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Innate Immunity in Coronary Disease. The Role of Interleukin-12 Cytokine Family in Atherosclerosis

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ABSTRACT

Atherosclerosis is a chronic, progressive, and multifactorial disease modulated by genetic and environmental factors. In recent years, the paradigm that explained atherosclerosis as resulting from a complex interaction between factors not accessible to medical intervention, and modifiable risk factors has changed. In this paradigm, alterations in lipid metabolism were the pivotal concept of atherosclerosis as a chronic degenerative disease. In the last years, an increasing number of observations have shown that the innate and adaptive immune responses to lipoprotein deposition and oxidation in the arterial wall significantly influence atherosclerosis. Currently, it is well recognized that the pathogenesis of atherosclerosis and its complications involves the inflammatory process, which includes the participation of several cytokines. Besides the classic cytokines involved in this process, the role of the interleukin-12 (IL-12) family has been recently demonstrated. This review describes our current understanding about the role of the family of IL-12 in atherosclerosis considering the participation of the genes that encode these cytokines in the genetic susceptibility to developing this disease.

Key words: Atherosclerosis. Inflammation. Interleukin 12 cytokine family. Genes. Genetic susceptibility.

IMMUNOLOGY OF ATHEROSCLEROSIS

Inflammation is a process that plays an important role for the initiation and progression of atherosclerosis. In this process, multiple cell types, including macrophages, T-lymphocytes, endothelial cells, smooth muscle cells, and mast cells, are involved[1]. It is well known that the innate and adaptive immune systems participate in atherosclerosis from its early stages to plaque erosion[2]. The innate immune response in atherosclerosis is represented by the immune-inflammatory cells, mainly monocytes and macrophages, which respond to the excessive uptake of lipoproteins, while the adaptive immune response is represented by antigen-specific T-cells[3]. In the first studies, only the presence of macrophages in the atherosclerotic lesion was reported; however later studies reported the presence of other cells such as mast cells, myeloid cells, CD4+, and regulatory T-cells. CD4+ cells are the most abundant T-cells present in atherosclerotic lesions and play an important role for the development and progression of the

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disease, whereas regulatory T-cells have a protective effect against the development of atherosclerosis[4]. Using atherosclerosis-prone models, it has been demonstrated that the development and progression of atherosclerosis are related to an imbalance of anti- and pro-inflammatory cytokines[5,6]. Anti- and pro-inflammatory cytokines produced by vascular and immune cells participate in atherosclerosis development through the modulation of cellular functions in the arterial wall in a paracrine and autocrine fashion[6]. Cytokines are involved at all stages of atherosclerosis and have a relevant influence on the pathogenesis of this disease. Cytokines participate in foam cell and fatty streak formation, in the development of complex lesions, and in plaque stability and rupture[7]. Inflammatory cells and pro-inflammatory cytokines have been detected in early and severe lesions, as well as in plaque rupture and thrombosis.

INTERLEUKIN-12 (IL-12) FAMILY

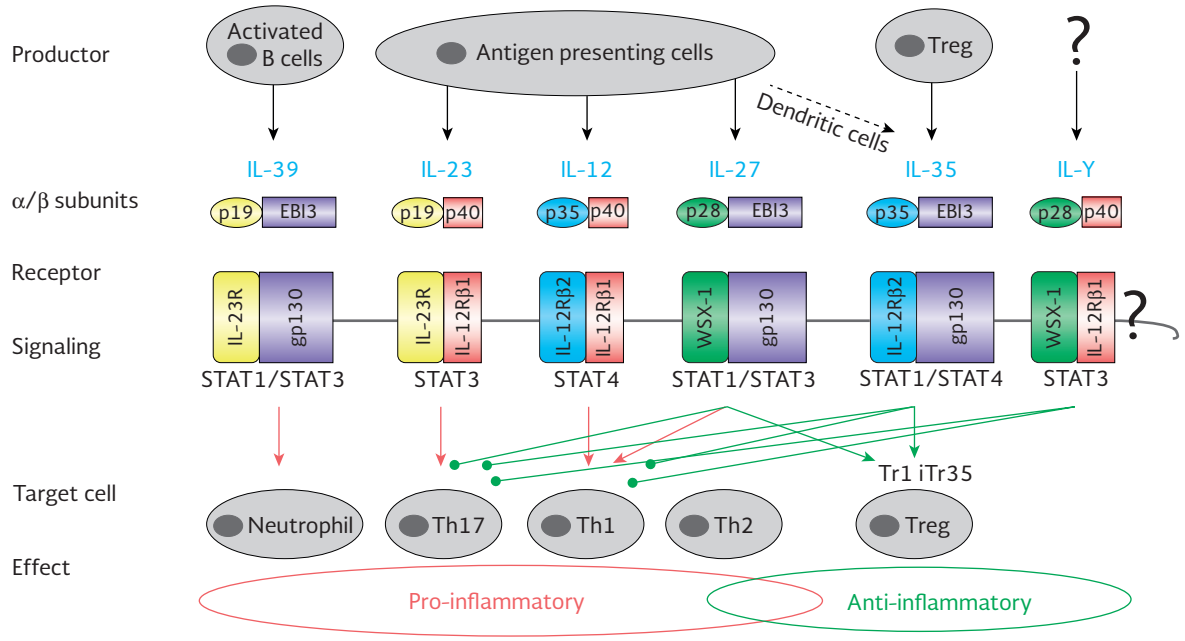
The IL-12 family is evolutionarily linked to the IL-6 cytokine superfamily and consists of a single group of α/β heterodimeric cytokines composed of one out of three possible α chains (p19, p28, or p35) and one out of two β chains (p40 or Epstein-Barr virus-induced gene 3 [*EBI3*])[8] (Fig. 1). The α subunit is a four-helix bundle structurally similar to the type I cytokine IL-6, whereas the β chains are composed of two tandem fibronectin type II domains that form a cytokine-binding homology region and a N-terminal immunoglobulin domain homologous to the soluble IL-6 receptor form[9]. Different combinations of α and β subunits resulted in six heterodimeric cytokines (Fig. 1). The IL-12 family members are key players in both promotion and suppression of multiple immune responses under physiological, as well as pathological conditions. The IL-12 family members signal through cell-surface heterodimeric receptors[8]. Each chain binds individually to its corresponding receptor subunit.

IL-12 was independently discovered by both Kobayashi et al.[10] as a "natural killer (NK)-stimulating factor" and Stern et al.[11] as a "cytotoxic lymphocyte maturation factor." IL-12 is a disulfide-linked 75-kDa heterodimeric protein composed of p40 and p35 subunits[12] that bind the heterodimeric receptor complex IL-12Rβ1/IL-12Rβ2. Subunit β p40 also binds p19 through a disulfide bridge to form IL-23[13], which engages the receptor IL-12Rβ1/IL-23R. IL-27 is formed by p28 and EBI3 subunits, which are not covalently linked, and signals through a receptor complex WSX-1/gp130[14]. The binding of p35 and EBI3 subunits forms IL-35; this cytokine has a unique feature of signaling, not shared by other members of the family. It signals through four receptor complexes: IL-12Rβ2/gp130, IL-12Rβ2/IL12Rβ2, gp130/gp130, and IL-12Rβ2/WSX-1[15,16]. Recently discovered IL-39 consists of p19 and EBI3 chains and signals through IL-23R/gp130[17]. The heterodimer p28/p40, tentatively called IL-Y, has been demonstrated to antagonize the signaling by IL-12 and IL-27[18]. Signaling through the above-mentioned receptors is mediated by Janus kinase-signal transducer members and activators of transcription (JAK-STAT) family[19]. IL-12 mediates signaling through STAT4[20], IL-23 through STAT3/STAT4[21], IL-27 and IL-39 through STAT1/STAT3[9,22], IL-35 through STAT1/STAT4[16], and IL-Y through STAT3[9] (Fig. 1).

The coexpression of α and β chains is a prerequisite for secretion of the bioactive cytokine[23]. Expression of α chain limits the production of each heterodimeric cytokine. Compared with EBI3 and p40 p35 is expressed at lower levels thus limiting the assembly of IL-12 and IL-35. Similarly, the tissue and cell-restricted expression of p19 limits the secretion of IL-23[13]. The antigen-presenting cells, such as dendritic and macrophages, express and secrete IL-12, IL-23, and IL-27. Their expression is induced by the activation of innate receptors. IL-35 is mainly produced by FoxP3+ regulatory T (Treg) cells, but can also be produced by γδT cells, CD8+ T-cells, and placental trophoblast[24]. In addition, it has been demonstrated that activated B cells secrete IL-39[17].

IL-12 family cytokines can directly induce the development of T-cell subpopulations and alter the fate and function of many cell populations that dictate disease outcome; they thus act as an immunological play maker, shaping immune responses[8]. The cytokine IL-12 family members exhibit immunoregulatory properties due to their effects on T-cell differentiation and function. Two examples of pro-inflammatory cytokines are IL-12 and IL-23; the former can induce Th1 cells while the latter has a major role in the induction of Th17 cells. Further, IL-27 exhibits a dual functional phenotype capable of increased pro- and anti-inflammatory responses[14]. IL-35 is a suppressive cytokine inhibiting the development of Th17 cells, the

Figure 1. Interleukin 12 family cytokines. This interesting and fascinating family is composed by heterodimeric cytokines sharing three α-chains (p19, p28, p35) and two β-chains (p40 and Epstein-Barr virus-induced gene 3). Their receptors are also heterodimeric and they share their subunits as well. Signaling is mediated by the Jak-STAT family having different effects on T-eff cell subsets. Red arrows indicate pro-inflammatory effects, green arrows represent anti-inflammatory properties *(modified from Ringkowski S, et al.*[145]*)*.



proliferation of effector T-cells[25], and inhibiting effector T-cell's responses[8,24] in an IL-10 dependent way[26]. Alternatively, IL-39 was shown to induce differentiation and/or expansion of neutrophils[27] (Fig. 1).

In early-stage atherosclerosis, the inflammatory response is initiated by lipid accumulation in the vessel wall. The pro-inflammatory response is driven by Th17 and Th1 cells, associated with increased production of IL-6, IL-17, interferon-gamma (IFN-g), and IgG2a antibodies against modified low-density lipoprotein[28,29]. Indeed, the IL-12 cytokine family plays a critical role in priming dendritic cell-mediated differentiation of CD4+ naïve T-cells. In Table 1 we summarized the evidence in cell culture, animal model and in human studies regarding the participation of IL-12 family members in atherosclerosis.

IL-12

IL-12 is recognized as a master regulator of adaptive type 1 cell-mediated immunity, the critical pathway involved in protection against neoplasia and many viruses. It binds to naïve CD4+ T-cells through its IL-12β1/IL-12β2 receptor[30] and promotes the generation of pro-inflammatory Th1[31] and Th17 cells[32]. It also inhibits IL-4, antagonizes Th2 responses and can also limit IL-2 production[33]; consequently, it has been shown to have a negative impact on Treg cells[34]. In addition to its, noted effects on the priming of Th1 cell responses and IFN-γ production by T and NK cells, more recent studies support not only its critical role as a third signal for CD8+ T-cell differentiation[35] but also its ability to serve as an important factor in the reactivation and survival of memory CD4+ T-cells[36]. This finding is particularly relevant in the repolarization of CD4+ T-cells from dysfunctional antitumor Th2 into Th1 cells in the cancer setting[37]. Besides its regulatory function, it has been reported that IL-12 expression is increased in several inflammatory diseases such as rheumatoid arthritis (RA)[38,39].

IL-12, known as the Th1 response master controller, stimulates T and NK cells to produce IFN-γ, which induces multiple proatherogenic processes in the atherosclerotic lesion[40]. A marked expression of both IL-12 mRNA and protein has been demonstrated in human atherosclerotic plaques[41]. Similarly, it has been suggested that the production of IL-12 contributes to plaque progression[42]. Experimental data have shown an association between circulating IL-12 levels and

Table 1. Studies and their findings on IL-12 family members in atherosclerosis

Study	Finding	References
IL-12		
Animal model	Production of IL-12 contributes to atherosclerotic plaque progression	42
	Increased IL-12 production in models of atherosclerosis	45,46
Human	Increased expression of IL-12 mRNA and protein in human atherosclerotic plaques	41
	Circulating IL-12 levels are associated with arterial stiffness in healthy individuals	43
	IL-12 and IL-18 elevated levels are significantly associated with higher risk of cardiovascular events	44
	Increased production of IL-12 in newly diagnosed type 2 diabetic patients with cardiovascular complications	47
	Correlation between increased inflammatory responses and IL-12 serum levels in coronary artery disease patients	48
IL-23		
Cell culture	Lower IL-23 gene expression in unstimulated peripheral blood lymphocytes of coronary patients compared with subjects with normal coronary angiography	64
Animal model	In IL-23p19$^{-/-}$ mice, after myocardial infarction, IL-23 deficiency results in myocardial inflammation, lower cardiac fibroblast activation, impaired scar formation, adverse remodeling, ventricular rupture and increased mortality	63
	IL-23 levels are upregulated in brain and circulation in a murine model of stroke	65
Human	Increased IL-23 serum levels and higher IL-23 expression in human carotid lesions of peripheral arterial disease patients	66, 67
IL-27		
Cell culture	IL-27 reduces lipid accumulation in THP-1 derived macrophages and enhances cholesterol efflux	96
Animal model	The deficiency of IL-27 and its receptor accelerates atherosclerosis. Treatment with IL-27 recombinant inhibits atherosclerosis *in vivo* and macrophage activation *in vitro*	94
	In rat model of ischemia/reperfusion injury, the administration of IL-27 reduces damaged tissue and improves post-ischemia recovery	95
Human	IL-27 is expressed in atherosclerotic plaques	93
	Compared with controls, coronary patients have higher IL-27 plasma concentrations, that correlated with the severity of the coronary atherosclerotic lesion	97, 98
IL-35		
Animal model	IL-35 treatment reduces the atherosclerotic lesion in ApoE$^{-/-}$ mice	107
	ApoE$^{-/-}$ mice with established atherosclerotic lesion have lower IL-35 levels compared with control mice	108
Human	The co-expression of the IL-35 α and β subunits was demonstrated in human advanced atherosclerotic plaque	93
	Patients with acute coronary syndrome have decreased levels compared with subjects with chest pain syndrome	109
	IL-35 levels significantly correlated with left ventricular ejection fraction	
	Premature coronary patients have lower IL-35 levels compared with healthy controls	110

IL: interleukin; THP-1: human acute monocytic leukemia cell line.

arterial stiffness in healthy individuals[43]. In an observational study, Opstad et al. showed that IL-12 levels were not associated with cardiovascular events[44]; however, when the levels of IL-12 and IL-18 were analyzed simultaneously, they were found to be independently and significantly associated with a higher risk of cardiovascular events[44]. Increased IL-12 production has been shown in animal models of atherosclerosis[45,46] and in newly diagnosed type 2 diabetic patients with cardiovascular complications[47]. In fact, a correlation between advanced inflammatory responses and IL-12 serum levels has been demonstrated in murine models of atherosclerosis, as well as in coronary artery disease (CAD) patients[48].

IL-23

IL-23 is mainly produced by macrophages and dendritic cells after Toll-like receptor activation. IL-23 expression is further augmented by interactions between dendritic cells and T-cells after CD40-CD40L binding[49]. In addition, its secretion is limited by the tissue and cell-restricted expression of p19 subunit[13,23]. The action of IL-23 is generated after its interaction with the heterodimeric IL-23 receptor (IL-23R), composed of the IL-12Rβ1 unit and the specific IL-23R chain. IL-23 plays a central role in inflammation, since it promotes Th1 cell differentiation, stimulates the proliferation of the T memory cells and is critical in the development of

pro-inflammatory Th17 cell population[50]. Moreover, IL-23 can switch IL-17 secreting FoxP3+RORγt Tregs to the pro-inflammatory Th17 phenotype[51]. Naïve FoxP3+ Tregs start differentiation into Th17 cells in the presence of IL-1β and IL-2, and IL-23 is able to enhance this process[52,53]. IL-23 promotes the survival of Th17 cells induced by TGFβ and IL-6[54]. In fact, acting in concert with IL-12, IL-18, and IL-2, IL-23 promotes the production of INF-γ by NK cells[21]. Other innate immune cells, termed "type 17" cells, respond to IL-23; these cells compromise γδT cells, NK T-cells and innate lymphoid cells, which are important for resistance to infection and autoimmune pathology[55]. These capacities give IL-23 strong pro-inflammatory and proatherogenic properties. Indeed, IL-23 has been related to several inflammatory disorders, such as RA[56], ulcerative colitis[57], psoriasis[58], systemic lupus erythematosus[59], and inflammatory bowel disease[60], as well as cancer[61].

Interestingly, IL-23 has also been implicated in atherogenesis[62]. In IL-23p19$^{-/-}$ mice, Savvatis et al. showed that after myocardial infarction, IL-23 deficiency results in myocardial inflammation, lower cardiac fibroblast activation with impaired scar formation, adverse remodeling, ventricular rupture, and increased mortality[63]. In 25 consecutive individuals defined as having CAD after a coronary angiography, the expression of *IL-23* gene was evaluated in unstimulated peripheral blood monocytes cells (PBMCs) and compared to the *IL-23* gene expression in unstimulated PBMCs of 25 consecutive individuals with normal coronary angiography studies. Results showed a significantly lower *IL-23* gene expression in unstimulated PBMCs of patients with CAD compared to those without CAD after adjustment for age, sex, hypertension, and diabetes mellitus[64]. These results are in accordance with the data obtained in murine models. In addition, in a model of ischemic stroke, levels of IL-23 were upregulated in both brain and circulation[65]. Peripheral arterial disease patients also showed an increase of IL-23 serum levels and higher *IL-23* gene expression in carotid lesions[66,67]. Furthermore, in a follow-up study (mean 3.5 years) of 177 patients with carotid atherosclerosis and 24 healthy controls, IL-23 levels were significantly higher in patients when compared with controls, and higher levels of the cytokine were associated with disease progression and increased mortality[68]. The inconsistent results regarding the role of IL-23 in atherosclerosis may be partly due to different experimental setups in animal model and human studies. As for the animal model studies, wild-type mice with different genetic background have been used with a selection of different time points, making comparisons difficult since the inflammatory reaction in atherosclerosis and myocardial infarction is a continuously changing process. Concerning human studies, population sample size, diagnosis criteria, gender, and presence of other clinical phenotypes such as obesity and type 2 diabetes mellitus can generate different results. In sum, the role for IL-23 in atherosclerosis is still unclear.

IL-27

IL-27 is mainly produced by dendritic cells, monocytes, and macrophages after their activation by recognition of pathogen-specific patterns[22,69]. Moreover, LPS-activated macrophages express the highest levels of p28 and EBI3 in any tissue or cell type described[22,69,70]. CD40-CD40L interactions between dendritic cells and T-cells increase production of IL-27, IL-23 and IL-12[49]. IL-27 is considered a pleiotropic cytokine as it modulates anti-inflammatory and pro-inflammatory responses depending on the predominant type of immune response, disease type, and severity. The pleiotropic effects of this cytokine suggest that its wide participation in several inflammatory diseases could be due to its effect on common mechanisms involved in these pathologies[71]. At first, it was reported that IL-27 together with IL-12 initiate clonal expansion of naïve T-cells and increased IFN-γ production by this cell type and NK cells[22,69], suggesting that IL-27 sensitizes T-cells to IL-12 effects. As a consequence, it is critical for the early events leading to Th1 cell proliferation and differentiation[72]. Later, studies have demonstrated that IL-27 also has anti-inflammatory activity. In fact, IL-27 can inhibit Th1-driven infections[73], development of Th17 cells in several inflammatory settings[74], Treg formation induced by transforming growth factor-β[75], and secretion of IL-4. Interestingly, it also antagonizes IL-2 production; hence, limiting Th2 cell differentiation and effector cell function[76]. IL-27 converts activated inflammatory CD4+ T-cells into IL-10-producing Th1 or Tr1 cells[74,77]. It seems that IL-27 has a bidirectional function: while it induces a pro-inflammatory response in naïve cells, it does the opposite in activated cells. In addition, an enhanced IL-27 expression during fetal development has been suggested; for instance, IL-27, like IL-35, could have a role in fetal-maternal tolerance[78]. In this regard, IL-27 is the only IL-12 family

member to have both inflammatory and immunomodulatory activities. The IL-27 p28 subunit alone acts as an agonist of gp130-mediated signaling[79]. The p28 subunit blocks the signaling mediated by IL-6, IL-12, and IL-27, including IL-6-dependent Th17 responses.

The IL-27 receptor is a complex formed by a WSX-1 chain and a glycoprotein 130 (gp130) subunit[80]. WSX-1 and gp130 are coexpressed on different cell types, such as dendritic cells, mast cells, monocytes, macrophages, NK cells, endothelial cells, and B and T lymphocytes[69,81,82]. Indeed, the complexity of its receptor could explain the wide-ranging immunomodulatory functions of this cytokine. The promotion or suppression of inflammation by IL-27 may vary within different pathologies. In fact, the available evidence show that IL-27 suppresses inflammation in *Leishmania donovani* infection[83], intraocular inflammation[84], chronic inflammation of the central nervous system[85], autoimmune arthritis[86], experimental autoimmune encephalitis[87], and allergic asthma[88]. In contrast, it promotes inflammation in systemic sclerosis[89], experimental crescentic glomerulonephritis[90], experimental colitis[91], and hepatitis[92].

The participation of IL-27 in the regulation of the innate and adaptive immunity supports its part in atherosclerosis. IL-27 is expressed in atherosclerotic plaques[93], and its role in atherosclerosis has been reported in cultured cells, animal models and coronary patients with inconsistent findings. In IL-27-deficient (*Ldlr*$^{-/-}$*Ebi3*$^{-/-}$) and IL-27 receptor-deficient (*Ldlr*$^{-/-}$*WSX-1*$^{-/-}$) *Ldlr*$^{-/-}$ mice, Hirase et al. demonstrated that the deficiency of this cytokine and its receptor accelerates atherosclerosis and that IL-27 recombinant treatment inhibits atherosclerosis *in vivo* and macrophage activation *in vitro*[94]. In addition, in a rat model of ischemia/reperfusion injury, IL-27 administered 5 minutes before reperfusion reduced tissue damage and markedly improved post-ischemia recovery in isolated perfused hearts; this finding suggests that this cytokine protects the myocardium against ischemia/reperfusion injury, thus facilitating the recovery of damaged cardiomyocytes[95]. In an *in vitro* study, it has been demonstrated that IL-27 decreased lipid accumulation in THP-1 derived macrophages and markedly enhanced cholesterol efflux through increasing the expression of both *ABCA1* mRNA and protein[96]. These findings suggest that IL-27 reduces lipid accumulation of foam cells by upregulating *ABCA1* expression. In contrast, in a clinical study of 136 CAD patients and 29 controls, Jin et al. found that IL-27 plasma concentration was higher and correlated with the severity of the coronary atherosclerotic lesion in CAD patients[97]. Similarly, significantly higher IL-27 plasma concentrations were observed in Mexican premature CAD patients compared to controls[98]. Moreover, dendritic cells incubated with oxidized low-density lipoprotein produced IL-27, suggesting that these modified lipoproteins could play an important role in dendritic cell activation and IL-27 production[97]. Altogether, these studies suggest IL-27 could play a crucial role in the immunity and inflammation regulatory network in atherosclerosis.

IL-35

IL-35 is considered an anti-inflammatory and immunosuppressive cytokine[99]. IL-35 is constitutively secreted by CD4+ regulatory T (Treg) cells instead of CD4+ effector T-cells[15]. In human and mice, IL-35 can induce the conversion of conventional Treg cells into a suppressive IL-35-producing Treg cell population (termed iTr35)[100]. IL-35 does not have a constitutive expression in tissue[101]. The genes encoding IL-35 are also transcribed by activated B cells and to a lower degree by monocytes, smooth muscle cells, and vascular endothelial cells after activation with lipopolysaccharide and pro-inflammatory cytokines[101]. Although IL-35 shares structural features and binding partners with the IL-12 siblings, its function appears to be strictly regulatory. Contrary to the inflammatory effects of the IL-12 and IL-23, IL-35 can efficiently inhibit Th1 and Th17 cells through the expansion of Treg cells and IL-10 production; indeed, this cytokine is also important for optimal Treg cell function[15]. Devergne et al.[102] reported a high expression of EBI3 and p35 (subunits of IL-35) in placental trophoblast, suggesting that, like IL-27, IL-35 may be an immunomodulator at the fetal-maternal border. Thus, IL-35 plays critical roles in preventing autoimmunity, maintaining self-tolerance, and suppressing antitumor immune responses. In experimental models, IL-35 has been demonstrated to suppress the development of collagen-induced arthritis[25], autoimmune diabetes[103], autoimmune encephalomyelitis[104], inflammatory bowel disease[105], and IL-17-dependent allergic airway disease[106].

As mentioned before, the gene that encodes IL-35 is transcribed by vascular endothelial cells, smooth

muscle cells and monocytes when activated by stimulation[101]. Then, it may play a role in atherosclerosis development. In ApoE$^{-/-}$ mice previously fed with a high-fat diet, it has been reported that IL-35 treatment reduces the atherosclerotic lesion area by improving Treg-mediated suppression[107]. In addition, Wang et al.[108] have recently demonstrated that mice with atherosclerotic lesion, display lower levels of IL-35 compared to the age matched wild-type C57BL/6 mice without plaque. On the other hand, the expression of IL-35 increased significantly in ApoE$^{-/-}$ mice with attenuated plaque. In fact, coexpression of the two subunits of IL-35 has been demonstrated in human advanced atherosclerotic plaque[93]. Decreased levels of IL-35 have been reported in patients with acute coronary syndrome (unstable angina pectoris and acute myocardial infarction) compared to a chest pain syndrome group; IL-35 levels also positively correlated with left ventricular ejection fraction whose reduction is associated with heart failure[109]. In contrast, it has been recently reported that premature CAD patients have significantly higher IL-35 plasma levels compared to healthy controls with no personal o familial history of premature CAD, and without subclinical atherosclerosis evaluated by computed tomography (coronary calcium score = 0)[110].

Genes encoding α and β subunits of the IL-12 family members and their association with cardiovascular and other diseases

The genes encoding the IL-12 family subunits are polymorphic. In fact, some of the polymorphisms could have a functional effect that changes the expression of these subunits and, in consequence, the expression and production of the IL-12 cytokine family. Functional genetic polymorphisms that alter cytokine gene expression are candidate genetic factors that could modulate the development and progression of atherosclerosis and cardiovascular disease. Indeed, the genotypes of the polymorphisms of α and β chain genes of the IL-12 family members could influence cytokine production and activities, and may define the balance in Th response in atherosclerosis. The current findings of the association between the genes encoding the subunits of the interleukin-12 family members and atherosclerosis are summarized in Table 2.

IL-12B gene

The β subunit p40 (present in IL-12, IL-23 and IL-Y) is encoded by *IL-12B* gene on chromosome 5. Genome-wide association studies (GWAS) have become a powerful approach to identify genes involved in complex pathologies. Recent GWAS have pointed out a set of polymorphisms in *IL-12B* gene that is consistently associated with chronic inflammatory disorders. The rs3212227 (*A→C*) polymorphism present in the 3'UTR region of *IL-12B* has been associated with psoriasis[111], type 1[112] and type 2 diabetes mellitus[113], multiple sclerosis[114], ankylosing spondylitis[115], allergic rhinitis[116], asthma[117], and lepromatous leprosy[118]. However, the information regarding the association of CAD with polymorphisms present in the *IL-12B* gene is scarce. In a small Japanese cohort, Momiyama et al. failed to show an association between rs3212227 *IL-12B* polymorphism and the presence or severity of CAD diagnosed by angiography[119]. Although an important limitation in that study is the lack of a healthy control group, the results are in agreement with those reported by Mangino et al. in a British cohort[120] In conclusion, the data available so far do not support the association between the rs3212227 *IL12B* polymorphism and the development of cardiovascular disease.

IL-23A gene

The α subunit p19 (present in IL-23 and IL-39) is encoded by the *IL-23A* gene located on chromosome 3. Considering that IL-23 cytokine has an important role in the atherosclerosis process, the gene that encodes this cytokine could be a candidate for genetic association studies. Genetic studies have associated the rs2066808, rs2371494, and rs11575248 polymorphisms in the *IL-23A* gene with susceptibility to multiple sclerosis[121]. Alternatively, the rs11171806 polymorphism in the *IL-23A* gene has been significantly associated with susceptibility to Graves' disease in Han Chinese[122], but not in the northern Italian population[123]. In a Brazilian cohort, the *GG* haplotype of rs11171806 and 2066808 polymorphisms in the *IL-23A* gene was more frequent in control subjects than in diabetic patients, thus conferring protection against this disease[124]. In German and Chinese cohorts, the intronic rs2066808 *IL-23A* variant has been associated with psoriasis[125,126], whereas in Northern Spaniards it has been associated with psoriatic arthritis[127]. Finally,

Table 2. Studies and their findings on genes encoding α and β subunits of the interleukin-12 family members and atherosclerosis

Gene	Finding	References
IL-12B	Lack of association between rs3212227 *IL-12B* polymorphism and the presence or severity of CAD evaluated by angiography	119
	Lack of association between myocardial infarction and rs3212227 *IL-12B* polymorphism	120
IL-23A	So far, no studies have evaluated the association of the *IL-23A* polymorphisms with cardiovascular disease	
IL-27p28	*rs153109 G* variant was associated with atrial septal defects and ventricular septal defects	136
	rs181206, rs17855750, rs37833, and rs153109 single nucleotide polymorphisms were not associated with CAD, age at disease onset or severity	136
	rs26528 *T* and rs40837 *A* alleles were associated with a decreased risk of developing premature CAD after adjusting for confounding variables	98
IL-12A	*IL-12A* rs2243115 polymorphism was significantly associated with reduced risk of developing premature CAD after adjusting for confounding variables	110
EBI3	The *EBI3* rs428253 was associated with reduced risk of developing premature CAD	110
	The *EBI3* rs4740 and rs4905 genotype were associated with different levels of IL-35	

IL: interleukin; CAD: coronary artery disease; *EBI3*: Epstein-Barr virus-induced gene 3.

these associations have been confirmed in a Romanian cohort[128] and a Caucasian cohort (British and Irish patients)[129]. Unfortunately, at present, no studies have evaluated the association of the *IL-23A* polymorphisms with the development of cardiovascular disease.

IL-27p28 gene

The human *IL-27p28* gene that encodes the IL-27 and IL-Y α subunit is located on the 16p11 locus, spans 5 exons and is highly polymorphic[130]. Polymorphisms in the *IL-27p28* gene have been associated with risk of inflammatory bowel disease[131], RA[132], asthma[130], allergic rhinitis[133], chronic obstructive pulmonary disease[134], and with protection of ulcerative colitis[135]. In a Chinese case–control study, an association of atrial septal defects and ventricular septal defect with the *G* allele of rs153109 polymorphism was reported; no association was found with the rs*17855750* polymorphism[136]. In a large number of CAD cases belonging to the GeneID Chinese Han population, four *IL-27p28* tag polymorphisms (rs181206, rs17855750, rs37833, and rs153109) were detected and after adjusting for confounding variables, the polymorphisms were not associated with CAD, age at disease onset or severity[137]. These results concur well with the findings of the Genetics of Atherosclerotic Disease (GEA) Study in a Mexican population, in which the rs181206 and rs17855750 *IL-27p28* gene variants were not significantly associated with premature CAD[98]. However, Posadas-Sánchez et al. reported a significant association (after adjusting for age, gender, body mass index, smoking habit, total abdominal fat, homeostatic model assessment-insulin resistance, aspartate aminotransferase, adiponectin, and uric acid) of both the rs26528 *T* and rs40837 *A* alleles with a decreased risk of developing premature CAD[98]. In addition, luciferase assays showed that cotransfection of the rs40837 *A* allele and miR-379-5p significantly decreased luciferase gene expression. To the best of our knowledge, this is the first and only study reporting a significant association and with a functional approach of *IL-27p28* gene polymorphisms with premature CAD. In consequence, the study reporting these associations (rs26528, rs40837) should be repeated in other cohorts to confirm such results.

IL-12A gene

The α subunit p35 present in IL-12 and IL-35 is encoded by *IL-12A*; this gene is located on chromosome 3q25.33 and consists of seven exons. Previous studies have indicated the association of *IL-12A* gene polymorphisms with inflammatory diseases. In a Chinese population, the *IL-12A* rs2243115 polymorphism was associated with genetic susceptibility to chronic obstructive pulmonary disease[138]. Chen et al.[117] reported the association of the *IL-12A* rs568404 polymorphism with the risk of asthma development in a Chinese population. In a case-control association study, 5 tag polymorphisms (rs2243115, rs2243123, rs583911, rs568408 and rs2243143) in the *IL-12A* gene were determined in two independent Chinese cohorts (a pilot cohort study conducted in Shanghai, and a replicate cohort in the Xiamen Island); haplotype analysis showed that the haplotype of the five polymorphisms (*TTAAG*) was associated with a significant risk of Graves' disease in

both cohorts[139]. Recently, Shen et al. reported that the *IL-12A* rs2243115 *GG* genotype may increase the risk of RA in Chinese individuals negative for rheumatoid factor[140]. A case–control study showed that the haplotype *TA* (rs582054 and rs2243151) in the *IL-12A* gene was significantly associated with the atopic dermatitis phenotype in a Korean population[141]. Only one study has heretofore evaluated the participation of *IL-12A* gene polymorphisms in the susceptibility to premature cardiovascular disease. A very recent report of the Mexican GEA study determined four *IL-12A* polymorphisms (rs2243115, rs2243123, rs583911, and rs568408) in 1162 patients with premature CAD and 873 controls[110]. In this study, after adjustment for age, gender, body mass index, and current smoking status, it was described that the *IL-12A* rs2243115 polymorphism was significantly associated with reduced risk of developing premature CAD under different inheritance models. Considering that this is the first study that evaluates the role of *IL-12A* polymorphisms in premature CAD, the detected associations are not yet definitive, and replicate studies in independent populations are warranted to confirm these findings.

EBI3 gene

The β subunit (EBI3) of IL-35 and IL-27 is encoded by *EBI3* gene; it is located on chromosome 19q13.3 and contains 5 exons. A Chinese population-based case-control association study was designed to assess the risk of allergic rhinitis conferred by polymorphisms in *FOXP3* and *EBI3* gene regions. Logistic regression analyses, adjusted for age and gender, showed a significant association of the *EBI3* rs428253 polymorphism with decreased risk of developing allergic rhinitis[142]. In another Chinese cohort, the *EBI3* gene rs428253 polymorphism was also associated with chronic rhinosinusitis[143]. In a Mexican study, the *EBI3* rs428253, rs4740, and rs4905 polymorphisms were associated with decreased risk of developing ulcerative colitis[135]. Furthermore, Zheng et al.[144] found that the *EBI3* rs4740 polymorphism is closely associated with susceptibility to pulmonary tuberculosis in Chinese subjects. As it was described for the *IL-12A* gene, only one study has addressed the participation of *EBI3* gene polymorphisms in the susceptibility to premature cardiovascular disease[110]. In this study, three *EBI3* gene polymorphisms (rs428253, rs4740, and rs4905) were evaluated; results showed that the *EBI3* rs428253 polymorphism was associated with reduced risk of developing premature CAD. In this study, the authors also found that coronary patients showed significantly higher IL-35 levels than control subjects, and that the *EBI3* rs4740 and rs4905 polymorphisms were associated with different levels of IL-35[110]. Nevertheless, considering that this is the first report that evaluates the role of *EBI3* polymorphisms in premature CAD, the detected associations are not yet definitive, and replicate studies in independent populations are required.

CONCLUSIONS

The IL-12 family cytokines act as the immunological playmaker, shaping immune responses by directly inducing the development of T-cell subpopulations and altering the function and fate of many cell populations that dictate disease outcome. They are a unique group of heterodimeric cytokines composed of one out of the three α subunits, p19, p35, or p28, and one out of two β subunits, p40 or *EBI3*. Despite their common structures, the biological function of the members of this family is very diverse. While IL-12 and IL-23 are pro-inflammatory, IL-27 is bi-directional in terms of being both pro- and anti-inflammatory; alternatively, IL-35 is anti-inflammatory. Considering their effects on the immune response, these cytokines have been associated with the development of cardiovascular diseases in both animal models and humans. In the former, some of these cytokines have been administered and variation in the development of atherosclerosis has been observed. Moreover, the variation in the levels of these cytokines in humans has been detected in both patients and healthy controls. Association studies of the polymorphisms present in the genes that encode the IL-12 family subunits are recent, and many of them need confirmation. From the point of view of etiopathogenesis and genetics, it is important to consider that the information about the role of the IL-12 family of cytokines in the development of atherosclerosis is limited. Indeed, future studies could help define the true role of these cytokines in the development of this complex disease.

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