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Review

Heterogeneity of Tregs and the complexity in the IL-12 cytokine family signaling in driving T-cell immune responses in atherosclerotic vessels

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ABSTRACT

The importance of immune inflammation in the development and progression of atherosclerotic lesions is well recognized. Accumulated evidence shows striking features of heterogeneity of regulatory T cells (Tregs) and the importance of the IL-12 cytokine family in regulation of Tregs in atherogenesis. The present review briefly summarized the current knowledge about the impact of the IL-12 cytokine family in regulation of immune processes in atherogenesis.

1. Introduction

Atherosclerosis progression involves many subsets of proinflammatory cells, with a central contribution of effector T cells (Sasaki et al., 2012; Chistiakov et al., 2013; Ohkura et al., 2013), which are the major drivers of proatherogenic inflammation (Ait-Oufella et al., 2009). Dendritic cells (DCs) were shown to accumulate in atherosclerotic plaques and present self-antigens to immature T cells promoting their differentiation to effector T cells such as Th1, Th2, and Th17 (Perrins and Bobryshev, 2011). During the last decades the role of regulatory T cells (Tregs) and the importance of the IL-12 cytokine family in regulation of Tregs in atherogenesis become much debated topics.

1.1. Heterogeneity of regulatory T cells

Tregs show robust immunosuppressive and anti-inflammatory properties that indeed suggest for their atheroprotective role (Sasaki et al., 2012; Chistiakov et al., 2013; Tselios et al., 2014). Tregs inhibit a variety of immune cells such as CD4+ and CD8+ T cells [including naïve T cells and activated effector and memory T cells (Suvas et al., 2003)], B cells, NK T cells, and DCs. There are several populations of inducible and non-inducible Tregs. Natural CD4+CD25+Foxp3+ Tregs (nTregs) produced by thymus suppress immunity mainly *via* cell–cell contacts by down-regulating T cell receptor (TCR)-induced stimulatory signals and IL-2 expression in target T cells and through blocking CD80/CD86 expression in DCs (Langier et al., 2010). Compared to nTregs, inducible Tregs are generated in periphery, and therefore could be defined as peripheral Tregs (pTregs). A subset of pTregs such as CD4+CD25+FoxP3+ Tregs (also called Th3 Tregs) are generated from naïve CD4+CD25− T cells by IL-2 and transforming growth factor (TGF)-β after antigen-specific presentation or from "rescued" autoreactive effector T lymphocytes (Chen et al., 2003). Antigen-stimulated plasmacytoid DCs were shown to induce IL-10- and TGF-β-producing

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FoxP3+ Tregs from naïve CD4+CD25− T cells *via* direct cell–cell contact involving indoleamine 2,3-dioxygenase (IDO)-dependent induction of FoxP3 (Moseman et al., 2004; Chen et al., 2008). Furthermore, Tregs were found to educate naïve CD4+CD25− T cells to become immunosuppressive cells through secreting TGF-β and IL-2 (nTregs) and through cell–cell-contacts and/or IL-10 and TGF-β-mediated mechanisms (Th3 Tregs) (Zheng et al., 2004).

CD4+CD25+FoxP3-T regulatory type 1 cells (Tr1) producing high levels of IL-10 and low levels of IFN-γ, IL-2, and IL-4 also have an IL-10-dependent induction process (Roncarolo et al., 2006; Fujio et al., 2010). In the presence of IL-15 and to a lesser content IL-2, Tr1 cells could be induced from Th0 cells in the absence of TCR activation (Bacchetta et al., 2002). Notably, IL-27 was found to induce IL-10-producing Tr1 cells from naïve CD4+ T cells and inhibit expression of Th17-specific cytokines IL-17 and IL-22 by activated CD4+ T cells even under strong Th17-polarizing conditions (e.g. in the presence of IL-23 and IL-1β) (Murugaiyan et al., 2009). In addition, IL-27-treated DCs showed decreased IL-17 secretion suggesting on the ability of IL-27 to reduce Th17 polarization of DCs and suppress DC-mediated differentiation of naïve T cells into Th17. Indeed, IL-27 could act as a key factor regulating Th17/Tregs ratio (and hence triggering inflammation/anti-inflammation) in atherosclerotic vessels through direct and indirect (e.g. *via* induction of Tr1 cells) inhibition of proinflammatory Th17 cells and induction of immunosuppressive Tr1 populations (Mallat et al., 2003; Klingenberg et al., 2010; Pot et al., 2011; Koltsova et al., 2012; Hirase et al., 2013).

Interestingly, in the proinflammatory microenvironment (e.g. in the presence of IL-1β, IL-2, IL-21, IL-23), peripheral CD4+FoxP3+ T cells expressing high levels of C–C chemokine receptor 6 (CCR6) start to produce IL-17 upon TCR stimulation (Voo et al., 2009). These cells coexpress FoxP3 and RORγt transcription factors specific for distinguishing and maintaining Tregs and Th17 lineages respectively (Chen et al., 2011). IL-17-producing FoxP3+ RORγt+ T cells maintain regulatory properties by suppressing responder CD4+ T cells *via* cell–cell contact. IL-17-producing Tregs were found in inflamed tissue of patients with inflammatory bowel disease (Eastaff-Leung et al., 2010; Hovhannisyan et al., 2011), psoriasis (Bovenschen et al., 2011) and colon cancer (Li and Boussiotis, 2013). No doubt, these Tregs are involved in regulating Th17/Tregs balance. It would be intriguing to identify IL-17-producing Tregs in atherosclerotic lesions. Furthermore, in chronic inflammation, IL-23 is able to switch IL-17-secreting FoxP3+RORγt+ Tregs to the proinflammatory Th17 phenotype by inducing high RORγt levels and enhanced loss of Foxp3 (Bovenschen et al., 2011). In the presence of IL-2 and IL-1β, naive FoxP3+ Tregs start differentiation into Th17 cells that could be enhanced by IL-23 and TGF-β (Deknuydt et al., 2009; Valmori et al., 2010). Similarly, human memory CD4+CD25^{high}Foxp3+CD127−CD27+ Tregs upon stimulation with APCs (especially monocytes) in the presence of IL-2/IL-15 were shown to induce expression of Th17 lineage-specific marker RORγt and CCR6 and then differentiate into IL-17-producing cells. This differentiation process was enhanced by exogenous IL-1β, IL-23, and IL-21 (Koenen et al., 2008).

These findings suggest for significant heterogeneity and likely plasticity of Tregs that could change the phenotype depending on the microenvironment and activating/blocking stimuli. Unfortunately, not all Tregs subsets were found in human and experimental atherosclerosis and atherosclerosis-related conditions. Those include nTregs (Ait-Oufella et al., 2006; Meier et al., 2008), Tr1 (Mallat et al., 2003; Klingenberg et al., 2010), Th3 Tregs (Ji et al., 2009; Zhou et al., 2009), and TGF-β- and IL-10-producing FoxP3+ pTregs (Jiagang et al., 2011; Ghourbani Gazar et al., 2012; Lü et al., 2013). It would be intriguing to identify IL-17-producing Tregs as well as a phenomenon of switching T cell phenotype from immunosuppressive (Tregs) to acute proinflammatory (Th17) in atherosclerotic lesions.

1.2. A complexity in the IL-12 cytokine family signaling in driving T-cell immune responses in atherosclerotic vessels

The proinflammatory response driven by Th1 and Th17 cells and associated with increased production of interferon (IFN-γ), IL-6, IL-17, and IgG2a antibodies against modified low density lipoprotein (LDL) is predominant at early stages of atherosclerosis (Galkina and Ley, 2009; Ketelhuth and Hansson, 2011). The IL-12 family of cytokines plays a critical role in priming DC-mediated differentiation of CD4+ naïve T cells. IL-12, IL-23, and IL-27 produced by activated APCs including DCs and monocytes/macrophages act alone or synergistically in stimulating Th1 response and Th1-mediated production of IFN-γ (Gee et al., 2009).

IL-12 supports Th1 proliferation and differentiation. It also induces IFN-γ secretion by CD8+ T cells and NK cells (Nabioullin et al., 1994). IL-12 can be induced by IFN-γ but suppressed by IL-4, a Th2-type cytokine (Smeltz et al., 2002). IL-23 is able to promote Th1 differentiation, but it also stimulates proliferation of memory T cells and is critical in the development of proinflammatory Th17 populations (Aggarwal et al., 2003). IL-12 plays only a marginal role in Th17 differentiation. At early stages of the Th1 response, IL-27 directs clonal expansion of naïve CD4+ T cells and acts synergistically with IL-12 to induce IFN-γ secretion by naïve CD4+ T cells (Pflanz et al., 2002). However, IL-27 could play a two-sided role: inducing Th1 differentiation in naïve T cells, this cytokine can act as an attenuator of inflammatory cytokine production by suppressing IL-17 production by activated CD4+ T cells partially through STAT-3-dependent mechanism (Yoshimura et al., 2006).

Indeed, we observe a complexity in the IL-12 cytokine family activities in driving T-cell immune responses in atherosclerotic vessels. The interplay between the IL-12 family members becomes more complicated since these cytokines and their receptors share common subunits. The IL-12 receptor consists of the IL-12Rβ1 and IL-12Rβ2 chains (Presky et al., 1996). IL-23 also uses IL-12Rβ1 and the IL-23R that is unique to IL-23 (Parham et al., 2002). The receptor for IL-27 comprises a combination of gp130 with IL-27R (WSX-1) (Pflanz et al., 2004). The biological effects of IL-12 cytokines are mediated by the bioavailability of receptor subunits. For example, gp130, which is shared between multiple cytokines, is abundantly expressed, and full functional activity of IL-27 and IL-35 will be restricted by the availability of the WSX-1/gp130 and IL-12Rβ2/gp130 receptor heterodimers respectively (Fasnacht and Müller, 2008). For the IL-12 receptor, the amplitude of IL-12 signaling is limited by the availability of the IL-12Rβ2 subunit whose expression is significantly less than that of IL-12Rβ1 (Jones and Vignali, 2011).

Furthermore, whereas the standard members of the IL-12 family are heterodimers, some subunits can also act as monomers or homodimers. Structurally, the IL-12 family α-subunits (p19, p28, p35) are homologous to IL-6 family cytokines and pair with β subunits (p40 and EBI3) to form IL-12 (p35/p40), IL-23 (p19/p40), IL-27 (p28/EBI3), and IL-35 (p35/EBI3) (Jones and Vignali, 2011). The IL-12 subunit p40 was reported to assemble into homodimers that serve as potent IL-12 antagonists (Wang et al., 1999).

The monomeric p28 subunit could function independently on EBI3 as an antagonist of IL-6 and IL-27 signaling through gp130 and IL-6-mediated production of IL-17 and IL-10 (Stumhofer et al., 2010). Moreover, p28 was shown to form a complex with the soluble cytokine receptor cytokine-like factor 1 (CLF1) secreted by DCs (Crabé et al., 2009). The p28/CLF1 cytokine is functionally relevant being able to activate and increase IL-12- and IL-2-induced IFN-γ production in human NK cells through the IL-6 receptor-dependent mechanism (Crabé et al., 2009) and support B cell differentiation