

## The bronchodilatory capacity of imiquimod: the existence of two mechanisms

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TO THE EDITOR: We recently published a study investigating the bronchodilatory capacity of the Toll-like receptor 7 (TLR7) agonist imiquimod, establishing the presence of a nonneuronal, TLR7-independent mechanism of dilation (9). Our study adds new information to the previously published reports, which demonstrated that relaxation can be mediated by a TLR7-dependent release of nitric oxide (NO) from innervating nerves (2, 5). In a Letter to the Editor, Drake (2a) now argues that the data we report do not provide sufficient evidence to support our conclusion of a nonneuronal TLR7-independent mechanism. We would instead argue that our results are sound and reflect the presence of an additional mechanism for imiquimod-mediated bronchodilation.

In our study, we demonstrated that administration of the neurotoxin tetrodotoxin (TTX) in vitro had no effect on imiquimod-induced bronchodilation and thus concluded that neuronal pathways were not involved in dilation (9). Due to the presence of TTX-resistant nerves, particularly those originating from the nodose ganglia (8), Drake suggests that this is not sufficient to discount neuronal involvement. However, the contribution of TTX-resistant nodose-derived sensory nerves to relaxation is unlikely. Although these nerves contain relaxatory mediators, namely pituitary adenylate cyclase-activating polypeptide (PACAP) (1, 3) and substance P (SP) (4, 12), SP induces relaxation via the epithelium (4) and PACAP relaxes independently of nitric oxide synthase (NOS) (7). This argues against Drake's proposed mechanism of epithelium-independent and NO-mediated relaxation. In addition, activation of sensory neurons by imiquimod has been shown to be TLR7 independent (6), which suggests that a TLR7-dependent neuronal mechanism of relaxation does not occur via sensory neurons. We could further demonstrate in our study that all nerve-mediated relaxation was blocked by TTX (9). NO-releasing inhibitory nonadrenergic, noncholinergic (iNANC) fibers have repeatedly been shown to be TTX sensitive (10), suggesting that our use of TTX was sufficient to evaluate and discount the possible contribution of NO-mediated neuronal relaxation, the mechanism proposed by Drake and colleagues.

Noticeably, there are discrepancies between our results and those published previously by Drake and colleagues, particularly from experiments assessing the roles of TLR7 and NO, using the interventions IRS661 and *N*<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME)/*N*<sup>G</sup>-monomethyl-L-arginine (L-NMMA), respectively. Using the same concentration of IRS661 as previously used in vitro (5), we were unable to see any effect on imiquimod-induced bronchodilation. Although we did not confirm the ability for IRS661 to inhibit TLR7 in this study, we

have previously confirmed its ability to inhibit TLR7-mediated responses in isolated airway smooth muscle cells (11). In addition, we showed that the TLR7 agonist CL264 failed to induce relaxation or inhibit histamine-induced Ca<sup>2+</sup> flux, which would dispute a role for TLR7. The discrepancy between our and Drake and colleagues' in vitro L-NMMA or IRS661 results may be related to the dissection technique, which can affect the extent of neuronal innervation. Indeed, the fact that we identified a small role for NO in vivo, as highlighted by Drake, could suggest that the relative presence or absence of intact nerves is of importance in identifying a neuronal TLR7-dependent mechanism for dilation.

We would like to reiterate that our study does not dispute that of Drake and colleagues but purely supplies an additional mechanism for imiquimod-mediated bronchodilation. Using a nerve-free setup with isolated airway smooth muscle, we identified that imiquimod could additionally directly influence histamine-induced Ca<sup>2+</sup> flux in smooth muscle cells in a TLR7-independent manner. In addition, as shown previously by Drake and colleagues (5), inhibition of NO or TLR7 does not fully reverse the dilatory effects of imiquimod, suggesting that another mechanism feasibly exists. The relative contribution of respective mechanisms may be dependent on the model used, the route of administration of imiquimod, and the relative presence of neuronal innervation. The complexity of imiquimod-mediated bronchodilation is fascinating and is likely to be of use in the development of novel fast-acting, bronchodilatory compounds.

## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

## AUTHOR CONTRIBUTIONS

O.J.L., M.L.M., and L.-O.C. conception and design of research; O.J.L. and S.K.G. drafted manuscript; O.J.L., M.L.M., M.S., B.F., M.A., S.K.G., and L.-O.C. edited and revised manuscript; O.J.L., M.L.M., M.S., B.F., M.A., S.K.G., and L.-O.C. approved final version of manuscript.

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