IS THERE A CRTH2 FUNCTIONAL RESPONSE IN THE DOG?

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CRTh2 is a G protein-coupled receptor activated by prostaglandin D_2 (PGD_2). It has been identified on human Th2 lymphocytes, eosinophils and basophils. The selective CRTh2 ligand 13,14-dihydro-15-keto-PGD_2 (DK-PGD_2) has been reported to induce shape change and chemotaxis in human eosinophils. However, functional responses with CRTh2 ligands have been difficult to generate in rats and mice. Thus the role of CRTh2 cannot be fully investigated in these species, which prompted us to re-evaluate previously unexplained functional effects of PGD_2 in non-rodent species. Emery *et al*., (1989) reported that PGD_2 induced an eosinophilia in the perfused dog trachea, a response that may be explained by the presence of CRTh2. AstraZeneca have recently cloned, expressed and characterised the ligand binding of dog CRTh2 (Carrillo *et al*., this meeting). This has confirmed that DK-PGD_2 can be used to probe the functional role of CRTh2 in the dog. The aim of this study was to investigate CRTh2 functional responses in dog eosinophil shape change assays and *in situ* in the perfused dog trachea using DK-PGD_2 as the agonist.

DK-PGD_2-induced shape change of eosinophils was measured as an increase in forward scatter (FS) of high autofluorescent granulocytes using a Becton Dickinson Facscan flowcytometer. Dog blood (n=4) was treated with DK-PGD_2, BW45C (DP agonist) or U46619 (TP agonist) (10^{-11} M to 10^{-5} M), for 15 min at 21°C. The reaction was stopped on ice and the red blood cells lysed. The remaining cells were fixed and FS read on the flowcytometer. PGD_2 and DK-PGD_2 induced-eosinophil recruitment to the tracheal lumen was assessed in propofol anaesthetised, ventilated, female beagle dogs (n=5;~15 kg) (approved by AstraZeneca ethical review). Before DK-PGD_2 perfusion, the ability of each dog to respond to PGD_2 was assessed. Using a double-cuffed endotracheal tube, a tracheal segment was perfused with PBS/polymixin B containing 0.01% EtOH (vehicle) or PGD_2 (10^{-5} M) for 3 h. Perfusate was changed every 30 min and the number of eosinophils and neutrophils counted (other cell types were not detected). Four of the dogs showed a repeatable eosinophil response (>5x10^4 eosinophils per animal on 3 consecutive weekly occasions). The tracheas of the PGD_2 responsive dogs were perfused with DK-PGD_2 (10^{-5} M) on one subsequent occasion. Statistical analysis was by paired t-test and $P<0.05$ was considered significantly different.

DK-PGD_2 increased the FS of eosinophils (EC_{50} 6.8 nM) but not other granulocytes gated on the FACS plot. BW245C or U44619 did not increase FS of any granulocytes. In tracheal perfusions DK-PGD_2 had no significant effect on neutrophil numbers but induced significant eosinophilia when compared to vehicle (Table 1, * $P<0.01$).

Table 1. Vehicle or DK-PGD_2 (10^{-5} M) induced tracheal eosinophilia (x10^4 per animal)

Time (min)	0-30	30-60	60-90	90-120	120-150	150-180
Vehicle	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.3	1.5±1.5	2.0±2.0
DK-PGD2	6.5±5.1	5.7±3.5	7.2±1.4*	15.8±4.1*	49.6±11.7*	83.9±19.4*

In conclusion a CRTh2 functional response was observed in canine cells. Thus the dog may be a suitable non-rodent species in which the role of CRTh2 in allergy and inflammation might be investigated.

Emery, DL *et al.*, (1989). *J. Appl. Physiol.* **67**:959-962.