Commentary

Th2/Th17 reciprocal regulation: twists and turns in the complexity of asthma phenotypes

Jason P. Lynch[1], Manuel A. Ferreira[2*], Simon Phipps[1,3*]

[1]Laboratory for Respiratory Mucosal Immunity, School of Biomedical Sciences, The University of Queensland, Queensland 4072, Australia; [2]QIMR Berghofer Medical Research Institute, Queensland 4029, Australia; [3]Australian Infectious Diseases Research Centre, The University of Queensland, Queensland 4072, Australia

*These authors contributed equally to this work.

Correspondence to: Dr. Simon Phipps. School of Biomedical Sciences, The University of Queensland, St. Lucia, Queensland 4072, Australia. Email: s.phipps@uq.edu.au.

Submitted Sep 25, 2016. Accepted for publication Sep 28, 2016.
doi: 10.21037/atm.2016.10.69
View this article at: http://dx.doi.org/10.21037/atm.2016.10.69

Asthma is an inflammatory disease of the airways that affects around 300 million people worldwide and has profound socio-economic costs. Asthma is characterized by reversible airway obstruction, airway hyperreactivity (AHR), airway remodelling and chronic inflammation, but it is now recognized that the cellular and molecular mechanisms driving these features vary between patients, resulting in distinct inflammatory sub-phenotypes, of which eosinophilic and neutrophilic asthma are the most commonly reported (1). Despite this heterogeneity, most patients are treated with beta-agonists (relievers) and corticosteroids (preventers), irrespective of the underlying pathogenic mechanisms. This treatment strategy is not always effective: for example, approximately 10% of patients are steroid-resistant (2). The lack of efficacy for existing asthma drugs in particular groups of asthmatics has been the catalyst for studies that elucidate the molecular pathways that promote different asthma subtypes, and for the development of drugs that target these pathways.

In both mice and humans, eosinophilic asthma is associated with Th2 inflammation, with a key role for the Th2 effector cytokines IL-4, IL-5, IL-13, and upstream, the Th2 instructive cytokines IL-33, IL-25, TSLP, and HMGB1 (3,4). In turn, this knowledge has translated into a series of new experimental drugs for asthma that target individual cytokines, some of which have recently been approved by the FDA [reviewed (1)]. The processes that underlie neutrophilic inflammation in asthma remain more obscure, but are likely to depend upon IL-1β- and IL-6-driven Th17 immunity (5,6). Although a number of clinical trials for drugs against Th2-driven inflammation have shown promising results, they have not been designed to determine the long-term effects of such targeted therapies. For example, can these Th2-targeted therapies restore epithelial homeostasis and correct the dysregulated epithelial responses that are now recognized to shape inappropriate immune responses to environmental stimuli? And while such treatments will block the designated cytokine or cytokine receptor, will the altered cytokine microenvironment promote another module of immunity that is similarly deleterious or unfavourable?

In a recent report published at *Science Translational Medicine*, Choy *et al.* seek to address this latter issue by exploring the reciprocal regulatory processes between Th2 and Th17 immunity in asthma (7). Previous studies have shown that IL-13 stimulation of bronchial epithelial cells (BECs) promotes a distinct gene signature including *POSTN*, *CLCA1* and *SERPINB2* ("IL-13 inducible genes"), all of which are upregulated in asthmatic BEC brushings (8,9). Using a similar approach, the authors initially confirmed that BECs up-regulate the same Th2 genes in response to IL-13 stimulation, and five Th17 genes in response to IL-17A stimulation. But of greater interest, IL-13 stimulation repressed the expression of the Th17 genes, with a trend for a similar repressive effect of IL-17A stimulation on Th2 genes. These results, which were mirrored by a negative correlation between the expression of Th2 and Th17 genes in bronchial biopsies of 51 asthma patients, support the notion that IL-13 and IL-17A reciprocally regulate the expression of their target pathways

in the lung. However, the extent to which cell types other than BECs (e.g., neutrophils) might have contributed to the negative correlation between Th2 and Th17 pathways in bronchial biopsies was not investigated. It also remains unclear if the effect observed for the eight genes analysed extends to (I) protein levels in sputum, which would be more practical to measure; and (II) other genes that play a key role in Th2 and Th17 immunity.

Next, the authors attempted to stratify the 51 asthma patients based on the similarity of their expression profiles in airway biopsies for the three Th2 and five Th17 genes analysed. Most patients were atopic and on corticosteroid treatment, with 20%, 31% and 49% classified as mild, moderate and severe, respectively. The best fitting model was consistent with three distinct groups: Th2-high (n=13), Th17-high (n=13), and Th2/Th17-low (n=25). The Th2-high and Th17-high subsets were mutually exclusive, i.e., there were no patients who were both Th2-high and Th17-high. These findings provide compelling evidence of reciprocal regulation between Th2 and Th17 pathways in asthma patients.

Extending on this, the authors next determined whether physiologic measures of eosinophilic and neutrophilic inflammation differed between the three groups of patients, identified based on the Th2 and Th17 gene expression profiles. Consistent with the expectation that markers of eosinophilic and neutrophilic asthma would be more common in the Th2-high and Th17-high groups, respectively, patients in the Th2-high group had significantly greater FeNO and sputum eosinophils when compared to both the Th2/Th17-low and Th17-high groups. However, for other markers of Th2 inflammation (e.g., serum periostin levels), patients in the Th17-high group had similar or slightly higher levels when compared to those in the Th2-high group. A possible explanation for this unexpected observation is that cytokines other than IL-13, for example TGF-β (10) (a cytokine also implicated in Th17 polarisation), can also induce the expression of periostin (10). Strikingly, tissue eosinophils were prevalent in many of the patients classified as Th17-high. The molecular basis for this remains obscure and needs to be elucidated because it is now clear, after many decades of contention, that eosinophil ablation is beneficial (11-13). Similarly, measures of neutrophilic inflammation were not significantly different between the three groups. As the authors note, it is possible that neutrophil function may be more important than their number in asthma, an important consideration for future studies.

To address the cellular and molecular processes that govern reciprocal regulation between Th2 and Th17 pathways *in vivo*, the authors employed a preclinical model of chronic asthma. They first addressed the effect on Th17 inflammation of blocking IL-13, IL-4 or both cytokines simultaneously. Relative to the control treatment, all three anti-Th2 interventions significantly increased *Il17* gene expression and CD4+ IL-17+ cell numbers in the lung and draining lymph nodes. Strikingly, the frequency of neutrophils in the lung or BALF increased significantly with anti-Th2 interventions. Moreover, blockade of IL-4, but not IL-13, led to an increase in expression of the Th17 signature genes *Cxcl1* and *Cxcl3*, pointing to IL-4 as a potent suppressor of Th17 responses. Whether these differences in gene expression translated into differences in protein expression (e.g., in BALF) was not determined. The shift to an elevated neutrophilic response, reminiscent of the effects of steroids in man [which ablate Th2 but not Th17 immunity (14)], raised the possibility that dual antagonism of Th2 and Th17 responses would be a more efficacious approach to ablate AHR, airway remodelling and both eosinophilic and neutrophilic inflammation. Treatment with both anti-IL-13 and anti-IL-17 decreased *Muc5ac*, *Clca3* expression and AHR. However, while the fraction of neutrophils in BAL decreased, consistent with the neutralization of IL-17A, the fraction of eosinophils increased, despite the neutralization of IL-13. These data suggest that part of the inhibitory effects of anti-IL-13 on eosinophil numbers were indirectly mediated by the increased expression of IL-17A and its counter-regulatory effects.

Collectively, these findings have important implications with regard to Th2-targeted therapies for asthma, particularly as longer term phase III/IV clinical trials of anti-Th2 biologics commence. For example, it will be important to determine if neutrophilic/Th17 inflammation develops in patients undergoing prolonged treatment with anti-IL-4/IL-13. The findings of this study certainly suggest that this is a possibility, and that dupilumab (IL-4R-alpha blocker) may have a greater propensity than lebrikizumab (anti-IL-13) to induce a shift towards Th17 immunity.

More importantly, what are the clinical implications of skewing an inflammatory response towards Th17? The teleological role of Th17 immunity is to protect against extracellular bacteria, but it is unclear whether a Th17-primed airway would protect against infection or promote an exaggerated and deleterious response. Moreover, the majority of asthma exacerbations are

triggered by viruses (15), and hence a Th17 cytokine microenvironment may increase susceptibility and loss of asthma control. This, and the possible development of steroid resistance, will need to be closely examined in clinical studies. If Th17 skewing is observed with anti-Th2 interventions in humans, then it would seem logical to block IL-17 cytokines simultaneously. However, the results from this study do not seem to support such a strategy, as dual IL-13/IL-17 blockade led to an increase in eosinophils when compared to single IL-13 blockade, an observation that warrants further investigation.

If Th17 skewing is observed with anti-Th2 interventions in humans, then perhaps the targeting of cytokines upstream of both pathways will provide a solution. For example, IL-33, a known epithelial-derived Th2-instructive cytokine, also induces the release of IL-17 by BECs (16). Thus, the Th17 eosinophilic phenotype may be promoted in part because IL-33 is sufficient to drive eosinophils, as well as promoting IL-17. It will be interesting to see the effectiveness of anti-IL-33 or anti-IL-33R across Th2-high and Th17-high asthma when Phase II clinical trials begin. Additionally, targeting of this pathway is likely to restore robust antiviral immunity (17,18).

The study by Choy *et al.* also highlights the importance of identifying new, non-invasive biomarkers that not only diagnose the phenotype, but more critically point to the underlying endotype. Future studies will need to identify reliable and non-invasive biomarkers for Th17 inflammation and attempt to replicate the results reported by Choy *et al.* using such biomarkers. High serum periostin levels were not a distinct feature of the Th2-high group, but did appear to associate with tissue eosinophilia. In a study of paediatric and adolescent asthmatics, periostin and blood eosinophils were found to be influenced by factors other than type 2 inflammation, including age, negating the use of periostin as a biomarker for childhood asthma (19). Thus, the fate of periostin as a biomarker of Th2-asthma per se remains unclear.

In conclusion, Choy *et al.* show that Th2 and Th17 immunity counter-regulate one another in the lungs of asthmatics, although more work is required to understand why the Th2-high, Th17-high, and Th2/Th17-low gene signatures failed to align with the expected type of granulocytic inflammation. Inevitably, the classification into the three patient groups will be shown to be too simplistic. Nonetheless, the study highlights the potential for anti-Th2 interventions to skew to neutrophilic inflammation, and more importantly, warns of the complexity and consequences of dual IL-13 and IL-17 antagonism.

Acknowledgements

None.

Footnote

Provenance: This is a Guest Commentary commissioned by Section Editor Bingrong Zhou, MD, PhD (Department of Dermatology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China).
Conflicts of Interest: Simon Phipps has received grants from Pfizer and GSK. The other authors have no conflicts of interest to declare.

Comment on: Choy DF, Hart KM, Borthwick LA, *et al.* TH2 and TH17 inflammatory pathways are reciprocally regulated in asthma. Sci Transl Med 2015;7:301ra129.

References

1. Fajt ML, Wenzel SE. Asthma phenotypes and the use of biologic medications in asthma and allergic disease: the next steps toward personalized care. J Allergy Clin Immunol 2015;135:299-310; quiz 311.
2. Barnes PJ. Corticosteroid resistance in patients with asthma and chronic obstructive pulmonary disease. J Allergy Clin Immunol 2013;131:636-45.
3. Lambrecht BN, Hammad H. The immunology of asthma. Nat Immunol 2015;16:45-56.
4. Ullah MA, Loh Z, Gan WJ, et al. Receptor for advanced glycation end products and its ligand high-mobility group box-1 mediate allergic airway sensitization and airway inflammation. J Allergy Clin Immunol 2014;134:440-50.
5. Ullah MA, Revez JA, Loh Z, et al. Allergen-induced IL-6 trans-signaling activates γδ T cells to promote type 2 and type 17 airway inflammation. J Allergy Clin Immunol 2015;136:1065-73.
6. Simpson JL, Phipps S, Baines KJ, et al. Elevated expression of the NLRP3 inflammasome in neutrophilic asthma. Eur Respir J 2014;43:1067-76.
7. Choy DF, Hart KM, Borthwick LA, et al. TH2 and TH17 inflammatory pathways are reciprocally regulated in asthma. Sci Transl Med 2015;7:301ra129.
8. Woodruff PG, Boushey HA, Dolganov GM, et al. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. Proc

Natl Acad Sci U S A 2007;104:15858-63.
9. Sidhu SS, Yuan S, Innes AL, et al. Roles of epithelial cell-derived periostin in TGF-beta activation, collagen production, and collagen gel elasticity in asthma. Proc Natl Acad Sci U S A 2010;107:14170-5.
10. Horiuchi K, Amizuka N, Takeshita S, et al. Identification and characterization of a novel protein, periostin, with restricted expression to periosteum and periodontal ligament and increased expression by transforming growth factor beta. J Bone Miner Res 1999;14:1239-49.
11. Flood-Page P, Menzies-Gow A, Phipps S, et al. Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. J Clin Invest 2003;112:1029-36.
12. Haldar P, Brightling CE, Hargadon B, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. N Engl J Med 2009;360:973-84.
13. Nair P, Pizzichini MM, Kjarsgaard M, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. N Engl J Med 2009;360:985-93.
14. McKinley L, Alcorn JF, Peterson A, et al. TH17 cells mediate steroid-resistant airway inflammation and airway hyperresponsiveness in mice. J Immunol 2008;181:4089-97.
15. Johnston SL, Pattemore PK, Sanderson G, et al. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. BMJ 1995;310:1225-9.
16. Fujita J, Kawaguchi M, Kokubu F, et al. Interleukin-33 induces interleukin-17F in bronchial epithelial cells. Allergy 2012;67:744-50.
17. Lynch JP, Mazzone SB, Rogers MJ, et al. The plasmacytoid dendritic cell: at the cross-roads in asthma. Eur Respir J 2014;43:264-75.
18. Lynch JP, Werder RB, Simpson J, et al. Aeroallergen-induced IL-33 predisposes to respiratory virus-induced asthma by dampening antiviral immunity. J Allergy Clin Immunol 2016. [Epub ahead of print].
19. Anderson HM, Lemanske RF Jr, Arron JR, et al. Relationships among aeroallergen sensitization, peripheral blood eosinophils, and periostin in pediatric asthma development. J Allergy Clin Immunol 2016. [Epub ahead of print].

Cite this article as: Lynch JP, Ferreira MA, Phipps S. Th2/Th17 reciprocal regulation: twists and turns in the complexity of asthma phenotypes. Ann Transl Med 2016;4(Suppl 1):S59. doi: 10.21037/atm.2016.10.69

