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COMMENTARY

R2D$_2$ for C$_4$Eo: an 'alliance' of PGD$_2$ receptors is required for LTC$_4$ production by human eosinophils

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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$_{\alpha s}$ increase adenylate cyclase activity and cAMP/ protein kinase A–dependent formation of lipid bodies, and DP$_2$ receptors coupled to G$_{\alpha i}$ 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 β_2-adrenoceptor agonists, which inhibit rather than enhance LTC$_4$ production.

LINKED ARTICLE

This article is a commentary on Mesquita-Santos *et al.*, pp. 1674–1685 of this issue. To view this paper visit http://dx.doi.org/10.1111/j.1476-5381.2010.01086.x

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$_2$

The eosinophil possesses a potent armoury of preformed and *de novo* synthesized mediators and can respond to a wide variety of stimuli. The complexity of the mix of cells, mediators and receptors and their interactions in immune responses has limited progress in determining the individual contributions of eosinophils in normal and abnormal immune function. It has been broadly accepted that the eosinophil is protective in a variety of parasitic helminthic infections. Eosinophil mediators such as leukotriene C$_4$ (LTC$_4$) are accorded a role in removal of nematodes by influencing the tissue environment and eosinophil-derived degranulation products are directly toxic to the parasite. However, the dominant role of eosinophils in controlling helminthic infections has recently been questioned, based on results from newer eosinophil-deficient mouse models that have failed to show the uniform and substantial increases in infectivity that would be consistent with eosinophil dominance in parasite control (Behm *et al.*, 2000). A greater significance is being accorded to the network of Th2-associated mechanisms, involving recruitment of innate and adaptive immune cells, as well as cells resident within the infected tissue (Anthony *et al.*, 2007).

Eosinophils have also been considered central to the pathobiology of asthma, a disorder characterized in textbooks as 'airway eosinophilic inflammation'. Indeed, this role has been the most common clinical driver for research on eosinophil biology. However, markedly elevated numbers of eosinophils are not always seen in asthma, and in some cases, sputum eosinophil counts are now used as a biomarker to direct more patient-specific asthma therapy. Interleukin-5 (IL-5) is a cytokine critical to the proliferation and differentiation of eosinophils. The anti-IL-5 antibody, mepolizumab leads to a marked decline in blood eosinophil counts in asthmatics. Disappointingly, initial studies in patients with moderate, persistent asthma showed no improvement in airway function following treatment with this anti-IL-5 antibody. However, more recent trials in selected patient groups with severe asthma have shown that mepolizumab reduces the frequency of asthma exacerbations, albeit without improvement in airway function (see Bochner *et al.*, 2010

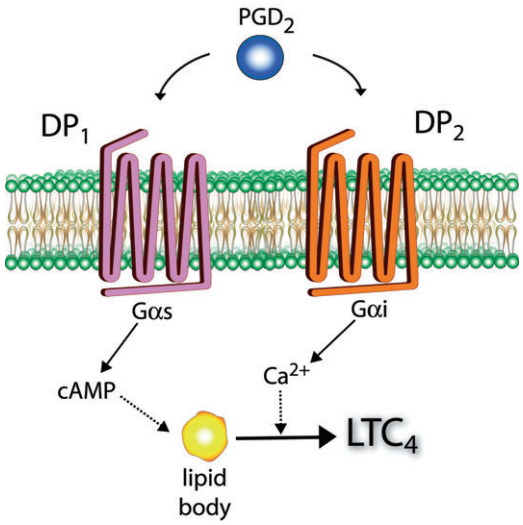

Figure 1

Illustration showing the necessity of agonism at both DP receptors by PGD_2 for effective LTC_4 production by eosinophils. (Mechanism based on the article of Mesquita-Santos *et al.* (2011) in the current issue). PGD_2, prostaglandin D_2; LTC4, leukotriene C_4.

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 $G_{\alpha s}$ to elevate cAMP levels, well known as a signal that generally reduces granulocyte activity. In many, but not all, cell systems, the DP_2 receptor acts via $G_{\alpha i}$ 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, laropripant, 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 $pg{\cdot}mL^{-1}$ (~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 laropripant rests with PGD_2 actions through DP_2 receptors that are unconnected to LTC_4 production. Prominent amongst these effects is the eponymous chemoattractant effect of activation of these DP_2 (CRTH2) receptors. Moreover, when testing the clinical effectiveness of laropripant, 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 cys-LT production have not been established, but EP_4 adenylate cyclase–coupled receptors are expressed (Mita *et al.*, 2002). Could the β_2-adrenoceptor agonists 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 LTC_4 synthesis (Dent *et al.*, 1994). Earlier studies with salmeterol suggested that it blocked salbutamol regulation of LTC_4 (Munoz *et al.*, 1995), whereas more recently, a direct inhibitory effect of this long-acting β–agonist on fMLP-induced eosinophil LTC_4 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 LTC_4 release in eosinophils. Compartmentalization of cAMP formation and action (Calaghan *et al.*, 2008) offers one explanation for stimulus-specific effects on LTC_4, 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 Gαs 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 LTC_4 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, DP_1 and DP_2 internalization, induced by PGD_2, has been shown to be differentially regulated (Gallant *et al.*, 2007). Factors controlling the expression of DP_1 and DP_2 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.

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