Interleukin-32 in chronic inflammatory conditions is associated with a higher risk of cardiovascular diseases

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ABSTRACT
Cardiovascular diseases (CVD) are the most frequent cause of death in developed countries. Their prevalence is higher in several chronic inflammatory conditions. This is likely due to the substantial contribution of the inflammatory status of the patients to the development and stability of atherosclerotic plaques. Recent evidence suggests that interleukin (IL)-32 may be involved in the conditions that contribute to CVD. IL-32 not only modulates important inflammatory pathways known to contribute to the pathogenesis of both inflammatory diseases and atherosclerosis, including tumor necrosis factor (TNF), IL-6 or IL-1; but it has been also suggested to modulate endothelial cell function and the serum concentration of high-density lipoprotein (HDL). In this review, we highlight the recent advances in the field of IL-32 in relation to chronic inflammatory disorders and argue for a role of IL-32 in the increased prevalence of CVD in these patients.

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1. Introduction

In general, acute inflammation is a positive reaction of the body to insults such as traumatic tissue injury or invading pathogens. When an immune response is triggered, it can lead to eradication of the pathogen or facilitate turnover, adaptation and repair of the tissues involved. In contrast, during chronic inflammation, these responses are usually low grade but persistent, resulting in tissue damage and degeneration [1]. Circulating elevated markers such as interleukin (IL)-1β and IL-6 are upstream of the biomarker C-reactive protein (CRP) and are known to be associated with ongoing low grade inflammation [2,3]. Moreover, immune cells from both innate- and adaptive immunity (monocytes, neutrophils, macrophages and T/B cells) have been suggested to play a role in chronic inflammatory conditions and are responsible for the main production of a wide spectrum of pro-inflammatory cytokines such as TNFz, IL-1β and IL-6 and IL-17. These pro-inflammatory cytokines may trigger other inflammatory responses and immune cell activation, leading to an ongoing inflammatory process.

The pathophysiology of many chronic inflammatory diseases, including rheumatoid arthritis (RA), chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD) and atherosclerosis, has been thoroughly studied in the past years. Interestingly, the development of cardiovascular diseases (CVD) have emerged as a serious concern in these patients [4]. For patients, it represents the leading cause of death, accounting for approximately one-third to one-half of all RA-related deaths (1; 2) [4–6]. It seems that persistent inflammation plays a major role in the development of CVD and atherosclerosis. Over the past years, atherosclerosis has been increasingly regarded as an inflammatory disease, with dyslipidemia representing only one (albeit important) of its inducers [7]. Furthermore, a direct interaction between inflammation and lipids has been well-documented. Circulating...
cholesterol levels may correlate with disease activity, which can result in a change in lipid composition during periods of a higher inflammatory state, favouring formation of atherosclerotic plaques in the case of RA patients [8–10].

Interleukin-32 has been described as a pro-inflammatory cytokine involved in the pathogenesis of several inflammatory diseases. It is known to play a role in RA due to its capacity to induce TNFα, which is a major cytokine in RA. Additionally, IL-32 contributes to the induction of other pro-inflammatory mediators such as IL-1β-induced ICAM-1 upregulation in human umbilical endothelial cells (HUVECs) and was later found to be expressed in atherosclerotic plaques [11,12]. IL-32 is also highly expressed in T cells, known to play an important role in the late stages of atherosclerosis, characterized by plaque instability and rupture. Given these facts, one may hypothesize that IL-32 could be an important contributing factor to the development of CVD in individuals suffering from chronic inflammatory conditions. Knowledge on the involvement of the innate and adaptive immune systems in the pathogenesis of chronic inflammatory conditions and CVD has greatly expanded in the last years. Nevertheless, the higher incidence of CVD in chronic inflammatory conditions is still an important unmet clinical need. Therefore, it is essential to get a better understanding of this complex interaction in these conditions.

In this review, we discuss the recent insights of the role of IL-32 in the pathogenesis of autoimmune diseases focusing on RA, COPD and IBD. Additionally, the potential role of IL-32 in the development of atherosclerosis, as pre-clinical condition of CVD, will be discussed. Finally, we will argue for an important role of IL-32 in the increased prevalence of cardiovascular diseases within these chronic inflammatory conditions.

2. Interleukin-32: history and function

In 1992 Dahl et al. discovered a molecule that was called natural killer cell transcript 4 (NK4). Gene expression of NK4 was increased when peripheral blood mononuclear cells (PBMCs), especially NK or mitogen-stimulated T cells, were exposed to a high dose of interleukin-2 (IL-2) [13,14]. Moreover, it was hypothesized that NK4 could play a role in cell adhesion since it contains an Arg-Gly-Asp (RGD) sequence, which is important for mediating cell adhesion for a variety of proteins [15]. Nevertheless, the function of NK4 remained unknown until 2005. Kim et al. then showed that re-combinant NK4 induced TNFα production in Raw 264.6 macrophages showing classical pro-inflammatory properties leading to renaming the molecule to interleukin-32 (IL-32) [16]. Additionally, the genomic structure and localization of IL-32 was explored. In humans, IL-32 resides on chromosome 16 p13.3 and consists of eight small exons. Besides mammals, IL-32 is expressed in other species like bovine and swine, but not in mice. Moreover, IL-32 can be spliced at mRNA level by alternative splicing, generating at least six different isoforms: IL32γ, IL32β, IL32α, IL32β, IL32ε, and IL32a, of which not all functions are known yet [17]. The receptor for IL-32 has not yet been discovered, although binding of IL-32 to proteinase 3 and integrins, αVβ3 and αVβ6, has been described [17,18]. In addition, in the presence of functional αvβ3, recombiant IL32γ induced in vitro endothelial cell tube formation showing a role for IL-32 in angiogenesis [19]. The total IL-32 protein can be induced by IFN-γ and besides TNFα, IL-32 can also stimulate IL-8 production by activation of signaling pathways nuclear factor-κB (NF-κB) and p38 mitogen-activated protein (MAP) kinase [16]. The intra-cellular nucleotide-binding oligomerization domain (NOD)1 and NOD2 ligands can also synergize with IL-32 for IL-6 and IL-1β production through a caspase 1-dependent mechanism [20] (Fig. 1). In contrast, silencing of endogenous IL-32 shows attenuated IL-1β, IL-6, IL8 and TNFα production in endothelial cells and monocytes [11]. IL-32 acts as a pro-inflammatory cytokine and an endogenous regulator of cytokine production. Although the knowledge on the functions of IL-32 has expanded significantly in the last decade, many aspects regarding the exact mechanism of action in health and diseases still remain unknown (see Table 1). In this review, we further discuss the current viewpoints and discoveries in the field of IL-32, focusing on the role of IL-32 in chronic inflammatory diseases (RA, COPD and IBD) and atherosclerosis (Fig. 2).

3. IL-32 and inflammatory diseases

IL-32 has been reported to be involved in the pathogenesis of chronic inflammatory diseases such as IBD, COPD and RA [21,22]. RA is often described as a standard prototype autoimmune disease. It affects about 1% of the world population but the exact mechanisms leading to RA still need to be defined [23]. Cytokines play an important role in the pathogenesis of this disease, with IL-6 and TNFs being ones of the most studied so far. Therapeutic blockade of these latter cytokines or their receptors improves the signs and symptoms of RA, but is not able to cure the disease. Therefore, the search for new mediators and pathways involved in the disease onset and progression is worthwhile and could lead to new therapeutic targets.

In line with this, a few years ago research started to focus on the role of IL-32 in RA pathogenesis. A clear correlation between the IL-32 protein expression in synovial tissue biopsies of RA patients and disease activity has been demonstrated [8]. Apart from synovial tissue macrophages, synovial fibroblasts can produce TNFα, which by itself is a potent inducer of IL-32 mRNA. Moreover, when IL-32 was overexpressed in these cells, mRNA transcripts of IL-1β and TNFα were stabilized. Besides, when RA patients started anti-TNF therapy, significant lower levels of IL-32 were detected in synovial tissue biopsies, suggesting a link between TNFα and IL-32 in RA patients [24]. As a proof of concept, Josteen et al. injected recombinant human IL-32 into the joints of mice, inducing joint swelling, influx of pro-inflammatory cells and cartilage damage. This was remarkably decreased when mice deficient for TNFα were used [8]. Furthermore, to confirm the pro-inflammatory effects of IL-32 in human samples, experiments were performed using synovial tissue biopsies and CD14+ monocytes obtained from both RA patients and healthy individuals. mRNA levels of the most biologically active isoform of IL-32, IL32γ, were significantly upregulated in specimens of RA patients compared to healthy individuals [25]. Another finding was the fact that IL-32 can synergize with soluble receptor activator of NFκB ligand (sRANKL) [26]. In cell cultures of CD14+ monocytes from healthy volunteers treated with human recombinant IL-32γ, the presence of sRANKL resulted in an induced osteoclast generation and tissue resorption. IL-32 alone was later also found to be able to stimulate osteoclastogenesis without RANKL [27]. Moreover, IL-32 levels were higher in fibroblast-like synoviocytes (FLS) from RA patients compared to FLS from osteoarthritis (OA) patients, strengthening the importance of IL-32 in osteoclast differentiation and maturation from osteoclast precursors and activity [28]. In 2012, Moon et al. explored into more detail the TNFα-dependent IL-32 induction in RA synovial fibroblasts [27]. They found that the spleen tyrosine kinase (Syk)/protein kinase Cδ (PKCδ)/c-Jun N-terminal kinase (JNK) pathways are involved in the regulation. Inhibitors of Syk, PKCδ and JNK were able to suppress the TNFα-mediated induction of IL-32.

Besides this clear link with TNFα, IL-32 is also positively linked to other inflammatory markers in RA [29]. IL-32 affects the production of thymic stromal lymphopoietin (TSLP) in a monocyte THP-1 cell line and PBMCs and by doing so influences monocyte differentiation into macrophage-like cells, which can affect the immune response [30]. In THP-1 cells or RA synovial fibroblasts,
overexpression of a splice-resistant IL-32γ mutant resulted in more IL-32γ secretion and increased the level of the important cytokine IL-1β. However, when IL-32β was overexpressed in these cells, secretion of IL-32β was decreased and no clear induction of IL-1β was observed [24]. These results suggest that IL-32β is a less active pro-inflammatory mediator and that IL-32γ splicing towards IL-32β serves as a rescue mechanism to reduce inflammation.

Another isoform of IL-32 lacking exon 3 and 4 of the IL-32γ isoform is described as a less active pro-inflammatory mediator and that IL-32γ splicing towards IL-32β serves as a rescue mechanism to reduce inflammation.
isoform was identified in human colonic subepithelial myofibroblasts (SEMFs) and named IL-32ε [31]. This isoform was enhanced in the inflamed mucosa of inflammatory bowel disease (IBD) patients. Currently, the exact mechanisms underlying IBD are also not completely known. Previously unidentified mediators such as IL-32ε or components of the gut microflora have lately received increasing attention in an attempt to understand the cascades of inflammatory pathways during IBD [32,33]. Accordingly, Netea et al. found that muramyl dipeptide (MDP), a peptidoglycan fragment from bacteria and a potent NOD2 ligand, could induce the expression of IL-32 in a caspase-1-dependent manner. Eventually, this would result in an increased production of IL-6 and IL-1β [20,34]. Interestingly, NOD2 mutations in Crohn's disease (CD) patients were found to cause enhanced IL-1β and NF-κB activity, importantly contributing to disease onset and its activity. These findings suggest an important role for IL-32 in the progression and pathophysiology of IBD, particularly in Crohn's disease (CD) [35]. In IBD, IL-32 also correlates with TNFα levels. TNFα by itself was shown to induce the IL-32ε isoform in these patients in a dose- and time-dependent manner. However, IL-32ε was found to reduce TNFα-induced IL-8 transcript, whereas IL-32α did not show this effect on IL-8 levels in the transfected human colon cancer cell line HT-29 [21,31]. Additionally, in IL-32γ transgenic mice, IL-32γ seemed to cause a greater and faster acute inflammation compared to wild-type mice. However, there was a lower amount of colonic inflammation and better survival rate upon dextran sodium sulphate (DSS)-induced colitis in these mice [32,36]. The lower amount of inflammation could be explained by splicing of IL-32γ into IL-32β, which can induce IL-10 and creates a more anti-inflammatory response [37]. Besides this, IL-32 in general not only stimulates the production of pro-inflammatory cytokines from monocytes, but also causes these monocytes to differentiate into macrophages or dendritic cells [38]. Moreover, IL-32 can stimulate neutrophils directly to produce IL-8 and IL-6 and indirectly recruits T cells to inflamed areas in CD and IBD by production of other pro-inflammatory cytokines by differentiated macrophages and DCs. Eventually, infiltrating neutrophils will release a variety of neutrophil proteinases resulting in mucosal tissue damage, augmenting inflammation in CD and IBD patients [21]. These data show a wide variation in the effects of IL-32 isoforms on disease progression and pathophysiology of IBD, and open new areas of investigation into the pathophysiology of this disease. It has been reported that IL-32 plays a role in chronic obstructive pulmonary disease (COPD). COPD is described as a progressive destructive disease occurring as an inflammatory response to toxic particles or gases, mostly linked to excessive smoking. It is characterized by airflow obstruction and persistent inflammation with more than 90% of cases currently being caused by chronic cigarette smoking [39]. Nevertheless, recent data shows a significant prevalence of COPD in non-smokers, suggesting the contribution of other factors to the pathogenesis of this disease, including environmental factors [40]. Pathogenically, COPD is also not fully elucidated. However, it is known that immune cells such as macrophages, T cells and neutrophils are important players in the disease. Moreover, epithelial cells are described to be the major source of inflammatory mediators in COPD. These cells are also known to produce IL-32. Various factors including infection, proteases and smoking can activate epithelial cells, triggering them to produce growth factors as well as pro-inflammatory mediators, such as TNFα, IL-12 and chemokines [41]. It has been indicated that IFNγ is able to induce IL-32 in human epithelial cells in a time-dependent manner [16]. Moreover, in human bronchial epithelial cells, IFNγ induced IL-32 expression via the signaling pathway involving c-Jun N-terminal kinases (JNK) but the induction was independent of p38 or mitogen-activated protein kinase (MEK) [39]. Interestingly, smoking can also modulate IL-32 in epithelial cells in the airways. Accordingly, COPD-affected smokers had higher IL-32 protein and mRNA levels in lung tissue than non-COPD smokers and non-smokers [22]. A correlation was shown between IL-32 lung tissue levels and airflow obstruction in vivo. Increased IL-32 expression
levels showed a clear increase in immune response in COPD. Finally, another study showed oxidative stress augmented the earlier described IFN$\gamma$-induced IL-32 expression in human bronchial epithelial cells [39].

Taken together, these studies underline the potential pathophysiologic role of IL-32 in various chronic inflammatory diseases. In general, many IL-32 effects are TNF$\alpha$ dependent, but also direct pro-inflammatory actions of IL-32 have also been suggested. It is of high importance to elucidate the exact role of IL-32 in the pathologic disorders presented above and other chronic inflammatory processes.

4. Chronic inflammation and cardiovascular disease

Cardiovascular (CV) morbidity and mortality are likely to be increased in patients suffering from chronic inflammatory conditions, e.g. RA, IBD and COPD. Traditional CV risk factors, however, do not fully account for the increased CVD risk in these patients. Only in the last decade, a clear relationship between chronic and systemic inflammation and an increased CV risk has been established. An increased burden of atherosclerosis in these patients has been indicated to be responsible for the observed higher CV risk. Subsequently, common pathophysiologic pathways to be shared by atherosclerosis, as well as RA, IBD and COPD as inflammatory conditions, have been hypothesized to explain the association of the latter three with CVD. Atherosclerosis is increasingly seen as an active inflammatory disease in which immune cells and systemic markers of inflammation such as circulating antibodies, pro-inflammatory cytokines such as IL-6, TNF$\alpha$ and IL-1$\beta$ and immune complexes play an important role in blood vessel pathology. The hypothesis behind atherosclerosis being driven by inflammation is consistent with the composition of the plaque and unstable coronary lesions in which inflammatory cells and immune cells are present at the shoulder region [42]. Many cell types that are involved in both early and late-stage atherosclerosis also play a role in the onset of RA, including endothelial cells, monocytes and macrophages, B and T cells [43]. Additionally, increased pro-inflammatory cytokine production and alterations in the Th1/Th2 cell ratio occur both in atherosclerosis and chronic inflammatory diseases such as IBD and COPD [44]. Moreover, during chronic inflammatory conditions such as RA, IBD and COPD, a non-resolving inflammatory response is present in the human body. This involves a dysbalance between various anti-inflammatory or resolution factors and pro-inflammatory cytokines/mediators. In more detail, this non-resolving status can be caused by impaired function or numbers of anti-inflammatory or regulatory cells, inadequate production of resolution factors such as IL-10, persistence of initiating stimuli like lipids or excessive inflammatory responses that form a positive feedback loop, maintaining inflammation [45,46]. Over time, this leads to endothelial damage of the arterial wall

Fig. 3. Schematic overview of the link between chronic inflammation–IL-32 and cardiovascular disease. Chronic inflammation is the cause of these three diseases (RA, COPD, IBD) and is characterized by a persistent pro-inflammatory state. Various immune cells are involved, which produce a wide variety of cytokines including IL-6, TNF$\alpha$, IL-1$\beta$, IL-17, but also IL-32. These cytokines all contribute to changes in lipid levels and lipid composition resulting in an increased risk for cardiovascular diseases.
initiating and causing progression of atherosclerosis.

In acute coronary syndromes, plaques abound in IFNγ, which is mainly produced by CD4+ T cells that lack the co-stimulatory molecule CD28 [47]. CD4+ T cells lacking CD28 are clonally expanded in acute coronary syndromes and are known to invade unstable atherosclerotic plaque regions resulting in weakening of the fibrous cap. Besides, CD4+ T cells can kill endothelial cells in vitro, maybe leading to the described endothelial injury in coronary plaques. Correspondingly, these CD4+ T cells lacking CD28 are also present in peripheral blood of RA patients, secreting Th1 associated-cytokines and possibly contributing to atherosclerotic damage in these patients too [47,48]. Moreover, T cells can be skewed toward the production of type II interferons (IFN) such as IFNγ. Together with the well known effects of matrix metalloproteinases (MMPs), these cells play a major role in joint destruction as well as rupture of unstable plaques [43].

The effects of immune dysregulation and chronic inflammation are associated with endothelial activation and dysfunction, another important contributor to atherosclerosis. When the endothelial cells are exposed to these factors, the cells start to express adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1), selectins and vascular cell adhesion molecule 1 (VCAM-1) on their cell surface [49]. Expression of adhesion molecules is induced by pro-inflammatory mediators like TNFα, CRP and IL-1β, which are also elevated in RA. In this respect, high-sensitivity CRP (hsCRP) has been shown to be an independent risk factor for stroke and myocardial infarction. Whether or not CRP has biological effects on the development of atherosclerosis remains complex. Data on hsCRP and its role in inflammation is massive and suggests a role for hsCRP in the increased risk for atherosclerosis and CVD. Additionally, CRP may induce complement system activation and endothelial cell production of VCAM-1, ICAM-1, IL-6, monocyte chemotactic protein 1 (MCP-1) and endothelin-1 (ET-1) [50].

Another pro-atherogenic property of CRP is its capacity to stimulate LDL uptake by macrophages, favouring foam-cell formation. However, Mendelian randomization studies have shown that hsCRP is not a causal factor of CVD and even though it can be a useful biomarker for risk prediction, hsCRP itself is unlikely to be a target for intervention [51,52].

Next to these above-mentioned mechanisms, other very important risk factors related to inflammation and auto-immunity have been described as possible players in atherosclerosis. This also includes endogenous self-molecules such as oxidized low-density lipoprotein (ox-LDL), heat shock proteins (hsp), antibodies against high-density lipoprotein cholesterol (HDLc) and apolipoprotein A-1 (ApoA-1). The latter two lipoproteins are known as anti-atherogenic, and therefore antibodies against them are thought to favour the development and progression of atherosclerosis [42].

In general, cholesterol is an essential lipid in vertebrates, which is crucial in, for example, cellular membranes, and cholesterol metabolites like steroid hormones are important in physiological functions [53]. Moreover, without the presence of excess cholesterol in the circulation and cholesterol accumulation in the vessel walls, there would be no atherosclerosis. Various types of lipoproteins and cholesterol take part in this process, with LDL cholesterol (LDLc) being a well-known mediator contributing to CVD. Traditionally, the atherogenic lipid profile is made up of increased total cholesterol (TC), LDLc, triglycerides (TG), and decreased HDLc. In chronic inflammatory diseases such as RA, however, different concentrations of lipids can be found throughout different stages of the disease: increased TC and LDLc in the years prior to disease onset, reduced levels of TC and HDLc during early active disease, different patterns in established RA [54,55]. It can, therefore, be concluded that inflammation is able to directly affect cholesterol levels [56]. Perhaps even more interesting, the ongoing inflammation is able to modulate the cholesterol composition and may shift it to a more pro-atherogenic profile. In line with this, multiple groups have shown that HDL becomes less anti-atherogenic in RA patients and this is associated with an inflammatory status [57,58]. Therefore, during chronic inflammatory conditions, HDL may lose its anti-atherogenic properties. Some of these functions include the capacity of HDL to prevent oxidation of LDL, preventing inflammasome activation and suppressing the expression of adhesion molecules such as VCAM-1 on arterial endothelial cells. Unfortunately, it is known that HDL levels may not reliably predict its composition and function and that cholesterol efflux capacity is a better marker for HDL function [59]. Besides HDL, other cholesterol structures such as cholesterol crystals (CC’s), which are formed upon accumulation of free, unesterified cholesterol inside the cells, also play a role in initiation of atherosclerosis [60]. Cholesterol crystals can actually be found in all stages of atherogenesis and are present in early atherosclerotic lesions [61]. They can be phagocytosed by macrophages, which can result in lysosomal damage and then trigger the activation of the NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome. Eventually, this leads to activation of caspase-1 and activation and secretion of IL-1β family cytokines [61,62]. Oxidized LDL particles can also activate the NLRP3 inflammasome and together with CC are potent inducers of IL-1β. Several studies involving IL-1β deficiency in atherosclerotic mouse models have shown attenuated disease progression, suggesting that cholesterol crystal-induced NLRP3 inflammasome activation and IL-1β production can drive atherogenesis [63,64].

Taken together, these studies support the important implication of inflammation to the development of atherosclerosis and cardiovascular diseases (CVD), both directly as well as by modulating other organs (endothelial cells) and factors (lipoproteins) on which the process of atherosclerosis is dependent. Various chronic inflammatory conditions such as RA, IBD and COPD are likely to share common pathophysiologic pathways with atherosclerosis, possibly explaining the increased CVD morbidity and mortality in these patients.

5. The role of IL-32 in cardiovascular diseases/atherosclerosis

As mentioned before, atherosclerosis is a chronic inflammatory process being the most studied determinant of CV morbidity and mortality. Besides inflammation, risk factors like dyslipidemia also play a role in progression. Knowing that inflammatory pathways involved in the pathogenesis of chronic inflammatory diseases like RA, IBD and COPD accelerate atherosclerosis, it is important to study the role of the newly described pathways and the corresponding cytokines involved also in the context of CVD and atherosclerosis.

As already described, IL-32 is a pro-inflammatory cytokine involved in the pathogenesis of RA. It promotes inflammation by induction of other pro-inflammatory cytokines like IL-6, IL-1β, IL-8 and TNFα. Moreover, these cytokines are known to play a role in atherogenesis. Additionally, in RA, as well as IBD and COPD, pro-inflammatory cytokine TNFα is an important mediator of the disease. TNFα by itself is known to be associated with CVD and atherosclerosis [67,68]. Furthermore, it was described previously that IL-32 can induce TNFα and vice versa, creating a positive inflammatory loop which might cause a non-resolving inflammatory response contributing to a pro-atherogenic environment. In this way, IL-32 by itself, and via induction of other pro-inflammatory cytokines, is suggested to maintain an inflammatory response.

Besides this role for TNFα in atherogenesis, IL-6 is known to contribute to thrombocytosis whereas IL-8 recruits immune cells to the endothelial intima during all stages of plaque formation [69]. IL-
1β plays a role in atherogenesis by acting on various aspects involved in vascular inflammation. The endothelium is the primary target of IL-1β by which its pro-inflammatory capacities influence systemic inflammation. Additionally, IL-1β sets off the production of IL-6, inhibitors of fibrinolysis, prostaglandin E2 (PGE2) and adhesion molecules in the endothelium, all of them participating in pathological conditions [70]. Within these processes, an unexpected role for IL-32 was found. When IL-32 levels were decreased by siRNA, the pro-coagulant, pro-inflammatory and cytotoxic effects of IL-1β, like IL-1β-induced ICAM-1 production were also reduced remarkably [11]. Therefore, IL-32 is hypothesized to also be an important player in atherosclerosis. IL-32 expression was detected in arterial vessel walls and another study showed expression in endothelial cells induced by Akt, a protein kinase B involved in apoptosis, cell proliferation, transcription and glucose metabolism [12,71]. In hematopoietic cells, IL-32α is the most abundant form, however, in endothelial cells, IL-32β was found mostly present [16,71]. Knowing that the vascular endothelium is an important mediator in inflammation, high levels of a pro-inflammatory isoform of IL-32 could contribute to its inflammatory state.

In addition to that, IL-32 is expressed in macrophages, which are highly present in atherosclerotic lesions. Macrophages showed a highly increased mRNA and protein expression of IL-32 when stimulated with pro-inflammatory mediators that are known to be involved in atherosclerosis, including interferon-gamma (IFNγ) and a Toll-like receptor (TLR3) ligand Poly I:C [72,73]. Moreover, macrophages are key players in controlling cholesterol levels in blood vessel walls. They take up cholesterol, can turn into foam cells, and participate in the reverse cholesterol transport (RCT) by expressing functional ATP-binding cassette transporter A1 (ABCA1) and ATP-binding cassette transport G1 (ABCG1) [74]. Since macrophages are able to express high levels of IL-32 and in the same time actively regulate circulating cholesterol levels, one could hypothesize that IL-32 may be somehow related to cholesterol concentrations. Possibly, the intra-cellular capacities of IL-32 can interfere with the regulation of cholesterol transporters or foam cell formation and therefore influence circulating cholesterol levels. Despite previous work describing the various isoforms of IL-32, the function of each isoform is not completely clear yet. In addition, not a lot is known about genetic mutations in IL32, especially at functional level. Recently, it was described that a single nucleotide polymorphism (SNP) in the promoter region of IL32 that seems to affect the expression of the isoforms in peripheral blood mononuclear cells (PBMCs). RA patients with this SNP had lower basal levels of IL-32β compared to RA patients with the other genotypes. This was also linked to lower cytokine production of PBMCs after stimulation [75]. Intriguingly, it was also demonstrated that this promoter SNP in IL32 is associated with higher levels of high-density cholesterol (HDLc) [76]. This mutation in the IL32 gene could therefore contribute to a lower risk for CVD in patients with chronic inflammatory diseases like RA and IBD in which IL-32 is known to play a role.

6. Concluding remarks and future perspectives

In this review, important advances in the field of chronic inflammation and cardiovascular diseases with respect to IL-32 are discussed (Fig. 3). A light was shed on the role of a relatively new cytokine IL-32 in chronic inflammatory disorders. On the basis of recent reports, IL-32 may prove to be a crucial player when it comes to reducing the burden of systemic inflammation in the future. Another important finding was the identification of a genetic polymorphism in the promoter of IL-32, which affects cholesterol metabolism, possibly decreasing CVD risk. Interestingly, this SNP also affects the isoform expression in PBMCs, including macrophages. Future studies should be focused on whether individuals bearing this SNP would be less prone to develop cardiovascular diseases. In addition, studies on the function of IL-32 in cholesterol metabolism should be initiated well in the near future. Of high interest, possible therapeutic effects of specific inhibitors against IL-32 isoforms to interfere with the development of atherosclerosis should be considered.

In conclusion, the above results not only provide novel insight in the cytokine IL-32 and its role in inflammation, but also propose important reasons why IL-32 should be taken into account when studying the development of cardiovascular diseases. Eventually, this could expand our understanding of the processes underlying CVD in both health and disease.

Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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