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SUPPLEMENTAL MATERIALS AND METHODS

Harvesting of intimal cells from the LC, GC, and DT regions of the mouse aorta for real-time PCR

The main focus of our study was on the intima, and gene expression was assessed using real-time PCR only in intimal cells. The vascular tree was perfused with 20 ml of cold PBS containing 1% heparin, which was injected into the left ventricle with a sterile syringe and 23-gauge needle. The ascending aorta and DT were harvested, and adipose tissue was removed using a dissecting microscope (SMZ-U; Nikon) in a Petri dish containing ice-cold PBS and 1 mM of the RNase inhibitor aurintricarboxylic acid. Segments of aorta opened and were pinned with the endothelial surface facing upwards on a dish with a black silicone base. Nuclei were stained for 5 min at 22°C with modified Harris hematoxylin solution (Accustain; Sigma-Aldrich), rinsed, and digested for 8 min at 37°C with Liberase Blendzyme 2 (1:100; Roche Diagnostics) in Ca^{2+}/Mg^{2+}-containing PBS. In the ascending aorta and arch, the LC and GC regions were identified under a dissecting microscope by their location and nuclear morphology. Intimal cells from these regions and from the DT (excluding the ostia) were gently scraped with a 25-gauge needle and transferred with a pipetman (P10; Gilson) directly into RNA extraction buffer. For each experiment, intimal cells were pooled from two to four aortae. We estimate that <1,000 intimal cells were harvested per pooled sample.

RNA isolation and reverse transcription

Total RNA was isolated using an RNA isolation kit (PicoPure; Arcturus Inc.). Reverse transcription into single-stranded cDNA was performed using random primers (Invitrogen) and the Powerscript kit (CLONTECH Laboratories, Inc.).

Normalization of real-time PCR data

Gene expression is directly proportional to the number of cells that are harvested per sample, and in the intima this is reflected by mRNA levels of endothelial cells that constitute the majority of cells in the mouse intima. Real-time PCR data were normalized to CD31 to account for differences in the abundance of RNA isolated from each intimal region. CD31 was selected because this gene is expressed primarily by endothelial cells and, to a lesser extent, by leukocytes. Furthermore, in our experience CD31 is not substantially regulated by inflammatory stimuli. Thus, dividing real-time PCR data for a sample by the CD31 value of the same sample normalizes for the abundance of endothelial cell RNA. In addition to CD31, other normalizer genes such as HPRT or ICAM-2 can be used, and we obtained identical results when using these genes to normalize our data (see Fig. S3). HPRT is a housekeeping gene that is expressed by multiple cell types, and its expression is very uniform in many tissues (de Kok, J.B., R.W. Roelofs, B.A. Giesendorf, J.L. Pennnings, E.T. Waas, T. Feuth, D.W. Swinkels, and P.N. Span. 2005. *Lab. Invest.* 85:154–159).

Comparison of intimal cell gene expression in different regions of the thoracic aorta

For each gene, expression levels in the GC and DT regions were compared with the LC region (values determined by real-time PCR in all regions were divided by the LC value; thus, LC value = 1). The LC region was selected because immunostaining revealed that very few if any leukocytes were found in the GC region. For some genes (e.g., CD11c), the PCR values obtained from the GC and DT regions were near or below detection, and for other genes (e.g., CD68) the GC and DT PCR values likely represent low level expression by vascular cells (Andreeva, E.R., I.M. Pugach, and A.N. Orekhov. 2005. *Atherosclerosis.* 135:19–27; Pulford, K.A., A. Sipos, J.L. Cordell, W.P. Stross, and D.Y. Mason. 1990. *Int. Immunol.* 2:973–980) or potential trace contamination by blood leukocytes.

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