

## The importance of keratinocyte–dendritic cell interactions in understanding fragrance allergy

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Fragrance allergy is one of the most common causes of allergic contact dermatitis,<sup>1</sup> and affected individuals report a reduced quality of life.<sup>2</sup> Compounds with low sensitizing potency can be activated abiotically, through air oxidation, or, biotically, through enzyme activity in the skin to generate allergens. This typically involves the formation of hydroperoxides, which can form further free radical intermediates. Experimental and clinical studies have demonstrated that hydroperoxides of different fragrance compounds form specific antigens.<sup>3</sup> Allergic reactions to oxidized products are often not detected in standard testing with fragrance markers, suggesting that the prevalence of fragrance allergy is higher than recognized.

In this issue of the *BJD*, Lichter *et al.* interrogate the radical intermediates generated in human epidermis by common hydroperoxide sensitizers derived from limonene, and explore the contribution of primary human keratinocytes to dendritic cell (DC) responses following exposure to limonene hydroperoxides.<sup>4</sup> The authors found distinct DC activation profiles for the limonene hydroperoxides, limonene-1-OOH and limonene-2-OOH, indicating specificity. Using electron paramagnetic resonance spectroscopy combined with a spin trapping technique the authors were also able to measure the short-lived intermediate radicals derived from limonene-1-OOH and limonene-2-OOH in reconstructed human epidermis, with limonene-1-OOH predominantly generating methyl radicals, contrary to what was previously believed. They also show that variability in DC responses to limonene-1-OOH, in particular, appear to be attributed primarily to the keratinocytes. As yet, there is no direct proof that the free radical intermediates identified in the three-dimensional (3D) human epidermal models are responsible for the DC activation by the limonene hydroperoxides, but while the evidence is preliminary, it does support an important contribution of keratinocytes to DC activation. Abiotic and biotic processes within keratinocytes, responsible for free radical cleavage, could underlie the observed human variation in allergic susceptibility to fragrance compounds.

Currently, the most well established method of assessing sensitizing activity and the potency of chemicals is to use the local lymph node assay, but in an effort to reduce animal usage new-approach methodologies are being sought.<sup>5</sup> The study by Lichter *et al.* highlights the need for experimental models that take into account the complexity of cellular interactions in the skin when studying the sensitizing

potential of chemicals. *In vitro* models adopted by the Organisation for Economic Cooperation and Development, such as the KeratinoSens™ skin sensitization assay,<sup>6</sup> is based on the immortalized HaCat cell line, which is not fully representative of human skin metabolomics. However, the 2016 report of the European Union Reference Laboratory's European Centre for the Validation of Alternative Methods expert group on 'Ability of non-animal methods for skin sensitisation to detect pre- and pro-haptens' concluded that negative *in vitro* data should be accepted unless there is compelling evidence that the substance is likely to be exclusively metabolically activated.<sup>7</sup> In summary, this work supports the need to further evaluate the abiotic and biotic mechanisms involved in the activation of fragrance chemicals in combination with analytical technologies that can identify metabolic products that may act as potent new sensitizers. While 3D skin constructs may currently have limited potential for high-throughput screening of sensitizers, this research highlights the advantages of more complex, physiological co-culture models.

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## 2 Commentary

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