Dendritic cells (DCs) are professional antigen-presenting cells (APC) that facilitate the development and progression of atherosclerosis. However, DCs also function as novel “switches” between immune activation and immune tolerance and represent a heterogeneous hematopoietic lineage, with cell subsets in different tissues that show a differential morphology, phenotype, and function. Regulatory DCs, depending on their immature state, can be induced by immunosuppressive modulation, which plays an important part in the maintenance of immunologic tolerance via suppression of the immune response. In this review, we describe the current understanding of the generation of regulatory DCs. The novel role of selectins in the modification of DCs in atherosclerosis is also discussed.

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**Keywords:** Dendritic cells; Selectins; Atherosclerosis; Immune tolerance

**Introduction**

Atherosclerosis is the primary cause of coronary heart diseases and stroke, which are the leading causes of death and disability worldwide. Atherosclerosis is a chronic disease of the arterial walls. Besides lymphocytes, macrophages and dendritic cells (DCs) can be found in atherosclerotic lesions and contribute to atherogenesis. In general, DCs are located in the aortic intima. In those predisposed to atherosclerosis, DCs tend to accumulate in plaque shoulders, which rupture relatively easily. The underlying role of DCs in atherosclerosis is well-established and atherosclerosis includes lipid accumulation, foam cell formation, secretion of proinflammatory cytokines, and antigen presentation.

As professional antigen-presenting cells (APCs), DCs are the main orchestrators of the immune response; they patrol the body to capture antigens and migrate to the secondary lymphoid organs, while the internalized antigen is processed and presented to other immune cells. Additionally, immature DCs can differentiate into a mature state. During maturation, the capacity of DCs to take up and process antigens decrease, but they become progressively powerful with active, naive T cells for increased expression of MHC molecules and costimulatory molecules such as cluster of differentiation CD 80 and CD86. It is now recognized that DCs
exhibit complicated plasticity and heterogeneity in different tissues by showing a differential morphology, phenotype, and function. Regulatory DCs, depending on their immature state, can be induced by immunosuppressive modulation and retain the ability to present antigens to T cells; however, they decrease the expression of costimulatory molecules and proinflammatory cytokines, upregulate anti-inflammatory cytokines, and are resistant to maturation-inducing signals under specific physiologic or pathologic conditions. Thus, advances in understanding the immune characterization of the DC system could reveal novel possibilities for atherosclerosis treatments. In this review, we discuss strategies to generate tolerogenic or regulatory DCs and introduce the concept of the immunomodulatory role of selectins on DCs.

### Strategies for the generation of immunomodulatory DCs

Studies have investigated the critical role of DCs in the maintenance of immune tolerance and regulation. The strategy of DC-mediated tolerance has become a promising tool to treat many types of autoimmune diseases. The mechanisms by which regulatory DCs promote immunologic tolerance are diverse. Regulatory DCs downregulate the expression of costimulatory molecules (B7), upregulate expression of inhibitory molecules (e.g., indoleamine 2,3-dioxygenase (IDO)), reduce expression of proinflammatory cytokines (e.g., interleukin (IL)-12 and tumor necrosis factor (TNF)-α), and increase the expression of anti-inflammatory cytokines (e.g., IL-10 and transforming growth factor (TGF)-β). Also, regulatory DCs can induce T cell apoptosis to decrease the number of effector T cells and induce the unresponsiveness of T cells (“anergy”). In addition, regulatory DCs facilitate the generation and expansion of regulatory T cells.

Recently, various tolerance-inducing, DC-based treatments have been implemented for atherosclerosis. The tolerogenic characteristics of DCs depend on their immature state and are induced by specific stimuli (Table 1).

### Drugs

Drug treatment has been recognized as a mechanism to build the immunomodulatory properties of DCs. Aspirin reduces the immunoreactivity of DCs by inhibiting the nuclear factor-kappa B (NF-κB) pathway and decreasing the number of costimulatory molecules. Oral administration of calcitriol (an active form of vitamin D3) has been reported to increase the number of Foxp3(+) regulatory T cells, as well as

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**Table 1** Strategies for the generation of regulatory DCs.

| Modulation Strategy | DC function | Cytokines or mediators | T cell |
|---------------------|-------------|------------------------|-------|
| Aspirin             | Maintain the immature state | Low IL-2 and IL-12 | Suppress proliferation of naive allogeneic T cells |
| Calcitriol          | Decrease number of CD80 + CD86 + DCs | Low IL-10 and high IL-12 | Increase Foxp3 Treg |
| Captopril           | Induces CD103, CD80, CD86, and MHC-II DCs | Promotes IL-10 and TGF-β; decreases IL-6 and IL-12 | Promotes polarization of Foxp3 Tregs |
| OxLDL, ApoB100 and IL-10 | Does not affect DC maturation | Increases oxLDL-specific IgG | Reduces Th1 profile |
| IL-10/TGF0          | Induces insulin-specific tolerance to DCs | Stimulates T cells acquired in IL-2 (low) IFN-γ (low) IL-10 (high) cytokine profiles | Reduces proliferation of effector T cells |
| IDO                 | Tolerogenic pDCs | Increases IL-10 | Decreased CD4+ T-cell infiltration |
| OxLDL-induced apoptotic DCs | CD103 + tolerogenic splenic DCs | Increases circulating CCL2 | Stimulates T cells acquired in IL-2 (low) IFN-γ (low) IL-10 (high) cytokine profiles |
| PD-L1               | DCs are more effective in activating CD4+ T cells | No | Increases numbers of Tregs |

DCs: dendritic cells; IL: interleukin; Foxp3: forkhead box P3; Tregs: regulatory T cells; TGF: tumor growth factor; oxLDL: oxidized low-density lipoprotein; ApoB: apolipoprotein B; IFN: interferon; IDO: Indoleamine 2, 3-dioxygenase; PD-L: programmed death-ligand.
decrease the number of differentiated CD80(+) CD86(+) DCs, the proliferative activity of T cells, and the expression of IL-12 mRNA. Captopril is widely used for the treatment of hypertension and congestive heart failure. Captopril treatment has been shown to increase the production of IL-10 and TGF-β, but decrease that of IL-6 and IL-12 in splenic DCs; it also inhibits DC maturation and promotes polarization of regulatory T cells in atherosclerotic rats.

**Antigen modification**

Inflammation, due to self-antigens, plays an important part in atherogenesis. Restoring the immune tolerance to self-antigens attenuates atherosclerosis development. In atherosclerosis, deposition of low-density lipoprotein (LDL), apolipoprotein B100 (ApoB100), or oxidized LDL (oxLDL) in vessel walls results in the accumulation of immune cells and, subsequently, chronic inflammation. Modified LDL-loaded DCs have been used for immunomodulation of atherosclerosis, because they dampen the immune reaction for autoantigens. Indeed, oxLDL and ApoB100 have been shown to inhibit the production of proinflammatory cytokines and induce the generation of regulatory T cells.

**Immunosuppressive factors**

Anti-inflammatory factors (e.g., IL-10 or TGF-β) or immunosuppressive enzymes (e.g., IDO) induce a tolerogenic phenotype in DCs, impair activation of T cells, and increase the generation of regulatory T cells. Also, transfer of oxLDL-pulsed DCs aggravates atherosclerosis, whereas DCs can become tolerogenic to LDL upon treatment with IL-10 during the loading of ApoB100.

**Apoptotic cells**

Apoptotic cells are effectively cleared from human systems through efferocytosis. Clearance of apoptotic cells is essential to avoid inflammation, but the atheroprotective role is impaired, owing to the decreased expression of CCR7 in DCs in a high cholesterol environment, which reduces the emigration DCs from atherosclerotic lesions. Thus, the antigen-specific tolerance is lacking in the absence of DCs. Several studies have suggested that apoptotic cells can inhibit antigen presentation and the maturation of DCs, reduce the expression of the proinflammatory cytokine IL-12, and induce expression of TGF-β and IL-10 recently. Frodermann et al modified DCs with epoxomicin or oxLDL for apoptosis, which showed that an atherosclerotic-specific antigen could be generated through regulatory signals. Then, they transferred the modified DCs into LDLR−/− mice, which resulted in increased numbers of CD103 (+) tolerogenic DCs and regulatory T cells, as well as fewer Ly-6Cmonocytes and lower circulating levels of chemokine ligand (CCL) 12.

**Genetic manipulation**

The genetic manipulation of DCs enables them to constitutively express low levels of immunostimulatory genes, such as IL-12 and NF-κB, and overexpress immunosuppressive genes, such as IL-10 and TGF-β. Programmed death-1 (PD-1) and PD-L2 are widely expressed on APCs, which inhibits T cell activation by binding to programmed death (PD)-1 on T cells; PD-L1/2-deficient DCs are more effective at activating naïve T cells. These genetically modified DCs display tolerogenic phenotypes and function through various mechanisms, some of which show a therapeutic potential for atherosclerosis.

**Selectins modulate DCs**

Selectins are type-I transmembrane proteins with an N-terminal C-type lectin domain, an epidermal growth factor-like domain, and a complement control protein. Selectins consist of three members: P, E, and L. P-selectin is expressed by megakaryocytes and activated endothelial cells. E-selectin is constitutively expressed on the surfaces of endothelial cells. L-selectin is constitutively expressed on the surfaces of all leukocytes. It is well known that the interactions between selectins and their ligands are responsible for the initial adhesion of hematopoietic cells to vascular surfaces and to each other, which is the first step of leukocyte recruitment to inflamed tissues. Interestingly, several recent studies have indicated that selectins may be a novel modification of DC plasticity, and selectin-induced signaling may be a new target for the regulation of the immune response.

**Selectins mediate adhesion**

Selectins are Ca²⁺-dependent lectins. The common recognition domain is sLeα, a terminal structure of O- or N-glycans. P-, E-, and L-selectins bind cooperatively to a sLeα capping O-glycan, particularly the N-terminus of P-selectin glycoprotein ligand (PSGL)-1, which is widely expressed on DCs. Silva et al found
that treatment of DCs with sialidase, which causes destruction of sLeα in DCs and leads to abrogated DC tethering to immobilized, purified P-, L-, or E-selectin under flow conditions or TNF-α-activated endothelial cells under static conditions; this indicated that sLeα is required for the adhesion of DCs.28 During inflammation, DCs and other leukocytes migrate to a specific location in response to a stimulus. Interactions between selectins and their ligands mediate rolling. Slow rolling reduces the velocity of neutrophils passing through venules, which provides cells with more opportunities to encounter endothelial-bound chemokines, such as CCL21 and CCL19.29 In addition, the slow rolling enables the activation of integrins, which causes leukocytes to roll slower, stop moving, and undergo adhesion.30

Selectins stimulate inflammation

Selectins and their ligands mediate the rolling of leukocytes and additional adhesion. DCs, like other leukocytes, become activated during both processes and secrete many potent inflammatory mediators, including proinflammatory cytokines (e.g., TNF-α and IL-6), chemotactic agents (monocyte chemoattractant protein (MCP-1), adhesive compounds (lymphocyte function associated antigen (LFA)-1), proteolytic agents (matrix metalloproteinases (MMPs)), and tissue factors.31,32 In addition, classical monocytes (Ly6Chi and dendritic cell precursor cells) preferentially migrate into the activated endothelium and infiltrate developing lesions to become atherosclerotic macrophages, inflammatory DCs, or foam cells, which display high levels of PSGL-1 and a higher binding affinity for E-/P-selectin expressing cells than other monocytes.33,34 However, inflammation is characterized by interactions among platelets, leukocytes, and endothelial cells. The progression of leukocyte activation causes the release of inflammatory mediators to the local microenvironment, thereby altering the chemotactic, adhesive, agglutinating, and proteolytic properties of endothelial cells and platelets. These interactions induce alterations of the phenotypes of the endothelial cells or platelets, which supports the chemotaxis, adhesion, and transmigration of the circulating monocytes and DCs to the site of inflammation.35

Selectins regulate the plasticity and signaling of DCs

Recently, some studies have shown that selectins or their ligands, especially PSGL-1, can regulate the maturation and generation of tolerogenic DCs. sLeα (the terminal domain of PSGL-1) was found to be expressed on immature DCs, but not involved in DC maturation.36 Intriguingly, we found that L-selectin could promote DC maturation and the immune response, which coincided with upregulated expression of CD80, CD83, CD86, and Toll-like receptor (TLR)-4 on DCs. Hence, we suggested that L-selectin could induce DC maturation via the TLR-4 signaling pathway.37 PSGL-1−/−ApoE−/− and L-SEL−/−ApoE−/− mice are currently being utilized to explore the connection between DCs with L-selectin and their ligands. TLR-4 has a pivotal role in the inflammatory activation of atherosclerotic lesions. Circulating monocytes overexpress TLR-4 in acute coronary syndrome and can release powerful proinflammatory cytokines, such as TNF-α and IL-12, if activated by TLR ligands.38,39 In addition, DC maturation via the TLR-4 signaling pathway was demonstrated in our in vivo study. Transfer of exogenous DCs increased the inflammatory response and accelerated atherosclerosis in recipient ApoE−/− mice, with maturation of endogenous DCs and upregulated expression of TLR-4.40

Conclusions and future considerations

As the most professional APCs, DCs represent a "bridge," linking the innate and adaptive immunity. DCs also function as novel switches between immune activation and immune tolerance. Based on the evolving pool of knowledge that has been obtained from preceding studies, the development of DC-based immunotherapy is now ongoing and takes advantage of their tolerogenic potential for the treatment of autoimmune diseases. A better understanding of the precise cellular mechanisms and immunity of DCs, especially in the field of inflammatory diseases, will provide essential insights into the causes and mechanisms of autoimmunity and valuable information for the improved design of DC-based vaccines for the control of autoimmune diseases, such as atherosclerosis.

Previously, we found that selectin can stimulate DCs for immune activation and that inhibition of the connection between DCs and selectin may aid immunosuppression. Fig. 1 shows that the multifunctional role of selectins in modification of DCs. However, a dissimilar role of selectins in the regulation of DCs has been reported; selectins (and their ligands) facilitate the efficacy of DC-based vaccines, depending on their ability to migrate and hone-in.28 In addition, bone marrow-derived DCs or splenic T cells from PSGL−/− mice show reduced immunosuppression with low levels of IL-10 or TGF-β, and PSGL-1 deficiency exacerbates the development of inflammation.41,42 Further investigation is required to address the development, plasticity,
and genetic programs of DCs. Indeed, an improved understanding of the pathogenesis of atherosclerosis and the mechanisms of action of regulatory DCs will aid the success of DC-based, tolerance-inducing therapies for atherosclerosis.

Conflicts of interest

No potential conflicts of interest, relevant to this article, are reported.

Acknowledgments

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