

METHODS

CRTH2 pharmacologic inhibitor

We also performed experiments with ramatroban (a pharmacologic inhibitor of CRTH2, the receptor for PGD₂) (Cayman Chemicals).^{E1,E2} In these experiments, PBMCs containing ILC2 were preincubated with either diluent or 10 μM ramatroban for 30 minutes at 37°C, and then the PBMCs were placed in the upper chamber with 25 nM PGD₂ in the lower chamber to assess ILC2 chemotaxis.

PGD₂ added to upper and lower chambers

In selected experiments, the same concentration of PGD₂ (5 or 25 nM) was added to both the upper chamber and the lower chamber.^{E3}

DISCUSSION

Previous studies examining the effects of PGD₂ on chemotaxis/chemokinesis of cell types other than ILC2 important to allergic inflammation (ie, eosinophils, basophils, and T_H2 cells) have demonstrated that the effect on eosinophils and basophils is predominantly chemotactic^{E4} but also chemokinetic especially at low concentrations of PGD₂ (1-10 nM).^{E5} Studies of T_H2 cells suggest that the predominant effect of PGD₂ is mostly chemotactic.^{E4} CRTH2 levels in blood eosinophils are increased in patients with atopic dermatitis than in healthy controls, although there is

considerable overlap between levels of eosinophil CRTH2 in healthy controls and patients with atopic dermatitis.^{E6} In this study, levels of CRTH2 in ILC2 were not increased in allergic compared with nonallergic individuals (data not shown).

REFERENCES

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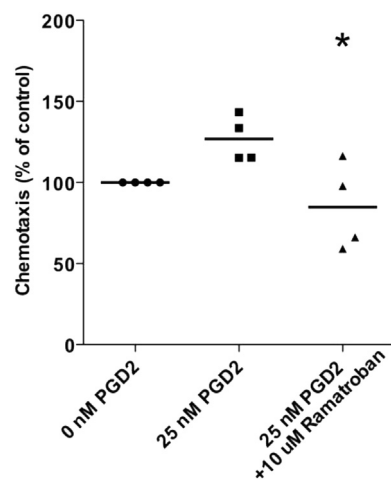


FIG E1. CRTH2 antagonist. PBMCs were preincubated with or without 10 μ M ramatroban ($n = 4$ nonallergic controls) for 30 minutes and then placed in the upper chamber of a 5- μ m pore-size plate. PGD2 (25 nM) was placed in the lower chamber. The number of ILC2 in the lower chamber was assessed by FACS at 90 minutes. FACS, Fluorescence-activated cell sorting. * $P = .03$, 25 nM PGD2 vs 25 nM PGD2 + ramatroban.

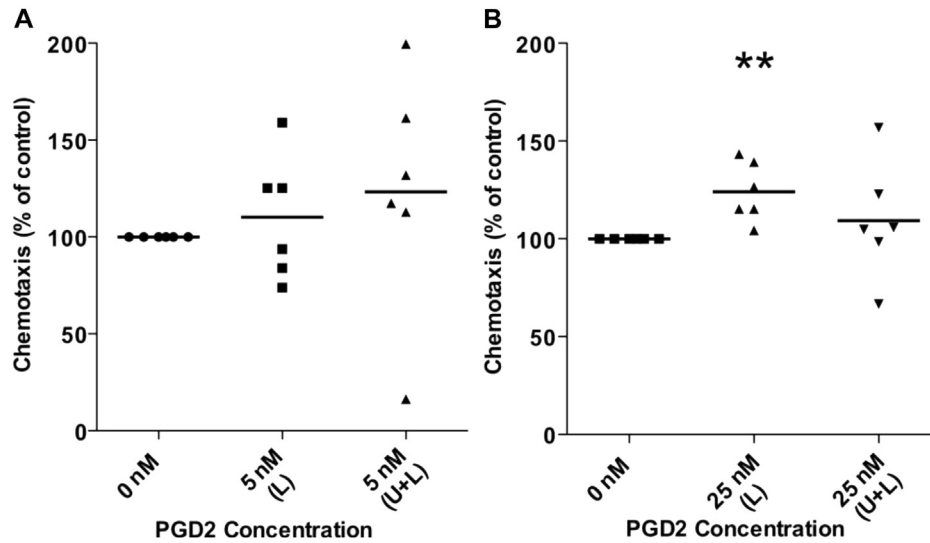


FIG E2. PGD2 upper and lower chambers. PGD2 5 nM (**A**) or PGD2 25 nM (**B**) was added to either the lower (*L*) chamber alone or to both the upper and lower (*U+L*) chambers. The number of ILC2 in the lower chamber was assessed by FACS at 90 minutes. FACS, Fluorescence-activated cell sorting. ****** $P < .01$.