Muscle Wasting in Chronic Obstructive Pulmonary Disease: Not Enough Autophagy?

Chronic obstructive pulmonary disease (COPD) is an inflammatory lung disease characterized by airway obstruction and inflammation. Pulmonary emphysema, a form of COPD, represents a major public health problem worldwide (1). Patients with COPD frequently experience exercise intolerance, which negatively affects their quality of life and worsens morbidity and mortality (2). Growing evidence indicates that patients with emphysema develop locomotor skeletal muscle wasting and weakness, which contributes to adverse clinical outcomes, independent of lung function impairment (2, 3). The etiology of skeletal muscle dysfunction in patients with COPD is multifactorial and includes systemic inflammation, oxidative stress, malnutrition, and enhanced proteolysis.

Recent studies have reported that dysregulation of autophagy may be involved in the pathogenesis of skeletal muscle dysfunction in patients with COPD (4, 5). Autophagy is the catabolic process by which cytosolic proteins or damaged organelles, such as mitochondria and peroxisomes, are enclosed by a double membrane autophagosome and subsequently delivered to lysosomes for degradation. It plays a key role in numerous physiological and pathological processes, including maintenance of muscle mass, contractile function, and myofiber integrity (6, 7).

In this issue of the *Journal*, Balnis and colleagues (pp. 623–637) report on the role of autophagy in the impairment of the myogenesis program of muscle satellite cells (SCs) using a murine model of emphysema in which $IL13^{TG}$ (T-helper cell type 2 cytokine IL-13) is overexpressed in pulmonary cells (8). They conclude that inhibiting autophagy impairs myogenic and replicative capacities of muscle SCs in emphysematous mice, leading eventually to muscle atrophy and contractile dysfunction. The $IL13^{TG}$ model was chosen because it replicates most features of COPD in patients, including pulmonary emphysema, decreased oxygen saturation, and subsequent development of limb muscle atrophy and weakness.

The authors demonstrate that the development of emphysema triggers significant fiber atrophy and leads to decreases in the maximum isometric force of the extensor digitorum longus (EDL) muscle (*see* Reference 8 Figure 1). Specific maximum isometric EDL force (force normalized per surface area) did not vary in comparison with control mice ($IL13^{WT}$), suggesting that muscle atrophy is the primary cause of limb muscle weakness in this model. This conclusion is supported by previous observations by the same group that the muscle-specific ubiquitin E3 ligases atrogin-1 and MuRF1, which play important roles in muscle atrophy in various catabolic conditions, are significantly upregulated in EDL muscles from $IL13^{TG}$ mice (9).

The first question the authors attempt to answer is whether autophagy is impaired in the skeletal muscles of emphysematous mice. In line with previous reports in patients with COPD (4, 5), they found that several autophagy-related genes (*Ulk1*, *Beclin1*, *Lc3*, *Sqstm1*, *Atg7*, and *Atg3*) were upregulated in the limb muscles of these mice. Of note, though, when quantifying autophagy, measuring autophagy-related gene expression alone is not an adequate method, given the fact that impaired autophagosome–lysosome fusion or lysosome dysfunction may develop with no accompanying changes in autophagy gene expression (10). Thus, one of the shortcomings of the study is that it lacks *in vivo* muscle autophagic flux measurements. Future studies should be designed to address this issue. Skeletal muscle autophagic flux can be measured in experimental emphysema models by using autophagy inhibitors such as leupeptin or colchicine, as previously reported by our group (11).

The second question the authors attempt to answer is whether the regenerative capacity of an SC is dysregulated in emphysematous mice. They demonstrate that differentiation of primary SCs derived from $IL13^{TG}$ mice significantly decreases relative to those derived from $IL13^{WT}$ mice, suggesting that the *in vitro* myogenic capacity of SCs is indeed impaired by emphysema. Furthermore, using a barium chloride injury model to elicit fiber necrosis in the tibialis anterior (TA) muscle, they showed that the fiber size of regenerating TA muscles from $IL13^{TG}$ mice was significantly smaller that that from wild-type mice, suggesting impairment of the *in vivo* myogenesis program. Interestingly, in the regenerating muscles of both the $IL13^{TG}$ and $IL13^{WT}$ mice, the expressions of desmin and embryonic myosin heavy chain (markers of regeneration) were similar, indicating the degree of impairment in the emphysematous mice was relatively mild.

The third question the authors attempt to address is whether the replicative capacity of an SC is disrupted in the muscles of emphysematous mice. Ethynyl-deoxyuridine incorporation assays resulted in significant decreases in the proliferation of $IL13^{TG}$ SCs relative to $IL13^{WT}$ SCs. To establish that altered regeneration and replication are intrinsic defects of $IL13^{TG}$ SCs rather than related to the COPD environment, they conducted transplantation experiments in which red fluorescent protein–labeled SCs were injected into barium chloride–injured muscles. The injured muscles were examined 2 weeks later, and lineage tracing confirmed that the defects in $IL13^{TG}$ SCs were indeed intrinsic. To identify the degree to which autophagy contributed to these defects, the authors measured Beclin1 protein concentrations and counted the number of LC3 punctae in these cells. Both appeared to be significantly higher in $IL13^{TG}$ SCs relative to $IL13^{WT}$ SCs, but direct measurements of *in vivo* autophagic flux using bafilomycin (inhibitor of lysosomal degradation) suggested that the rate of autophagosome formation in $IL13^{TG}$ SCs was actually significantly lower than in $IL13^{WT}$ SCs. So, they then administered spermidine (inducer of autophagy) to the drinking water of $IL13^{TG}$ mice to confirm that decreased autophagy was, in fact, the mechanism underlying the observed impairments to the myogenic

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and replicative capacities of emphysematous SCs. They reported improved autophagosome–lysosome fusion in isolated $IL13^{TG}$ SCs and normalization of myogenic potentials when transplanted into *in vivo* injured TA muscles.

Although this article advances intriguing new information relating to autophagy in SCs in emphysema, the mechanism by which autophagosome formation decreases in $IL13^{TG}$ SCs remains unclear. The authors speculate that acute hypoxia is likely, as hypoxia reportedly causes upregulation of histone deacetylase C9, which inhibits the expressions of Atg7, Beclin1, and LC3 by binding directly to their promoters (12). Hypoxia can also attenuate the regenerative capacity of SCs by decreasing the expressions of MyoD or myogenin via HIF1α (hypoxia-inducible factor 1-α)-dependent (12) and -independent mechanisms (13). We speculate that another mechanism may also explain why autophagy decelerates in $IL13^{TG}$ SCs. The key nutrient sensor SIRT1 (sirtuin 1) is a nicotinamide adenine dinucleotide–dependent deacetylase that plays an important role in stimulating autophagic flux during SC activation (14). Increased metabolic demands during SC activation are sensed by SIRT1, which then activates autophagy to provide the amino acids required for synthetic activity (14). The exact role of SIRT1 in defective regeneration of SCs in COPD needs to be investigated in future studies.

Balnis and colleagues have produced interesting and novel results regarding the roles of autophagy and emphysema in impaired regenerative and replicative capacities of SCs and are to be commended for their work. Their findings are in accordance with previously reported roles of autophagy in skeletal muscle regeneration (15). It also concurs with research that shows that autophagy plays a critical role in the activation phase of SCs upon muscle fiber injury. For example, in a mouse model of Duchenne muscular dystrophy, decreased SC regenerative capacity was associated with decreased autophagy (16). In addition to SCs, autophagy also improves the regenerative capacities of other kinds of stem cells (17). This positive effect of autophagy is mediated through mitophagy (removal of dysfunctional mitochondria), the prevention of oxidative stress, and a delay of senescence (15). ■

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