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Attenuation of murine allergic airway inflammation with a CXCR1/CXCR2 chemokine receptor inhibitor

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To the Editor:

More than two decades ago, we were amongst the first to report the presence of neutrophils in the airways in severe asthma[1]. However, the nature of contribution of neutrophils to allergic inflammation remained a scientific enigma until quite recently. Neutrophils have long been viewed as terminally differentiated cells that clear extracellular pathogens. However, a growing body of literature indicates that neutrophils regulate innate and adaptive immune responses, and contribute to allergic diseases[2–4]. Adoptive transfer or recruitment of neutrophils to the skin stimulates allergic skin inflammation[2]. Likewise, neutrophils are required for both the sensitization and elicitation phase of allergic contact dermatitis[3]. Neutrophil activation occurs *in vivo* during anaphylaxis, and neutrophil depletion inhibits active and passive systemic anaphylaxis[5]. Importantly, mouse and human neutrophils each restore anaphylaxis in anaphylaxis-resistant mice, demonstrating that neutrophils are sufficient to induce anaphylaxis in mice, and suggesting that neutrophils can contribute to anaphylaxis in humans[5]. *Tlr4*KO mice are unable to mount pollen-induced allergic airway inflammation[4]; adoptive transfer of neutrophils to the lungs of *Tlr4*KO mice increases IL-33 secretion and reconstitutes pollen-induced allergic airway inflammation[4]. Exposure of the airway epithelium to allergenic extracts recruits reactive oxygen species (ROS)-generating neutrophils to the lungs[4]. Additionally, inhibiting ragweed pollen extract (RWPE)-induced neutrophil recruitment in mice by administration of CXCR2 small molecule inhibitor SB225002 attenuates allergic airway inflammation and sensitization[4]. Together, these studies provide compelling evidence for a role of neutrophils in the pathogenesis of allergic inflammation[2–4].

An unexpected development in this story of the role of neutrophils to pathogenesis of allergic inflammation came from a report that administration of the CXCR2 inhibitor AZD5069 in humans failed to improve severe uncontrolled asthma even though it reduced

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the levels of neutrophils in sputum and blood[6]. Neutrophils have CXCR1 and CXCR2 on their surfaces, and these receptors have distinct non-redundant roles in its recruitment and activation. Thus CXCL8 monomer and dimer differentially activate and regulate function of CXCR1 and CXCR2 on neutrophils[7]. Inhibition of CXCR1 but not CXCR2 pathway suppresses neutrophil's bacterial killing functions[8]. We identified the CXC motif in chemokines functions as a conformational switch for high affinity binding and activation of CXCR1 and CXCR2 receptors. Based on these reports[4,7,8], here we hypothesized that a dual CXCR1 and CXCR2 pharmacologic inhibitor maybe effective in attenuating allergic airway inflammation.

Reparixin is a noncompetitive allosteric inhibitor of both CXCL8 receptors CXCR1 and CXCR2 that is safe for human use. Administration of reparixin in humans in a phase 2 randomized pilot study improved outcome of transplant of allogeneic islets in type 1 diabetes[9]. To test our hypothesis, we selected reparixin to perform a proof-of-concept study to elucidate the role of the CXCR1/CXCR2 inhibitor in an animal model of cat dander extract (CDE)-induced innate and allergic lung inflammation. While the functionality of CXCR2 has been extensively reported in mice, it was the recent demonstration of functional CXCR1 receptors in mice[10] that provided the critical rationale for using mice to test our hypothesis. Wild-type (WT) naïve mice were subjected to a CDE single challenge model, CDE-SCM, Fig. 1A, by challenging them once with CDE and evaluating markers for innate inflammation were quantified 16 hrs later in BALF[4]. To induce allergic inflammation, WT mice were sensitized to CDE by subjecting them to CDE multiple challenge model, CDE-MCM, Fig. 2A, as previously described[4]. Following a rest period of one week, the mice were challenged again on Day 11, and allergic inflammation was quantified in the lungs on Day 14. Two doses of reparixin or vehicle were administered one hour before and after CDE challenge in a subset of the CDE-SCM group or CDE-MCM group on Day 11, and the effects of the inhibitor on innate or allergic lung inflammation were elucidated.

In the CDE-SCM model (Fig. 1A), administration of reparixin (15 mg/kg body weight) suppressed neutrophil recruitment into the lungs (Fig. 1B). Building on this observation, we used the same dose of reparixin in the CDE-MCM model (Fig. 2A). As expected, CDE challenge in the CDE-MCM model stimulated eosinophil recruitment, allergic inflammation and sensitization (Fig. 2B–F). Administration of reparixin inhibited eosinophil, neutrophils, and total cell numbers in BALF (Fig. 2B), serum levels of total IgE and CDE-specific IgE (Fig. 2C), airway epithelial mucin secretion (Fig. 2D), levels of Th2 inflammation-associated genes *periostin* and *muc5ac* (Fig. 2E), and the BALF levels of IL-4, IL-13, IL-33, and TSLP in BALF (Fig. 2F). These results indicate that pharmacological inhibition of CXCR1/2-axis by administration of reparixin inhibits allergen-induced innate and allergic airway inflammation in mice. Taken together with the earlier report demonstrating that neutrophils recruited and activated by the CXCR1/2 axis contribute to allergic sensitization and inflammation[4], our data from the present study suggest that reparixin attenuates allergic airway inflammation by inhibiting allergen-induced recruitment and activation of neutrophils.

To our knowledge, this is the first report showing that administration of dual CXCR1/2 inhibitor suppresses allergen-challenge induced neutrophilic inflammation and allergic

airway inflammation. As second generation CXCR1/2 dual inhibitors such as ladarixin are developed and tested, some of these inhibitors may prove to be even more effective than reparixin in inhibiting allergic inflammation. Since asthma is a heterogeneous disease, administration of these new generation CXCR1/2 inhibitors may prove to be effective as controller therapy in neutrophil-dominant forms of asthma, such as the one described by us earlier[1].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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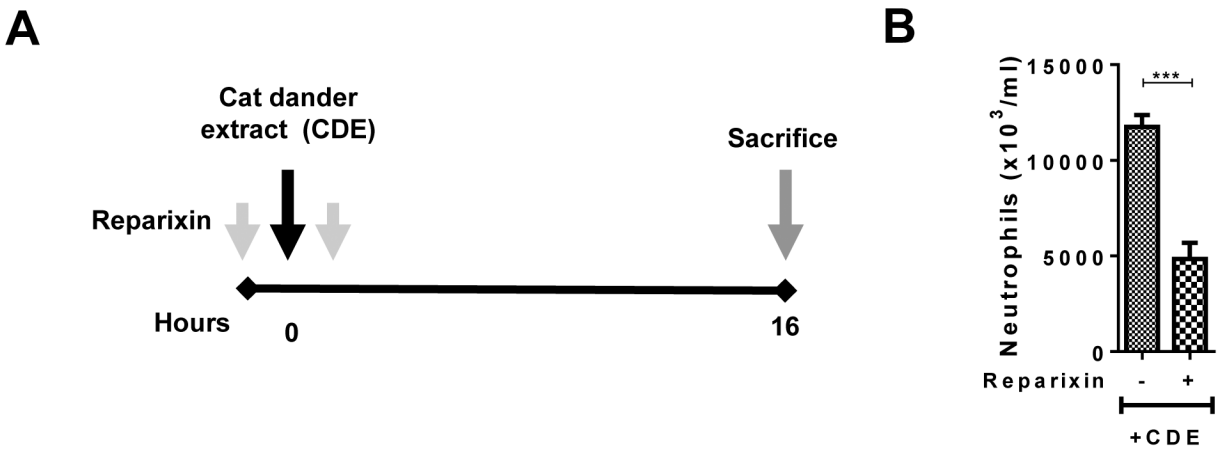

Fig. 1. Effect of Reparixin on CDE challenge-induced innate inflammation

(**A**) Protocol for CDE Single Challenge Model, CDE-SCM in naïve WT mice with or without reparixin. (**B**) BALF neutrophil numbers in WT mice in a single CDE challenge model.

For all groups n =6–8 mice per group were used.

Data are expressed as means ± SEM. *=P< .05, **=P< .01, ***=P< .001, *** *=P< .0001.

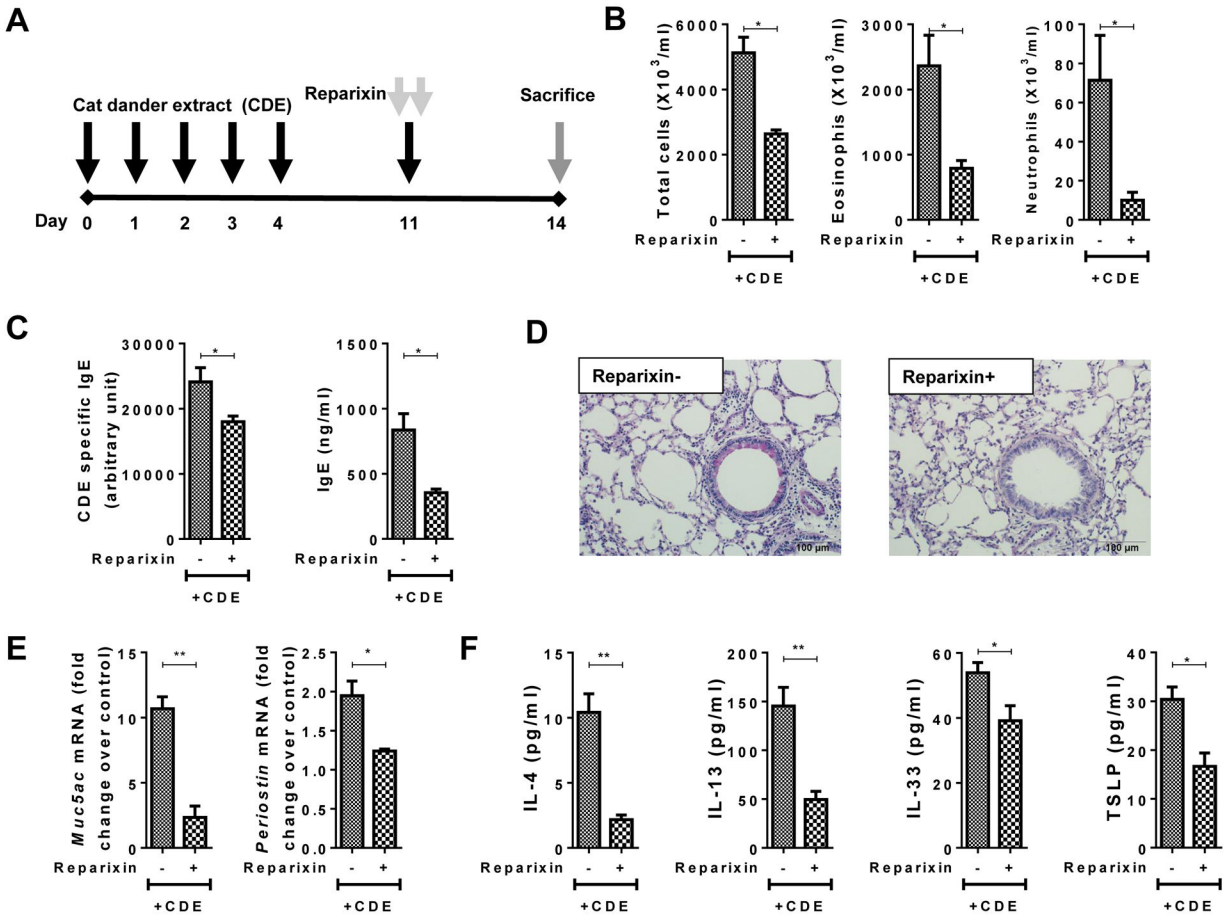

Fig. 2. Effect of Reparixin on CDE challenge-induced allergic inflammation

(**A**) Protocol for CDE Multiple Challenge Model, CDE-MCM in naïve WT mice with or without reparixin. (**B-F**) Effect of multiple CDE challenge in the presence or absence of reparixin in WT mice. (**B**) BALF total inflammatory cell, eosinophil, and neutrophil numbers. (**C**) Serum total IgE and CDE-specific IgE. (**D**) Mucin secretion in airway epithelial cells. (**E**) *Muc5ac* and *Periostin* mRNA expression in lungs. (**F**) Th2 cytokines in BALF

For all groups n =4 mice per group were used.

Data are expressed as means ± SEM. *=P< .05, **=P< .01, ***=P< .001, *** *=P< .0001.