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Lung Injury Sphingosine-1-Phosphate to the Rescue

Recent interest in the immunodulatory features of sphingomyelin metabolism products establishes a beachhead in pulmonary science. For many years sphingomyelin, which is abundant in the cell membrane, was essentially considered a structural lipid. It is now clear that through the sequential actions of sphingomyelinase, ceramidase, and sphingosine kinase, sphingomyelin is degraded to ceramide and then sphingosine to form the lysophospholipid, sphingosine-1-phosphate (S1P), a potent inducer of cell signaling. Acting as an extracellular ligand, S1P binds several G protein-coupled cell surface receptors (GPCRs), previously called Edg (endothelial differentiation gene) receptors, but now renamed S1P receptors (S1PR). Expression of S1PR in both the lung vasculature as well as the airway suggests that S1P could induce a wide variety of responses in the lung.

Pulmonary interest in S1P stems from two sets of novel findings. First, S1P as well as the drug FTY720, an S1P analog that binds S1PR in its phosphorylated form, have immunosuppressive properties, in that they block T cell egress from lymph nodes and Peyer's patches. This finding now forms the basis of stage III clinical trials for FTY720 as therapy of organ rejection after kidney transplantation (1). It is exciting to think that the drug may be applicable to therapeutic management of lung transplantation, because FTY720 given in combination with other agents prevented obliterative airway disease resulting from cross-strain lung transplantation in mice (2). One hopes that there is more research to come in this area. Second, a landmark paper from the Hla laboratory shows that S1P promotes endothelial adherens junction assembly (3). This finding is relevant to the hyperpermeability issues associated with lung injury and pulmonary edema.

The adherens junction critically determines endothelial barrier properties. Enhanced adherens junction assembly in lung endothelial cells augments barrier function and blocks pulmonary edema formation induced by intratracheal acid instillation (4, 5). The question of whether the Hla data (3), which were obtained in umbilical vein endothelial cells, are relevant to the lung has been settled by Garcia and colleagues, who showed that S1P not only enhanced barrier properties in cultured lung endothelial cells but also abrogated the hyperpermeability effect of thrombin (6, 7).

In this issue of the *Journal* (pp. 987–993), McVerry and co-workers report that S1P has protective effects against lung injury caused by high-volume mechanical ventilation and intratracheal endotoxin installation in animal models (8). Importantly, the S1P effect was tested in a large animal model of lung injury. Because of issues relevant to the height-dependent distribution of microvascular filtration and blood flow in lung, the pattern of pulmonary edema formation in a large animal suitably replicates the human disease. These experiments show that S1P blocks exudation into the alveolar space when given concomitantly with endotoxin and also abrogates pathologic patterns of

pulmonary edema formation, as detected through computer tomographic analyses. These findings bring the S1P story full circle, giving translational credence to what was previously shown only for cultured endothelial cells (3, 6)

Despite the fact that S1P was given as a single intravenous injection, McVerry and colleagues found that the protective effect persisted for several hours, indicating that barrier-enhancing processes activated by S1PR are robustly sustained over prolonged periods. This intriguing result raises several mechanistic considerations regarding the dynamics of S1PR ligation. The duration over which GPCR-mediated effects remain effective depends on multiple factors including receptor-recycling time, interrelationships among activated signaling pathways, and the extent of ligand internalization. These issues require clarification for S1PR. Moreover, it should be noted that S1P is secreted by several cell types, but most abundantly by platelets. This results in steady state plasma S1P levels of approximately 400 nM, an order of magnitude higher than S1PR binding affinity (K_d) values (9), suggesting that the receptors are tonically ligated by endogenously secreted S1P. Hence, the efficacy of exogenous S1P binding to available S1PR needs to be defined. These mechanisms remain inadequately understood for S1P–S1PR interactions, but are clearly important for the therapeutic evaluation of this agent.

A number of S1P-related issues are relevant to its therapeutic use. By activating Akt and increasing cellular calcium levels, S1P induces NO production (10). Although lung NO production may be hemodynamically advantageous, NO potentially mediates the generation of nitration products that might promote injury. S1P's actions in the airway also demand attention. These effects may be proinflammatory because S1P induces the secretion of interleukin-8, a neutrophil chemoattractant, from bronchial epithelial cells (11). By modifying the alveolar pool of sphingomyelin hydrolysis products, S1P impairs the biophysical properties of alveolar surfactant (12). Because mast cells express S1PR, S1P may act as an inflammatory mediator in the airway (13, 14).

From the standpoint of clinical therapy, an important question relates to the efficacy of *post hoc* treatment with S1P. To what extent would the barrier strengthening effects of S1P be curative rather than preventive in a lung injury scenario? Despite these questions, the Garcia group has shown that S1P must be seriously considered as a candidate for the smart therapy of lung injury.

Conflict of Interest Statement: J.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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Smoke and Mirrors

Mouse Models as a Reflection of Human Chronic Obstructive Pulmonary Disease

In this issue of the *Journal* (pp. 974–980), Guerassimov and colleagues exposed five strains of mice to cigarette smoke and found impressive variability in the development of emphysema between strains (1). Questions that some readers of the *Journal* might be asking are: Why are mice “smoking,” and why am I reading about mice in the Blue journal?

Why the mouse? The mouse has been an invaluable tool to dissect disease pathways in humans. Mice are becoming the animal of choice for many types of research because: (1) like us, they are mammals; (2) we have great understanding of mouse biology and many molecular probes to study the mouse; (3) breeding is rapid; (4) genetic homology maps are available to allow translation of genetic findings in laboratory mice to the human genome; and (5) we can manipulate the murine genome eliminating (“knock-outs”) or enhancing (transgenics) the expression of individual gene products or introducing specific variants within a gene of interest (“knock-ins”). Thus, controlled genetic experiments in mammals can be performed. Disadvantages of the mouse include their small size, which precludes some surgical models. Their small size also makes physiologic assessment more difficult, although much progress has been made in this regard. In fact, the phenotyping in the Guerassimov manuscript, which includes quantitative measurements of morphometry and physiology, is a major strength. The biggest criticism of the mouse, of course, is that mice are not (wo)men (note the whiskers). Mouse biologists would argue that basic biological processes are usually conserved in mammals. Overall, information derived from studies in mice is best used to guide studies in humans, many of which have been incredibly informative, including important insights into obesity and cancer (2, 3).

The marked strain-to-strain variability in mice exposed to the same environmental conditions strongly suggests that genetic differences between the strains influence the differential susceptibility to develop emphysema. These results provide further evidence that genetic factors can predispose to emphysema, and

more importantly, that these genetic factors are amenable to genetic dissection by careful study of the histologic and physiologic quantitative phenotypes examined by Guerassimov and colleagues. This type of detailed phenotyping is crucial in human chronic obstructive pulmonary disease (COPD) studies as well, because phenotypic heterogeneity is a potential contributor to the inconsistent results of previously reported human COPD candidate gene association studies (4). Although lung histology is not usually readily available from patients with emphysema, high-resolution chest CT scans may provide a useful surrogate.

It is unclear how many genes influence the development of emphysema in these mouse strains, where they are located, and what their individual effect size is. The next steps will likely involve localization of broad genomic regions that influence these quantitative phenotypes; this can be performed using crosses between susceptible and nonsusceptible strains and assessment of the relationship in subsequent generations between the phenotype and genetic variants across the genome that differ between the parental strains—traditional quantitative trait locus (QTL) mapping. However, the investigation of complex traits in mice has been accelerated with the development of new analytic methods and computer software, and *in silico* gene mapping using comparative SNP maps between inbred mouse strains is now an important addition to QTL mapping (5). Using a bioinformatic approach to relate known genetic variation between strains to phenotypic variation may speed the discovery of disease genes in COPD, as it eliminates some of the time and cost involved in classical QTL approaches. In either case, the size of the genomic region identified will depend on the magnitude of the experiment. In classical QTL analysis, phenotyping and genotyping a larger number of mice in crosses between strains will increase the number of recombination events and lead to a narrower genomic region of interest. Including a larger number of strains for bioinformatic comparisons will similarly lead to a more refined estimate of QTL localization.