Dynamic regulation of alveolar morphogenesis in mature lungs

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The article by Massaro et al., one of the current articles in focus (Ref. 6, see p. L896 in this issue), provides several fascinating intellectual challenges. Since the pioneering work of Sahebjami et al. (10, 11) almost 25 years ago, it has been known that caloric restriction can lead to loss of alveoli. However, the molecular mechanisms underlying this alteration in lung parenchyma have remained obscure, and little is known about how this alveolar septation is dynamically regulated. What Massaro et al. have shown in this issue is that in adult male mice, not only do alveoli begin disappearing in as few as 3 days of calorie restriction, but even more remarkably, in just 3 days after refeeding, the alveoli have been restored. They have identified several acute molecular changes that occur in the destruction process, in particular, granzymes A and B, several caspases, TNF ligands and receptors, and other proteins from cytotoxic lymphocytes and natural killer (NK) cells. As recent measurements have found evidence of altered granzymes in patients with chronic obstructive pulmonary disease (COPD) (9), these results observed in mice may have relevance to the events underlying changes in COPD. Despite these remarkable structural and molecular observations, the paper is perhaps even more significant for raising the host of questions that may lead to even further insights into the dynamic regulation of alveolar septation in lung development and lung disease. In the following brief discussion, we consider several of these provocative issues.

One key fundamental question is whether the alveoli that reform after refeeding are the same, or at least in the same location, as in the original lung. This is obviously a difficult question to answer, but it may have implications for other emphysema models. How different are the processes involved in loss of alveoli after calorie restriction from those that are involved in alveolar loss from other environmental insults? Initial investigation into these processes of programmed destruction and regeneration was published last year by Massaro et al. (8). If these two structural processes have common mechanistic pathways, then acute caloric restriction may provide a convenient model to evaluate new ways to promote alveolar septation. On the other hand, if we consider the question of the dynamic progression of alveolar destruction, the similarities seem less obvious. Is there a steady loss of structural elements, until a critical state is reached where the entire vasculature in the wall suddenly collapses? With regard to the recovery of normal structure, is there a steady increase in surface area, or does a new wall suddenly pop up? Imagining how these events could occur more likely leads to the speculation that these two structural changes must involve quite different mechanisms.

In the present work, Massaro et al. (6) did not find any gross tissue necrosis: the alveolar walls simply seemed to disappear. This all-or-nothing loss of alveoli also seems to occur in humans with COPD (19, 21). Is this evidence convincing enough to presume that there really is no progressive loss of alveolar tissue? In this regard, the modeling of Suki et al. (14) may be quite relevant. These investigators suggest that with progressive destruction of structural elements, there may be little gross structural change until a level of destruction is reached that can no longer sustain the increased stresses associated with lung inflation. At such a critical juncture, there would be a sudden destruction of one or more septal walls. Of course, we are still left with the issue of what happens when the alveolar structure regenerates. At the present time, there is neither experimental evidence nor modeling to deal with this critically important process.

Another point overlaying this whole issue of alveolar formation and destruction is the level of complexity of the alveolar wall. A functional alveolar wall is a complex structural arrangement of capillaries supported by collagen and elastin fibers. What happens to these blood vessels? How are they reformed so quickly with refeeding? Recent work has clearly shown that transient inhibition of VEGF in early postnatal life leads to an emphysematous adult lung (3, 18). This suggests an important interaction of alveolar walls with pulmonary vascular growth, that with hindsight, now seems obvious. Thus it is perhaps surprising that Massaro et al. (6) did not find any alteration of VEGF signaling. New or reformed alveolar septa might contribute to lung elastic recoil, but without signaling to induce vascular growth, they would not be functional for gas exchange. Whether such a structure is even possible, however, is questionable, because vascular growth may be a prerequisite for alveolar septation.

One can only wonder at this time about the several mechanistic pathways that underlie the findings in this study. The rate of alveolar loss is a bit staggering, and there is some question as to whether it reflects real destruction of tissue. Might the septa be folded up (17), so that you would need electron microscopy to properly visualize this? If the loss of alveoli observed histologically with light microscopy is, in fact, only a manifestation of alveolar crumpling in the corners, this could partially address the question of how the alveoli reform with refeeding in the proper structural architecture. As noted already, this reforming issue otherwise seems a very complex problem. If alveolar walls are totally destroyed, how does the lung know where to grow them back? This, perhaps, could be addressed by keeping the mice calorie restricted for extended periods (assuming they could survive), but some electron microscopy may still be required.

This question is, in fact, part of a much broader question, since where alveoli form in the neonatal lung is hardly much better understood. More broadly, this signaling for an alveolar septal wall to form varies across mammalian species, and this variation in signaling is what leads to broad variation in alveolar size in different species (16) or even within a species (13). The issue has been addressed by Massaro’s group (8) in previous work, which has attempted to address what deter-
mines the site at which a septum is formed. Other work by this team has demonstrated a potential role for all-trans retinoic acid in promoting alveolar septation (7). They believe that this chemical is released by lung retinol-storing cells and that the concentration gradient established in the interstitium induces the formation of a septum at the point of highest concentration. Whether retinoic acid plays a role in the reseptation observed after caloric restriction and refeeding remains to be determined.

The question of dose response is also an important area not yet addressed. That is, how does the lung respond to a lesser degree of caloric restriction? Is there some threshold of food intake above which no lung changes occur? This gets into the complex area of caloric restriction on life span. There is very solid evidence in all mammalian species studied that chronic caloric restriction can extend life span by substantial degrees and decrease the incidence of many age-related diseases and pathologies (2, 12, 20). In such observations and studies, the degree of such chronic calorie restriction is often substantially less (25–50%) than the 67% used by Massaro et al., but when different levels have been studied (20), life span continually lengthens with increasing calorie restriction up to the magnitude used by Massaro (6). Lung structure is generally not studied in these life span studies, but if there is a concomitant loss of alveoli, it would seem curious for a longer life to be associated with an emphysematous-like lung. On the other hand, it has been known for some time that there is good correlation between alveolar surface area and oxygen consumption across mammalian species (16). So perhaps such a loss of alveolar surface area would be consistent with the decreased oxygen consumption that would be expected with calorie restriction. How this process would be regulated is unknown, but before any mechanism is sought, morphological examination of such super-aged lungs is needed.

One related physiological question is whether diet composition plays any role. Total caloric restriction is a pretty gross hammer, but at least for the effect of caloric restriction on life span, this is the variable that correlates better than diet composition (5, 20). What mechanisms might be operative by just lowering total calories? Current thinking on the mechanisms related to extended life span center on a lessening of the normal oxidative damage that occurs with aging (12). This altered balance between oxidants and antioxidants in aging is reminiscent of the altered balance between proteases and antiproteases that is often cited as the cause of emphysema. One wonders whether there might be some interaction between these two balances that might originate from some mechanistic link between aging and emphysema.

More specifically, one can experimentally investigate the role of specific dietary components. Might the changes in lung structure be linked to the lack of some essential protein? This question was specifically addressed in a rat model (4), but these investigators found the surprising result that dietary protein restriction actually ameliorated the alveolar destruction. Are carbohydrates the culprit? Would weight loss, by putting the mice on an Atkins diet, have the same effect? Clearly, we have many questions but few answers. Perhaps a productive way to investigate this effect of nutritional balance is in the refeeding phase, by selectively adding specific dietary components after the loss of alveoli has already occurred.

Also related to this interaction between diet and alveolarization are the results of Tankersley et al. (15), who examined the lungs of genetically obese (ob−/ob−) mice. For reasons that are still not entirely clear, these mice have stiffer and smaller lungs than wild-type mice. Leptin administration in these obese mice was also shown to increase lung volume. Although the alveolar structure was not studied, can the results with refeeding in the present study of Massaro et al. (6) be extrapolated to suggest that these obese mice should have increased alveolarization? Such increased alveolarization and concomitant energy expenditure would be consistent with experimental results in ob−/ob− mice, showing that their life span could be extended by as much as 50% with calorie restriction (1). These studies raise all sorts of interesting speculative questions. Will leptin administration increase alveolarization? Is there increased alveolarization with overfeeding? If not, then what regulatory feedback mechanisms might there be that link caloric intake to lung structure? How does the body sense whether there is sufficient alveolarization in the lung? Might poor nutrition in patients with COPD contribute to the progression of the pathology? These provocative questions provide motivation for new experimental studies that can be most easily attacked using these mouse models.

Finally, perhaps most remarkable, is the speed at which the molecular changes with calorie restriction are observed in the lung. Even recognizing that mice do not regulate their lives around human breakfast, lunch, and dinner, but rather eat in a continual grazing pattern, to see activation of NK cells in just 2 h, is indeed quite astonishing. Although it is unlikely that human lungs will respond as quickly, it does suggest the possibility that changes in lung signaling with variations in diet might be occurring even before body weight changes. Overall, the challenging results from Massaro et al. (6) provide motivation for a variety of new studies that will surely help to clarify the process of alveolar septation, and such understanding may ultimately lead to new therapies for emphysema.

REFERENCES


