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Interleukin 10 knockout frail mice develop cardiac and vascular dysfunction with increased age ☆

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ABSTRACT

Cardiovascular dysfunction is a primary independent predictor of age-related morbidity and mortality. Frailty is associated with activation of inflammatory pathways and fatigue that commonly presents and progresses with age. Interleukin 10 (IL-10), the cytokine synthesis inhibitory factor, is an anti-inflammatory cytokine produced by immune and non-immune cells. Homozygous deletion of IL-10 in mice yields a phenotype that is consistent with human frailty, including age-related increases in serum inflammatory mediators, muscular weakness, higher levels of IGF-1 at midlife, and early mortality. While emerging evidence suggests a role for IL-10 in vascular protection, a clear mechanism has not yet been elucidated.

Methods: In order to evaluate the role of IL-10 in maintenance of vascular function, force tension myography was utilized to access ex-vivo endothelium dependent vasorelaxation in vessels isolated from IL-10 knockout IL-10(tm/tm) and control mice. Pulse wave velocity ((PWV), index of stiffness) of vasculature was measured using ultrasound and blood pressure was measured using the tail cuff method. Echocardiography was used to elucidated structure and functional changes in the heart.

Results: Mean arterial pressures were significantly higher in IL-10(tm/tm) mice as compared to C57BL6/wild type (WT) controls. PWV was increased in IL-10(tm/tm) indicating stiffer vasculature. Endothelial intact aortic rings isolated from IL-10(tm/tm) mice demonstrated impaired vasodilation at low acetylcholine doses and vasoconstriction at higher doses whereas vasorelaxation responses were preserved in rings from WT mice. Cyclo-oxygenase (COX-2)/thromboxane A2 inhibitors improved endothelial dependent vasorelaxation and reversed vasoconstriction. Left ventricular end systolic diameter, left ventricular mass, isovolumic relaxation time, fractional shortening and ejection fraction were all significantly different in the aged IL-10(tm/tm) mice compared to WT mice.

Conclusion: Aged IL-10(tm/tm) mice have stiffer vessels and decreased vascular relaxation due to an increase in eicosanoids, specifically COX-2 activity and resultant thromboxane A2 receptor activation. Our results also suggest that aging IL-10(tm/tm) mice have an increased heart size and impaired cardiac function compared to age-matched WT mice. While further studies will be necessary to determine if this age-related phenotype develops as a result of inflammatory pathway activation or lack of IL-10, it is essential for maintaining the vascular compliance and endothelial function during the aging process. Given that a similar cardiovascular phenotype is present in frail, older adults, these findings further support the utility of the IL-10(tm/tm) mouse as a model of frailty.

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1. Introduction

Aging is inevitable, yet its physiologic consequences are, to some degree, modifiable. Cardiovascular (CV) dysfunction is the final common pathway of many acquired disease states and hence the most common cause of age-related deaths in the United States (Godwin, 2005; Heron, 2011). Frailty is a geriatric syndrome of late-life vulnerability to adverse outcomes and early mortality associated with declines in multiple physiological systems, the activation of inflammatory pathways, skeletal muscle decline, and subclinical cardiovascular disease(Dato et al., 2012; Espinoza and Walston, 2005; Ko et al., 2012). Given that frailty is such an important marker of adverse outcomes, the identification of etiological pathways that influence frailty-related vulnerability will greatly facilitate the development of improved risk assessment and better preventive and treatment modalities.

Interleukin (IL) 10 was originally demonstrated to be an anti inflammatory product of T-helper 2 cells (Fiorentino et al., 1989). Genetic deletion of IL-10 in mice (Kuhn et al., 1993) leads to a series of IL-10 associated pathologies. An increased risk of developing enterocolitis and colorectal cancer (Berg et al., 1996), inflammatory bowel disease (Das et al., 2003), development of osteopenia, decreased bone formation, mechanical fragility of long bones (Dresner-Pollak et al., 2004), and exacerbation of fatigue and motor deficits (Krzyszton et al., 2008) have been demonstrated in IL-10 deficient mice. The IL-10 mouse has been proposed as a mouse model of frailty, as aging IL-10(tm/tm) mice develop increased inflammatory pathway activation, decreasing skeletal muscle strength, and declining activity as well as early mortality. This phenotype is consistent with frail older humans. (Ko et al., 2012; Walston et al., 2008).

Further studies have shown that IL-10 inhibits LDL/Ox-LDL dependent monocyte-endothelial interaction thereby inhibiting atherogenesis and hence preventing the development of atherosclerotic plaque in mice (Caligiuri et al., 2003; Mallat et al., 1999; Pinderski Oslund et al., 1999). Furthermore, plasma IL-10 levels have been shown to decrease in patients following myocardial infarction (Heeschen et al., 2003). Additionally, data demonstrate that plasma IL-10 levels are directly correlated with good prognosis and remain an independent predictor of long-term adverse cardiovascular outcomes in Acute Coronary Syndromes (Cavusoglu et al., 2011). IL-10 levels also have a strong inverse correlation with stroke mortality, as shown in the Leiden 85-Plus study (van Exel et al., 2002).

It is well established that the endothelium is critical in mediating vasorelaxation to agonists such as acetylcholine (ACH) through nitric oxide (NO) and Endothelial Derived Hyperpolarizing Factors such as hydrogen sulfide (Mustafa et al., 2011). Equally important are the Endothelium Derived Contractile Factors (EDCF). Indeed, arachidonic acid derivatives produced by endothelial COX mediate constriction or relaxation in different vascular beds (Moncada and Vane, 1978). Recent studies have reinforced the idea of endothelial and COX dependent vasoconstriction, induced by mechanical or chemical stimuli. These cholinergic or stretch-mediated stimuli lead to increased intracellular calcium concentration (Miller and Vanhoutte, 1985; Katusic et al., 1987, 1988; Ihara et al., 1999; Okon et al., 2002; Yang et al., 2004). It is also known that senescence increases expression of physiologic and inflammatory isotypes of COX protein, COX-1 and inducible cyclo-oxygenase (COX-2) respectively (Heymes et al., 2000; Matz et al., 2000; Stewart et al., 2000), endothelial COX-2 mRNA (Voghel et al., 2007), and mRNA and protein expression of inducible NOS (iNOS) (Tabernero et al., 2000; Chou et al., 1998). IL-10 is known to impair production of inflammatory TNF and iNOS produced by liver CD11b1/Ly6C1 cells (Bosschaerts et al., 2011). Interestingly, iNOS binds to and S-nitrosylates COX-2, leading to activation and increased catalytic activity (Kim et al., 2005).

Given the support for the use of this mouse as a model of human frailty, and the knowledge that IL-10 as well as inflammatory mediators profoundly effect cardiovascular function, we hypothesized that the IL-10(tm/tm) mice would develop an age-related change in cardiovascular phenotype, develop endothelial dysfunction and vascular stiffness consistent with that reported in frail older adults. We explored the role of IL-10 in vasoregulation and maintenance of cardiac function in this model of aging, frailty and inflammation.

2. Methods

2.1. Animals

Age and background matched, IL-10 deficient (IL-10(tm/tm)); B6.129P2-Il10tm1Cgn/J and control mice (C57BL6; WT) were obtained from Jackson Laboratories (Bar Harbor, ME, USA). IL-10(tm/tm) mice used are homozygous for the Il10tm1Cgn targeted mutation. These mice were housed in Association for Assessment and Accreditation of Laboratory Animal Care International accredited facilities and pathogen contact prevention (prophylaxis from infections, inflammatory bowel disease and early mortality) was achieved under specific pathogen-free (SPF) barrier conditions until terminal experiments were carried out. It is known that the pro-inflammatory potential achieved by the lack of IL-10 in this mouse model can be attributed to activation of TNF-α and IL-1β synthesis via IFN-γ, which is produced in massive amounts and also is important in antigen presentation and pathogen death via activation of macrophages (Ko et al., 2012). Animals with any signs of inflammatory/infectious disease were ruled out of the study. The study was performed at approximately 3–4 months (young) and 9 months of age or greater (old).

2.2. Vascular function

2.2.1. Endothelial function

Endothelial function was assessed using force-tension myography. Mouse aortas were isolated and cleaned in ice-cold Krebs-Ringer-bicarbonate solution containing the following (in mM): 118.3 NaCl, 4.7 KCl, 1.6 CaCl2, 1.2 KH2 PO4, 25 NaHCO3, 1.2 $MgSO_4$, and 11.1 dextrose. Vascular tension changes were determined as previously described (Winters et al., 2000). Briefly, one end of the aortic rings was connected to a transducer, and the other to a micromanipulator. The aorta was immersed in a bath filled with constantly oxygenated Krebs buffer at 37 °C. Equal size thoracic aortic rings (2 mm) were mounted using a microscope, ensuring no damage to the smooth muscle or endothelium. The aortas were passively stretched to an optimal resting tension using the micromanipulator, after which a dose of 60 mM KCl was administered, and repeated after a wash with a Krebs buffer. After these washes, the vessels were allowed to equilibrate for 20–30 min. Phenylephrine (1 μM) was administered to induce vasoconstriction. A dose-dependent response (1 ηM to 10 μM), with the muscarinic agonist, ACH, was then performed. The responses were repeated in the presence of inhibitors. Relaxation responses were calculated as a percentage of tension following pre-constriction. Sigmoidal dose–response curves were fitted to data with the minimum constrained to 0.

2.2.2. Pulse wave velocity

Pulse wave velocity (PWV) was measured non-invasively using a high-frequency, high-resolution Doppler spectrum analyzer (DSPW). Mice were anesthetized with 1.5% isoflurane, placed supine on the heated (37 °C) plate. The animals were maintained at a physiologic heart rate of approximately 500 BPM. 10 MHz probe was used to record the aortic pulse waves at thorax and abdomen separately at a distance of 4 cm. EKG was recorded simultaneously and the time taken by the wave to reach from thoracic aorta to abdominal aorta was measured using R wave of the EKG as a fixed point. Subsequently, the velocity was calculated.

2.2.3. Blood pressures

Blood pressures were measured invasively through high fidelity solid-state transducer. The animals were anesthetized using 1.5–2%