Uridine diphosphate–glucose/P2Y$_{14}$R axis is a nonchemokine pathway that selectively promotes eosinophil accumulation

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Allergic asthma is a chronic inflammatory airway disease characterized by dysregulated type 2 immune responses, including degranulating airway eosinophils that induce tissue damage and airway hyperresponsiveness (AHR). The type 2 cytokines interleukin 5 (IL-5) and IL-13 and the eosinophil-specific chemokine CCL11/CCL24/CCL26 axis recruit, activate, and regulate eosinophils in the airways. In this issue of the *JCI*, Karcz et al. identified a mechanism involving the nucleotide sugar UDP-glucose (UDP-G) and the purinergic receptor P2Y$_{14}$R in amplifying eosinophil accumulation in the lung. During type 2 inflammation, UDP-G activates P2Y$_{14}$R on eosinophils, inducing the cells to move and migrate into the lung. Pharmacologically or genetically inhibiting P2Y$_{14}$R on eosinophils attenuated eosinophil infiltration and AHR. Future experiments, including identifying additional type 2 factors regulating P2Y$_{14}$R expression on lung eosinophils, are necessary to ascertain the impact of targeting P2Y$_{14}$R as an alternative or adjunctive therapy to current type 2 biologics for the treatment of asthma.

Asthma

Asthma is a chronic inflammatory disorder of the airways with mixed lymphocytic and myelogenous (e.g., granulocytes/monocytes) infiltrates (1). Although the inflammatory response is complex, many patients with asthma have an aberrant type 2 immune response, characterized by the presence of CD4$^+$ T helper type 2 lymphocytes (Th2 cells), group-2 innate lymphoid cells (ILC2s), and eosinophils. Activation of these inflammatory cells predisposes to many pathognomonic features of asthma. Th2 cells and ILC2s secrete interleukin 5 (IL-5) and IL-13, which can cooperate with the eosinophil-specific chemokines CCL11, CCL24, and CCL26 (formerly known as eotaxin-1, -2, and -3, respectively), to regulate eosinophil accumulation in the lung (2). Structural cells of the lung, such as epithelial and endothelial cells, primarily produce CCL11, CCL24, and CCL26, which bind to the CC chemokine receptor type 3 (CCR3) on eosinophils to induce chemotaxis (3, 4).

Regulation of eosinophil trafficking

Allergen exposure of the asthmatic lung induces the production of IL-5, IL-13, and CCL11, -24, and -26 to form a cytokine/chemokine network that regulates eosinophil expansion, homing, and accumulation (5). IL-5 regulates eosinophil development, activation, migration, and survival (6, 7). IL-5 also facilitates eosinophil expansion in the bone marrow and their egress into the blood. IL-13 upregulates the expression of adhesion pathways on vascular endothelial cells and increases expression of CCL11, -24, and -26, which recruit eosinophils from the blood into airways. IL-13 also induces a range of proinflammatory molecules and activates inflammatory cells to induce the histopathological features of asthma, airway hyperresponsiveness (AHR) and mucous hypersecretion (8–12). Corroborative data from human- and mouse-based studies demonstrate that eotaxin chemokines regulate the temporal and spatial accumulation of eosinophils in the airways. CCL11 is thought to regulate the early recruitment of eosinophils into the lung, while CCL24 and CCL26 regulate eosinophil tissue localization and persistence (13, 14). CCL24, like CCL11, can synergize with IL-5 to promote accumulation of eosinophils in the pulmonary compartment and the subsequent production of IL-13 (15, 16). Eosinophils express other chemokine receptors, including CCR1, -3, -6, -8, -9, and CXCR2–CXCR4, and respond to chemokines derived from other cell types (e.g., CCL5, -7, -8, and -12). These alternative chemokine pathways are thought to be ancillary, amplifying or cooperating with the IL-5/IL-13/CCR3 axis to promote eosinophil accumulation within the inflammatory milieu (5).

UDP-G/P2Y$_{14}$R axis promotes selective eosinophil migration

In this issue of the *JCI*, Karcz et al. (17) reveal a role for the uridine diphosphate–glucose (UDP-G)/P2Y$_{14}$R axis in the regulation of eosinophil migration and the induction of AHR in a mouse model of protease-induced asthma (Figure 1). UDP-G is an intracellular nucleotide sugar that is involved in several metabolic pathways, including glycogen biosynthesis and glycolysis. However, stressed or damaged cells can release UDP-G to act as a nucleotide alarmin (18), triggering a broad range

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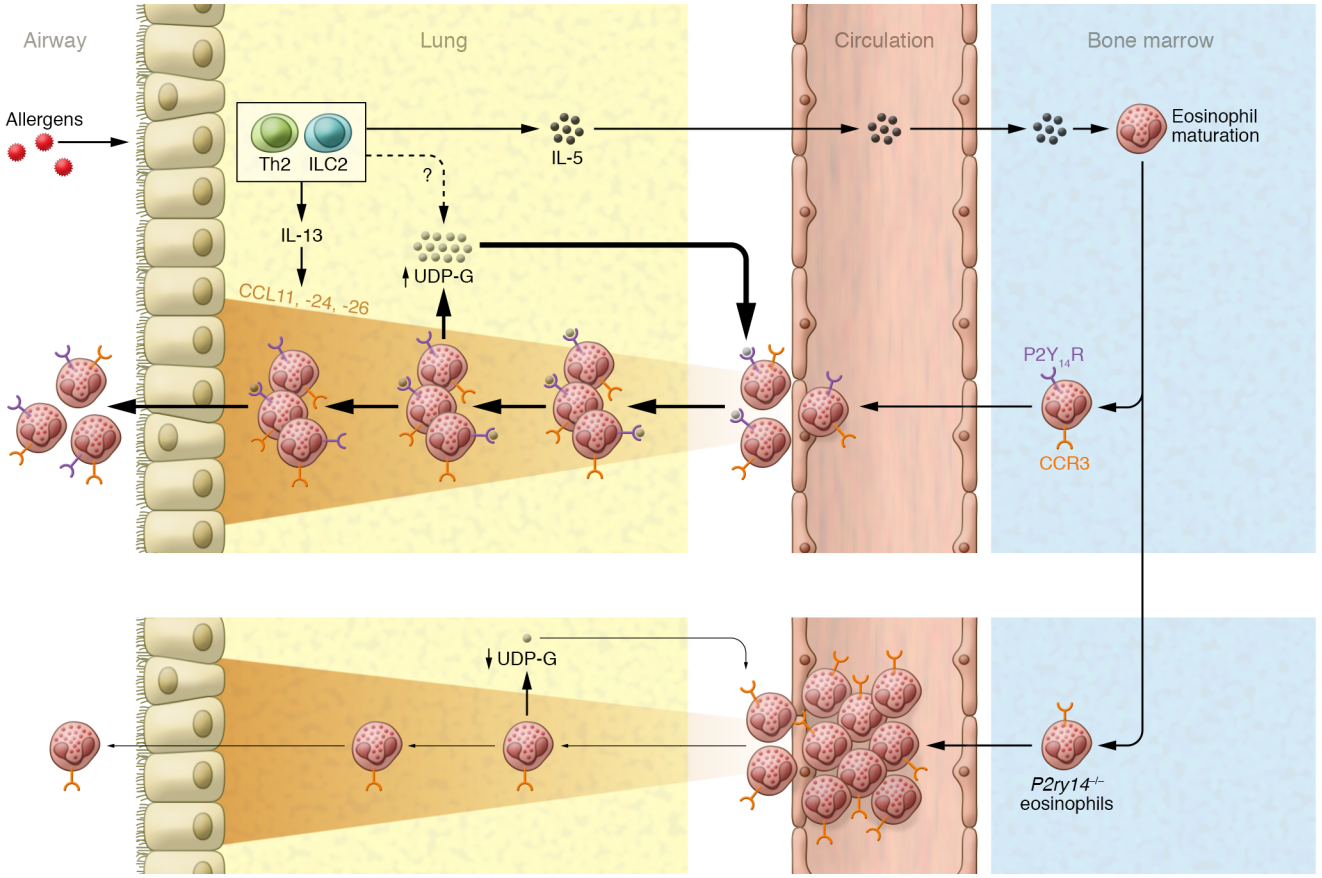

Figure 1. The uridine diphosphate–glucose (UDP-G)/P2Y$_{14}$R axis selectively regulates eosinophil migration by promoting chemokinesis and amplifying chemotaxis. In response to allergen challenge, group-2 innate lymphoid cells (ILC2s) and CD4$^+$ T helper type 2 lymphocytes (Th2 cells) expand and secrete interleukin 5 (IL-5) and IL-13, promoting allergic inflammation. IL-5 promotes eosinophil differentiation, expansion in the bone marrow, and egress into the circulation. In the lung, IL-13 promotes the local production of eosinophil-specific chemokines CCL11, CCL24, and CCL26, which signal through CCR3 to promote chemotaxis and eosinophil migration to the airways. This IL-5/IL-13/CCR3 axis is the primary mechanism, in conjunction with vascular adhesion molecules, for recruiting eosinophils into the lung during type 2 inflammation. UDP-G accumulation in the lung following allergen provocation signals through P2Y$_{14}$R to induce eosinophil chemokinesis and amplify chemokine-directed eosinophil migration and accumulation in the lung. Loss of the UDP-G/P2Y$_{14}$R signaling in *P2ry14*$^{-/-}$ eosinophils diminishes CCR3-mediated eosinophil chemokinesis and eosinophil accumulation in the lung and induction of the asthma manifestations.

of physiological and metabolic functions. The cellular functions of adenine and uridine nucleotides are mediated by two large classes of receptors, ligand-gated ion channels (P2X) and the G protein–coupled receptor family (P2Y). UDP-G binds with high affinity to P2Y$_{14}$R (19). While it is reported that P2Y$_{14}$R may regulate neutrophil trafficking, airway epithelial cytokine release, and mast cell degranulation, the roles of UDP-sugars and P2Y$_{14}$R in regulating pulmonary inflammation are largely unexplored (20).

Karcz et al. (17) employed an asthma model in which mice were sensitized by administering *Aspergillus oryzae* protease (ASP) concurrently with the antigen ovalbumin (OVA) to the oropharyngeal cavity and then challenged again with OVA (ASP/OVA). OVA challenge induced an eosinophil-rich inflammatory infiltrate, and elevated allergen-specific IgE, IL-5, and IL-13. Further, the mice developed AHR, mucus hypersecretion, and all the hallmark pathognomonic features of allergic asthma. Notably, challenging *P2ry14*-deficient mice (*P2ry14*$^{-/-}$) with ASP/OVA induced mucus hypersecretion, allergen-specific IgE, and elevated IL-5 and IL-13 levels; however, eosinophil numbers in bronchoalveolar lavage fluid (BALF) were reduced by approximately 50%, and airway reactivity returned to baseline. The authors showed that the reduction in eosinophil levels was not due to decreased expression of eotaxin chemokines or lower expression of IL-5R or CCR3 on eosinophils. Further, in a series of elegant adoptive transfer and pharmacological inhibition experiments using the P2Y$_{14}$R antagonist 4-[4-(piperidin-4-yl)phenyl]-7-[4-(trifluoromethyl) phenyl]naphthalene-2-carboxylic acid (PPTN), Karcz et al. established that the P2Y$_{14}$R pathway was dispensable during allergic sensitization, as loss of P2Y$_{14}$R function did not directly impact CD4$^+$ Th2 cell activation and expansion (17).

P2Y$_{14}$R expression on pulmonary eosinophils regulates migration

To begin to determine the mechanism by which P2Y$_{14}$R regulates eosinophil recruitment, the authors first examined the requirement for P2Y$_{14}$R expression on structural or hematopoietic cells in eosinophil accumulation and AHR. Recipro-

cal bone marrow–chimera experiments using WT and *P2ry14*$^{-/-}$ mice revealed that $P2Y_{14}R$-expressing hematopoietic cells and not stromal cells were required for the accumulation of eosinophils. Molecular analyses identified several hematopoietic cell populations, including neutrophils, conventional CD103^{+} dendritic cells, alveolar macrophages, and eosinophils that express *P2ry14* mRNA; however, expression was highest in eosinophils. Notably, *P2ry14* was not expressed by eosinophil precursors, although appreciable mRNA levels were observed in mature splenic and pulmonary eosinophils at steady state and those infiltrating during type 2 inflammation. Furthermore, stimulating eosinophils with IL-5 and CCL24 also increased *P2ry14* mRNA expression, linking activation to upregulation of eosinophil regulatory pathways. In a series of bone marrow–chimera experiments and studies utilizing conditionally mutant mice that selectively deleted $P2Y_{14}R$ in myeloid cells, dendritic cells, or eosinophils (*LysM-Cre*, *Cd11c-Cre*, or *Eo-Cre*), Karcz et al. revealed an eosinophil-intrinsic role for $P2Y_{14}R$ in recruitment. Moreover, selective deletion of *P2ry14* only in eosinophils reduced the number of airway eosinophils during allergic inflammation (17).

These observations clearly established an eosinophil-intrinsic role for $P2Y_{14}R$ in the recruitment of eosinophils to the allergic lung. However, the connection with UDP-G remained elusive. To explore UDP-G involvement, Karcz and colleagues first examined the levels of UDP-hexose in allergic mice following allergen challenge and revealed that challenging the sensitized mice with allergen triggered the release of UDP-hexoses into BALF. Furthermore, the authors demonstrated that the temporal increase in UDP-hexose levels in the BALF was associated with eosinophil accumulation in the lung. These studies connected extracellular UDP-G in BALF with eosinophil recruitment in the allergic lung. Importantly, the observation that peak UDP-hexose levels in the airway preceded peak eosinophil accumulation suggested that eosinophils may also secrete UDP-G, and that eosinophil-derived UDP-G may in turn promote more eosinophilia in a positive feedback loop. These studies firmly linked UDP-G with the $P2Y_{14}R$-eosinophil phenotype in the allergic lung (17).

Karcz et al. next examined the spatial distribution of eosinophils in the lungs of WT and *P2ry14*$^{-/-}$ mice during the allergic inflammatory response. *P2ry14*$^{-/-}$ eosinophils had reduced mobility when compared with WT cells. Moreover, the authors observed higher quantities of eosinophils in the blood, with large numbers of eosinophils marginating around blood vessels compared with that observed in WT mice. Furthermore, the frequency of *P2ry14*$^{-/-}$ eosinophils in the airspace was reduced compared with WT mice. Fewer eosinophils in the airspace were found despite having a sufficient primary eosinophilic recruitment/chemotactic pathway (IL-5/IL-13/CCL11 and -24 or CCR3). Collectively, these experiments suggested that in the absence of $P2Y_{14}R$, eosinophils were unable to efficiently cross the vascular endothelium and that migration was impaired at the level of the eosinophil. RNA-seq analyses of WT and *P2ry14*$^{-/-}$ eosinophils failed to reveal major differences in genes governing cell adhesion and migration. In vitro migration studies showed that UDP-G elicited eosinophil chemokinesis (nondirected movement) and CCL24 amplified chemotaxis (directed movement). Consistently, instillation of UDP-G into the allergic lung increased the number of infiltrating eosinophils, which directly associated with total UDP-hexose levels. Thus, the UDP-G/$P2Y_{14}R$ axis played a role in amplifying eosinophil accumulation in the allergic lung (17).

The translational importance of Karcz et al. (17) is currently unclear. Gene-based analyses have identified *P2ry14* as an asthma risk gene (21). Furthermore, $P2Y_{14}R$ protein is expressed by human blood eosinophils (22). The authors reported that they did not detect differences in UDP-G levels between people with mild asthma and healthy volunteers; however, UDP-G levels may only increase during asthma exacerbations, when eosinophil numbers increase. Interestingly, it has been reported that patients with obesity and mild-to-moderate and severe asthma have a higher submucosal eosinophil burden than nonobese patients with asthma (23). That study showed increased plasma UDP-G levels in mice fed a high-fat diet, suggesting that obesity might contribute to chronic UDP-G/$P2Y_{14}R$ activation and increased asthma risk and severity.

Whether pharmacologically blocking the UDP-G/$P2Y_{14}R$ signaling axis has therapeutic potential also remains unclear. The authors observed that treating mice with the $P2Y_{14}R$ antagonist PPTN prior to allergen airway exposure prevented eosinophils from accumulating, suggesting that prophylactic exposure to $P2Y_{14}R$ antagonists may protect against some components of the asthma phenotype. However, it currently remains unknown whether the reduced eosinophil accumulation following treatment with the $P2Y_{14}R$ antagonist was associated with a reduced mucus hypersecretion or AHR (17). Determining whether treatment with $P2Y_{14}R$ antagonists could alleviate an established disease phenotype would have translational implications.

Concluding remarks

Karcz et al. (17) have identified a role for the UDP-G/$P2Y_{14}R$ axis in the amplification of eosinophil accumulation in the lung during allergic inflammation (Figure 1). During type 2 inflammation, UDP-G released from the damaged epithelium and/or from infiltrating eosinophils activates $P2Y_{14}R$ on eosinophils to enhance chemokinesis and promote chemotaxis. Interestingly, IL-5 and CCL24 induced the expression of $P2Y_{14}R$ on eosinophils. This IL-5–CCL24 interaction may regulate the selective expression of $P2Y_{14}R$ on resident and inflammatory eosinophils during allergic inflammation, and subsequently facilitate UDP-G amplification of the IL-5/CCL11/CCL24 axis, which controls the spatiotemporal distribution of eosinophils in the lung. Important next steps include determining (a) the requirement of IL-5 and IL-4/IL-13 (IL-4R) in the mechanism of $P2Y_{14}R$ expression and induction of eosinophil recruitment and AHR, (b) the possible role of other nucleotide sugars in amplification of allergic inflammatory cell recruitment, and (c) the signaling processes that mediate $P2Y_{14}R$ amplification of CCR3-dependent migration. Answering these questions would give insights into whether current biologics may inhibit activation of the UDP-G/$P2Y_{14}R$ pathway or if this axis represents an independent pathogenic pathway. Furthermore, with the recent rise in other eosinophil pathologies, such as eosinophil-associated gastrointestinal diseases, it will be intriguing to determine whether

the UDP-G/$P2Y_{14}R$ axis also contributes to eosinophil recruitment and effector function in these disease processes.

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