Eosinophils play important roles in limiting parasitic infection and in allergic inflammation in the asthmatic airways. Activation of eosinophils by diverse stimuli, including prostaglandin D$_2$ (PD$_2$), leads to leukotriene C$_4$ (LTC$_4$) synthesis that contributes to the expulsion of parasites and to epithelial injury in allergic inflammation. Mesquita-Santos et al. in this issue of the journal describe a collaboration between the two PGD$_2$ receptors, DP$_1$ and DP$_2$ [also known as CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 lymphocytes)] that is required to trigger LTC$_4$ synthesis. DP$_1$ receptors coupled to $G_s$ increase adenylate cyclase activity and cAMP/protein kinase A–dependent formation of lipid bodies, and DP$_2$ receptors coupled to $G_i$ increase calcium. Each of these signals is required for LTC$_4$ production. These observations lead to consideration of the effects of other stimuli for eosinophil cAMP, such as the $\beta_2$-adrenoceptor agonists, which inhibit rather than enhance LTC$_4$ production.

**Abbreviations**
CRTH2, chemoattractant receptor-homologous molecule expressed on Th2 lymphocytes; IL-5, interleukin-5; LTC$_4$, leukotriene C$_4$; PDE4, type IV phosphodiesterase; PGD$_2$, prostaglandin D$_2$
and Wenzel, 2009 for a more complete discussion). Findings such as these reinforce the notion that asthma is a syndrome comprised of many phenotypes, with each one potentially requiring distinct treatment regimens.

Which mediators might be driving eosinophil activation in severe asthma? A recent study has shown that concentrations of the predominantly mast cell-derived eicosanoid, prostaglandin D$_2$ (PGD$_2$), are selectively elevated in the bronchoalveolar fluid in severe asthma (Balzar et al., 2010). PGD$_2$ recruits and activates eosinophils (Sandig et al., 2007) and directly produces smooth muscle shortening, leading to airway obstruction. PGD$_2$ has two well-defined receptors, DP$_1$ and DP$_2$ [the latter also known as CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 lymphocytes)]. The interest in PGD$_2$ as a therapeutic target for asthma and allergic disease has catalyzed the development of many well-characterized, selective pharmacological tools to examine the activity and importance of these two receptor types (Pettipher, 2008). The prevailing view of the regulatory activities of these two receptors, at least as regards eosinophil chemotaxis, was that they exerted opposing actions with final responses representing a balance between activation of both receptor types (Monneret et al., 2001). The DP$_1$ receptor acts through G$_{ai}$ to elevate cAMP levels, well known as a signal that generally reduces granulocyte activity. In many, but not all, cell systems, the DP$_1$ receptor acts via G$_{ai}$ to inhibit cAMP production, enhance intracellular Ca$^{2+}$ levels and activate phosphoinositide-kinase producing responses such as chemotaxis and degranulation. The study by Mesquita-Santos et al. in this edition has now shown that the same cannot be said for the release of LTC$_4$ induced by PGD$_2$ in eosinophils, in which these pathways synergize to stimulate LTC$_4$ synthesis (Figure 1).

Mesquita-Santos et al. (2011) demonstrated that rather than inhibiting LTC$_4$ production, activation of DP$_1$ signals via a protein kinase A (PKA)–dependent pathway to enhance the generation of lipid bodies in eosinophils, thus facilitating the DP$_2$-driven LTC$_4$ synthetic pathway. Engagement of both DP receptor subtypes was obligatory for effective LTC$_4$ generation. Their findings suggest that the synergy is manifest over a relatively narrow concentration range, with the effect being subthreshold at 5 nM, evident at 25 nM, and the bell-shaped curve indicates no effect of PGD$_2$ at 625 nM (see figure 4 in Mesquita-Santos et al., 2011). The bell-shaped curve may be explained by both synergistic and antagonistic interactions between different signals recruited by the DP receptors at different parts of the concentration–response relationship. Bell-shaped concentration–response curves are not unusual for inflammatory mediators, but they require some consideration as regards mediator antagonism. Specifically, partial antagonism of PGD$_2$ actions at the DP$_1$ receptor by shifting the curve to the right could have a paradoxical effect to increase PGD$_2$ action. The recent failure of the DP$_1$ receptor antagonist, laropiprant, in treatment of allergic airways disease (Philip et al., 2009) leads Mesquita-Santos et al. (2011) to suggest that dual antagonism may be required to block the full contribution of PGD$_2$. However, it is reasonable to predict, based on the findings of Mesquitas-Santos et al. that antagonism of either DP$_1$ or DP$_2$ receptors should be sufficient to antagonize the contribution of PGD$_2$. One reason for deviation from this expectation is that it is engendered by observations in short-term models of allergic pleuritic inflammation in the mouse. The relationship of this and other models of allergic airways/lung disease to human asthma is constantly being questioned.

The temporal concentration profile of the DP$_1$ antagonist may be another factor in its likely success in allergic disease, given the paradoxical consequences of shifting a bell-shaped concentration–response curve to the right. It is also important to consider that the levels of PGD$_2$ observed in bronchoalveolar fluid from asthmatics of 3–7 pg/mL$^{-1}$ (–10–25 pM) (Balzar et al., 2010) suggest that high nanomolar concentrations of PGD$_2$ are only likely to occur in discrete microenvironments. A further, and possibly more likely, explanation for the disappointing results obtained with laropiprant rests with PGD$_2$ actions through DP$_2$ receptors that are unconnected to LTC$_4$ production. Prominent amongst these effects is the eponymous chemotactant effect of activation of these DP$_2$ (CRTH2) receptors. Moreover, when testing the clinical effectiveness of laropiprant, it may be important to stratify patient selection in the same manner as has ultimately been done for those showing benefit from anti-IL-5 therapy (Bochner et al., 2010).

One other important issue raised by the observation that cAMP drives the formation of lipid bodies that enable effective LTC$_4$ production is whether other agents that elevate cAMP levels do likewise. The effects of PGE$_2$ on eosinophil cyclic AMP production have not been established, but EP$_1$ adrenolyte–coupled receptors are expressed (Mita et al., 2002). Could the β$_2$-adrenoceptor agonist used in the treatment of asthma be priming lung eosinophils for heightened LTC$_4$ production induced by PGD$_2$ or other eosinophil activators? Eosinophils express β$_2$-adrenoceptors (Yukawa et al., 1990). The short-acting β$_2$-adrenoceptor agonist, salbutamol, reduces LTC$_4$ synthesis and eosinophil peroxidase release induced by the formyl peptide, f-Met-Leu-Phe (fMLP) (Munoz et al., 1994). Inhibition of the predominant type IV phos-
phodiesterase (PDE4) in eosinophils also reduces LTC₄ synthesis (Dent et al., 1994). Earlier studies with salmeterol suggested that it blocked salbutamol regulation of LTC₄ (Munoz et al., 1995), whereas more recently, a direct inhibitory effect of this long-acting β-agonist on MRP4-induced eosinophil LTC₄ production has been unmasked by concurrent incubation with the selective PDE4 inhibitor rolipram (Meliton et al., 2003). Thus, not all CAMP-elevating agents have a similar effect on LTC₄ release in eosinophils. Compartmentalization of CAMP formation and action (Calaghan et al., 2008) offers one explanation for stimulus-specific effects on LTC₄, another being the context of the CAMP signal. The extraordinary impact of signal compartmentalization has recently been highlighted in studies of attomolar activity of relaxin acting on RXFP1 receptors coupled to CAMP formation, which show a preformed signalosome of, amongst other things, PKA-activated PDE4D3 associated with Goα and the Gβγ β-arrestin-2 (Halls and Cooper, 2010). The study by Mesquita-Santos et al. (2011) opens the way to address whether similar mechanisms are important in mast cell LTC₄ synthesis, in which the presence and functional relationship of lipid bodies to eicosanoid synthesis has been discussed (Dvorak, 2002); there are many stimuli that increase CAMP in mast cells. In addition, are DP receptor populations influenced by disease states, thus biasing functional responses one way or another? In HEK293 cells, DP₁ and DP₂ internalization, induced by PGD₂, has been shown to be differentially regulated (Gallant et al., 2007). Factors controlling the expression of DP₁ and DP₂ on eosinophils are currently little understood. The study of Mesquita-Santos et al. (2011) provides new insights into the interplay between DP receptors in regulating eosinophil function. However, the study also raises many questions of potential therapeutic importance that warrant further work.

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**Conflict of interest**

Neither of the authors has any conflict of interest to declare in respect of the content of the submitted commentary.

**Reference**


