Gene Expression Signatures in Tuberculosis Have Greater Overlap with Autoimmune Diseases Than with Infectious Diseases

To the Editor:

Tuberculosis (TB) continues to be depressingly persistent as a global pandemic, killing more than any other infectious agent (1), and the mechanisms underlying pulmonary pathology remain elusive (2). Diverse clinical phenomena and experimental observations recently led us to hypothesize that an infection-initiated autoimmune phenomenon is part of the pathological process (3), and others have since noted this association (4, 5). To investigate this hypothesis, we performed an analysis of differentially expressed genes (DEGs) in patients with TB and patients with autoimmune or infectious diseases, using within-study control groups. We obtained gene expression datasets from the Gene Expression Omnibus (accession numbers GSE19444 and GSE22098, Illumina platform) to reanalyze a major published study (6).

All members of the TB cohort had culture-confirmed pulmonary disease. The autoimmune diseases studied were adult and pediatric systemic lupus erythematosus, and adult Still's disease. The infectious diseases were culture-confirmed staphylococcal or streptococcal bacteremia. All samples from patients with TB were taken before treatment was initiated. No information was available to the authors regarding the treatment status of the autoimmune cases, which could potentially introduce confounding factors. To control for variability between different microarray experiments, we first defined all DEGs in each condition relative to the healthy control subjects for each dataset. DEGs were identified using the *limma* package in R on quantile normalized raw expression data obtained from the Gene Expression Omnibus datasets using a moderated *t* test statistical filter (Benjamini-Hochberg [false discovery rate (FDR)]-adjusted $P < 0.05$) to define differential expression.

Our analysis identified 2,468 DEGs in TB, 8,134 DEGs in infection, and 11,348 DEGs in autoimmune disorders relative to controls, resulting in disease-specific gene signatures (Figure 1). A total of 1,481 DEGs were common to all three conditions, thus representing a generic inflammatory response, and comprised 60.0% of the TB signature. Only 96 DEGs were shared exclusively between TB and infection (3.8% of the TB signature), whereas TB and autoimmune disease had 810 DEGs in common (32.8% of the TB signature). Notably, once the genes shared by infectious and autoimmune diseases were accounted for, only 81 (3.3% of total DEGs) were exclusive to TB. We further refined our disease DEG lists by quantitative filtering (fold change >2 or <-2), which reduced the number of genes identified, and the observed disease-overlap relationships were broadly maintained (Figure 1, values in parentheses). By this more stringent analysis, 48.0% of the total TB signature was common to the autoimmune signature, whereas only 3.9% genes were common to the infectious signature. All of the 35

Figure 1. Venn diagram demonstrating overlap in differentially expressed genes relative to healthy controls in tuberculosis, infectious disease, and autoimmune disease. Total differentially expressed genes analyzed by *P* value are shown, and values further filtered by a fold change of $>\pm 2$ are shown in parentheses.

common genes differentially expressed in both TB and the autoimmune conditions were found to be modulated in the same direction (i.e., up- or down-regulated) between TB and autoimmunity.

Next, we studied the pathways that were communal between TB and autoimmune diseases. We used ToppFun functional enrichment in the ToppGene suite to determine gene ontology for disease associations for the TB–autoimmune overlap genes. Using the FDR-corrected *P* value–filtered common TB–autoimmune DEG list, we annotated significant (FDR *P* value < 0.05) associations with a number of autoimmune diseases, such as systemic lupus erythematosus, Behçet's disease, and rheumatoid arthritis. After an additional filter for fold change (fold change >2 or <-2) was applied, the 35 common TB–autoimmune DEGs were also annotated to infer biological processes common to both autoimmune disease and TB (Table 1). These gene ontology associations were dominated by the type I IFN signaling pathway.

These analyses support the hypothesis that an autoimmune process contributes to pathology in pulmonary TB (3). DEGs in TB predominantly overlap with autoimmune disease and less so with infection. Furthermore, combining infection and autoimmune disease signatures explains 96.7% of the differentially expressed TB signature, suggesting that pathology in TB results from an interplay between infection and a currently unrecognized autoimmune process. Our analyses identified a predominant role for type I IFN signaling, which for many years has been known to play a critical role in autoimmune disease (7) and has recently emerged as a key factor in the gene expression signature for TB (8). Our study is both strengthened and limited by the fact that the gene expression signatures were all derived from samples taken from whole blood, as opposed to the site of disease. A benefit of this approach is that it allowed us to compare different diseases by sampling the same compartment. The fact that autoimmune disease and TB have major commonalities in the circulating gene expression signatures remote from the site of disease supports the concept that underlying pathological mechanisms may be shared.

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Table 1. Biological Process Gene Ontology Analysis: Tuberculosis–Autoimmune Differentially Expressed Gene Overlap

Identity	Name	FDR BH	Hits to Annotation
GO:0060337	Type I IFN signaling pathway	1.39×10^{-12}	8/81
GO:0071357	Cellular response to type I IFN	1.54×10^{-12}	8/82
GO:0034340	Response to type I IFN	2.28×10^{-12}	8/86
GO:0034097	Response to cytokine	3.27×10^{-12}	15/825
GO:0045087	Innate immune response	4.77×10^{-12}	15/847

Definition of abbreviations: BH = Benjamini-Hochberg; FDR = false discovery rate.

Gene ontology associations of the common differentially expressed genes between tuberculosis and autoimmune diseases. Biological processes were annotated from a differentially expressed gene list filtered statistically and quantitatively ($P < 0.05$, fold change $> \pm 2$). Disease ontology associations were annotated using ToppFun functional enrichment in the ToppGene suite, and we report the BH-adjusted P value to account for FDR.

An important next step is to compare expression signatures at the respective sites of disease to identify common pathways that can underpin dissection of the fundamental pathological process. This approach may elucidate the disease mechanisms in pulmonary TB, which continues to be poorly understood and for which standard treatment has remained unchanged for 40 years and vaccination for almost 100 years (9). Defining an autoimmune process that exacerbates pathology in TB would help inform emerging host-directed therapies, to limit the lung-tissue destruction that results in mortality and potentially to accelerate cure (10), as well as avoid vaccination strategies that may inadvertently exacerbate lung destruction and transmission. ■

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Randomized Double-Blind Controlled Trial of Roflumilast at Acute Exacerbations of Chronic Obstructive Pulmonary Disease

To the Editor:

Chronic obstructive pulmonary disease (COPD) exacerbations are critical events that lead to poor health status (1), lung function decline (2), and mortality (3). However, treatment options for acute exacerbations remain limited. Treatment failure with the current standard of care is 27% at 30 days (4) and rises to 37% at 90 days (5). Thus, new therapies for the management of acute exacerbations are urgently needed.

Roflumilast is a phosphodiesterase 4 inhibitor and a nonsteroidal antiinflammatory agent designed to target both the systemic and pulmonary inflammation associated with COPD. Roflumilast reduces neutrophilic airway inflammation (6), improves lung function, and reduces exacerbations in stable patients with COPD (7–9), but it has not previously been studied as an acute exacerbation therapy.

Therefore, we conducted a randomized, double-blind, placebo-controlled study to test the hypothesis that roflumilast reduces neutrophilic inflammation during exacerbations of COPD when added to usual medication. Faster resolution of neutrophilic inflammation is associated with shorter clinical recovery (10). Thus, this study served as a proof-of-mechanism study to examine whether roflumilast can improve clinical recovery after COPD exacerbations.

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