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# A New Twist to Myopathy of Critical Illness

SEPSIS, with its associated complications, is a leading cause of death in intensive care units, with a mortality rate exceeding 40%.[1] An important untoward effect of such a critical illness is muscle wasting and muscle weakness, resulting in ventilator dependence with increasing morbidity and mortality rates.[2] The origin of these functional changes can be broadly classified as neural, neuromuscular (junctional), and/or muscular,[3,4] and these critically ill patients, particularly of the "surgical" type, may exhibit signs of one or more of the following: fade with repetitive stimulation, altered tendon reflexes, elevations of creatine phosphokinase levels, and electrophysiologic evidence of myopathy or polyneuropathy.[3,4] Rodent models of surgical critical illness include denervation,[5] immobilization,[6] burns,[7] and endotoxic state[8] and have all demonstrated loss of muscle mass and/or weakness. The loss of muscle mass and the associated functional changes have been attributed to immobilization,[6] myofibrillar degeneration,[9] and use of concurrent medications such as steroids and muscle relaxants.[10] Apoptosis or programmed cell death of muscle cells may also contribute to loss of function in conditions such as immobilization, sepsis, and burns.[11–13]

In addition to the changes in muscle function, many clinical and laboratory studies have consistently reported a surprising pharmacologic phenomenon in skeletal muscles of critically ill surgical intensive care patients. Intuitively, one would assume that a functionally weak muscle would have increased sensitivity to curare-like drugs. This, however, has not been the case in reports following denervation,[5] burns,[7] immobilization,[14] and endotoxicosis,[8] in which a decreased sensitivity (resistance) to nondepolarizing relaxants (*e.g.*, *d*-tubocurarine) and increased sensitivity to depolarizing relaxants (*e.g.*, succinylcholine) have consistently been reported.[15] The increased requirement for curare-like drugs has been explained by the qualitative (isoform expression) and quantitative (upregulation) changes in the number of acetylcholine receptors (AChRs) at the neuromuscular junction (endplate).[15] In this issue of ANESTHESIOLOGY, however, Tsukagoshi *et al.*[16] document some interesting, provocative and contradictory findings in a rodent model of sepsis.

An accepted *in vivo* system to study sepsis is the cecal ligation and puncture (CLP) rat model, in which one can produce acute and subacute forms of sepsis by altering the size of the cecal puncture.[17] Tsukagoshi's group from Japan has produced a mild form of sepsis with a 90% survival rate. Rats at 7, 14, and 21 days following CLP demonstrated muscle weakness, which was tested by forced exercise and evoked twitch responses. The surprising features were that *in vivo* dose-response curves to *d*-tubocurarine demonstrated increased sensitivity (decreased ED50), and there was a decreased number of AChRs; both findings were most prominent at days 7, 14 and 21 after CLP. Moreover, the authors demonstrated the presence of antibodies to AChR in the sera of the CLP rats first evident at day 4 after CLP and persisting to periods beyond. The timings of these changes suggest that antibodies to AChRs may have caused the loss of AChR and the increased sensitivity to *d*-tubocurarine.

Antibodies to AChRs are diagnostic of myasthenia gravis, a disease in which muscle weakness and fatigue follows loss of AChRs from the neuromuscular junction, and these antibodies have never been reported in critically ill patients without myasthenia. These new findings, therefore, not only challenge existing data on *d*-tubocurarine responses and AChR numbers relative to surgical critical illness but also provide preliminary evidence for a myasthenia gravis–like syndrome occurring with sepsis that could explain some of the muscle weakness seen in critically ill subjects. The results, despite some methodologic limitations, have to be taken seri-

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ously, because the experiments have been done carefully.

There are two situations in which a downregulation of AChR numbers is found: in myasthenia gravis and in chronic agonist stimulation of the AChR as seen with chronic administration of cholinesterase inhibitors.[15,18] In the latter condition the persistent high levels of acetylcholine downregulate AChR numbers, as seen in any receptor system stimulated by an agonist. The downregulation of AChRs following myasthenia, by contrast, is clearly caused by an immunoglobulin (Ig)G-mediated autoantibody process.[18,19] Passive transfer of IgG from myasthenia patients to mice mimics the symptoms and signs of myasthenia; immunization of animals against AChRs can produce experimental myasthenia from frogs to primates; and a reduction in antibody levels by immune suppression or plasmapheresis results in clinical improvement.[18] The level of antibody against AChR, however, does not correlate well with clinical severity, and a proportion of patients with myasthenia gravis do not have detectable antibodies. Involvement of the thymus is suggested by the presence of AChR on muscle-like cells in the thymus, the existence of plasma cells secreting antibodies to AChR, and the beneficial effects of thymectomy, but the cause of the disease is unknown.[18,19]

There are several theories that seek to explain autoimmunity. One such hypothesis is that an infectious agent induces an autoimmune response because of shared antigens or epitopes between the infectious agent and the host. This theory of "molecular mimicry" was advanced 50 yr ago[20] and has been entertained for several of the neuromyopthies,[21,22] which often follow an acute illness. In about 30% of cases, enteric *Campylobacter jejuni* infection precedes Guillain-Barré syndrome[20] or its variant, Miller-Fisher syndrome.[19] In both syndromes, there are IgG antibodies to glycolipid antigens that are expressed by the bacteria and are also found on neuronal tissues.[19] A similar mechanism for myasthenia gravis was suggested in 1985. Stefansson *et al.*[23] demonstrated that monoclonal antibodies to torpedo-fish AChR also bound (on western blots) to bacterial protein from *Escherichia coli, Kebsiella pneumoniae*, and *Proteus vulgaris*. This sharing of epitopes, or cross-reactivity, was seen between the bacterial proteins and the $\alpha$-subunit of the AChR, which is thought to be the main target for antibodies in myasthenia gravis.[18,23] However, there was no proof that antibodies in patients with myasthenia gravis bound to bacterial proteins, and there have been no further reports confirming Stefansson's findings. Nevertheless, *E. coli, K. pneumoniae*, and *P vulgaris* are all enteric bacteria that can be released into the peritoneum following CLP. Thus, Tsukagoshi *et al.* hypothesize that the primary event following CLP is the release of these bacteria and formation of antibodies, as part of the normal immune response, some of which happen to cross react with AChRs. The immune (antibody) response to bacterial antigens released following CLP could explain the increased AChR antibody levels in sera and the decreased AChR number on the muscle membrane in their study.

One reason for previous skepticism regarding the role of bacteria in inducing myasthenia is that the antibodies against AChR in myasthenia gravis do not behave as if directed primarily against bacterial antigens. However, it is possible that antibacterial antibodies might be the initiating insult, and that subsequent "determinant spreading" leads to the high-affinity antibodies specific for the AChR that typify the neurologic disease; some of these possibilities have been recently reviewed.[24] Thus, the relevance of the results of Tsukagoshi *et al.* to the etiology of myasthenia gravis could be particularly exciting, and, if confirmed, this model may prove useful for further evaluations of the pathogenesis of myasthenia gravis.

The studies of Tsukagoshi *et al.* are not conclusive, and there are certain methodologic limitations. The curare dose-response curve, AChR assays, and anti-AChR antibody assessments were all determined in different groups of animals, limiting inferences from the results. The assay used for the quantification of AChR was not very sensitive, and a large amount of nonspecific binding resulted in a low signal-to-noise ratio. Furthermore, the authors report total AChR numbers in whole muscle, rather than measuring AChRs specifically at the endplate regions, which is far more accurate. The specificity of the anti-AChR antibody assay could also be open to question. The assay involves immunoprecipitation of radiolabelled $\alpha$-bungarotoxin-bound AChR from rat denervated muscle, and the authors did test AChR specificity. However, the amount of precipitated AChR was not high, and one wonders whether sera from sick CLP animals might become stickier and precipitate AChRs in a nonspecific manner. Autoantibodies to AChRs have been reported in another chronic inflammatory state: In Chagas' disease (*Trypanosoma cruzi* infection), autoantibodies (IgG) are directed against $\beta$-adrenoceptors[25] and muscarinic receptors[26] in the myocardium as well as against AChR.[27] It is important, therefore, to obtain independent validation of the anti-AChR antibody levels in

the sera in laboratories with recognized expertise in the assay.

The investigators have no doubt already initiated experiments to demonstrate a cause-and-effect relationship between subacute bacterial infection (inflammation), the resultant autoimmune response to some bacterial proteins, cross-reaction with AChR, and reduction in AChR numbers. Despite the reservations expressed, this preliminary study challenges us to explore the possibility that a downregulation in AChR at the junction can account for some of the muscle weakness of critical illness and should stimulate a search for antibodies to human AChR in critically ill patients.

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