

Free Immunoglobulin Light Chains in Chronic Obstructive Pulmonary Disease

In this issue of the *Journal*, Braber and colleagues (pp. 817–824) describe the unexpected and provocative observation of elevated free immunoglobulin light chains (IgLC) in experimental and clinical chronic obstructive pulmonary disease (COPD) (1). This is not the first article to link IgLC to lung disease: Nijkamp, Redegeld, and colleagues did that for asthma a decade ago (2, 3). And neither is it the first article to link free light chains to human inflammatory diseases (4). Why, then, is it so interesting and important? The reason is there is now increasing evidence to suggest that the B lymphocyte can be pathogenic in COPD. Their findings take us one step closer to understanding B-cell immunodysregulation and may possibly provide a new conduit for research into autoimmune effector pathways in the condition.

We conventionally envisage the five major classes of immunoglobulins in their familiar Y-shaped structure composed of two heavy chains and two light chains. Classical proteolysis experiments identified functional substructures: the two Fab (fragment antigen binding) containing the antigen-binding V (variable) regions just above a hinge region and the Fc (fragment crystallizable) that can interact with effector molecules (e.g., complement) and with cells via immunoglobulin Fc receptors. It would be natural, therefore, to imagine that IgLC are also formed proteolytically from intact immunoglobulin, but this is not the case.

Instead, IgLC are produced directly by B cell–derived plasma cells and are actually found naturally in health in a range of body fluids—we make about half a gram a day physiologically (5). B cells make about 40% more IgLC than needed to complement heavy chain production and can make much more when stimulated or in disease. Given that evolution is frugal, that excess is no accident.

Using several mouse models, the researchers found that levels of IgLC were elevated in animals with emphysema and right heart enlargement caused by chronic cigarette smoke inhalation (or after i.p. injection of smoke extract with adjuvant, a model found serendipitously by others to induce emphysema). Extending these findings to patients with moderate to severe COPD, they found increased serum levels of IgLC compared with controls, and they found IgLC in lung tissues around airways and, importantly, lymphoid follicles. Binding of IgLC to human neutrophils was shown to induce the chemokine IL-8 (CXCL8). They also used an experimental inhibitor, F991, to reduce smoke-induced neutrophilia in mice and decrease IgLC binding to human neutrophils. F991 is a derivative of Tamm-Horsfall protein, a monomeric glycoprotein secreted in the ascending limb of the loop of Henle in the kidney (IgLC have rapid renal clearance), and works by binding specifically to IgLC, neutralizing its activity.

At the molecular level, human light chains come in two forms, designated lambda (λ) and kappa (κ) (Figure 1). In humans, the normal kappa:lambda ratio is 2:1, and each plasma cell makes only one chain type. What is interesting is that the genes encoding λ and κ chain sequences are on different chromosomes and are arranged differently (chromosomes 22 and 2, respectively) (6). Other light chains are known in other species. This indicates differential evolutionary pressure and, even though there are no important known differences in the antibody-binding ability of lambda or kappa containing mature immunoglobulins, different functions of free lambda and kappa light chains have been discovered. IgLC are enzymatic proteases and can

directly activate leukocytes and increase their survival. In contrast, IgLC induce oxidative stress in diverse cells and tissues and are directly damaging to cardiomyocytes and renal epithelial cells, raising suspicion that IgLC might contribute to cardiac failure and impaired kidney function in COPD, common comorbidities (7). The likely receptor(s) for IgLC, which may differ by isotype, have yet to be identified.

IgLC can also polymerize, forming damaging aggregates that vary by isotype: most light chain amyloidosis is due to lambda chains, whereas almost all light chain deposition disease is due to kappa chains. Amyloid assessed by Sirius red staining was not found in COPD lung in a recent study, but the possibility is not excluded (8). Similarly, direct interaction with bacterial L protein differs by isotype. Braber and colleagues measured Kappa chains in blood and found both chains in tissue in patients but did not systematically study if there is a difference in light chain isotype expression ratio. This is an issue that needs to be resolved in future studies in much larger patient cohorts.

Most importantly, however, is what this study may reveal about the nature of immune dysregulation in COPD and in the role of autoreactive antibodies. This is for two reasons. First, expanded B-cell numbers and the appearance of lymphoid follicles in advanced COPD have been known for several years. B cells acting as IL-10–secreting “Bregs” can calm inflammation, but B cells can secrete enough IL-6 to drive autoimmunity (9). Autoantibodies have now been demonstrated by several groups in COPD, which means that the normally very effective mechanisms that operate to prevent their emergence and maintain peripheral tolerance must have failed. Light chain editing is a major mechanism silencing B-cell autoreactivity. There is evidence that light chains differentially *suppress* the formation of autoantibodies—IgLambda chains are more effective silencers of autoreactive B-cell antibody production than kappa chains (10). Has this protection via light chains been lost in COPD?

Second, the study brings us to the central issue—what is driving the B-cell→plasma-cell production of these excess free light chains. We do not know if the IgLC are produced by oligoclonally expanded B cells (as might occur if a self-antigen is driving B-cell proliferation) or from polyclonal populations (as might occur in response to a nonspecific mitogen).

It might be both. In their study, Braber and colleagues found that IgLC were increased rapidly in response to smoke—presumably well before any chance of autoreactive B cells developing in their mice. It is known from other studies that any stimulus that increases NF- κ B activity, which is common in inflammation, will directly affect IgLC expression. But they also found that levels fell on cessation after long-term smoking but did not revert to normal, and that might reflect an ongoing autostimulus.

It seems almost certain that in the future we will understand IgLC as part of more complex B-cell immune regulatory networks as more is learned of their biology. By inference from other areas of B-cell biology, that is likely to involve effects on B-cell lineage development and functional specification, germinal center formation, isotype class switching, suppression of autoreactivity, T $\leftrightarrow$ B-cell interactions, and innate immunity, including effects on dendritic cell populations.

It should also be kept in mind that we should not necessarily assume that IgLC are detrimental. There is good evidence for a role in innate bacterial defense, and when F991 was used

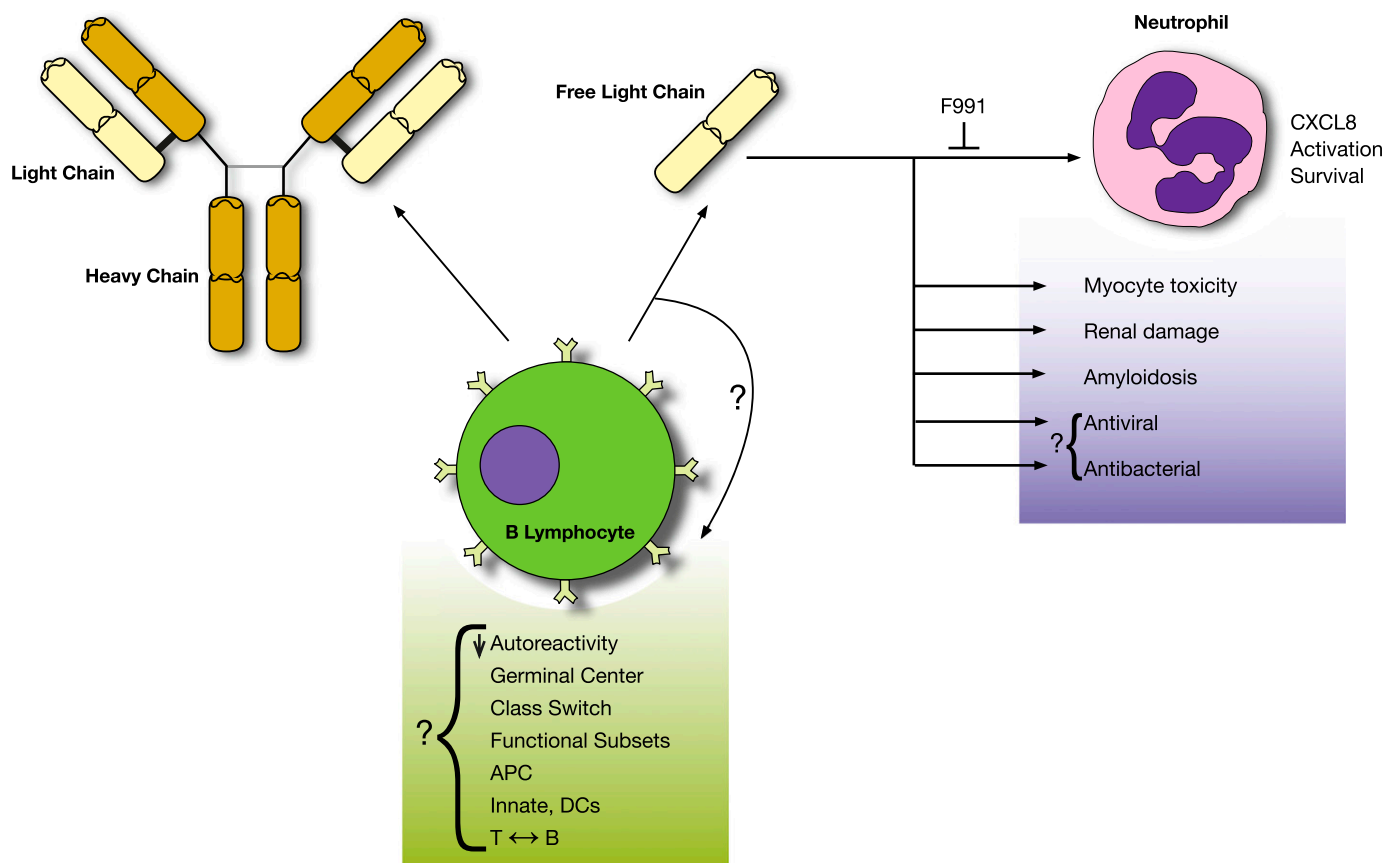


Figure 1. B-cell accumulation and formation of lymphoid follicle/aggregates is a feature of advanced COPD but remains of controversial significance. Antibody-secreting B cells (plasma cells) assemble mature immunoglobulins by covalently linking two lambda or two kappa light chains with two heavy chains. These cells produce about 40% more light chains than needed to complete immunoglobulins, and these are secreted as free light chains (IgLC). Braber and colleagues show here that IgLC, which can be blocked by an antagonist, F991, are elevated in experimental and clinical COPD, are present in human COPD lung tissue, and bind to human neutrophils inducing CXCL8 chemokine production. Others have shown that excessive IgLC can damage the heart and kidneys and deposit in tissue as amyloid. However, IgLC may also confer some antibacterial and antiviral defense. Speculatively, IgLC, which Braber and colleagues found in lymphoid aggregates in COPD lung, may feed back to affect diverse aspects of B-cell (patho)biology relevant to impaired host defense and B cell-mediated immunopathology. Autoantibodies have been found in COPD, reflecting a failure of central and peripheral tolerance: in health, light chain editing is one of the main mechanisms mediating protection from self-harming autoantibodies. APC = antigen-presenting cell; DC = dendritic cells; $T \leftrightarrow B$ = interaction of B cells with T lymphocytes.

in a mouse model of viral myocarditis, it worsened disease (11). The effect of F991 in the present study was clear but not striking, suggesting that IgFCs are not the dominant driver of neutrophilic inflammation, at least in the mouse models that were used. Perhaps in the future we may be looking for ways to eliminate autoreactive immunoglobulins while *stimulating* free chain production.

Whichever way the story unfolds, the intriguing work of Braber and colleagues indicates that we need to know much, much more about the B-cell (patho)biology of COPD.

Author disclosures are available with the text of this article at www.atsjournals.org.

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Lord of the RING: Ubiquitination and Directed Degradation of Skeletal Muscle in Acute Lung Injury

Skeletal muscle wasting is frequently observed in patients in the ICU, and its severe manifestation is independently associated with prolonged duration of mechanical ventilation and higher hospital mortality (1, 2). ICU-acquired weakness may also contribute to impaired long-term function and quality-of-life outcomes in survivors of acute lung injury/acute respiratory distress syndrome (ALI/ARDS) (3). In addition, patients with chronic obstructive pulmonary disease (COPD) often present with skeletal muscle atrophy, resulting in enhanced morbidity and mortality (4).

It is increasingly evident that muscle wasting in respiratory diseases is an active event mediated by ubiquitination and subsequent directed degradation of muscle fibers. Ubiquitination is a post-translational modification during which ubiquitin, a small protein of 76 amino acid residues, covalently conjugates to lysine residues of target proteins using an enzymatic cascade (5). During this process, an ATP-dependent E1 ubiquitin-activating enzyme activates ubiquitin, which is subsequently transferred by an E2 ubiquitin-conjugating enzyme onto the target protein. Binding of ubiquitin to the target is facilitated by E3 ubiquitin ligases, which, depending on the subtype of the E3 ligase, may bind ubiquitin and subsequently transfer it to the substrate, or directly bind the substrate, thereby facilitating the transfer of ubiquitin from the E2 to the target protein (5). Once ubiquitinated, proteins may undergo proteasomal or lysosomal degradation or nonproteolytic events. The hierarchical nature of this cascade plays a pivotal role in the diversity and specificity of ubiquitination and allows regulation of a large spectrum of cellular functions (5). Particularly, recent studies established an important role for the ubiquitin–proteasome pathway in the pathomechanism of ICU-acquired muscle weakness (6–8).

In this issue of the *Journal*, Files and coworkers (pp. 825–834) provide compelling evidence that muscle RING (really interesting new gene)-finger protein-1 (MuRF1) plays a central role in muscle atrophy secondary to lipopolysaccharide (LPS)-induced ALI in mice (9). MuRF1 is an E3 ubiquitin ligase whose activity depends on its zinc finger-containing RING domain that facilitates transfer of ubiquitin to substrates (10). The study convincingly demonstrates that MuRF1 is rapidly up-regulated after intratracheal LPS administration leading to ubiquitination of myosin and subsequent muscle fiber degradation and atrophy. Importantly, suppression of MuRF1 by genetic inactivation or microRNA-based short hairpin RNA prevents muscle fiber atrophy in LPS-induced ALI. The study of Files and coworkers is in line with recent research associating muscle wasting with ubiquitination and particularly MuRF1 activity in critically ill patients. In a model of an experimental rat ICU, where animals were ventilated over 14 days, MuRF1/2-mediated degradation of myosin was observed leading to skeletal muscle wasting and a significant decrease in muscle fiber force generation capacity (7). Other recent studies have identified ubiquitination as an important mediator of

diaphragmatic dysfunction in mechanically ventilated patients, showing high expression of ubiquitin and other proteolysis-related factors, degradation of myosin resulting in myofiber atrophy, and thus reduced diaphragm force-generating capacity (6, 11, 12). Moreover, both limb and diaphragm muscle atrophy have been recently related to MuRF1 signaling in patients with COPD (4, 13). Thus, the study of Files and coworkers is the latest addition to a growing body of evidence implicating MuRF1 as a potential regulator of skeletal muscle remodeling in pulmonary disease.

Another muscle-specific E3 ubiquitin ligase, atrogin-1, may also play an important role in muscle catabolism (14). Recently, it has been associated with LPS-induced skeletal muscle atrophy mediated by Toll-like receptor 4 in mice (15). Also, atrogin-1, and not MuRF1, has been shown to be the predominant E3 ubiquitin ligase in other human models of muscle atrophy (16, 17). In contrast, the current study demonstrated that atrogin-1 was not involved in the muscle degradation pathway in LPS-induced ALI. Intriguingly, pair-fed animals, in which calorie intake was matched to mice with ALI, also showed similar muscle atrophy and dysfunction; however, MuRF1, although transiently activated, was not involved in the atrophy signaling. Thus, various degradation pathways exist and further research will be necessary to dissect their specific role under different atrophying conditions.

It has been described that slow-twitch (type I) muscle fibers are more vulnerable in skeletal muscles of patients with COPD than fast-twitch (type II) fibers (18). Interestingly, Files and coworkers describe that type II fibers are more sensitive to ALI. This finding is consistent with the notion that MuRF1 is expressed primarily in type II fibers (19). The fact that ALI selectively causes degradation of type II fibers suggests that specific interventions may be designed to inhibit induction of MuRF1 and thus ALI-associated muscle atrophy.

The current study also provides important insights into the signaling mechanisms contributing to muscle wasting secondary to ALI. Since previous studies established important roles for nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and Forkhead box “Other” (FoxO) proteins in MuRF1-mediated muscle degradation (4), Files and coworkers set out to identify the upstream mediator of MuRF1 in LPS-induced ALI focusing on these candidates. Their data demonstrate that both NF- κ B and FoxO signaling were markedly activated during ALI and identified NF- κ B as a potent factor driving transcription of the MuRF1 gene. Although this study suggests a role for NF- κ B signaling in ALI-induced MuRF1 induction, additional *in vivo* studies will be required to confirm the role of NF- κ B in ALI-induced muscle wasting. Furthermore, although the magnitude of FoxO activation was not as pronounced as that of NF- κ B, its function in ALI-induced muscle atrophy warrants further investigation, particularly as a recent study identified FoxO-mediated ubiquitin–proteasome activity and subsequent myosin degradation as key

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