ASTHMA

T cell types that take your breath away

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A new T helper cell signature in asthma patients highlights the potential impact of a personalized approach to asthma care (Choy *et al.*, this issue).

Affecting nearly 300 million people worldwide, asthma is a common, chronic disease characterized by airway inflammation, bronchial hyperresponsiveness, and reversible airway obstruction. The use of molecular tools and advanced cellular immunophenotyping to define subgroups of patients with asthma (so-called asthma "endotypes") has enhanced our understanding of disease pathogenesis and allowed for the identification of distinct patient populations that can most benefit from specific targeted treatments (*1*). Only a minority of patients with asthma have severe asthma, although these patients have considerable morbidity and mortality and are expensive to treat (*2*). Therefore, the use of molecular profiling to guide management decisions for even a small subset of patients has the potential to have a large effect on the overall burden of disease (*1*). The new work by Choy *et al.* described in this issue of *Science Translational Medicine* marks an important advance toward establishing a putative T helper 17–high (T_H17-high) cell signature in asthma and highlights the potential impact of a personalized approach to asthma care (*3*).

Although asthma has long been understood to be characterized by type 2 cytokines [interleukin-4 (IL-4), IL-5, and IL-13], large clinical studies that used microarrays to define gene expression patterns in the lung found that only 50% of patients displayed an up-regulation of mRNA transcripts in the pulmonary epithelium that was consistent with immune activation by IL-13, a canonical type 2 cytokine. This T_H2-high subset of asthmatics was defined by an increase in epithelial cell expression of the *POSTN, CLCA1,* and *SERPINB2* genes, which correlate highly with *IL13* and *IL5* expression, and is characterized by greater airway hyperreactivity, eosinophilia, and, importantly, a treatment response to glucocorticoids (*4*). Close examination of one of the up-regulated transcripts, *POSTN,* revealed that it encodes for a protein named periostin, an integrin ligand that is secreted by pulmonary epithelial cells and can be detected in the bloodstream. A recent clinical trial of the humanized IgG4 monoclonal antibody to IL-13, lebrikizumab, revealed that T_H2-high patients with elevated periostin levels and poorly controlled disease despite steroid therapy had a significant clinical response to IL-13 blocking therapy (*5*). In view of the cost and potential morbidity of performing bronchoscopy procedures and airway biopsies, the discovery of periostin's role as a potentially meaningful noninvasive biomarker to guide treatment was particularly important.

Other treatments have also shown success by similarly targeting subjects on the basis of molecular profiles. Mepolizumab is a humanized monoclonal antibody against IL-5, a type 2 cytokine that has a central role in promoting the maturation and activation of eosinophils. Clinical trials that specifically targeted patients with high blood-eosinophil counts despite steroid therapy have shown that mepolizumab significantly decreases asthma exacerbations and reduces the need for treatment with systemic steroids (*2*, *6*). The success of lebrikizumab, mepolizumab, and other biologic agents clearly has validated molecular approaches to asthma care and underscored the promise it has for identifying new drug targets, developing biomarkers, and targeting select patients for therapy.

However, despite progress in establishing T_H2-high patients as a distinct group of patients and the promising results of clinical trials testing type 2 cytokine therapies, many challenges remain. Because some type 2-high asthma patients continue to suffer asthma symptoms despite treatment and represent only 50% of total asthma subjects, there has been intense interest in discovering the other molecular phenotypes (*1*). The current study by Choy *et al.* marks an important advance toward establishing a putative T_H17-high signature in asthma and provides insight into the mechanisms by which T_H2 immunity, glucocorticoids, and type 2 cytokine therapy promote a lung microenvironment that supports the emergence of this new molecular phenotype.

Since the discovery of T_H17 cells more than a decade ago, there has been tremendous interest in understanding whether this T helper cell subset and other IL-17 secreting cells participate in asthma pathogenesis, especially given the central role T_H17 cells play in mucosal immunity. Indeed, several studies in animal models have demonstrated a pathogenic role of IL-17 in experimental asthma through direct effects on the airway epithelium and smooth muscle (*7*). Because IL-17 cytokines are known to induce the release of neutrophil chemoattractants, researchers have long suspected a primary role for T_H17 immunity in patients with moderate to severe neutrophilic or mixed neutrophilic/eosinophlic asthma. Highlighting the complexities of attempting to implicate a single T cell lineage as the dominant effector in disease, recent studies have suggested that IL-4/IL-17–secreting $CD4^+$ T cells that coexpress GATA-binding protein 3 (GATA3, a transcriptional regulatory factor for IL-4–secreting T_H2 cells) and retinoic acid receptor–related orphan receptor-γt (RORγt, a transcriptional regulatory factor for IL-17–secreting T_H17 cells) just might be the crucial cell type that drives asthma pathogenesis in patients with severe, steroid-resistant disease (*8*).

JUST SEVENTEEN

To investigate T_H17 in asthma, Choy *et al.* (*3*) took an approach similar to the one used previously to both identify and validate the *POSTN, CLCA1, SERPINB2* T_H2-high signature (*4*). They first defined a T_H17 gene signature in human bronchial epithelial cells based on the ability of IL-17A and IL-17F to induce the expression of genes that encode neutrophil chemoattractants CSF3, CXCL1, CXCL2, CXCL3, and IL-8 and the neutrophil hematopoetic factor CSF3. The authors showed that IL-13 suppressed the expression of IL-17A–inducible mRNA transcripts and, to a lesser extent, IL-17A suppressed the expression of IL-13–inducible transcripts by human bronchial epithelial cells in vitro. A transcriptional analysis of airway biopsies from the authors' patient cohort for T_H2-high and T_H17-high signatures revealed three sets of patients: T_H2-high, T_H17-high, and T_H2/T_H17-low, indicating the presence of three distinct cytokine profiles that drive disease (Fig. 1). Surprisingly, the T_H2-high and T_H17-high subgroups were mutually exclusive; that is, there were no patients who were T_H2/T_H17-high, suggesting that, despite reports of T_H2/T_H17 double-positive cells in patients

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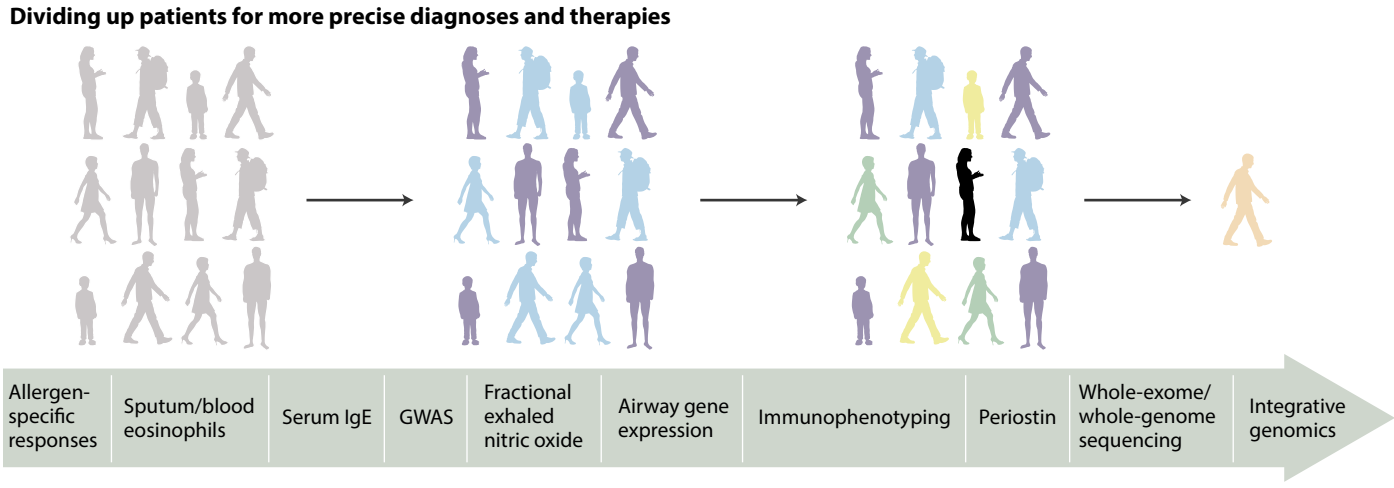

Fig. 1. Divide and conquer. As researchers developed new technologies and defined new biomarkers, a population of asthma patients (gray) has been subdivided, first into T_H2-high (purple) and T_H2-low (blue) groups. A T_H17-high group has now emerged, possibly from the T_H2-low group. Also shown are a recently identified interferon-γ (IFN-γ)–high group (black) (19) and a yet-to-be-subdivided group (yellow). In the future, additional groups will be defined, with the ultimate goal of precision medicine in an individual asthma patient (orange). GWAS, genome-wide association studies.

with severe asthma (*8*), the gene signature of the lung epithelium might reflect the dominant immune-effector phenotype within the lung at the specific time at which the biopsy was taken, thus indicating which immune pathways should be targeted for treatment. These findings also further emphasize the importance of noninvasive biomarkers such as periostin and the potential impact that a noninvasive T_H17 biomarker could similarly have in patient management.

Having established a T_H17-high population of patients, the authors next evaluated whether there was an association between this group and a history of glucocorticoid therapy. Prior studies have shown that steroids dampen T_H2 cytokine release but have a limited effect on IL-17 production. Furthermore, type 2 cytokines have been shown to negatively regulate T_H17 differentiation whereas IL-17 negatively regulates T_H2 differentiation. All of the patients with an IL-17 signature had previously been exposed to steroids, suggesting that steroids might have a contributory role in promoting a T_H17 signature in these subjects, either directly or through inhibition of the T_H2 pathway.

In order to establish potential mechanisms for the T_H2-high and T_H17-high subjects in their cohort, the authors turned to a mouse model of experimental asthma to study the cross-regulation of T_H2 and T_H17 responses and the effect of glucocorticoids on T helper subsets in vivo. Specifically, they sought to test whether a T_H2-low environment generated in the setting of anti-cytokine therapy or dexamethasone treatment leads to an up-regulation of T_H17 immunity. Using a mouse model in which the house dust mite serves as an allergen to cause asthma, Choy *et al.* observed airway hyperreactivity and a strong induction of T_H2 responses that was accompanied by a modest increase in IL-17. After the mice were given doses of anti–IL-4, anti–IL-13, or anti–IL-4+anti–IL-13 antibodies, the authors found that airway hyperreactivity was markedly improved and IL-17 responses were significantly increased, particularly after giving anti–IL-4 treatment.

The authors then analyzed gene expression profiles in lung tissue isolated from the differentially treated mice and detected an induction of IL-17–dependent genes involved in neutrophil chemotaxis, which mirrored their findings in T_H17-high subjects. The effects of the anti–IL-13 blockade on up-regulation of IL-17 immune responses were largely reversed with the concomitant administration of anti–IL-17 therapy, indicating that coadministration of anti–IL-13 and anti–IL-17 might provide some additive benefit over single therapy. Despite in vitro studies that previously showed a direct role for dexamethasone in promoting T_H17 development (*9*), Choy *et al.* found that steroid treatment was unable to increase IL-17A expression in their mouse model, despite a reduction in IL-13 and IL-4. Steroid treatment in the mice did, however, correlate with an up-regulation of mRNA transcripts that encode neutrophil chemoattractants and an increase in neutrophil inflammation, two hallmarks of T_H17 immunity.

These studies provide strong experimental evidence in support of mutual cross-regulation of T_H2 and T_H17 immune responses by steroids and T_H2 and T_H17 cytokines, two possible mechanisms that account for the mutually exclusive T_H2-high and T_H17-high populations the authors observed in their patient cohort. Ideally, future studies in humans will examine the immune cells in patients' bronchoalveolar lavage samples and bronchial biopsies in parallel, taking into account both the status of the patient's disease and various treatment regimens. Despite the obvious practical challenges of doing such a study, an evaluation of the relationship between the $CD4^+$ effector T cell phenotype (or other immune cells such as innate lymphoid cells) and the transcriptional profile of the epithelium is likely to provide tremendous insight into the relative utility of each parameter in guiding treatment decisions. Because the lung epithelium is the source of mucus production and other key inflammatory cytokines and chemokines that are critical drivers of disease, the epithelial gene signature might indeed hold primary importance for establishing which molecular subgroup a patient belongs to and ultimately what therapies will be most effective. Nevertheless, an evaluation of both the immune cell subsets and the epithelium would allow one to answer, for instance, whether dual-positive T_H2/T_H17 cells can give rise to a mutually exclusive T_H2 or T_H17 epithelial gene signature and provide insight into how the epithelial gene signature might change over time (Fig. 1).

Given the known plasticity of CD4+ T cells, it is plausible that a patient might experience a shift from a T_H2- to a T_H17-predominant immune response as a result of changes in the local microenvironment that can arise from viral infections, environmental factors, drugs, or different phases of disease (for example, acute exacerbation versus chronic, stable disease). Indeed, the authors suggest that the association, in T_H17-high patients, of high numbers of eosinophils and high levels of periostin (T_H2 biomarkers that might be degraded or cleared slowly over time) along with low fractional exhaled nitric oxide (FeNO, a T_H2 biomarker that likely is washed out of the airways rapidly) might reflect a shift from a T_H2 to a T_H17 phenotype. It is intriguing to speculate how biomarker kinetics might be used in the future to noninvasively follow the dynamics of a patient's T cell response or epithelial signature over time, particularly in response to therapy. The elevated eosinophils found in the T_H17-high subjects also raise the possibility that these patients might benefit from IL-5–directed therapies.

The results of this paper have clear implications for the use of anti-cytokine therapy in asthmatics going forward, particularly as these treatments become more widely adopted. Given the number of cytokines and other immune modulators implicated in asthma pathogenesis both upstream [for example, thymic stromal lymphopoietin (TSLP) and IL-33] and downstream (for example, NF-κB) of type 2 responses, a clear understanding of functional cytokine hierarchies could be valuable for predicting which immune checkpoints are effective targets and which changes in other inflammatory mediators are likely to occur in response to a particular treatment. Because levels of IL-17 and IL-13 mRNA transcripts in the lungs were difficult to detect in all but a handful of patients in this focused study, it remains unclear whether additional cytokines play a role in asthma. Along these lines, it would be interesting to examine, in steroid-treated mice, the role of other immune mediators that might contribute to the rise in neutrophil chemokines and increased neutrophil inflammation, given the repressed IL-17 mRNA levels in the lungs of these mice. Further evaluation of other inflammatory-response genes in human bronchial biopsies or mouse lung tissues could shed light on other important immune pathways that are activated or suppressed in response to either glucocorticoids or type 2 cytokine inhibitors. A recent report of a population of severe asthmatics with high IFN-γ (T_H1) responses (*10*) suggests that a comprehensive evaluation of other immune effector cytokines will likely be quite informative.

It will be exciting to see whether other researchers can replicate these findings in independent cohorts and identify additional patients who share the T_H17-high signature reported here. Comparisons across larger patient populations will help to discern whether the T_H17 signature commonly arises in response to a low T_H2 environment brought on by steroids or whether T_H17-high patients represent a distinct molecular phenotype with a singular disease pathogenesis process and thus define a truly new subcategory of asthma. Last, it would be instructive if future clinical studies of patients who have received type 2 cytokine therapies demonstrate an expansion of T_H17 immune responses to the same degree as the mice did in this study. If IL-17 levels do rise in type 2 cytokine–treated asthma patients, then it is possible that dual inhibition of type 2 cytokines and anti–IL-17 might provide a more durable and effective treatment response that allows for greater reductions in steroid use in these patients.

As we head full steam into the era of precision medicine, it is likely that advances in genomic-sequencing technologies and integrative-genomic analyses will prove useful adjuncts to the molecular tools being used for phenotyping and studying patients. Given the dynamic nature of asthma and the complex regulatory networks that control inflammatory disease, this study is an important example of the vital role in vivo animal modeling has in testing treatments and their potential consequences. Targeted genome editing of primary cells from patients also holds great promise as another powerful tool for similar types of functional analyses. Our increasing appreciation for the many molecular mechanisms underlying asthma pathogenesis is allowing us to redefine the disease. By integrating several translational research approaches with clinical care, we may one day be able to precisely tailor treatments based on the distinct molecular signature that we define for each and every patient walking through our doors.

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