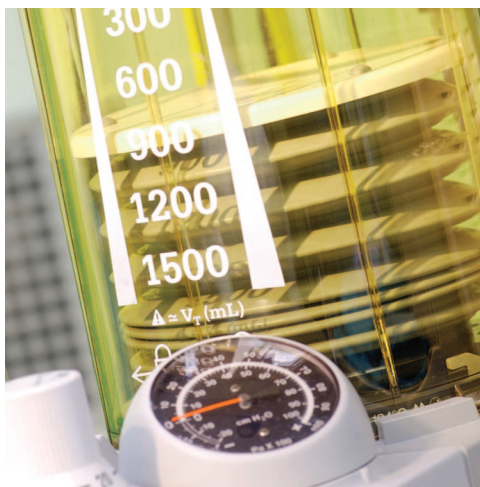


Do We Need to Pay Toll on the Bridge from Innate Immunity to Ventilator-induced Diaphragm Atrophy?

MECHANICAL ventilation (MV) is frequently mandatory in patients with respiratory failure. However, MV has the potential to induce respiratory muscle dysfunction, and diaphragm weakness is associated with a prolonged need for MV. Human studies confirm results from animal studies showing that inactivity of respiratory muscles with controlled MV leads to structural injury and atrophy of diaphragmatic fibers.¹ The exact upstream mechanisms that initiate muscle injury and fiber atrophy are unclear. In this month's issue of *ANESTHESIOLOGY*, Schellekens *et al.* present the results of an animal study that tested the role of Toll-like receptor 4 (TLR4) in the development of ventilator-induced diaphragm atrophy.² In their experiments, wild-type and TLR4 knockout mice were ventilated for 8 h. Controlled MV in wild-type mice resulted in a reduction of the diaphragm myosin heavy chain content by approximately 50% as compared with spontaneously breathing mice, which was associated with increased local concentrations of the inflammatory mediators interleukin-6 and keratinocyte-derived chemokine, the mouse analog of human interleukin-8. In TLR4 knockout mice, MV neither affected myosin content nor diaphragm interleukin-6 and keratinocyte-derived chemokine levels.

Muscle fiber atrophy can be a consequence of decreased protein synthesis as well as increased proteolysis. The former was demonstrated in a rat model in which 6 h of controlled MV decreased the synthesis of diaphragmatic mixed muscle protein (*i.e.*, an average synthesis rate for all muscle proteins) and myosin heavy chain protein because of impairment of posttranscriptional events.³ Four proteolysis pathways have also been reported to play a role in ventilator-induced diaphragm atrophy. Animal and human studies demonstrate up-regulation of 1) the calpain (calcium-dependent proteases) and 2) caspase-3 pathways, both important for the initial step of sarcomeric protein degradation, and 3) the



“[This study] suggests a potential new therapeutic target, TLR4 [for ventilator-induced diaphragm atrophy].”

ubiquitin-proteasome pathway, central for further processing of partially cleaved and disassembled myofilament proteins.¹ A more recent human study revealed up-regulation of the 4) autophagy pathway during MV. Autophagy, which literally means self-eating, is characterized by the degradation of cytoplasmic organelles and proteins in phagolysosomes. This pathway is important for cell survival by recycling old organelles and proteins during normal homeostasis, but can be quickly up-regulated when increased energy is needed. In Schellekens *et al.*'s analysis of activation of the caspase-3, ubiquitin-proteasome, and autophagy pathways, the latter was found associated with TLR4 mediated ventilator-induced diaphragm atrophy.

TLRs are important pattern recognition receptors crucial for the detection of invading pathogens. TLR4, for example, recognizes lipopolysaccharide present in the outer membrane of Gram-negative bacteria. In the last decade it became clear that TLRs are also activated by endogenous danger signals. These so-called damage-associated molecular patterns are molecules released from injured tissue that also initiate an innate immune response. Therefore, TLR4 activation is not only important during infections but can also play a central role during sterile inflammation. Schellekens *et al.* suggest TLR4 to be important in the pathogenesis of ventilator-induced diaphragm atrophy,² but how?

A previous animal study demonstrated ventilator-induced lung inflammation to be dependent on TLR4 activation.⁴ Interestingly, endogenous TLR4 agonists were found in bronchoalveolar lavage fluid. Schellekens *et al.* hypothesize that these endogenous TLR4 ligands might be responsible for activation of TLR4 in ventilator-induced diaphragm atrophy.² But how can these damage-associated molecular patterns reach the diaphragm? Are these ligands also present in plasma? Does the injured diaphragm itself release damage-

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Accepted for publication May 3, 2012. The authors are not supported by, nor maintain any financial interest in, any commercial activity that may be associated with the topic of this article.

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◆ This Editorial View accompanies the following article: Schellekens W-JM, van Hees HWH, Vaneker M, Linkels M, Dekhuijzen PNR, Scheffer GJ, van der Hoeven JG, Heunks LMA: Toll-like receptor 4 signaling in ventilator-induced diaphragm atrophy. *ANESTHESIOLOGY* 2012; 117:329–38.

associated molecular patterns? These questions need to be addressed in future animal studies. Future studies should also focus on whether other pattern recognition receptors are involved in ventilator-induced diaphragm atrophy.

Although early cytokine release is important for the normal immune response, it may also cause collateral tissue damage. Numerous experimental studies support this scenario in skeletal muscles, linking the actions of cytokines to muscle weakness and wasting.⁵ Notably, increased systemic cytokine levels during sepsis may amplify local cytokine release in muscles.⁵ The study by Schellekens *et al.* does not provide direct evidence for the contribution of up-regulated cytokines to the reduced myosin heavy chain content.² The use of cytokine antagonists in ventilated wild-type mice, or administration of cytokines to TLR4-deficient mice (to test if the phenotype can be reverted back to wild-type), might further elucidate this issue.

As mentioned above, Schellekens *et al.* showed 8 h of MV to trigger the autophagy pathway in the diaphragm of mice.² These data are, at least in part, in line with results from a human study demonstrating prolonged MV (from 15 to 276 h) to trigger diaphragm autophagy and proteasomal degradation.⁶ Indeed, controlled MV increased the expression of various autophagy-related genes and proteins, and induced autophagosome vesicles in muscle fibers of the diaphragm. Oxidative stress was described as the main trigger of autophagy and proteasomal degradation pathways.⁶ Whether oxidative stress plays a role in the study by Schellekens *et al.* was not analyzed, but is of potential interest because activation of TLR4 has been suggested to induce reactive oxygen species production in an *in vitro* study.⁷ Downstream of TLR4, activation of p38 mitogen-associated protein kinases is necessary for the activation of the ubiquitin proteasome pathway and autophagosome formation in lipopolysaccharide-induced muscle degradation.⁸ Thus, intracellular signaling pathways should also be studied in models of ventilator-induced diaphragm atrophy in future investigations.

Weaning difficulties often occur when weakened respiratory muscles cannot cope with the increased respiratory workload present in patients with a high pulmonary resistance and decreased compliance because of lung injury. Physicians frequently focus on improving lung function, probably because pulmonary problems are easier to diagnose (using chest radiographs) and more knowledge on treatment possibilities is present. Scientific understanding of ventilator-induced diaphragm atrophy is limited, and concrete guidelines for patient management are lacking. However, several animal studies indicate that the degree of injury can be modulated by how the ventilator is set. Maintenance of some respiratory muscle activity, for example by using assist-control ventilation, alleviates impairment of diaphragmatic contractility.¹ Clinicians might therefore want to use modes that allow some degree of respiratory muscle activity while maintaining patient comfort and adequate gas exchange. Never-

theless, studies are needed to determine the optimal level of diaphragmatic activity. Whether the use of newer ventilation modes, like adaptive support ventilation, neutrally adjusted ventilatory assist, and proportional assist ventilation has impact on ventilator-induced diaphragm atrophy is of great interest.¹

It is important to notice that even with the use of partial support settings, impairment of the diaphragmatic force is not completely prevented.¹ Pharmacological support might play an additional role herein. The study of Schellekens *et al.* brings new insights into the molecular mechanisms involved in ventilator-induced diaphragm atrophy. Moreover, it at least suggests a potential new therapeutic target, TLR4. Of course, beneficial effects of TLR4 blockade should be carefully studied because the innate immune response is of utter importance to combat infections.

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