Toll-like receptor 4, a potential therapeutic target of lower limb ischemic myopathy that raises further questions

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Chronic limb-threatening ischemic (CLTI) affects ≤1 in 50 Americans[1,2] and generally demands reconstructive procedures, while medical management has a limited role. Without prompt revascularization, ≤25% of patients need an amputation at 1 year. In addition to limb threat, CLTI also includes a poorly understood, complex, and progressive lower limb muscle fibrosis and myopathy encompassing numerous pathophysiologic mechanisms, including mitochondriopathy, oxidative damage, autophagy, and microvessel thickening. In CLTI patients with unreconstructable disease, the need for alternatives to mitigate these processes is even more critical.

Sachdev et al previously studied Toll-like receptor 4 (TLR4) in murine models of hindlimb ischemia showing both the protective effects of TLR4 agonism in preventing muscle necrosis after hindlimb ischemia, but also harmful results with prevention of arteriogenesis and angiogenesis.[3] Furthermore, they explored relationships between TLR4, TLR2, MyD88, NLRP3, and HMGB1 and their effect on perfusion and ischemia recovery.[4-6]

In this issue of *JVS: Vascular Science*, Navi et al[7] extend on these reports and present work based on investigations using tissue harvested from patients with CLTI to understand TLR4 immunomodulation under these conditions, and then apply their findings in a murine model of hindlimb ischemia to understand the effects of targeting this mechanism. In short, the authors show that CLTI muscle had significant upregulation of TLR4 and its downstream inflammatory cytokine compared with control muscle. From myotubules grown from CLTI muscle, they demonstrate upregulation of TLR4 and its downstream cytokine in response to ischemia, while this response was downregulated by an antagonist. Finally, in the murine model with either TLR4 knockout or antagonism, perfusion, overall function, and muscle histology improved, with decreased inflammatory cell loads over time after 21 days.

Although convincing, the study does not unravel in which context, whether acute, chronic, after revascularization, claudication, or CLTI, immunomodulation from TLR4 inhibition would best apply. The findings obtained from analyses of human material from patients with tissue loss requiring amputation may also infer confounders from inflammatory processes coupled with gangrene and tissue damage, unrelated to muscle ischemia. The myotubule experiments are executed in an in vitro model that does not necessarily reflect the more complex environment of the extremity musculature in vivo. The murine model better represents the effect of TLR4 inhibition or alteration on the recovery from acute limb ischemia rather than the effects in the long-term ischemic environment.

The strength and novelty of their work lies in the demonstration that TLR4 and its downstream signaling is upregulated in human CLTI muscle and how this is validated and explored further both in vitro and in their mouse model. Here, the authors confirm the inflammatory upregulation of TLR4 and improvement in perfusion recovery after TLR4 inhibition as previously shown.[3] Given the established correlation between human tissue and a murine model by the authors, TLR4 remains an interesting potential pharmaceutical target. However, further studies in small or large animal models of limb ischemia are needed to further understand TLR4 mechanisms, and in which context TLR4 would be most useful as a therapeutic target.

The opinions or views expressed in this commentary are those of the authors and do not necessarily reflect the opinions or recommendations of the JVSeVascular Science or the Society for Vascular Surgery.

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DISCLOSURES

None.

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