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Macrophages dictate the progression and manifestation of hypertensive heart disease☆,☆☆

David Kain [a,b,c], Uri Amit [a,b,c], Chana Yagil [d], Natalie Landa [a,b,c], Nili Naftali-Shani [a,b,c], Natali Molotski [a,b,c], Vered Aviv [c], Micha S. Feinberg [a,b], Orly Goitein [e], Tammar Kushnir [e], Eli Konen [e], Fredrik H. Epstein [f], Yoram Yagil [d], Jonathan Leor [a,b,c,*]

[a] Tamman Cardiovascular Research Institute, Sheba Medical Center, Tel-Hashomer, Israel
[b] Neufeld Cardiac Research Institute, Tel-Aviv University, Sheba Medical Center, Tel-Hashomer, Israel
[c] Sheba Center for Regenerative Medicine, Stem Cell, and Tissue Engineering, Tel Hashomer, Israel
[d] Laboratory for Molecular Medicine and Israeli Rat Genome Center, Faculty of Health Sciences, Ben-Gurion University, Barzilai Medical Center Campus, Ashkelon, Israel
[e] Diagnostic Imaging Department, Sheba Medical Center, Tel-Hashomer, Israel
[f] University of Virginia, Department of Biomedical Engineering, Charlottesville, VA, USA

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ABSTRACT

Background: Inflammation has been implicated in the initiation, progression and manifestation of hypertensive heart disease. We sought to determine the role of monocytes/macrophages in hypertension and pressure overload induced left ventricular (LV) remodeling.

Methods and results: We used two models of LV hypertrophy (LVH). First, to induce hypertension and LVH, we fed Sabra salt-sensitive rats with a high-salt diet. The number of macrophages increased in the hypertensive hearts, peaking at 10 weeks after a high-salt diet. Surprisingly, macrophage depletion, by IV clodronate (CL) liposomes, inhibited the development of hypertension. Moreover, macrophage depletion reduced LVH by 17% ($p < 0.05$), and reduced cardiac fibrosis by 75%, compared with controls ($p = 0.001$). Second, to determine the role of macrophages in the development and progression of LVH, independent of high-salt diet, we depleted macrophages in mice subjected to transverse aortic constriction and pressure overload. Significantly, macrophage depletion, for 3 weeks, attenuated LVH: a 12% decrease in diastolic and 20% in systolic wall thickness ($p < 0.05$), and a 13% in LV mass ($p = 0.04$), compared with controls. Additionally, macrophage depletion reduced cardiac fibrosis by 80% ($p = 0.006$). Finally, macrophage depletion down-regulated the expression of genes associated with cardiac remodeling and fibrosis: transforming growth factor beta-1 (by 80%) collagen type III alpha-1 (by 71%) and atrial natriuretic factor (by 86%).

Conclusions: Macrophages mediate the development of hypertension, LVH, adverse cardiac remodeling, and fibrosis. Macrophages, therefore, should be considered as a therapeutic target to reduce the adverse consequences of hypertensive heart disease.

1. Introduction

Hypertension and the subsequent development of left ventricular hypertrophy (LVH) and heart failure remain a major public health concern [1–3]. Hypertrophy leads to LV wall thickening and stiffening [3]. Based on Laplace law, short-term LVH is adaptive and helps to normalize wall stress and oxygen demand [4]. However, long-term LVH is unfavorable as the myocardium is prone to inflammation, cell death, fibrosis, diastolic and systolic heart failure [5,6]. Although the mechanisms underlying the switch from adaptive LVH to maladaptive failure remain unclear, animal studies have suggested that reducing LVH, without deterioration in contractile function, is possible even in the presence of chronic pressure overload [5,7–9]. Thus, selective suppressing of pathological hypertrophy could be a new therapeutic target in preventing heart failure [5].

Fibrosis is a central feature of pathological LVH and adverse structural remodeling of the myocardium in hypertensive heart disease [1,10]. Deposition of collagen contributes to the development of LVH, stiffness, arrhythmias, and progressive failure [10]. Therapeutic options to prevent cardiac fibrosis are limited. Thus, the development of new therapies becomes increasingly relevant [5].

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* Corresponding author at: Neufeld Cardiac Research Institute, Sheba Medical Center, Tel-Hashomer 52621, Israel.
E-mail address: leorj@post.tau.ac.il (J. Leor).

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The immune system has been linked to the development and progression of hypertension and LVH [5,11,12]. Although macrophages are implicated in many features of human health and disease, including regulation of inflammation, angiogenesis, and fibrosis [13], their role in the pathogenesis of hypertension and LVH is less clear and controversial. For example, a few studies have suggested that macrophage protects against hypertensive cardiac damage [14], as macrophage depletion increases blood pressure [15], and accelerates the development of cardiomyopathy [15]. In view of these conflicting reports, we aimed to determine the role of monocytes/macrophages in hypertension, LVH, and subsequent cardiac fibrosis. A better understanding of the role of macrophages in hypertension and pressure overload may contribute to the development of novel, specific therapies to reduce LVH, fibrosis, and heart failure.

2. Materials and methods

All animal experiments complied with the international standards stated in the Guide for the Care and Use of Laboratory Animals and were approved by the Sheba Medical Center Institutional Animal Care and Use Committee.

2.1. Rat model of hypertensive heart disease

To induce hypertensive heart disease, we fed high-salt diet to 6-week old male salt-sensitive SBH/y rats (Fig. 1a), from the Israeli Rat Genome Center, Barzilai Medical Center, Ashkelon, Israel (www.irgc.co.il).

To determine the role of macrophages in LVH, macrophage depletion was induced by a tail vein injection of 600 μl (20 mg/kg) liposome-encapsulated clodronate (CL), every 72 h for 30 days after systolic blood pressure (BP) elevation in SBH/y rats [16]. Based on our previous work [17], macrophage depletion by CL is transient and only effective for up to 72 h. The control groups were treated with IV PBS-liposomes at similar time intervals and injection volumes.

Systolic BP was measured at 4 time points: at baseline (prior to salt loading), 6 weeks after high-salt diet, 2 and 4 weeks after CL injections (Fig. 1a). Measurements were performed at a temperature of 27–28 °C in conscious rats by the tail-cuff method using an IITC photoelectric oscillatory detection device (IITC Life Science, Woodland Hills, CA, USA), as described previously [18]. At least 3 replicate BP measurements were obtained and averaged for each rat at each time point.

To assess LV remodeling and hypertrophy, echocardiography, including speckle-tracking based-strain analysis, and cardiac magnetic resonance (CMR) imaging were obtained, as detailed below and previously described. [17].

2.2. Pressure overload model in mice

To determine the role of macrophages in the pathogenesis of LVH, independent of high-salt diet, we subjected mice to continuous LV pressure over-load. Transverse aortic constriction (TAC) was performed in Balb/c female mice (20–25 g.). Animals were anesthetized with 1–2% Isoflurane in 100% oxygen delivered through a volume-cycled rodent respirator (HSE-HA MiniVent Mouse Ventilator, Harvard Apparatus, Holliston,

MA, USA). After the second left intercostal space incision, aortic constriction was carried out by ligating 6–0 silk suture against a 27G needle, which was then quickly removed. The chest wound was closed with a 5–0 absorbable suture. Mice were allowed to recover from anesthesia under warm conditions. Mortality during and immediately following the procedure was approximately 10%.

After TAC operation, mice were allocated to either IV CL (100 μl, 20 mg/kg) or PBS liposomes (100 μl) by tail vein injection immediately after surgery and every 72 h for a period of 21 days. Echocardiography studies were performed at 1 and 21 days after TAC and injections. Mice were euthanized 3 weeks after TAC.

2.3. Echocardiographic evaluation of cardiac remodeling and function

To assess changes in LV remodeling and function, we anesthetized rats with a combination of 40 mg/kg ketamine and 10 mg/kg xylazine. Echocardiography of both long- and short-axis views were obtained in all animals, using a small animal echocardiography system (Vevo 2100, VisualSonics, Toronto, Canada, equipped with a 22–55 MHz transducer for rats and a 13–24 MHz for mice). To optimize and validate LV remodeling and hypertrophy in hypertensive rats, examinations were performed before and 10 weeks after BP elevation. To assess the development and severity of LV remodeling and dysfunction in hypertensive rats following macrophage depletion, serial examinations were performed: before BP elevation, before CL injections, 6 weeks after BP elevation, and 10 weeks after BP elevation. In TAC mice, echocardiography studies were performed at day 1 and 21 after TAC. Hearts were imaged by 2-dimensional mode in the parasternal long- and short-axis views, through which the M-mode cursor was positioned perpendicular to the LV septum and posterior wall. All measurements were averaged for three consecutive cardiac cycles, and analyzed by an experienced, blinded technician.

For speckle-tracking based-strain analysis of strain quantification in the radial axes, short-axis echocardiography images were acquired at a frame rate of 280 frames per second. Three consecutive cardiac cycles were selected and their endocardium and epicardium borders were traced. Each LV image in short axis was divided into six segments for regional speckle -tracking based-strain imaging; global strain of the LV was calculated as the averaged peak strain obtained from all six segments.

2.4. CMR imaging

To confirm the changes in LV remodeling and function, CMR examinations were performed before BP elevation, and at 6 and 10 weeks after BP elevation. Scanning was performed under isoflurane anesthesia (1.5%), at a constant temperature of 37 °C, as previously described [19]. We used a whole-body clinical 3 T MRI system (3 T HDxt Ver.15 M4A, GE Healthcare Systems, Milwaukee, WI, USA) and a custom-built quadrature cylindrical radiofrequency volume coil, I.D. 77 mm × 178 mm length, (Doty Litzcage Coil-Doty Scientific, Inc., Columbia, SC, USA).

Clinical CMR protocols were adjusted and optimized for the hypertensive rat model, as previously described [19]. To assess LV anatomy and function, a cine CMR protocol was acquired in long- and short-axis views. For cine CMR, the total scan time for one slice was approximately 2 min (depending on the heart rate), and the entire heart was covered typically using eight slices in approximately 15 min. CMR images were analyzed using a dedicated post-processing CMR Workstation (Medis Medical Imaging Systems BV, QMass MR 7.4, Leiden, The Netherlands). Cine images acquired in the short-axis plane with multiple slices were used for functional analysis. CMR data were transformed into polar maps and subdivided using a standard 16-segment model.

2.5. Flow cytometry analysis (FACS)

We used single cell flow cytometry to characterize macrophage subsets in the hypertensive rat heart. Hearts were harvested before, and at 6 and 10 weeks after the initiation of the high-salt diet. Cells from the hearts were isolated using a previously described enzymatic digestion mixture [20]. Cells were suspended in fluorescence-activated cell sorting (FACS) wash buffer (PBS, supplemented with 0.1% BSA, 2 mM EDTA) and Fc block for 20 min at 4 °C, in order to prevent a non-specific binding of antibodies to Fc receptors. Cells were then stained with Alexa Fluor®647-conjugated anti-rat CD68 (AbDserotec, Kidlington, UK) (as a general macrophage marker), and either with PE conjugated anti-rat CD80 (BioLegend, San Diego, CA, USA) as a marker of pro-inflammatory M1-type macrophages, or FITC conjugated anti-rat CD163 (BioLegend, San Diego, CA, USA) as a marker of anti-inflammatory M2-type macrophages. Results were analyzed using FACS Calibur Cytometer (BD Bioscience, San Jose, CA, USA) with CellQuest software (BD Bioscience, San Jose, CA, USA).

2.6. Micro RNA analysis

To determine the potential role of miRNA in the pathogenesis of LVH, we analyzed and compared miRNA from the hearts of four groups: hypertensive vs. control hearts, and hypertensive hearts with and without macrophage depletion. Total RNA was isolated from LV, using TRIzol Reagent (Biological Industries, Beit Haemek, Israel). The amount and quality of the total RNA were determined by a spectrophotometer. miRNA analysis was performed using AffymetrixGeneChip arrays (Affymetrix, Santa Clara, CA, USA), in accordance with an Affymetrix data sheet. MiRs, which were up- or down-regulated 1.5 fold, were considered significant and selected for data analysis.

2.7. Histopathology

After the last imaging, rats/mice were euthanized and hearts were perfused with 4% formaldehyde and sectioned into 3 to 4 transverse slices parallel to the atrioventricular ring. Each slice was fixed with 4% buffered formalin, embedded in paraffin. Serial sections were stained with hematoxylin-eosin, Massons Trichrome, anti-ED1, elastin to evaluate blood vessel wall thickness, and wheat germ agglutinin (WGA) to evaluate cardiomyocte and extracellular hypertrophy and fibrosis. In the TAC mice, sections were also stained with elastin staining to evaluate blood vessel thickness and perivascular fibrosis.

Fig. 1. LV remodeling in hypertensive rats by echocardiography. (a) Systolic BP (mm Hg) measured by the tail-cuff method shows a significant elevation of BP after 6 weeks of high-salt diet (red) compared with controls (blue) SBH/y rats. Echocardiography analysis was performed before, and at 6 and 10 weeks after BP elevation. (b–c) BP elevation increased anterior wall diastolic/systolic thickness in rats after 10 weeks. (d–e) LV diastolic/systolic dimension was lower in hypertensive rats, compared with baseline and controls. (f) LV contractility, reflected by fractional shortening, was increased in hypertensive rats. (g) LV mass was also increased in hypertensive rats. Difference ($p < 0.05$) between hypertensive (n = 6) and control rats (n = 6), using 2-way analysis of variance (ANOVA). BP = Blood pressure, LV = Left ventricular, EF = Ejection fraction.