. In contrast, tumors that overexpress fucosylated structures interact with CD209 on myeloid-derived suppressor cells, presumably to enhance their tolerogenic function. However, CD209 is not only a signaling receptor, and it has been clearly demonstrated that glycan-conjugated antigens targeted to CD209 are efficiently presented in MHC-I and MHC-II to induce $CD8^+$ and $CD4^+$ effector T cells, respectively. Thus, expression of CD209 on $CD14^+$ and some subpopulations of $CD1a^+$ dermal DCs or $CD11c^+$ conventional DCs provide these cells with an antigen-dependent immunogenic pathway. The mice equivalent could be a subset of DCs that result from the exposure of monocytes to LPS from gram bacteria. This triggers a transcriptional profile that differentiates these cells into $CD14^+CD209a^+$ highly immunogenic DCs with $CD4^+$ and $CD8^+$ T cell activation capacity.

et al., 2010). Unfortunately, experiments aimed at investigating the function of CD209a on in vivo monocyte-derived DCs were not pursued in this manuscript and have not yet been reported. Evidence so far points in the direction of CD209a resembling the dual role reported for human CD209 in mediating both tolerance and immunity, depending on the context of CD209 triggering. Thus, the natural function of human CD209 would be the maintenance of immunological homeostasis through the interaction with multiple host glycoproteins in order to keep peripheral tolerance, while providing a pathway for antigen processing and presentation. The tolerogenic aspect of human CD209 might have been hijacked by pathogens and tumors, which have learned to upregulate the expression of CD209-ligands in order to take advantage of CD209-dependent tolerogenic signaling circuits as a strategy to escape the immune system. But at the same time, human CD209 is an extremely efficient internalization receptor that mediates routing to intracellular compartments involved in MHC-I and -II antigen presentation (Figure 1). The strategic localization of CD209 in both dermal DCs, as well as in paracortical DCs in the lymph nodes, and its sensitivity to be upregulated by growth factors, such as GM-CSF, ensures that, together with a proper adjuvant, antigens targeted to human CD209 are effectively presented to T cells and result in the generation of strong immune responses (Tacken et al., 2005; Unger et al., 2012), thus a definitely interesting option in vaccine development. As the main character in Stevenson's famed novel *The Strange Case of Dr. Jekyll and Mr. Hyde*, CD209 represents the allegory of good and evil contained within the same identity. It could be that the molecular context of the type of myeloid-derived antigen-presenting cell that expresses CD209 and the glycosylation microenvironment determines the balance between tolerance and immunity upon CD209 interaction. And in doing so, CD209 provides us with a sensitive and sophisticated way of manipulating the immune system in the desired direction.

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