Carotid artery wire injury model, a neutralising IL-11 antibody inhibits vascular hyperplasia

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ABSTRACT

In individuals with Coronary Artery Disease (CAD) and Peripheral Artery Disease (PAD), vascular restenosis is still a substantial issue (PAD). A key underlying disease is neointimal hyperplasia, which is characterised by post-procedure proliferation and migration of Vascular Smooth Muscle Cells (VSMCs). Interleukin 11 (IL-11) was examined in a mouse model of injury-related plaque formation. Apoe/ mice were fed a hyperlipidemic diet and had their right carotid wire injured. Anti-IL11 antibody (X203), IgG control antibody, or buffer were administered into mice. We used ultrasound to determine the thickness of the vessel wall and the rate of blood flow. When compared to control, X203 treatment reduced post-endothelial injury vessel wall thickness and injury-related plaque in mice with carotid wire injury. In compared to the control, immunofluorescence staining of the injury-related plaque revealed that X203 therapy reduced the number of VSMCs, lowered Matrix Metalloproteinase 2 (MMP2) levels, and decreased collagen content. In comparison to control, X203 therapy was linked with a considerable increase in Smooth Muscle Protein 22 (SM22) positive cells in injury-related plaque, implying that the contractile VSMC phenotype was preserved. In contrast to IgG, X203 lowered the collagen content of undamaged carotid arteries, indicating that hyperlipidemia-induced arterial remodelling can occur even in the absence of mechanical injury.

Key Words: *Coronary artery disease; Vascular smooth muscle cells*

INTRODUCTION

Vascular restenosis remains a major concern in patients with Coronary Artery Disease (CAD) and Peripheral Artery Disease (PAD) despite breakthroughs in stent design and revascularization therapy (PAD). In-stent restenosis can cause serious problems such heart ischemia and chronic limb ischemia, which necessitates the development of new therapeutic measures to prevent these issues. Switching from a contractile to a synthetic phenotype in Vascular Smooth Muscle Cells (VSMCs) is a primary contributor to neointimal hyperplasia, the fundamental disease driving vascular restenosis. The primary role of VSMCs, which are situated in the medial layer of the vasculature, is to regulate vascular tone and blood pressure. VSMCs proliferate, move into the tunica intima, and adopt a synthetic phenotype in response to vascular injury, which is an adaptive response but resulting in vessel wall thickening. Extracellular matrix secretion characterises the synthetic VSMC phenotype, which leads to fibrosis and inflammation [1]. Phenotypic switching is a cellular transition to a synthetic phenotype that plays a major role in arterial restenosis, aortic remodelling, and the development of atherosclerosis.

Transforming Growth Factor-beta (TGF) and angiotensin-II are two major factors linked to VSMC phenotypic flipping and vascular diseases such atherosclerosis and arterial restenosis (ANGII). Many similarities exist between fibroblast-to-myofibroblast differentiation and VSMC phenotypic flipping, including extracellular matrix secretion, cell proliferation, and migration, and both transitions can be initiated by the same stimuli [2].

MATERIAL AND METHODS

Mouse husbandry

The Biomedical Sciences Institute Singapore Institutional Animal Care Committee at A*STAR authorised all of the experiments (161165). Mice were fed a lipid-rich Western-Type Diet (D12079B, Research Diets, NJ) and were kept on a 12 h dark–light cycle with ad libitum access to water. Plasma was extracted from blood taken from the orbital sinus in EDTA-coated capillary tubes for all studies. Cobas c111 was used to determine plasma Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C), Triglycerides (TG), and total cholesterol (Roche Diagnostics, Switzerland).

Wire injury to the carotid artery as a model of vascular restenosis

Male Apoe/mice aged 10 to 12 weeks (C57BL/6J background, Charles River Laboratory, Italy) were fed a lipid-rich Western-Type Diet17 for three weeks: one week before wire injury and two weeks after. To avoid oestrogen effects on injury-related plaque interfering with our target of interest, IL-11, only male mice were employed in this investigation. Mice were anaesthetized (100 mg/kg ketamine hydrochloride, 10 mg/kg xylazine i.p.) and endothelial denudation of the left common carotid artery was performed using a 1 cm insertion of a flexible 0.36 mm guide wire through a transverse arteriotomy of the external carotid artery, as previously described. As previously described, X203 was produced in mice using a cDNA encoding human IL-11 amino acids 22-199 cloned into expression plasmids (Aldevron Freiburg GmbH, Freiburg, Germany). Its effectiveness in arterial remodeling16, myocardial infarction, hepatic fibrosis, and pulmonary fibrosis has already been proven [3].

Carotid arteries and heart ultrasound measurements

During the measurements, mice were sedated with 2% isoflurane and monitored to keep their heart rates over 500 beats per minute. B-Mode and M-Mode measurements were taken. Using a 40 MHz transducer and a small-animal ultrasound imager (Vevo 3100, FUJIFILM Visualsonics, Toronto, Canada) as well as the VevoLab Software, velocities were recorded and measured in B-Mode (2D-realtime) using angle correction and vessel diameters (wall thickness) were recorded and analysed in M-Mode using a 40 MHz transducer and a small-animal ultrasound imager (Ve (FUJIFILM Visualsonics, Toronto, Canada) [4].

Analytical statistics

The data is presented as a mean standard deviation. Prism 6.1 software was used for statistical analysis (GraphPad). We utilised 1-way ANOVA followed by Tukey's multiple comparison test for analyses involving more than two groups. P values of less than 0.05 were deemed significant.

RESULTS

Treatment with X203 for two weeks had no effect on blood lipids

Wire damage was used to test the effect of IL-11 suppression on neointimal hyperplasia in mice that were given either control IgG, the anti-IL-11 antibody X203, or no therapy. We started with plasma lipids since circulating lipids

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play a significant role in arteriosclerosis and neointimal hyperplasia. There were no differences in triglyceride, total cholesterol, LDL cholesterol, or HDL cholesterol levels in mice treated for a short period of time. Furthermore, all treatment groups had equivalent liver transaminases, indicating that systemic anti-IL-11 antibody injection had no effect on plasma lipids or liver function during the length of the experiment [5].

The use of X203 reduced neointimal hyperplasia after a wire injury

The X203-treated group had much thinner walls than the controls, although there were no variations in blood flow velocity. Surprisingly, ultrasound tests of the right-side, undamaged carotid artery indicate considerable thinning following X203 therapy, despite no variations in velocity, confirming the X203 treatment's influence on arterial remodelling. In addition, when X203-treated mice were compared to controls, injury-related plaque and neointimal regions were considerably reduced. The tunica media area did not differ across the treatment groups [6]. We detected no alterations in total vascular area, intima, or media in the right, undamaged carotid arteries. One out of every control carotid arteries and one out of every IgG-treated carotid arteries produced native atherosclerotic plaques, whereas none of the X203-treated carotid arteries did.

The use of X203 had no influence on the development of post-endothelial damage. plaque infiltration caused by macrophage injury

Arteriosclerosis and vascular restenosis are complicated by inflammation and macrophages. As a result, we looked at how inhibiting IL-11 affected the amount of macrophages invading the injury-related plaque region. The proportion of macrophages and the absolute number of macrophages in the treatment groups were not different. Except for one carotid artery from the control groups presenting with natural atherosclerotic plaque, the right carotid arteries showed no staining or isolated subendothelial staining for macrophage marker Mac2.

The use of X203 reduced the buildup of VSMCs after endothelial damage

We studied the effect of IL-11 suppression on VSMC accumulation and phenotype in post-endothelial injury neointimal hyperplasia neointimal hyperplasia because of the essential role of VSMC flipping to a synthetic phenotype characterised by proliferation and migration. When compared to controls, X203 treatment decreased the number of VSMCs in the injury-related plaque and increased the number of VSMCs expressing SM22, a contractile marker, implying that the majority of VSMCs in the injury-related plaques of the X203-treated mice were contractile in phenotype, implying atheroprotection. The arterial wall of the right undamaged carotid arteries was mostly made up of vascular smooth muscle cells [7].

Treatment with X203 decreased plaque fibrosis caused by post-endothelial damage

IL-1115,16 has been shown to have pro-fibrotic effects in several recent investigations. We studied the effect of IL-11 suppression on MMP2 expression and collagen content in post-endothelial damage neointimal hyperplasia, which is characterised by the production of extracellular matrix [8]. In comparison to controls, X203 therapy reduced MMP2 expression and collagen content in the injury-related plaque, implying that inhibiting IL-11 is atheroprotective through lowering VSMC phenotypic shift [9]. Interestingly, after X203 treatment, the collagen content of the undamaged right carotid arteries decreased significantly, suggesting the anti-fibrotic function of X203 treatment and protection against arterial remodelling. The right carotid arteries may seem thinner during ultrasound measurements because of this [10].

DISCUSSION

In a carotid wire-induced endothelial damage mouse model, we show for the first time that inhibiting IL-11 lowers artery wall thickness and neointimal hyperplasia. Reduced accumulation of VSMCs, increased proportion of contractile VSMCs, lower MMP2 expression, and reduced collagen content in the injury-related plaque were all associated with favourable effects on post-endothelial injury injury-related plaque remodelling. In contrast to either IgG or buffer control mice, suppression of IL-11 reduced the collagen content of undamaged carotid arteries, indicating an additional effect on hyperlipidemia-induced arterial remodelling in the absence of mechanical injury. In diverse cell types involved in Extracellular Matrix (ECM) synthesis, autocrine IL-11 signalling is a significant downstream effector of TGF1 and ANGII. IL-11 is necessary for ERK-dependent myofibroblast activation in cardiac fibroblasts, lung fibroblasts18, and hepatic stellate cells. TGF1 stimulates the production of IL-11 by VSMCs in the aortic and coronary arteries. We recently uncovered an autocrine loop of IL-11 activity in VSMCs, which is required downstream of both TGF1 and ANGII for phenotypic switching to occur in the context of aortic modelling.

However, little research has been done on the involvement of IL-11 in phenotypic switching and function of VSMCs in the setting of neointimal hyperplasia following endothelial damage. Treatment with X203 has been shown to improve arterial remodelling in hypertension, cardiac fibrosis and healing following a myocardial infarction, liver fibrosis, and idiopathic pulmonary fibrosis. In control mice, carotid wire damage resulted in neointimal hyperplasia, as demonstrated by an increase in artery wall thickness and tunica intima area, while IgG therapy had no influence on these parameters. Treatment with the anti-IL-11 antibody X203 reduced vascular wall thickness and overall injury-related plaque area while having no effect on carotid artery velocity (with a reduction in tunica intima area but no effect on media area). Reduced numbers of VSMCs and higher expression of SM22, a marker for VSMCs with a retained contractile phenotype, were linked to a reduction in injury-related plaque area with X203 therapy. This research backs up our previous findings, which showed that genetic or antibody-mediated suppression of IL-11 reduced VSMC phenotypic flipping. Macrophages have been found as key contributors to vascular restenosis in several investigations. However, with X203 therapy, there were no variations in macrophage injury-related plaque infiltration, implying that the positive effects of suppressing IL-11 on lowering neointimal hyperplasia were independent of macrophage accumulation into the injury-related plaque at this timepoint. Furthermore, in the same study16, expression of the VSMC contractile marker SMA22 was reduced in response to IL-11 antibody therapy. Treatment with X203 decreased injury-related plaque MMP2 levels and increased injury-related plaque SMA22 levels, indicating a beneficial effect of IL-11 inhibition on vascular remodelling following wire-induced endothelial injury, which is consistent with a pathological role for IL-11 on VSMC function. Previous research has demonstrated that IL-11 is anti-inflammatory, anti-fibrotic, and pro-regenerative, and that it inhibits VSMC proliferation and plaque development in the vasculature, which is the reverse of what we show here. The use of recombinant human IL-11 in mouse models of disease is one cause for the widespread misunderstanding of IL-11 function. Surprisingly, rhIL-11 has recently been demonstrated to be a competitive inhibitor of mouse IL-11 in mouse cells, implying that much of the previous work may need to be re-examined.

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