In this study, using sLC and CD11b-DTR techniques, monocytes were depleted from both peripheral blood by 70–95% and spleen by 50–70%. Administration of MC21 mAb removed 45% of the PBMs. Whether or not the three techniques have an influence on monocytes and neutrophils in the bone marrow was not reported. It is crucial to cautiously choose depletion strategies because monocytes are able to migrate among different pools (the peripheral blood, spleen, and bone marrow) during inflammation. Monocytes also play a requisite role in regulating the resolution of inflammation by different mechanisms. Otherwise, it is very difficult to interpret the experimental findings. This notion is supported by the following findings: (1) bone marrow can release $\text{Ly6C}^{\text{high}}\text{CCR2}^+$ monocytes to the blood stream in response to the LPS challenge (12); (2) the spleen is a storage site that contains 50% of monocytes ($\text{Ly6C}^{\text{high}}$) in the body, and splenic monocytes can be rapidly mobilized toward the peripheral blood and inflamed site during inflammation and injury (13); (3) the splenic monocytes are indispensable elements forming immune reflex, which controls production of systemic proinflammatory cytokines (for example, $\text{TNF-}\alpha$) (14) through the cholinergic- $\alpha 7\text{nAChR}$ pathway; (4) alternatively activated monocytes (M2) in the peripheral blood and spleen might be involved in the resolution of inflammation (15); and (5) activation of CD11b in monocytes is able to limit TLR4-dependent proinflammatory cytokine production (16).

Alveolar flooding is a hallmark of ALI and ARDS. It should be mentioned that the magnitude of lung injury in this study is not equivalent to the clinical conditions because the dosages of LPS (0.4 mg/kg) and bacteria (10^{-5} cfu) used in the experiments were relatively low. In the meantime, a clear link among PBM depletion, alveolar neutrophilia, and pulmonary edema has not been established. Alveolar neutrophilia and the slight increase of lung vascular permeability are not parallel to the formation of pulmonary edema. Therefore, the conclusion drawn from the current study should be verified in more severe conditions and the other ALI animal models that are neutrophil dependent, for example, acid aspiration, transfusion-related acute lung injury, and ventilator-induced lung injury. Nevertheless, this study contributes importantly to ongoing research on how neutrophils are guided to the airspaces of the lung to mediate lung inflammation and injury.

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Epigenomics of Idiopathic Pulmonary Fibrosis Evaluating the First Steps

The current model of idiopathic pulmonary fibrosis (IPF), a chronic, progressive, and frequently lethal interstitial lung disease, proposes multiple cycles of epithelial cell injury and activation, leading to

migration, proliferation, and activation of mesenchymal cells with the formation of myofibroblastic foci, accumulation of extracellular matrix, abnormal wound repair, and dramatically aberrant lung remodeling (1). Central to this model is the observation that both alveolar epithelial cells and fibroblasts exhibit distinct and profound

changes in their phenotypes: alveolar epithelial cells undergo hyperplasia and activation and serve as local regulators of the inflammatory and fibrotic response, and fibroblasts are activated and exhibit the contractile and secretory features of myofibroblasts, including excessive extracellular matrix production (1). The extensive and sustained changes in cellular phenotype, the diversity of pathways involved, and the relentless nature of the disease all suggest a profound and persistent alteration in the way cellular phenotypes/behavior are regulated in the lung.

Epigenetic regulatory mechanisms, summarized briefly as changes in DNA methylation, histone modifications, or microRNA expression, were traditionally defined as mechanisms that allow for heritable changes to be transferred without changes in DNA content. In recent years, epigenetic regulation has emerged as a key adaptive mechanism by which environmental or other stresses can induce lasting changes in the gene expression repertoire and thus in the phenotype of a cell or an organism (2). The mechanisms and evidence for epigenetic regulation of gene expression in the lung have been recently reviewed (3). In IPF, evidence for alterations in epigenetic regulation was provided by the observation of changes in microRNA expression (4, 5), changes in promoter methylation state in specific genes implicated in the disease (6–8), and the recent report of significant methylation changes in hundreds of CpG islands in IPF lungs (9).

The article by Sanders and colleagues in this issue of the *Journal* (pp. 525–535) provides significant support for the notion that changes in methylation may involve hundreds of genes in IPF (10). They compare the methylation status of 27,578 CpG islands that represent the putative promoters sites of close to 15,000 genes in 12 IPF and 7 control lungs and determine that 870 genes are differentially methylated, with a relatively balanced distribution of changes (53% hypermethylation and 47% hypomethylation). When they merge their methylation data with gene expression microarrays performed on the same samples, they identify only 16 genes with inversely related significant changes in gene methylation and expression, a number that is probably not different than that expected by chance. Importantly, 8 of these genes were previously shown to be associated with fibrosis. They also validate the results by demonstrating protein decreases in hypermethylated ZNF465 and CLDN5 and increases in hypomethylated TP53INPI and DDAH1.

The article by Sanders and coworkers (10) opens significant avenues for research. Follow-up studies testing the mechanistic roles of ZNF465, CLDN5, TP53INPI, or DDAH1 as well as the changes in methylation of IPF known genes such as MMP7 or COL3A1 will be of significant interest to the research community, as are the hundreds of methylation changes they did not validate. It is, in this context, worthwhile to compare the results with those of Rabinovich and colleagues (9). Although both articles identify statistically significant changes in the methylation state of hundreds of CpG islands or genes, and validate changes in methylation and the required inverse changes in gene expression in a small number of them, a brief observation of the tables reveals very little overlap. This may be because the studies used different methods to detect methylated DNA. Sanders and colleagues (10) used the *bisulfite* conversion assay that converts unmethylated cytosine into uracil, whereas Rabinovich and coworkers (9) used methylated DNA immunoprecipitation, a method that applies antibodies to methylated cytosine to identify differential methylation. It may also be because they used different array profiling platforms (Illumina and Agilent, respectively) that differ in their designs, coverage, and even genome build versions. Finally, it could be because the studies differ in their control groups. Sanders and coworkers (10) used as controls lung transplant rejects, and Rabinovich and colleagues (9) used normal histology edges of lung resected for cancer. In both studies, control groups were too

small to allow adjusting for potential confounders such as age and smoke exposure. Although it is possible that a formalized analysis of the raw data of both studies will demonstrate a better overlap, it is nearly impossible to predict now how many of the specific methylation changes will be replicated in larger, better powered studies.

A more important insight is derived from the relatively disappointing results of the exciting attempt at integrative genomics applied in this study. The authors try to use gene expression microarrays to identify whether methylation changes are associated with the expected inverse effects on gene expression and report only a small number of genes where degree of methylation predicts gene expression. These results put a question mark on the whole exercise: If changes in the methylation of hundreds of genes only affect the expression of a tiny fraction, why even bother? The answer of course is that it is unclear that this result is indeed representative of what happens in the lung. On a statistical level, one could argue that the study was underpowered to examine this interaction and that many more tissues should be studied. On a biological level, it is possible that whole-lung methylation and expression profiles represent a sum of the results of cellular admixtures and that these may cancel each other. In our view, these are important limitations, but they, considering the wealth of data, can explain some loss of data but not such a significant lack of intersection, especially when both lists (differentially expressed genes and differentially methylated genes) contain multiple fibrosis-relevant molecules.

The main reason for the lack of intersection between expression and methylation, we believe, lies in the design of the methylation arrays that focus on promoter regions and on CpG islands. Other methods, unbiased toward CpG islands, such as comprehensive array-based relative methylation or methylation sequencing, demonstrate that most methylation changes occur neither in promoters nor in CpG islands but in sequences up to 2 kb from the islands termed CpG island shores. In fact, changes in the “shores” were strongly correlated with expression in a small cohort of colon cancer samples and despite studying a mix of cells (11). More recently the same group, analyzing 290 samples that included five types of cancers and controls, and using multiple platforms (12), has shown methylation changes in large blocks (5–10 kb) of DNA in colon cancer, a phenomenon that would probably only be detected by sequencing.

In summary, there is now enough evidence to suggest that DNA methylation is altered in IPF. To really determine the extent of the alterations, their impact on gene expression, and their reproducibility, we should analyze methylation changes in significantly larger cohorts, apply integrative genomics approaches, and analyze methylation changes in both whole tissue and specific cell types. Most importantly, we need to apply global profiling methods that indeed measure the full methylation changes in the human genome. Based on the promising studies by Sanders and coworkers (10) and Rabinovich and colleagues (9), it is safe to assume that follow-up studies will indeed find that genome-scale methylation changes encompassing hundreds of genes determine the sustainability of the abnormal lung cellular phenotype in IPF by their impact on gene expression patterns. Identifying the extent of methylation and other epigenomic changes in IPF may have significant and immediate clinical impact. Drugs that interfere with aberrant DNA methylation and histone modifications are well known, and some, like 5-azacytidine or suberoylanilide hydroxamic acid, are now U.S. Food and Drug Administration approved (13). Thus, if future studies demonstrate that changes in methylations are indeed abundant and mechanistically relevant in IPF, studies that will evaluate the efficacy of these drugs will be indicated and highly feasible. In this case, the articles discussed in this editorial will be remembered as the first steps toward finding effective therapies for this devastating disease.

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Pentraxin 3 in Primary Graft Dysfunction

The Long and Short of It

Primary graft dysfunction (PGD), the earliest manifestation of lung allograft dysfunction, is a form of acute lung injury (ALI) resulting from ischemia-reperfusion injury and other processes endogenous to lung transplantation. Severe PGD affects 10 to 35% of lung transplant recipients (LTRs) and is a significant cause of morbidity and mortality (1). PGD also increases the risk for development of the bronchiolitis obliterans syndrome (BOS) or chronic rejection. Despite advances in our knowledge of the inflammatory mediators involved in PGD, a better understanding of the pertinent cellular and molecular pathways and identification of useful biomarkers is needed. The study by Diamond and colleagues in this issue of the *Journal* (pp. 546–552) advances this effort by identifying two single nucleotide polymorphisms (SNPs) in the long pentraxin 3 (PTX3) gene that are associated with an increased risk for developing PGD, with one SNP correlating with increased PTX3 plasma levels (2).

PTX3 is a member of the pentraxin superfamily, a group of highly conserved proteins characterized by a cyclic multimeric structure. Similar to other family members, C-reactive protein and serum amyloid P, the prototypical short pentraxins, PTX3 is an acute phase protein, yet it differs by gene organization, ligand recognition, and the structural presence of a long N-terminal domain (3). PTX3 is produced by multiple cell types, including dendritic cells, monocyte/macrophages, endothelial cells, bronchial epithelial cells, and fibroblasts in response to inflammatory mediators such as tumor necrosis factor (TNF)- α , IL-1 β , and various toll-like receptor (TLR) agonists, including LPS, as shown in Figure 1. As with TLRs, PTX3 is a pattern recognition receptor, and has been shown to play a critical role in humoral innate immunity to infectious pathogens such as *Aspergillus fumigatus*

and *Pseudomonas aeruginosa* (4). Additionally, PTX3 levels have been correlated with severity of critical illness and fatal bacteremia (5, 6). Moreover, high levels of PTX3 have been associated with worse outcomes in several animal models of ALI. In one model, transgenic mice overexpressing PTX3 demonstrated severe lung injury, decreased survival, and increased levels of inflammatory cytokines (TNF- α , IL-1 β , monocyte chemoattractant protein-1 [MCP-1], and keratinocyte chemoattractant [KC]) (7). In other studies, higher PTX3 levels or gene expression correlated with ventilator-induced lung injury (8, 9). Together, these studies suggest a role for PTX3 in ALI pathogenesis.

The current study by Diamond and colleagues extends their previous findings showing that high plasma levels of PTX3 were associated with PGD in LTRs with a pretransplant diagnosis of idiopathic pulmonary fibrosis (IPF) (10). Notably, this study included LTRs of diverse ethnicities, and population stratification was well-controlled using two principle components derived from ~1800 ancestral markers. Among the seven SNPs analyzed, the minor alleles of two tightly-linked markers, rs2120243 and rs2305619 ($r^2 = 0.83$), and their related haplotypes were associated with PGD, suggesting that PTX3 is a genetic predisposition factor for PGD. Moreover, they described a correlation between the rs2305619 genotype and PTX3 plasma levels in LTRs with the pretransplant diagnosis of IPF. Interestingly, homozygous subjects for this risk allele exhibited the highest plasma levels, along with increased risk of PGD. Together, these findings provide a novel genetic mechanism for their earlier findings correlating PTX3 levels and PGD.

Importantly, the authors recognized that a limitation of their study was the lack of replication of the PTX3 genotype association