

Interleukin-36 (IL-36) system in the 1-fluoro-2,4-dinitrobenzene (DNFB) mouse model of allergic contact dermatitis

To the Editor,

Inflammatory processes are critical for protective immunity against microbes, but can lead to allergies and autoinflammatory diseases when excessive. Consequently, anti-inflammatory mechanisms have evolved to restrain these responses. A well-characterised example of a complete self-regulated inflammatory pathway is the interleukin-1 (IL-1) system comprising the agonist cytokines IL-1 α and IL-1 β , and their receptor IL-1R1 (IL-1 receptor type I), which is inhibited by the IL-1 receptor antagonist IL-1Ra.¹ A related system involves the three IL-36 cytokines IL-36 α , IL-36 β and IL-36 γ , which are neutralised when the IL-36Ra binds the IL-36 receptor IL-1RL2 (also known as IL-36R). IL-36R is expressed on many epithelial, dendritic, monocyte and T-cell subtypes, and all five agonists activate MAP kinases and NF- κ B leading to increased expression of chemokines that promote recruitment of inflammatory cells.¹

Interest in how the IL-36 system may contribute to skin diseases has grown dramatically in recent years. Conflicting data are reported in the literature regarding the expression of the system in contact hypersensitivity. While some studies found increased expression of the three IL-36s,²⁻⁴ this was not observed in other independent experiments.^{5,6} A recent investigation conducted in mice suggested that IL-36 α might be involved in T-cell priming in the lymph nodes during the development of contact hypersensitivity; however, IL-36 α deficiency did not impact the disease phenotype in the skin as evaluated through ear thickness and gross histology.⁷ Since some human studies observed upregulation of all three IL-36s²⁻⁴ in affected skin and we previously demonstrated specific involvement of a single IL-36 in different mouse models of skin disease,⁸ we here examined the possible involvement of each of the IL-36s and their antagonist IL-36Ra in the well-established DNFB (1-fluoro-2,4-dinitrobenzene) mouse model of contact hypersensitivity.

All animal procedures were approved by the Temple University Lewis Katz School of Medicine Institutional Animal Care and Use Committee and performed in compliance with the US Department of Health and Human Services Guide for the Care and Use of Laboratory Animals. The previously described⁸ IL-36 cytokine knock-out (KO) mice and controls were treated as described in Figure 1A. Treatment with DNFB lead to similar ear swelling in all four strains (Figure 1B, data pooled from 5 independent experiments and analysed by ANOVA). Although male ears were significantly ($P < .01$) heavier than female ears, no statistically significant differences

between mouse strains were identified. In agreement with this, histological analyses of epidermal and dermal thickness did not reveal statistically significant differences between wild-type and IL-36 α -, IL-36 β - or IL-36 γ -deficient mice (Figure 1C-D). Similar preliminary analyses of shorter sensitisation (4 days) or challenge periods (6 and 18 hours) did not reveal strain-dependent differences either. While ear thickness is an outcome commonly used to determine disease severity, the approach has its limitations. Hence, we also evaluated expression levels of a number of cytokines linked to the disease phenotype⁶ and previously shown to be regulated by IL-36 directly or indirectly.¹ Inflammation was clearly induced in all four strains, as chemokines not measurable in normal ears were readily detected after DNFB treatment. However, in agreement with the gross ear swelling data (Figure 1B-D), no statistically significant differences could be detected between mouse strains (Figure 1E).

The balance between pro-inflammatory agonists to anti-inflammatory antagonists is naturally important for the final outcome in many situations. In allergic contact dermatitis patients, expression of IL-1 β , IL-1Ra, IL-36 α , IL-36 β and IL-36 γ is reported to be higher in involved skin than uninvolved sites.² Interestingly, expression of IL-36Ra appears unchanged, thus prompting the hypothesis that low IL-36Ra expression could be part of the pathogenesis.² Consequently, we next examined the effect of IL-36Ra deficiency in vivo in the DNFB mouse model (Figure 2). If IL-36Ra plays an important role in limiting contact hypersensitivity in the model used, we would expect more severe disease in the IL-36Ra KO mice compared to wild-type controls; however, all parameters measured in the model (ear weight (Figure 2A), epidermal and dermal thickness (Figure 2B-C) and cytokine expression (Figure 2D)) were found to be similar to those in wild-type mice. Hence, it appears that IL-36Ra is not an essential regulator of the immune response in at least the here used model at the examined timepoints.

A recent contact hypersensitivity study conducted in mice found an effect of deleting IL-36 α when T-cell priming was examined ex vivo; however, there was no effect upon disease outcome in vivo.⁷ We here confirm that IL-36 α deficiency does not impact severity of the DNFB-induced inflammation (Figure 1) and further establish that deletion of IL-36 β or IL-36 γ also does not impact the phenotype (Figure 1). It remains a possibility that the three IL-36 cytokines act together to amplify priming and/or inflammatory responses; further analyses in IL-36R KO mice could test this hypothesis. However, such

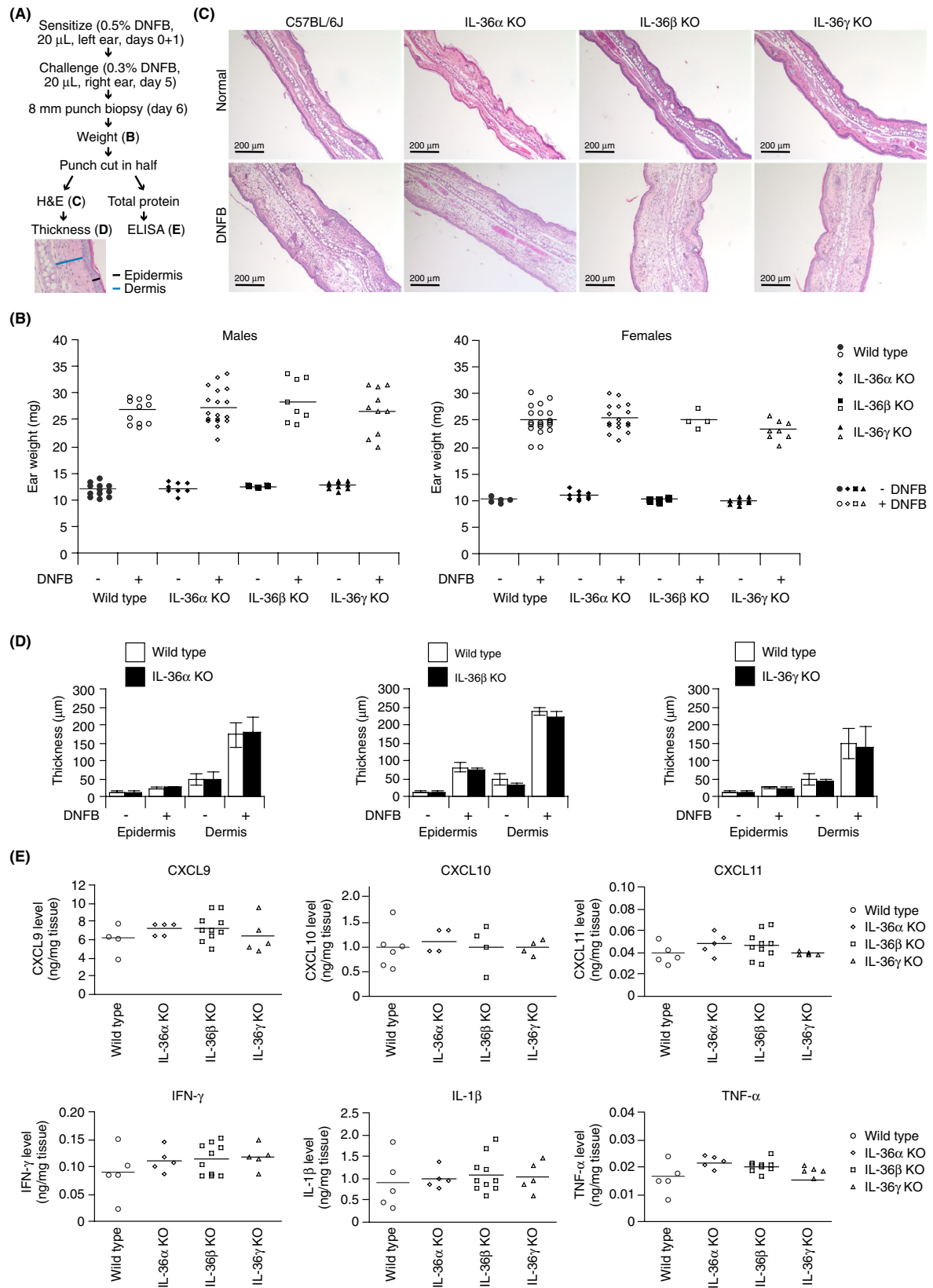
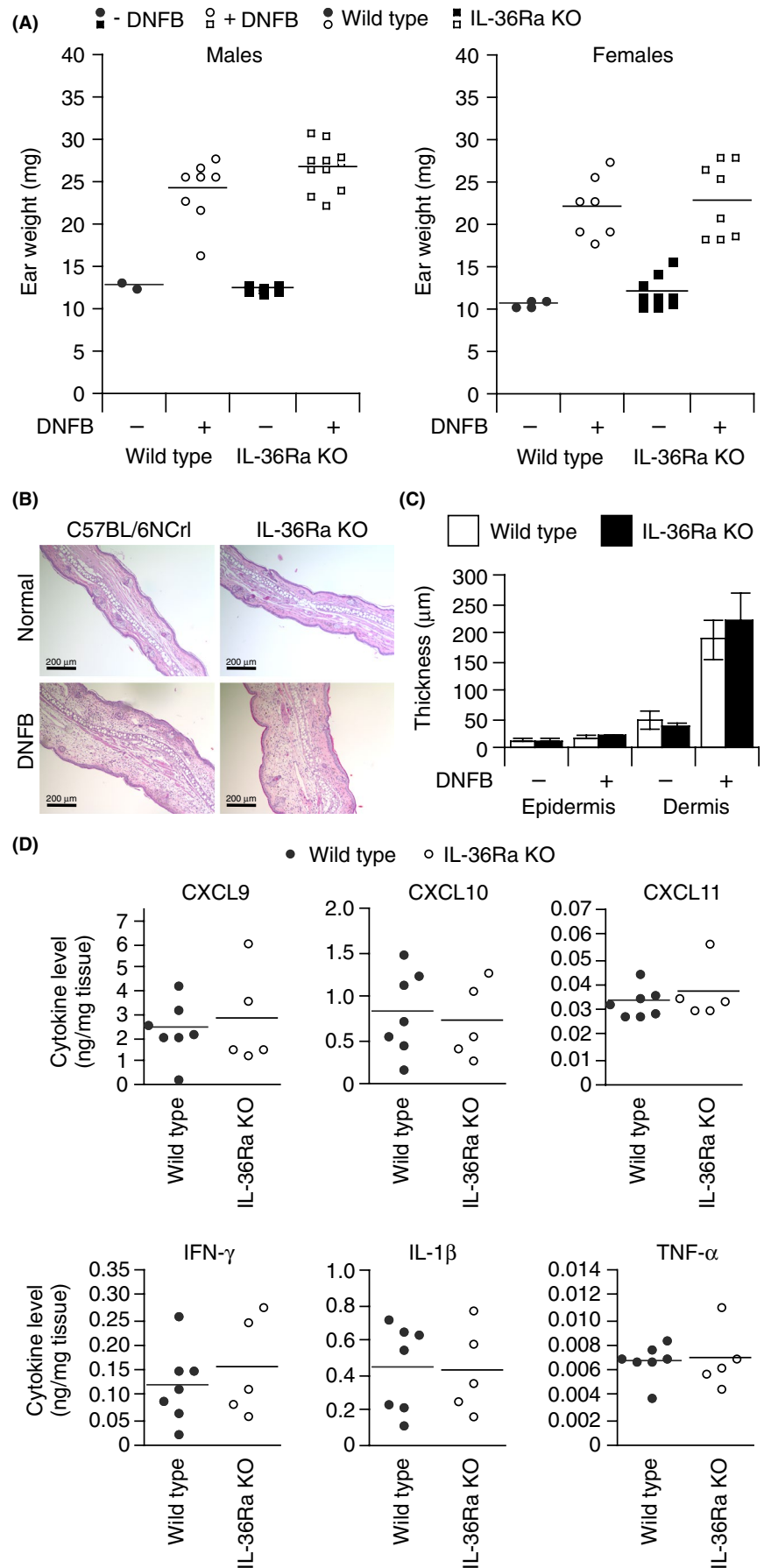


FIGURE 1 IL-36 deficiency does not impact acute DNFB-induced hypersensitivity. Mice were treated with DNFB (Sigma-Aldrich) in acetone/olive oil (3:1 vol/vol) and evaluated as outlined (A). Standardised ear samples were collected using punch biopsy tools. Tissue weight was determined (B) before the sample was cut into half. One half was used for histology (C) and evaluation of epidermal and dermal thickness (D); the other for ELISAs (R&D Systems, E). Thickness was determined using ImageJ software by drawing straight lines through the epidermis and dermis (A). Three sites were measured and averaged per tissue/mouse. No statistically significant differences between strains were identified

FIGURE 2 Acute DNFB-induced contact hypersensitivity is not aggravated in the absence of IL-36Ra. Mice deficient in IL-36Ra were obtained from the Mutant Mouse Resource & Research Centers (strain B6N.129S5-*Il1f5*^{tm1Lex}/Mmucd) and genotyped using primers Neo3a(II1f5) GCAGCGCATCGCCTTCTATC, UNQ1896-16 GAGCTCCATGATGTTCACTGG, UNQ1896-7 GAGCTGCATCCTGTCATCG and UNQ1896-8 CCTCTAGCTGATATAC ATCC. IL-36Ra KO and C57BL/6NCrI background-matched wild-type mice were treated and analysed as described in Figure 1. Tissue weight (A), histology (H&E staining, B), epidermal and dermal thickness (C) and cytokine levels (D) were evaluated. No statistically significant differences between strains were identified



a combined function cannot be regulated by IL-36Ra as we did not observe an effect of deleting IL-36Ra (Figure 2).

The previous study of hypersensitivity in IL-36 α KO mice found no increase in IL-36 α expression in the challenged inflamed skin, and this lack of induction was proposed as a possible explanation for the lack of an in vivo phenotype.⁷ In this context, it is noteworthy that while some human studies have observed increased expression of the IL-36 cytokines in affected skin compared to uninvolved sites,²⁻⁴ others did not identify such changes.^{5,6} Unlike the laboratory mice which are genetically identical and are challenged with a single agent in each experiment, human patients are genetically diverse and develop allergies to a wide range of allergens. The present (Figures 1-2) and previous⁷ study of hypersensitivity in mice employed the DNFB model in C57BL/6 mice and found no evidence for involvement of IL-36 or IL-36Ra in induction of acute disease as determined 24 hours after challenge. However, it remains a possibility that different allergens and/or genetic backgrounds could reveal a role for the IL-36 system in initiation of allergies. Interestingly, a more recent study revealed a role for IL-36Ra 48 hours post-challenge,⁹ which suggests involvement in chronic disease. Hence, future studies should be designed accordingly.

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CONFLICTS OF INTEREST

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