IL-1R2: A novel approach for gene therapy in atherosclerosis

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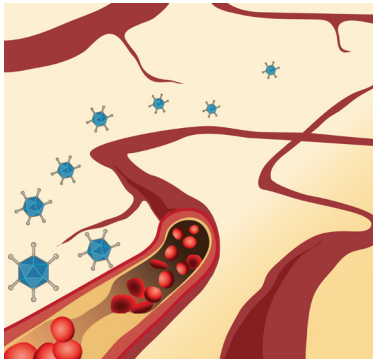

Illustration by Eloïse Kremer

ABSTRACT Atherosclerosis, a chronic inflammatory disease, is the result of an inflammatory process in the arterial intima. Pro- and anti-inflammatory cytokines and their receptors are involved in the atheromatous plaques. Interleukin-1 (IL-1) has been considered a pro-inflammatory cytokine in atherogenesis. IL-1α and IL-1β are members of the IL-1 family functioning as agonist molecules while IL-1 receptor antagonist (IL-1Ra) acts as antagonist. When IL-1α or IL-1β bind to type 1 IL-1 receptor (IL-1R1), an intracellular signal is activated, which can lead to the up-regulation of a mix of pro-inflammatory cytokine genes. By comparison, binding IL-1 to type 2 IL-1 receptor (IL-1R2) does not lead to intracellular activation. Therefore it is assumed to be a decoy receptor. IL-1R2 has been implicated in a number of inflammatory disorders, but so far no research has indicated IL-1R2 as a factor in the treatment of atherosclerosis. In the present hypothesis, it is proposed that IL-1R2 is useful in atherosclerosis treatment through neutralizing the IL-1 inflammation pathway in a mouse xenograft model of atherosclerosis.

INTRODUCTION Atherosclerosis-related cardiovascular circumstances lead to fatality in nearly 50% of cases in developed countries[1]. Atherosclerotic vascular disease is one of the main factors in myocardial infarction, stroke, unstable angina, and sudden cardiac death whose incidence has increased recently. The disease is initiated by the subendothelial retention of apolipoprotein B containing lipoproteins in focal areas of arteries and can activate inflammatory response. This response will stimulate the accumulation of endothelial and vascular smooth muscle cells (SMCs), monocytes, and lipid material in the arterial subendothelial space, or arterial intima. At the molecular level, many cells from the immune system, such as monocyte-derived macrophages, T cells, B cells, dendritic cells, and mast cells, as well as SMCs, contribute to myofibroblast formation. Furthermore, the atheroprone endothelium produces pro-inflammatory cytokines and chemokines, extracellular matrix proteins, and growth factors, which can promote a local proatherogenic environment. The pro-inflammatory cytokines secreted by type 1 CD4+ T-helper cells (TH1 cells) include IL-1, IL-2, IL-12, interferon-γ (IFN-γ), tumor necrosis factor (TNF)-α, and TNF-β and are involved in atheromatous plaques[2,3]. There are some novel and promising therapeutic approaches including antagonists/inhibitors of specific receptors, gene silencing via nanoparticles, blocking antibodies against chemokines or their receptors, and decoying ligands through binding to the receptors, which cannot

cause activation[4]. Studies of atherosclerotic plaques have distinguished a large indefinite number of pro-inflammatory cytokines. Cytokines of the IL-1 family have drawn more attention due to their early identification in human atherosclerotic plaques and investigations of mouse models of atherosclerosis[5]. Monocytes/macrophages, neutrophils, vascular endothelial cells, and SMCs are the main sources of IL-1[6]. IL-1α and β make lesion formation easier, through enhancing leukocyte adhesion and transmigration[7]. Although IL-1α has not often been taken into account, its role in atherosclerosis should not be ignored. The deletion of IL-1α creates more protection (about 60%) against atherosclerosis in ApoE–/– mice than the deletion of IL-1β (about 30%)[8]. IL-1 signaling is originated by the binding of IL-1α or IL-1β to IL-1R1. Following this binding, the IL-1 receptor accessory protein (IL-1RAP), with a greater affinity to MyD88 as an adaptor protein, forms a complex for signaling *(FIG 1)*. This process activates phosphorylation of the IL-1 receptor-associated kinase (IRAK) 4, which subsequently phosphorylates both IRAK1 and IRAK2 at residues. It leads to their interaction with the downstream adaptor tumor necrosis factor receptor associated factor-6 (TRAF-6), eventually resulting in the activation of the transcription factor NF-κβ [9]. IL-1R2 inhibits IL-1 activity by a variety of mechanisms[10]. First, membrane IL-1R2 plays a role as a non-signaling decoy receptor which can reduce IL-1 function by removing IL-1 from the signaling pathway of type I receptor[10]. Second, IL-1R2 hinders the binding between IL-1RAP and IL-1R1[11]. Third, the low binding affinity of IL-1R2 for the endogenous antagonist IL-1Ra lets these molecules to act in a synergistic way. Finally, soluble IL-1R2 (sIL-1R2) can lead to reducing IL-1's interaction with IL-1R1 by binding to IL-1, and therefore, provides another mechanism to decrease IL-1 bioactivity[12]. As for the ligands, IL-1R2 has a different capacity. IL-1R2 binds to IL-1β with a higher affinity than IL-1α (about 100×). However, it shows far lower binding affinity to the antagonist IL-1Ra. Meanwhile, sIL-1R2 practically

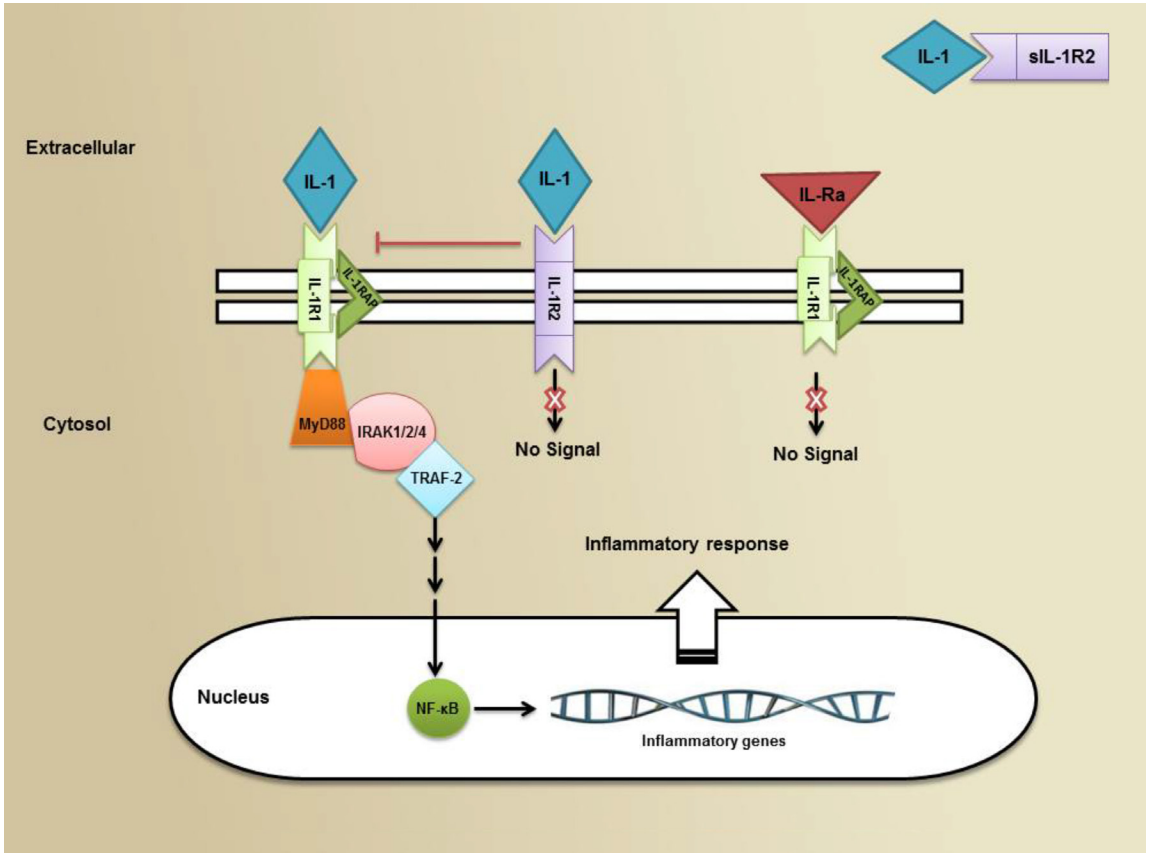

Figure 1 | A schematic design of the IL-1 signal transduction pathway and role of IL-1R2 in inhibiting IL-1 activity.

cannot bind to the IL-1Ra[13]. It indicates that sIL-1R2 is a better inhibitor of IL-1 than membrane IL-1R2, as it cannot bind to the antagonist ligand. Also, sIL-1R2 has been considered in the plasma of healthy individuals[14]. In addition, IL-1R2 has been mainly found on neutrophils, B-cells, monocytes and macrophages[15] which suggests another advantage of sIL-1R2. Macrophages from patients with familial combined hyperlipidemia lowered IL-1R2 mRNA and protein expression[16]. However, no research has examined the usefulness of exogenous IL-1R2 in atherosclerosis treatment[17]. In this hypothesis, it is proposed that IL-1R2 plays a role in modulating atherosclerosis via inhibiting IL-1α and β pathways in an atherosclerotic mouse model.

HYPOTHESIS Atherosclerosis is one of the most common causes of mortality in western countries[18]. Therefore, it still remains as a challenging disorder and needs efficient treatment strategies. Thus, taking advantage of gene therapy and cell therapy approaches, which have been emphasized in many different malignancies and diseases[19-25], can be helpful in the treatment or management of atherosclerosis as promising therapeutic methods.

In this hypothesis, we introduce the IL-1 neutralizing method as a novel target for atherosclerosis treatment. In fact, it is proposed that the inflammation pathway can be suppressed by IL-1R2 overexpression because of the inhibition of the IL-1 pathway. The basis of this proposal is to induce *IL-1R2* gene expression in the THP-1 monocytoid cell line and inject this cell line into a xenograft system so as to block the IL-1 inflammation cascade *(FIG 2)*.

EVALUATION OF HYPOTHESIS

- Recombinant adenoviruses: The cDNA sequence coding for the extracellular portion of IL-1R2 is proposed to be cloned by RT-PCR from mouse 70Z3 B cell mRNA. Having been confirmed by bidirectional sequencing, the cDNA will be subcloned in frame with an XbaI fragment, encoding the constant and hinge mIgG1 region (Fc-g fragment). The obtained cDNA sequence encoding the IL-1R2 fusion protein is likely to be subsumed to the transcriptional control of a CMV promoter into pC5 plasmid, yielding pC5-IL-1R2. Adenoviral vectors will be made using a new method grounded on direct DNA ligation[26]. Recombinant adenovirus AdsIL-1R2 can be created by co-transfection of pC5-sIL-1R2 with PacI-restricted DNA from AdP-EGFP, a Δ1-deleted recombinant adenovirus including a unique PacI site[26].
- Cell culture: Human THP-1 monocytoid cell line (TIB#3456, ATCC, MD, USA) will be maintained at 37°C and 5% CO_2 in HEPES buffered RPMI-1640 (Sigma Aldrich, St Louis, MO, USA) medium with 10% heat inactivated FBS, 50 µg/mL gentamicin and 100 U/mL penicillin (pH 7.2). THPs can be genotyped by ATCC to exclude contamination with other cell lines. Cells will be passed so that cell counts cannot exceed 106/ml.
- Transfection of hTHP-1 with ad-sIL-1R2
- Atherosclerotic xenograft in mice: C57BL/6 Apolipoprotein E-deficient (ApoE-/-) mice (Charles River Co.) are ideal models for atherosclerosis study. C57BL/6 ApoE-/- will be given low fat diets to induce atherosclerosis. Twenty C57BL/6 ApoE-/- mice will be prepared and divided into two classes of 10 animals each:
  (a) The first group, as control group, includes atherosclerotic animals, which will receive equal volume of normal saline.
  (b) The second group will be selected for cell-line inoculation and Ad– IL-1R2. Cells (5 × 106) in 200 µl of suspension mixture will be injected intravascularly in C57BL/6 ApoE-/- mice. The mitigation of atherosclerotic lesions will be measured weekly.
- The detection of sIL-1R2 expression in vitro: sIL-1R2 expression in macrophage cells pre-incubated with either AdsIL-1R2 will be measured by ELISA.
- The detection of sIL-1R2 functional activity in vitro: An in vitro functional assay will be used to show specific IL-1 inhibition by sIL-1R2[27].
- Detection of sIL-1R2, IL-1α, IL-1β, and IL-1Ra protein levels in vivo: Plasma levels of sIL-1R2, IL-1α, IL-1β, and IL-1Ra will be measured by ELISA[28,29].
- The absence/presence of atherosclerotic lesions will be supported by conventional Masson's trichrome or hematoxylin and eosin staining[30].

DISCUSSION AND CONCLUSION

Atherosclerosis is a chronic inflammatory disorder that is the rudimentary cause of most cardiovascular diseases. Endothelial dysfunctions, in addition to subendothelial lipoprotein retention, are the main factors in atherosclerosis initiation and progression. These stimulators can turn on the inflammatory response of the immune system leading to arterial thrombosis and end-organ ischemia[2]. At the molecular level, oxidized LDL, heat shock proteins, and infections have been identified as the triggers of

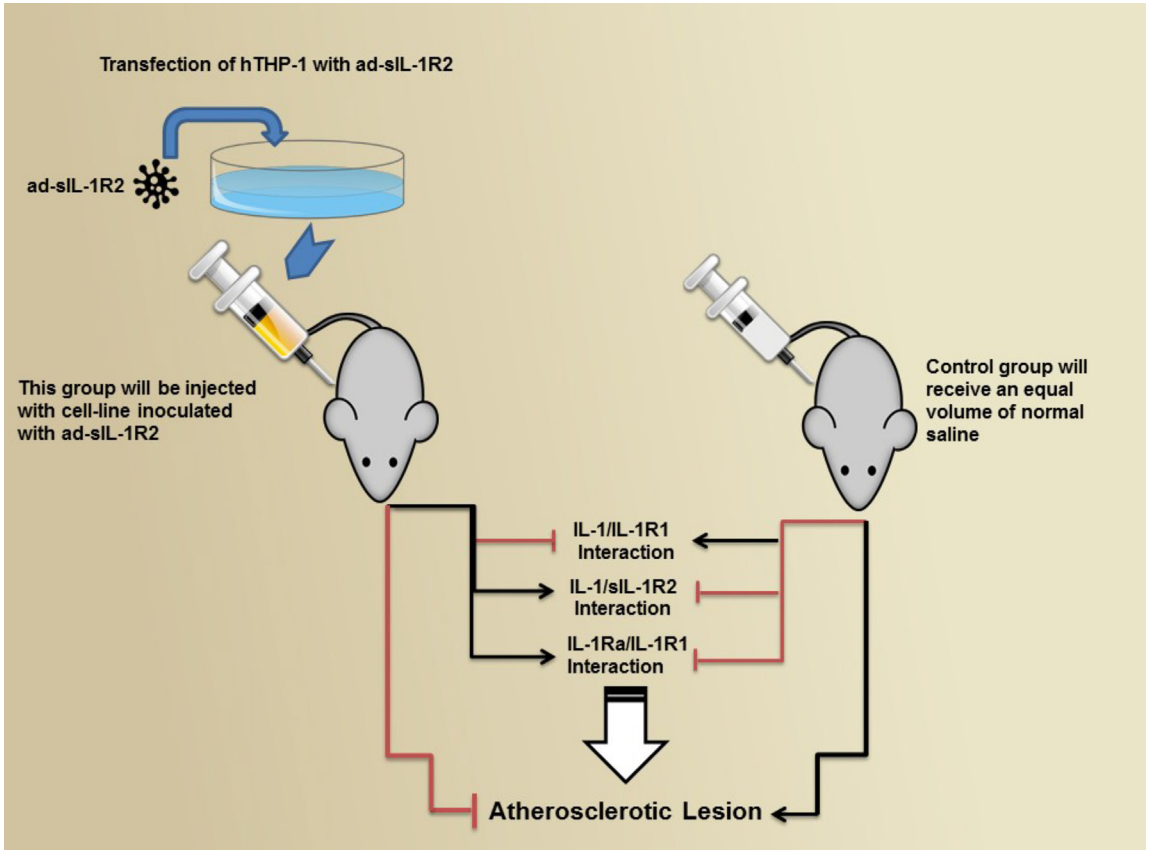

Figure 2 | A schematic design of the proposed experimental procedure indicating IL-1R2 as a decoy receptor in the gene therapy of atherosclerosis.

atherosclerotic inflammation[31]. Many vascular, metabolic, and particularly immune system molecules including T lymphocytes, macrophages, and activated platelets expressing P-selectin are involved at different stages of atherosclerosis. In addition, cytokines have pro-inflammatory (Th1-related cytokines) and anti-inflammatory (regulatory T cell–related cytokines) roles in atherosclerosis[32,33]. Many chemokines and cytokines, such as TNF-α, IL-1, IL-2, IL-3, IL-6, CXCL8, IL-10, IL-12, IL-15, IL-18, IFN-γ, M-CSF, transforming growth factor-β (TGF-β)1, TGF-β2, and TGF-β3 have been considered within atherosclerosis-prone vessels[33]. Atherosclerosis treatment includes classic medicines such as allopurinol, colchicine, methotrexate, and biologic therapies such as tumour necrosis factor inhibitors, as well as targeting of lipid mediators or intracellular pathways. The documents support the use of anti-inflammatory therapies for atherosclerosis[34]. In this hypothesis, it is suggested that IL-1R2 may be useful as another therapeutic target. Therefore, we propose that IL-1R2 as a decoy receptor can suppress the main inflammation pathway triggered by IL-1. The role of the IL-1 family in the pathogenesis of atherosclerosis has been detected in previous studies[5]. The IL-1/IL-1Ra ratio has a significant role in the pathogenic pathways of vascular inflammation and atherosclerosis in ApoE -/- mice[35]. IL-1, TNF-α and IFN-γ, promote SMCs and macrophage apoptosis. IL-1 also induces the expression of matrix metalloproteinases (MMPs)[32]. According to previous studies, MMPs are the main proteases in atherogenesis[36]. Mouse models of atherosclerosis have shown the proatherogenic properties of IL-1α and IL-1β, apart from the upregulation of endothelial adhesion molecules and activation of macrophages and vascular cells. IL-1Ra, a natural antagonist of IL-1R1, has anti-inflammatory properties, via the endogenous suppression of the IL-1 pathway. IL-1 receptor acts via MyD88, which contains a death domain, accelerating its interaction with IRAK proteins. IRAK-2 and IRAK-4 are critical signaling molecules

in the IL-1 receptor/Toll-like receptor family[32]. By activating IL-1R1, IL-1 acts as the main factor causing inflammatory response. IL-1R2 works as a molecular trap for agonist ligands of IL-1R1, and is effective in modulating inflammatory and adaptive responses intermediated by the IL-1 pathway. The anti-inflammatory role of IL-1R2 has been confirmed by in vivo studies. The overexpression of *IL-1R2* under the control of the human keratin gene promoter was protective in the murine models of PMA-induced chronic skin inflammation[37]. In a mouse model of collagen-induced arthritis, overexpressing sIL-1R2 played a protective role[38]. Recently, in an endometriosis mouse model composed of human endometrial tissue implanted in mice, human sIL-1R2 decreased the development of endometrial implants and inflammation[39]. In another study, investigators in a model of arthritis in rabbit demonstrated that endovenous administration of sIL-1R2 remarkably lowered joint swelling and corrosion[40]. In a rat model of heart transplantation, as a result of decreased penetrating macrophage CD4+ T cells and lowered level of TNF-α, TGF-β and sIL-1R2, as well as the inflammation and allograft rejection, were reduced[27]. IL-1R2, by blocking IL-1 and inhibiting the production of Th17 cells, IL-6, and TGF-β, controlled experimental autoimmune myocarditis[41]. As a whole, P-selectin inhibition, anti-interleukins, tumor necrosis factor antagonists, viral-derived serpins, and leukotriene synthesis inhibition have recently appeared as possible therapeutic approaches for atherosclerotic disease[42]. In this study, IL-1R2 is suggested to be used as a novel therapeutic target in atherosclerosis treatment. It is concluded that a proper animal model of atherosclerosis may be useful to assess the effect of IL-1R2 in the control or full recovery of atherosclerosis. IL-1 neutralization can suppress inflammatory response from the immune system and control this inflammatory disease by the overexpression of *IL-1R2*. The inhibitory effect of IL-1R2 on the IL-1 inflammation pathway can be assessed in a clinical trial as the next step in the evaluation of this hypothesis. In addition, the combinational therapy of IL-1R2 method can be more useful apart from other chemical and biological techniques. **END**

CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

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