

Finally, they developed a novel triple mTNF knock-in mouse model, in which “triple” refers to the substitution of nonsense nucleotides encoding for three distinct amino acids that are normally required for the Na⁺ uptake stimulatory activity of TNF- α , the so-called functional alveolar liquid clearance-stimulatory domain. The authors show that when these triple mTNF knock-in mice were exposed to pneumococcal cholesterol binding pore-forming toxin, a model of pulmonary edema, there was no change in the quantal generation of TNF- α in the bronchoalveolar lavage fluid, but there was reduced ENaC activity, decreased ENaC- α protein expression, and greater lung edema. This finding suggests a physiological role for the lectin-like domain of native TNF- α in alveolar fluid clearance and the resolution of pulmonary edema. Taken together, these basic science results provide new physiological insight into the potential role of the lectin-like domain of TNF- α and support the novel therapeutic use of TIP aerosols in patients with ALI/ARDS and ischemia reperfusion lung injury. ■

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Searching for Distinct Mechanisms in Eosinophilic and Noneosinophilic Airway Inflammation



Chronic rhinosinusitis (CRS) is an inflammatory disease of the upper respiratory tract affecting up to 30 million Americans annually. It is associated with a significant impairment in quality of life and places a large financial burden on the healthcare system, with more than \$6 billion spent annually on management (1–4). CRSwNP, a subset of CRS, is characterized by the presence of nasal polyps and chronic inflammation of the sinonasal mucosa. In European and American patients, CRSwNP is characterized by type 2 inflammation and eosinophilia. However, there is accumulating evidence, especially in China, that almost half of patients with CRSwNP in Asian countries have a noneosinophilic pattern of inflammation in their polyp tissue that is characterized by a mixed type 1 and/or type 3 response (5, 6). Although the mechanisms that drive these phenotypes are unclear, it has been suggested that differences in Th cell subsets found in polyps from eosinophilic and noneosinophilic patients may play an important role (7). Dendritic cells (DCs) are known to be important in skewing Th responses

in the mucosa (8), and thus may be important for skewing Th cells in polyps. However, there has been a lack of in-depth analyses of Th cell subsets found in polyps from different CRSwNP groups, and few studies have investigated the importance of DCs in CRSwNP pathogenesis (9, 10).

In this issue of the *Journal* (pp. 628–638), Shi and colleagues evaluated the function and phenotype of Th and DC subsets from polyps of eosinophilic and noneosinophilic patients with CRSwNP in China to assess any differences (11). Interestingly, many of the features examined in the Th and DC subsets isolated from polyps did not differ between the two groups of patients with CRSwNP. The researchers found similar elevations of IL-17A⁺ and IFN- γ ⁺ CD4⁺ cells in polyps from both groups compared with controls, confirming a recent study from Europe (12). Likewise, they found similar elevations of activated DC subsets (both myeloid DC [mDC] and plasmacytoid DC [pDC]), and these DCs produced equivalent elevated levels of IL-6 and

IL12p70 compared with DCs from control tissue. Moreover, they found that polyp-derived mDCs and pDCs had similar effects on Th responses, although mDCs were superior at skewing naive Th cells and expressed higher levels of activation markers compared with pDCs. Recent work has revealed the presence of at least two different subsets of mDCs in humans, mDC1 and mDC2, which can be differentiated by their expression of CD1c and CD141, respectively, and there is accumulating evidence that mDC2s may play an important role in allergic disease (13). Although Shi and colleagues did not differentiate between these two types of mDCs in this work, future studies aimed at elucidating the potential role of either of these subsets in CRSwNP pathogenesis would be of great value.

The authors did find some differences in inflammatory cell subsets between the two types of CRSwNP polyps. As expected, they found that IL-4⁺ CD4⁺ T cells were elevated in polyps from eosinophilic patients compared with polyps from noneosinophilic patients. Although these results do represent a step forward in the characterization of Th cells in nasal polyps, the authors may have missed an opportunity to identify potential key differences in Th subsets between the two groups of patients with CRSwNP. Recent work in asthma has revealed the presence of unique Th subsets that can coexpress distinct pro-inflammatory cytokines, such as IL-4 and IL-17 (14), indicating that Th cells are capable of expressing more than one type of cytokine profile. Thus, future studies examining Th subsets based on coexpression of cytokines may provide further insight into the differences between eosinophilic and noneosinophilic polyps.

Importantly, Shi and colleagues found that in *in vitro* co-cultures, DCs isolated from either eosinophilic or noneosinophilic polyps skewed autologous naive CD4⁺ T cells toward Th17 (IL-17A⁺) and Th1 (IFN- γ ⁺) phenotypes, but only DCs from eosinophilic polyps were able to skew naive Th cells toward a Th2 phenotype (IL-4⁺, IL-5⁺, or IL-13⁺). Although this finding is quite interesting, these assays were conducted in the absence of any specific antigenic stimulation. It is difficult to understand the mechanisms by which polyp-derived DCs could induce skewing of naive T cells in the absence of signal 1 from the T-cell receptor–major histocompatibility complex interaction that is classically required for the activation of naive Th cells. It has been established in asthma that DC subsets play a critical role in the maintenance of Th2 inflammation in the lung after the primary antigen challenge during chronic inflammation (15), and the DCs in polyp tissues are likely in a similar inflammatory environment. However, the asthma models demonstrate a role for activated tissue-resident DCs in the reactivation of memory T cells, as well as a requirement for specific antigen. Thus, activated DCs in polyps may play a similar role to that in asthma and help reinforce the inflammatory milieu during ongoing inflammation, through interactions with local memory Th cells. Shi and colleagues have not demonstrated a role for antigen directly with the experiments described in this work, leaving us to wonder whether the observed phenomena are truly noncognate or involve endogenous antigen peptides.

The authors went on to show that the frequency of DCs expressing either OX40 ligand (OX40L) or programmed death ligand-1 (PD-L1) was elevated only in eosinophilic polyps, although it was not clear whether these molecules were coexpressed on DCs or not. Moreover, the authors found that blockade of either OX40L or PD-L1 had no effect on Th cell production of IL-17A but

suppressed production of type 2 cytokines while enhancing IFN- γ . This supports previous work that suggested that OX40L on mDCs is important for skewing Th2 responses (16). However, this previous work found that the Th2 skewing ability of OX40L⁺ DCs was abolished in the presence of IL-12, whereas Shi and colleagues have shown that OX40L⁺ DCs from polyps can promote Th2 responses, even though they also produce elevated levels of IL-12. The reasons for this discrepancy are not clear, although they could be caused by differences in blood-derived versus tissue-derived DC subsets. Further, the authors demonstrate that OX40L and PD-L1 play an important role in the skewing of Th cells to produce type 2 cytokines, which has been previously established (16, 17), but they have not provided any insight into the factors that might be important for the induction of IL-17A or IFN- γ by Th cells. Given that a large proportion of patients in Asia with CRSwNP display a noneosinophilic phenotype, it will be important to understand the mechanisms that facilitate this phenotype, in addition to those that drive type 2 inflammation, to better treat all subsets of patients with CRSwNP.

Finally, the authors assessed expression of thymic stromal lymphopoietin (TSLP) and osteopontin, two cytokines known to play a role in skewing of Th responses (18). TSLP expression was elevated only in eosinophilic polyps, which is consistent with previous reports investigating TSLP in polyps from patients in America (19), and it was positively correlated with levels of OX40L⁺ DCs. This was not surprising because previous studies have shown that TSLP can directly induce OX40L expression on DCs (16). Previous studies have also suggested that TSLP has different effects on the ability of mDCs and pDCs to skew Th responses. Although TSLP-stimulated mDCs favored the induction of IL-13⁺ Th2 cells, TSLP-stimulated pDCs induced FoxP3⁺ and IL-10⁺ regulatory T cells (16, 20). In this work, Shi and colleagues found that both mDCs and pDCs from eosinophilic polyps, which have increased levels of TSLP, can induce Th2 responses, although they did not analyze expression of IL-10 or FoxP3 in the T cells, so it is not clear whether the pDCs from polyps have the potential to induce regulatory T cells as well. Osteopontin was elevated in polyps from both groups and was positively correlated with IL-6 and IL-12p70 expression by DCs, suggesting it may play a role in promoting type 1 and type 3 inflammatory responses in both subsets of polyps. Together, these data suggest that local factors within the polyp microenvironment, such as TSLP and osteopontin, can function to influence the polarization of DC subsets in the tissue, potentially by up-regulating expression of OX40L and/or PD-L1. Whether these polarized DCs then travel to draining lymph nodes to induce a skewed Th response or remain in the tissue to reinforce the established inflammatory environment is not clear at this time, but these studies would be of great value to the field.

Overall, Shi and colleagues have provided a more in-depth analysis of Th cell and DC subsets found in polyps of Chinese patients with eosinophilic and noneosinophilic CRSwNP than has been previously reported. This work has provided new insights into the complexity of the inflammatory milieu within polyps and has demonstrated that polyp-derived DC subsets have the potential to directly influence Th responses. In addition, it has shed light on some of the potential mechanisms DCs use to induce type 2 inflammatory responses, which could lead to the development of improved therapeutic strategies for patients with CRSwNP. ■

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The Art of Making Predictions: Statistics versus Bedside Evaluation

Cardiac arrest or intensive care unit admissions for deteriorating ward patients are common events. The observation that these events are often preceded by premonitory signs and symptoms of cardiopulmonary instability (1) led to the widespread deployment of medical emergency teams (METs), further stimulated by several reports of improved outcomes (2, 3). However, a large cluster randomized trial, the Medical Emergency Response and Intervention Trial (MERIT), failed to detect a decrease in cardiac arrests in intervention hospitals (4). Why did METs not achieve their purported claims? Possibilities include methodological concerns, such as a spill-over effect in the control group or lack of statistical power, and, of course, that METs do not work. To explore whether METs work, two important questions need to be

answered. First, are the trigger tools used to activate METs effective in identifying deteriorating patients in a timely manner, and second, does early identification lead to actions that can improve outcomes?

A study by Churpek and colleagues (pp. 649-655) published in this issue of the *Journal* (5) tried to solve the first of these questions. The authors used a large database to generate a new prediction score based on vital signs and laboratory values and compared it with one of the most commonly used scores, the modified early warning score, which is solely based on vital signs. They observed that the proposed score, the electronic Cardiac Arrest Risk Triage (eCART) score, had better discrimination than the modified early warning score, with an area under the curve of 0.83 versus 0.71, and led to significant gains in reclassifying

