European Journal of Immunology
Clinical·Basic·Translational

Notes and Insights

Influence of E-cadherin deficiency on the dendrite morphology of Langerhans cells

The homophilic adhesion molecule E-cadherin (E-cad) is highly expressed within the epidermis by keratinocytes and Langerhans cells (LC), which are members of the dendritic cell (DC) lineage within the epidermal layer of the skin. By breaching the tight junction barrier formed by epidermal keratinocytes, activated LC capture external antigens [1], mature in that process, and migrate to skin-draining lymph nodes for antigen presentation to T cells [2] (see also Suppl. Ref. 4 and 5). It was suggested that E-Cad is responsible for the attachment of LC to keratinocytes and thus their persistence in the epidermis [2]. This hypothesis was supported by three lines of evidence: (1) formation of adherens junction-like structures between E-Cad expressing LC-like DC and keratinocytes [3], (2) inhibition of LC maturation by E-Cad ligation [4] and downregulation of E-Cad during activation, maturation, and emigration of LC [5, 6], and (3) requirement of TGF-β for E-Cad expression in dendritic cells (DC) and lack of epidermal LC in TGF-β null mutants [7, 8]. Recent data have challenged this hypothesis by showing that in mice with a selective E-Cad deficiency in CD11c$^+$ cells generated by Cre/LoxP recombination (E-cad$^{fl/fl}$CD11c Cre$^+$) E-Cad deficient LC are found in normal numbers in the epidermis [9, 10].

In line with recently published data [10], we observed that LC were present in the epidermal layer of the skin in comparable numbers between mice with E-Cad deficient LC (Cre$^+$ mice) and littermate mice with E-Cad competent LC (Cre$^-$ mice) (Fig. S1A, B). The cell type-specific E-Cad deficiency was confirmed by flow cytometry that showed a selective lack of E-cadherin and β-catenin in LC of Cre$^+$ mice (Fig. S1C–F). Analyzing the morphology of LC by using confocal microscopy in whole mount ear skin we could not confirm the previously reported observation [10] that LC from Cre$^+$ mice appear to have a more rounded cell body (Fig. S2) and seem to have fewer dendrites than LC from Cre$^-$ mice in en face view (Fig. S1C). This difference in observation may be due to the different methods used for LC analysis, as the separation of the dermis and epidermis may have an impact on LC morphology. While analyzing LC in lateral view we observed that E-Cad deficient LC appeared to be plainer than E-Cad competent LC. Thus, we sought to quantify this observation and evaluated whether E-Cad deficiency has an influence on the morphology of LC by performing immunofluorescence staining of ear skin either from naïve ears or after activation by tape stripping. We confirmed the activation of LC by flow cytometry of co-stimulatory and co-inhibitory markers that demonstrated enhanced CD86 and MHC II and diminished PDL-1 expression on LC, while PDL-2, CD40, and CD80 expression was unaffected. We did not observe differences in the activation status when comparing LC from Cre$^-$ and Cre$^+$ mice (Fig. S3). The quantification of up-reaching dendrites of single LC demonstrated that indeed in Cre$^+$ mice significantly fewer LC dendrites reached the direction of the tight junctions, both under steady-state conditions and after activation by tape stripping (Fig. 1A, D). The LC count in the epidermis and LC dendrite count remained comparable in naïve and tape-stripped skin at the analyzed time point (Fig. 1B, C).

Furthermore, we confirmed the previous observation that E-Cad deficiency does not lead to impaired migration by using the in vivo FITC migration assay (Fig. S4B). In contrast to previously published data [10] we observed significantly fewer crawlouts from the skin of Cre$^+$ mice during spontaneous and CCL21-directed emigration in vitro (Fig. 2A). When analyzing the crawlouts, we demonstrated a reduced percentage of CCL21 receptor (CCR7) expressing LC in the absence of E-Cad (Fig. 2B), which may reflect the impaired in vitro emigration of E-Cad deficient LC. We could also observe this difference in CCR7 expression in the skin and to a lesser and statically not significant extend in skin-draining lymph nodes of naïve mice (Fig. S4A).

Given the observed difference in dendrite alignment of E-Cad deficient LC, we analyzed the ability of E-Cad deficient LC to take up external antigens. For this purpose, we painted cell impermeable biotin on the ear skin of the mice after limited (3×) tape stripping. 24 h later we obtained the ears and analyzed LC and as a control dDC for their biotin content by flow cytometry. In LC from Cre$^+$ mice, we detected significantly less biotin compared with LC from Cre$^-$ littermate controls. Biotin was also detected in other cell populations but to a much lesser extent than the LC of E-Cad competent mice. In contrast to the differences observed in the LC population, no differences in the biotin signal were observed between the two genotypes of the other DC populations analyzed (Fig. 1C and Fig. S5).

Finally, in an attempt to address the functional relevance of differences in antigen content of LC, mice of both genotypes were sensitized transcutaneously with biotin for three times every other day. Two weeks later, we obtained serum and analyzed it for biotin-specific IgG1 antibodies by ELISA. Transcutaneous sensitization with biotin induced a biotin-

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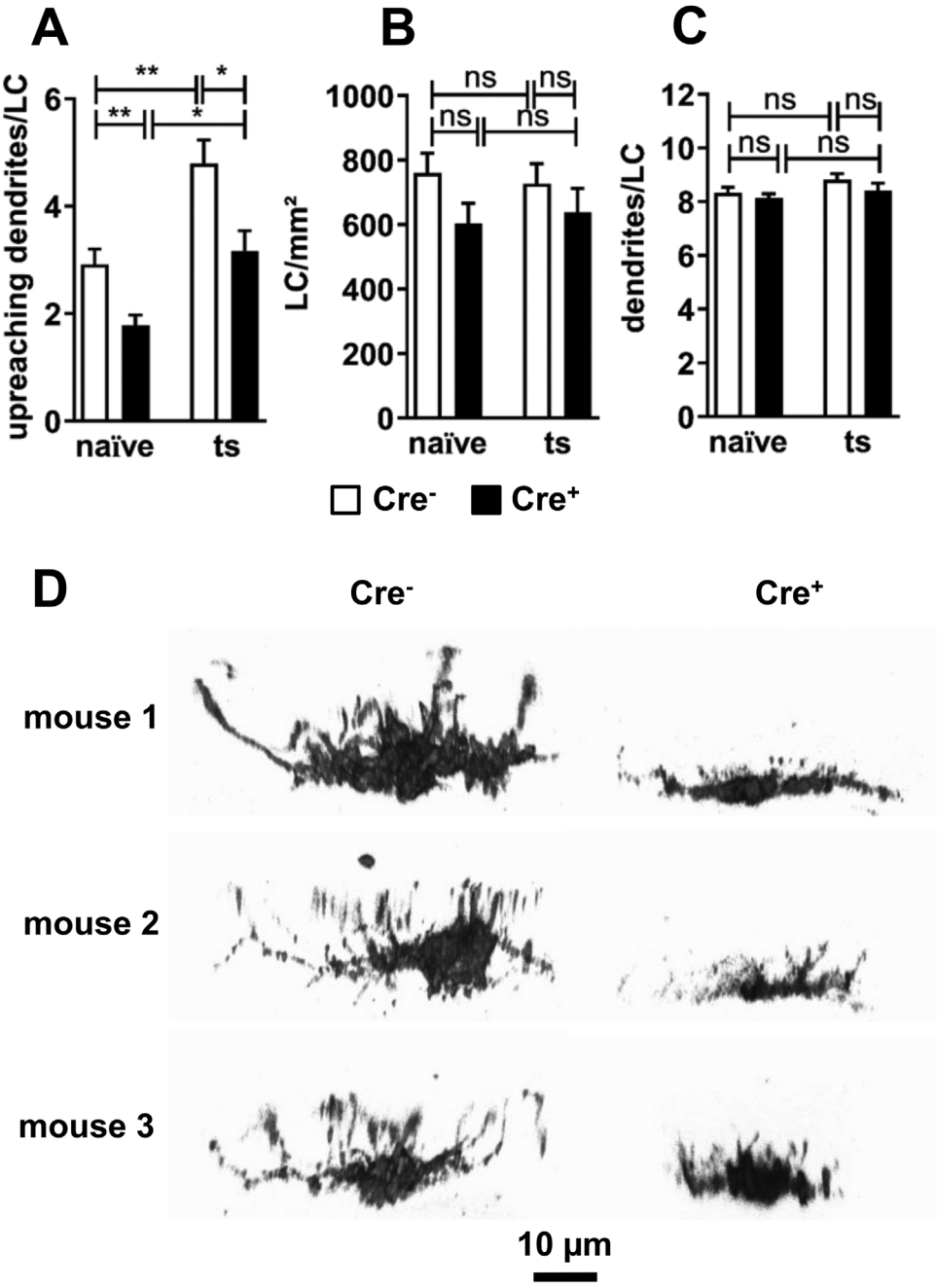

Figure 1. LC in the right ear of mice were activated by tape stripping (ts), and the left ear remained untreated, 8 h later LC were stained and 3D confocal images were used for analysis. (A) Quantification of dendrites reaching in the direction of the tight junctions. (B) LC counts per mm^2. (C) Dendrite count per LC. (A–C) Three independent experiments are shown with $n = 11$ Cre^- mice (white bars)/10 Cre^+mice (black bars), bars indicate mean ± SEM. (D) Example pictures in 3D lateral view, the scale bar indicates 10 µm.

specific IgG1 response in both Cre^- and Cre^+ mice, albeit without a significant difference between the two groups. (Fig. 2E).

In summary, here we confirm previously published results that show that the E-Cad expression on LC is not required for maintaining LC within the epidermis, does not regulate LC maturation but does affect dendrite morphology. In an extension of this, we demonstrate that E-Cad deficiency in LC leads to a significantly lower percentage of CCR7 expressing LC, a reduced LC emigration from skin explants in vitro, reduced numbers of upreaching dendrites, and diminished uptake of cutaneous applied antigens after tape stripping. The impact of the reported phenotype on percutaneous sensitization and tolerance induction remains to be investigated.

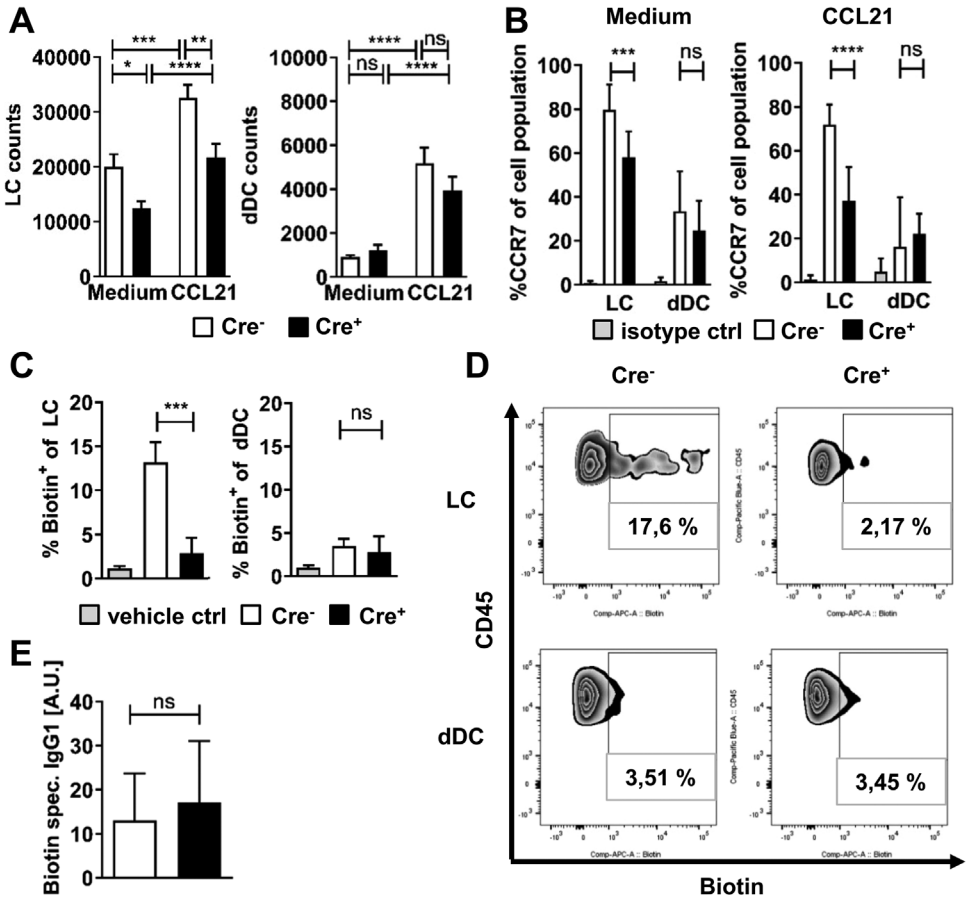

Figure 2. (A) LC and dDC emigrated spontaneously (medium) or CCL-21 directed from ear halves in vitro. Pooled data from four experiments with $n = 18$ (Cre^-)/19 (Cre^+). (B) CCR7 expression of LC/ dDC after in vitro emigration. Pooled data from two experiments with $n = 15$ (Cre^-)/11 (Cre^+). (C) Biotin content of LC and dDC 24 h after application to the ear. Pooled data from three experiments with $n = 10$ (Cre^-)/14 (Cre^+). (D) Representative flow cytometry blots. (E) Biotin-specific IgG1 in serum after cutaneous sensitization. Pooled data of two experiments with $n = 13$ (Cre^-)/7 (Cre^+). (A–C, E) Grey bars = isotype control/ vehicle treatment; white bars = Cre^- and black bars = Cre^+ mice. Bars indicate mean ± SEM or SD (E).

Acknowledgements: This work was financed by intramural funds of the Department of Dermatology and Allergology, University Medical Center Gießen, Justus Liebig University Gießen, Germany. Open access funding enabled and organized by Projekt DEAL.

Conflict of interest: The authors declare no conflict of interest.

Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Peer review: The peer review history for this article is available at https://publons.com/publon/10.1002/eji.202350797.

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Abbreviations: **Biotin**: EZ-link sulfo-NHS-LC-biotin · **Cre^+**: E-$cad^{fl/fl}$CD11c Cre^+ mice · **Cre^-**: E-$cad^{fl/fl}$CD11c Cre^- mice · **CHS**: contact hypersensitivity · **DC**: dendritic cells · **dDC**: dermal dendritic cells

· **E-Cad**: E-cadherin · **FITC**: fluorescein isothyiocyanate · **LC**: Langerhans cells

Keywords: Langerhans cells • Cadherin • Dendrites • Adherens • Migration

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Received: 29/9/2023
Revised: 26/4/2024
Accepted: 29/4/2024
Accepted article online: 12/5/2024

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