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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

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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.

Keywords

Interleukin 10; Cyclo-oxygenase; Thromboxane; Endothelial dysfunction; Acetylcholine; Aorta; Frailty; Aging; Prostanoid; Cytokines

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 intra-cellular 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-II10tm1Cgn/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 II10tm1Cgn 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-1 β 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 MgSO₄, 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. Phenyl-ephrine (1 µM) was administered to induce vasoconstriction. A dose-dependent response (1 nM 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% isoflurane for induction of anesthesia and then maintained at 1%. A midline neck skin incision was made and blunt dissection was carried out to access, clean and catheterize jugular vein for the purpose of saline infusion. Similarly carotid artery was catheterized with 1.2 F Scisence Pressure Catheter™. Data was recorded and analyzed using ADInstrument Labchart version 7.

2.2.4. Real time quantitative polymerase chain reaction—Total RNA was extracted from isolated mice aortas using Trizol and RNeasy Mini Kit (Qiagen) as described previously (Pandey et al., 2012). RNA was then reverse transcribed with oligo dT primers to obtain cDNA using SuperScript First Strand kit (Invitrogen), and quantitative real-time PCR (Applied Biosystems) was performed using SYBR Green Supermix (Applied Biosystems) and the following primer sets:

COX2: forward 5′ ACACACTCTATCACTGGCACC

COX2: reverse 5′ CAAACTGAGTGAGTCCATGTT

iNOS: forward 5′ GGCTTGCCCCTGGAAGTTTCTCTTCAA AGT C

iNOS: reverse 5′ AAGGAGCCATAATACTGGTTGATG

18s: forward 5′ AGAAACGGCTACCACATCCAA

18s: reverse 5′ GGGTCGGGAGTGGGTAATTT.

2.3. Cardiac function

2.3.1. Transthoracic echocardiography—Transthoracic echocardiography in conscious mice was performed using Sequoia Acuson C256 (Malvern, PA) ultrasound machine, equipped with a frequency bandwidth of 15 MHz (Yang et al., 1999; Olson et al., 2003). The two-dimensional (2-D) and M-mode echocardiogram were obtained in the parasternal short and long axis view of the left ventricle (LV) at the level of the papillary muscles and sweep speed of 200 mm/sec.

Using the M-mode echocardiogram image, four parameters were measured: (i) left ventricular posterior wall thickness at end of diastole (LVPWD), (ii) interventricular septal thickness at end of diastole (IVSD), (iii) left ventricle (LV) chamber diameter at end of diastole (LVEDD), and (iv) left ventricle chamber diameter at end of systole (LVESD). All measurements were performed according to the guidelines set by the American Echocardiography Society. For each mouse, three to five values for each measurement were obtained and averaged for evaluation. Using the LVEDD and LVESD, we derived the fractional shortening (FS) which represented the percent change in left ventricular (LV) chamber dimension with systolic contraction. We used the FS in the estimation of the LV wall contractility or the systolic function based on the following equation: $FS (\%) = [(LVEDD - LVESD) / LVEDD] \times 100$. The left ventricular mass (LVmass) was derived and used in the assessment of left ventricular hypertrophy and enlargement, using the following equation (Pollick et al., 1995): $LV \text{ mass (mg)} = 1.055 [(IVSD + LVEDD + PWTD)^3 - (LVEDD)^3]$ where 1.055 is the specific gravity of the myocardium.

2.3.2. Doppler imaging—Doppler imaging was used for evaluation of regional wall motion. Myocardial relaxation (diastolic) and contraction (systolic) velocities of the left ventricle were measured using the four-chamber view. The sample volume was positioned at the basal level of the inter-ventricular septum. The isovolumetric relaxation time (IVRT) was measured as an index of diastolic function.

All measurements were performed according to the guidelines set by the American Society of Echocardiography. For each mouse, three to five values for each measurement were obtained and averaged for evaluation.

2.3.3. Histological evaluation and cellular morphometry—A transverse section of heart tissue was utilized for measurement, with a superior axial view, after the atrium was removed. Myocardium was fixed in 10% formalin, processed by standard paraffin embedding and serially sectioned in 5–8 μm thicknesses. Myocyte diameters were determined at the nucleus from a longitudinal view of cells from digitized images of hematoxylin and eosin (H&E) stained slides and analyzed using Image J program (NIH, Bethesda, MD).

2.4. Statistical analysis

The results were expressed as mean and standard error (mean $\pm$ SEM). One-way analysis of ANOVA and the Bonferroni post hoc test for multiple-comparison were used for comparing all groups and pairs of groups respectively. A $p < 0.05$ was considered significantly different. All analyses were carried out using Graph Pad version 5 and Microsoft Excel version 14.1.3 statistical analysis software.

3. Results

3.1. Body mass

There was no significant difference in the body mass in age matched IL-10(tm/tm) and WT mice. Young IL-10(tm/tm) vs. WT mice average weight was measured to be 27 g vs. 31 g and in old IL-10(tm/tm) vs. WT mice group the average weights were 38 g vs. 36 g (Fig. 2E).

3.2. Vascular studies

In ex vivo myograph experiments, measured tension represents a balance between vasorelaxant and vasoconstrictor dependent function and mediators. In phenylephrine pre-constricted isolated mouse aorta, ACH stimulates the release of endothelial factors, which mediate vasorelaxation as a result of greater relaxation than constriction. In young animals the ACH dose response curves were no different in aortas from WT as compared to IL-10(tm/tm) (Emax, 80.9 ± 4.6 vs. $71.9 \pm 5.7\%$; EC50 125.9nM vs. 50.1nM) in IL-10(tm/tm) mice aortas (Fig. 1A). By contrast, in old mice ACH mediated vasorelaxation was markedly impaired in IL-10 as compared to WT age matched controls (Emax 30.7 ± 9.3 vs. $98.5 \pm 14.1\%$; EC50 39.4nM vs. 251nM; $p < 0.001$, $n = 6$) (Fig. 1C). Furthermore vasoconstriction was observed at higher doses ($> 1 \mu\text{M}$) of ACH in old IL-10 aortas (Fig. 1C,D).

Pre-incubation of aortic rings with $3 \mu\text{M}$ indomethacin (COX1/2 inhibitor), $5 \mu\text{M}$ COX-2 inhibitor (nimesulide), or 100 nM thromboxane receptor antagonist (SQ29548) abolished the vasoconstrictive responses and significantly improved endothelial dependent vasorelaxation in old IL-10(tm/tm) aortas (Emax $80.3 \pm 2.6\%$, $82.9 \pm 2.0\%$, $65.1 \pm 3.2\%$; EC50 171 nM, 240 nM, 265 nM respectively) (Fig. 1E,F).

Mean arterial blood pressure (MAP) was significantly increased in old IL-10(tm/tm) mice as compared to WT age matched controls (89 ± 18.6 mm Hg vs. 68 ± 6.5 mm Hg, $p < 0.05$, $n = 4$; Fig. 2A). Furthermore PWV a measure of vascular stiffness was also significantly increased in old IL-10(tm/tm) mice as compared to WT mice (3.72 ± 0.12 m/s vs. 3.23 ± 0.15 m/s, $p < 0.05$, $n = 7$) (Fig. 2B). There was no significant difference observed in the PWVs of young WT and IL-10(tm/tm) mice.

The abundance of COX2 mRNA was significantly increased in aortas of young IL-10(tm/tm) mice as compared to WT age matched controls (1.97 ± 0.13 $2^{\Delta\Delta\text{Ct}}$ vs. 0.99 ± 0.02 $2^{\Delta\Delta\text{Ct}}$, $p < 0.05$, $N = 6$). There was no statistical difference in abundance of COX2 mRNA in old age matched IL-10(tm/tm) mice as compared to WT aortas (0.63 ± 0.06 $2^{\Delta\Delta\text{Ct}}$ vs. 1.33 ± 0.32 $2^{\Delta\Delta\text{Ct}}$ ns, $N = 6$) (Fig. 2C).

The abundance of iNOS mRNA was significantly increased in aortas of young IL-10(tm/tm) mice as compared to WT age matched controls (2.06 ± 0.06 $2^{\Delta\Delta\text{Ct}}$ vs. 1.00 ± 0.07 $2^{\Delta\Delta\text{Ct}}$, $p < 0.05$, $N = 6$). There was no statistical difference in abundance of iNOS mRNA in old age matched IL-10(tm/tm) mice as compared to WT aortas (0.72 ± 0.01 $2^{\Delta\Delta\text{Ct}}$ vs. 0.90 ± 0.10 $2^{\Delta\Delta\text{Ct}}$ ns, $N = 6$) (Fig. 2D).

3.3. Cardiac studies

Cardiac echocardiography (Fig. 3) demonstrated no difference in LVEDD between the old IL-10(tm/tm) (3.5 ± 0.2 mm) as compared to old WT and young WT and IL-10(tm/tm) mice groups (3.3 ± 0.1 mm, 2.9 ± 0.1 mm, 3.0 ± 0.1 mm respectively). In contrast, left ventricular end-systolic diameter (LVESD) was significantly greater in old IL-10(tm/tm) mice (2.0 ± 0.2

mm) as compared to age matched WT (1.5 ± 0.1 mm), and young WT (1.2 ± 0.09 mm) and IL-10(tm/tm) (1.2 ± 0.06 mm) mice ($p < 0.01$, $n = 7$) (Fig. 3C).

A significant reduction in ejection fraction (EF) was also observed in old IL-10(tm/tm) mice ($73 \pm 3\%$) as compared to old WT ($84 \pm 1\%$; $p < 0.01$) mice, and young WT ($84 \pm 1\%$; $p < 0.01$) and IL-10(tm/tm) ($86 \pm 1\%$; $p < 0.001$) mice ($n = 7$) (Fig. 3D).

WT hearts undergo symmetric changes with no difference in IVSD/LVPWD ratio between young and old WT mice (IVSD/LVPWD = 1.06 vs. 1.04). Aging of IL-10(tm/tm) mice results in asymmetric cardiac hypertrophy; IVSD/LVPWD in old IL-10(tm/tm) mice is significantly higher than young IL-10(tm/tm) mice (IVSD/LVPWD = 1.14 vs. 1.05; $p < 0.05$, $n = 7$) (Fig. 3E).

LV mass was significantly increased in the old IL-10(tm/tm) (156.3 ± 9.0 mg) as compared to old WT (142.2 ± 10.1 mg; $p < 0.05$) and young WT (92.9 ± 10.5 mg; $p < 0.001$) and IL-10(tm/tm) (102.3 ± 5.1 ; $p < 0.001$) mice, suggesting LVESD dilatation and heart enlargement ($n = 7$); (Fig. 3F).

Also, H&E staining in old mice demonstrated an increase in myocyte size in IL-10(tm/tm) group as compared to age matched WT controls (14.3 ± 3.7 μ m vs. 10.9 ± 2.8 μ m; $p < 0.001$, $n = 45$) (Fig. 4).

Isovolumic relaxation time (IVRT), an index of diastolic function, was significantly increased in old IL-10(tm/tm) mice (36.3 ± 3.4 ms) as compared to age matched WT controls (25.0 ± 2.0 ms) and young WT (21.50 ± 1.89 ms) and IL-10(tm/tm) mice (24.50 ± 1.71 ms) ($p < 0.01$, $n = 7$) (Fig. 3G).

4. Discussion

The role of inflammatory pathway activation and elevation of serum inflammatory cytokines in age-related disease states, frailty, and functional decline is an active area of investigation (Singh and Newman, 2011; Chen and Frangogiannis, 2010). Chronic activation of NF- κ B induced inflammatory cascades, such as that induced via deletion of IL-10, influences the frailty phenotype and the associated vulnerability to multi-systemic decline in these mice, similar to that observed in frail older human adults (Hanada and Yoshimura, 2002; Kuhn et al., 1993; Rennick et al., 1995; Lira et al., 2012). These conditions include hypertension, congestive heart failure, metabolic and endocrine abnormalities, among other conditions (Kassan et al., 2011; Barzilay et al., 2007; Newman et al., 2001). Our efforts in this paper were, in part, meant to determine whether the loss of IL-10 influences the cardiovascular pathophysiology observed in frailty and in aging, and help to determine if these changes may be a potential target for modifying age-related cardiovascular mortality and morbidity.

This study has established a relation between the loss of IL-10 and associated age related cardiovascular dysfunction. The inability of the aortas of old IL-10(tm/tm) mice to relax with muscarinic stimulation can be attributed to endothelial dysfunction. We also observed an increased blood pressure and vascular stiffness in old IL-10(tm/tm) as compared to age matched WT mice. Additionally, the hearts of the old IL-10(tm/tm) mice also undergo dynamic changes causing asymmetric hypertrophy, and both systolic and diastolic dysfunction.

Our data suggest that the vascular endothelial dysfunction and stiffness may potentially be important in the development of early mortality observed in the frail mouse (Ko et al., 2012). With IL-10(tm/tm), we observed that cholinergic agonists cause vasoconstriction in mice aortas despite the fact that all pathways of endothelial dependent relaxation are not

inhibited. It is not clear whether this is caused specifically by a lack of IL-10 or due more generally to the activation of chronic inflammatory pathways. Interestingly, however, we demonstrated that this impaired endothelial vasorelaxation is reversible with both COX inhibitors and TXA₂ antagonists.

The unchecked activation of endothelium causes activation of multiple signaling cascades. This especially includes the eicosanoids, the signaling molecules produced by the substrate arachidonic acid, specifically via prostaglandin H₂ (PGH₂) synthase (COX1/2 and peroxidase) (Gryglewski, 2008). These enzymes are committed to production of prostaglandins, prostacyclin and thromboxane. Different cell types convert PGH₂ to different end products, which may also depend on the cell stress and conditions. The peroxidase in PGH₂ synthase can produce peroxide, which oxidizes heme iron. The resulting heme is capable of accepting electron from tyrosine residue (385) and hence the resulting tyrosine residue is supposed to extract a hydrogen atom from arachidonic acid to produce reactive oxygen species (Shimokawa et al., 1990). On the other hand, vascular endothelial cells express both isoforms of COX, COX-1 (constitutive) and COX-2 (inducible), which produce PGH₂, a substrate for both PGI₂ and TXA₂ (Marnett et al., 1999; Smith et al., 1996). While PGI₂ causes vasorelaxation, TXA₂ causes vasoconstriction (Bunting et al., 1983). Indeed, it is interesting to consider the potential beneficial effect of COX inhibitors in endothelial protection as we age.

We consider IL-10 to be more than just the cytokine synthesis inhibitory factor; it might very well also contain and check the unregulated production of eicosanoids and their activation in response to local inflammatory processes such as in infection, systemic conditions like sepsis and chronic inflammatory processes such as aging. The frail and immune compromised phenotypes of IL-10(tm/tm) mouse model reinforce the same. Unexpressed under most normal conditions and inducible under inflammatory stress, COX-2 is known to be nitrosylated and activated via iNOS (Kim et al., 2005), and IL-10 decreases TNF and iNOS production (Bosschaerts et al., 2011). Hence, IL-10 could have the ability to suppress the activity of COX by checking NOS activation. Indeed, our study also suggests that in youth the abundance of iNOS mRNA is 2 fold higher in the aortic tissue of IL-10 depleted mice as compared to WT controls. Similarly, this iNOS induction is possibly able to drive the abundance of COX2 mRNA, which is also significantly higher in young IL-10(tm/tm) mouse aorta as compared to WT counterparts. Interestingly, the abundance of COX2 and iNOS was not different in old IL-10(tm/tm) and WT mice aortas. This may be due to differences in protein synthesis, post-translational modification or degradation. Moreover, it is known that both iNOS and COX2 are regulated via NF- κ B (Baeuerle and Baltimore, 1996) and its role in IL-10(tm/tm) mouse model needs to be elucidated. Previous research suggests that in a few studies IL-10 levels have been inversely correlated with cardiovascular morbidity and mortality in a population suffering with myocardial infarction, stroke, acute coronary syndrome and atherosclerosis (Heeschen et al., 2003; Cavusoglu et al., 2011; Caligiuri et al., 2003; Mallat et al., 1999; Pinderski Oslund et al., 1999).

Here we demonstrate a significantly greater increase in blood pressure and vascular stiffness in aging IL-10(tm/tm) mice as compared to WT mice. Thus, it is possible that the loss of compliance may not be a direct effect of IL-10 depletion but an effect of rise in blood pressure caused by endothelial dysfunction. A pressure independent integral of stiffness needs to be studied in order to resolve this question.

IL-10(tm/tm) mice undergo early cardiac remodeling and have impaired function. The fact that the septum gains more mass than the posterior wall represents asymmetric hypertrophy and raises a new question as to what could be the cause of this phenomenon.

Our data, along with proposed mechanisms and the available literature, should open avenues in which IL-10 can be studied in accordance with prostanoids, not only to predict age related cardiovascular changes, but also to modify and therapeutically target cardiovascular syndromes such as age-related systolic hypertension and non-systolic heart failure.

5. Conclusion

We conclude that the older IL-10(tm/tm) mouse model of frailty has stiffer vessels and a loss of endothelial vascular relaxation, possibly due to an increase in eicosanoids, especially due to the increase in activity of COX 2 and resultant thromboxane A2 receptor activation, but not due to the increase in prostacyclin. Our results also suggest that this model of frailty has increased heart size but also a worsening of cardiac function. Finally, further studies will be required to determine whether it is the lack of IL-10 per se that causes these changes or the related chronic inflammatory pathway activation.

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Fig. 1.

(A) Acetylcholine (ACH) dependent vasorelaxation recorded via force tension myography, is not different in young Interleukin (IL)-10(tm/tm) and wild type (WT) mouse aortas. (B) Example tracing: young IL-10(tm/tm) aorta in the presence and absence of indomethacin (above) and young WT aorta in the presence and absence of indomethacin (below). (C) Endothelial dependent vasorelaxation is markedly diminished in old IL-10(tm/tm) mice compared to old WT controls. Insets within (A, C) represent diminishing ACH dependent relaxation at a 1 μ M in IL-10(tm/tm) but not WT aorta. (D) Example tracing: old IL-10(tm/tm) aorta in the presence and absence of indomethacin (above) and old WT aorta in the presence and absence of indomethacin (below). (E) ACH dose response curve: treatment with 5 μ M COX-2 inhibitor (nimesulide), and 100 nM thromboxane receptor antagonist (SQ29548) enhanced endothelial dependent vasorelaxation in IL-10(tm/tm) aortas. (F)

Endothelial counter vasoconstriction in IL-10(tm/tm) aortas at a dose of 1 μ M is abolished on treatment with COX-2 and TxA2 antagonists.

Fig. 2.

Noninvasive arterial stiffness and invasive carotid artery pressures measured in old IL-10(tm/tm) and WT mice. (A) The mean arterial pressure in old IL-10(tm/tm) mice is 89 ± 18.6 mm Hg as compared to age matched WT mice, 68 ± 6.5 mm Hg. (B) Pulse wave velocity recorded at a heart rate of approximately 500 BPM is higher in old IL-10(tm/tm) as compared to the WT controls (3.72 ± 0.12 m/s vs. 3.23 ± 0.15 m/s). (C) COX2 mRNA measured via qPCR is higher in young IL-10(tm/tm) as compared to WT controls. (D) iNOS mRNA measured via qPCR is higher in young IL-10(tm/tm) as compared to WT controls. (E) Body mass (g) of young and old IL-10(tm/tm) and WT mice.

Fig. 3.

(A) Echocardiography images from old WT (left) and IL-10(tm/tm) (right) mice. Graphical representation of echocardiography and Doppler studies showing (B) left ventricle end diastolic diameter (LVEDD), (C) left ventricle end systolic diameter (LVESD), (D) ejection fraction (EF), (E) ratio of septal thickness (IVSD) and wall thickness (LVPWD) (F) left ventricle mass (LV mass) and (G) isovolumic relaxation time (IVRT), in young and old wild type and IL-10(tm/tm) mice.

Fig. 4.

(A) High resolution digitized images of H&E stained slides to determine myocyte cross-sectional diameter. (B) Cardiac myocyte size measured in old WT and IL-10(tm/tm) groups.