A transgenic mouse with vascular endothelial overexpression of the non-muscle myosin light chain kinase-2 isoform is susceptible to inflammatory lung injury: role of sexual dimorphism and age

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We have generated genetically engineered mice that are uniquely susceptible to lipopolysaccharide (LPS)-induced and mechanical ventilation–induced lung injury in a sex-specific and age-specific manner. These mice express a nonmuscle isoform of the myosin light chain kinase gene (nmMLCK2) targeted to the endothelium. Homozygous mice have significantly reduced fecundity and litter survival until weaning, and they are initially growth delayed but eventually exceed the size of wild-type littermates. Mice at all ages show increased protein transport across the lung barrier; however, the phenotype is most discernible in 8–12-week-old male mice. When subjected to a clinically relevant LPS-induced lung injury model, 8–12-week-old young females and 30–36-week-old males seem to be the most significantly injured group. In contrast, 30–36-week-old males remain the most significantly injured group when mechanically ventilated at high tidal volumes, which is a clinically relevant model of mechanical stress lung injury. These data reveal that nmMLCK2 overexpression in the endothelium exacerbates lung injury *in vivo* in a sexually dimorphic and age-dependent manner. (Translational Research 2008;151:141–153)

Abbreviations: ALI = acute lung injury; ANOVA = analysis of variance; ARDS = acute respiratory distress syndrome; BAL = bronchoalveolar lavage; EC = endothelial cell; ELISA = enzyme-linked immunosorbent assay; HBEC = human bronchial epithelial cell; HPAEC = human pulmonary artery endothelial cell; HTAB = hexadecyltrimethyl ammonium bromide; KRP = kinase-related protein; LPS = lipopolysaccharide; MLCK = myosin light chain kinase; nmMLCK = nonmuscle myosin light chain kinase; ORF = open reading frame; PBS = phosphate buffered saline; RIPA = radioimmunoprecipitation assay; RT-PCR = reverse transcription-polymerase chain reaction; SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis; smMLCK = smooth muscle myosin light chain kinase; VE = vascular endothelial

Actin microfilaments generate force by virtue of their ability to contract away from their site of attachment to the plasma membrane, and they do so via traction of the myosin light chain head groups attached to actin filaments by cross-bridges. The cross-bridge pulling action of myosin involves a conformational change in the molecule initiated by energetic phosphorylation of Ser19 on the myosin light chains.[1] The key enzyme involved in this phosphorylation event is myosin light chain

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AT A GLANCE COMMENTARY

Background

All inflammatory disorders are marked by the disruption of the semi-permeable endothelial barrier that results in increased vascular permeability. The chief mechanism involved is dynamic perturbation of the actomyosin assembly that is governed largely by the enzyme myosin light chain kinase (MLCK). Knockout of the nonmuscle MLCK (nmMLCK) results in protection from lung injury.

Translational Significance

We demonstrate that overexpression of a splice variant of the same nmMLCK protein isoform (nmMLCK2) in the endothelium results in increased lung injury. This converse result confirms the criticality of nmMLCK in the endothelium, reveals a role of age and gender in the process, and allows us to direct potential therapies in a tissue-specific manner.

kinase (MLCK), which exists in different tissues, and it may be characterized broadly as skeletal/cardiac, smooth muscle, and nonmuscle myosin light chain kinase (nmMLCK) isoforms. Although biochemical evidence for the existence of a Ca^{2+}/calmodulin-dependent nonmuscle isoform in cultured endothelial cell (EC) was suggested earlier,[2,3] the controversy as to whether this enzymatic activity is identical to the 110-kDa protein found abundantly in smooth muscle preparations was resolved when we provided conclusive proof of a larger MLCK protein isoform (210 kDa) by cloning the gene (*MYLK*) from a human EC–derived cDNA library.[4] The gene was subsequently mapped to chromosome 3q21.[5]

Detailed characterization of the gene revealed that a single 217-kb gene, which spans 31 exons (upgraded to 272 kb and 33 exons in RefSeq build 36; all exon numbers in this manuscript are denoted according to build 36) produces both the 1914 amino acids (210 kDa) nonmuscle and the 1091 amino acids (108 kDa) smooth muscle isoforms.[6] The gene also encodes a ~19-kDa protein known as kinase-related protein (KRP) or telokin that is transcribed from the C-terminal exons 30–33, and it serves to stabilize actin filaments.[7] In addition to smooth muscle myosin light chain kinase (smMLCK) and KRP, 5 splice variants were identified that use the start codon of the 210-kDa isoform, but they have internal exon deletions compared with the longest variant (nmMLCK1).[6] Both smMLCK and nmMLCK isoforms contain catalytic domains (eg, calmodulin binding motif) and structural domains (myosin light chain binding motif), and both isoforms exhibit robust MLC kinase activity. A major distinguishing feature is the 922 amino acids N-terminal stretch unique to nmMLCK1, which exhibits distinct cellular functions through unique interactions with other contractile proteins.[8,9]

Despite the uniqueness of the nmMLCK N-terminus, information is limited regarding the tissue distribution or physiologic roles of the splice variants that use the nmMLCK ATG. Northern and reverse transcription-polymerase chain reaction (RT-PCR) data[6] suggest that splice variant 2 (nmMLCK2) is the most abundant isoform in many tissues, including the endothelium. In gastrointestinal epithelium, however, nmMLCK1 accounts for 97% of MLC kinase activity in the perijunctional actomyosin ring.[10] Kinetically, nmMLCK1 or 2 do not significantly differ in V_{max} or $K_{0.5CaM}$, although only nmMLCK1 can undergo $p60^{Src}$-catalyzed phosphorylation on Tyr^{464} and Tyr^{471}, which are posttranslational modifications that significantly increases V_{max}(3-fold increase). Interestingly, both tyrosine residues are encoded within the exon 11 that is spliced out in nmMLCK2.[11]

Complete targeted deletion of the nmMLCK (MLCK210) isoforms (but not smMLCK or KRP) resulted in protection of the mice from the combined stress of bacterial product lipopolysaccharide (LPS) challenge and mechanical ventilation,[12] which raised the question as to the target tissue for nmMLCK protective effects in acute lung injury (ALI) (ie, epithelium, leukocytes, or vascular endothelium). In this study, we report the role of the nmMLCK2 splice variant in vascular barrier regulation, and we describe the consequences of overexpressing this isoform specifically in murine endothelium. Furthermore, as several acute and chronic inflammatory pulmonary disorders, which include ALI and acute respiratory distress syndrome (ARDS), are influenced by gender,[13-22] age,[23-27] or gender–age interactions,[28-30] we designed our study to evaluate the influence of these comodifiers on postulated nmMLCK2-mediated increases in vascular paracellular transport. This is supported by findings in mice, which like humans, have varying respiratory properties that differ between sexes[31-33] and with age,[34,35] information often neglected when describing a pulmonary phenotype. We have further applied standard observational and pathologic approaches to initially phenotype this line—an approach that is now becoming *de rigueur* in the postgenomic era.[36,37]