Doctoral Program W 1248-B30:
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Epidermal delivery of polyinosinic-polycytidylic acid in a human skin explant model activates the MDA5/MAVS pathway in Langerhans cells

Pooja Tajpara[1], Christopher Schuster[1], Elisabeth Schön[1], Philip Kienzl[1], Michael Mildner[2], Adelheid Elbe-Bürger[1]

[1]Division of Immunology, Allergy and Infectious Diseases, Department of Dermatology, Medical University of Vienna, Vienna, Austria
[2]Research Division of Biology and Pathobiology of the Skin, Department of Dermatology, Medical University of Vienna, Vienna, Austria

Introduction and Aims

Together with keratinocytes (KCs) and the dense network of Langerhans cells (LCs), the epidermis is an ideal portal for vaccine delivery. Pattern recognition receptor (PRR) agonists, in particular polyinosinic-polycytidylic acid [p(I:C)], are promising adjuvant candidates for therapeutic vaccination to generate protective T cell immunity. As the efficacy of PRR agonists in skin cells has predominantly been tested *in vitro*, we have applied a human skin explant model and administered p(I:C) onto barrier-disrupted human epidermis to study the expression and activation of dsRNA-sensing PRRs in LCs and KCs in their natural environment.

Results

Schematic diagram of a barrier-disrupted human skin model and experimental set up

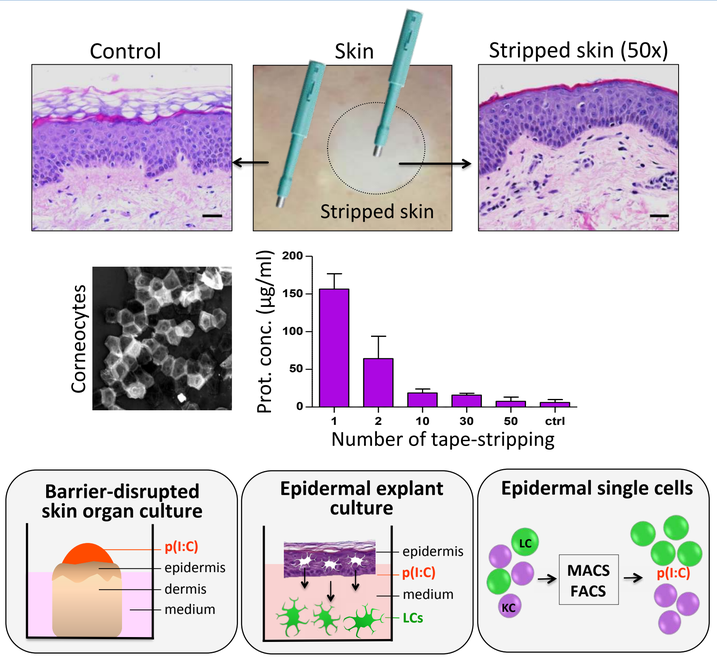


Fig 1. H&E staining of paraffin sections from untreated (control) and tape-stripped healthy human skin (upper panel). One representative strip disc with corneocytes is demonstrated and protein estimation of each disc using the Bicinchoninic acid protein assay (n=4) is shown. The experimental set up is illustrated in the lower panel. Low molecular weight (LMW) p(I:C) has been used. KCs, keratinocytes; LCs, Langerhans cells. Scale bar = 50 µm.

LCs upregulate CD83 but do not regulate TLR3, PKR and RIG-1 upon stimulation

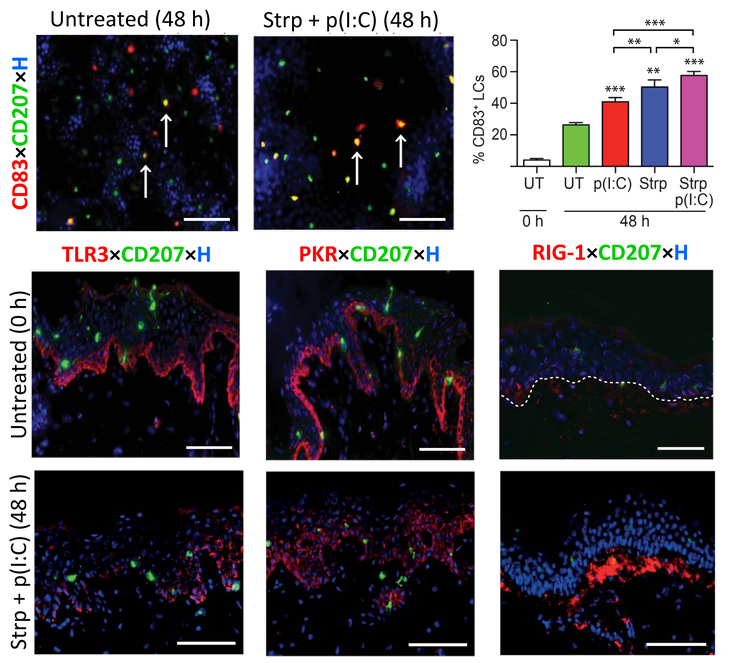


Fig 3. Immunofluorescence double labeling of 48 h untreated and stripped, p(I:C)-treated skin was performed on epidermal sheets with an anti-CD83 mAb (upper panel) and on cryostat sections with anti-TLR3, -PKR, and -RIG-1 antibodies. Counter staining was performed with an anti-CD207 mAb. Nuclear counterstain was performed with Hoechst. Scale bar = 50 µm. Values are mean ± standard error of mean (n=3). One-way ANOVA test; *P<0.1, **P<0.01, ***P<0.001.

Differential expression of downstream signalling pathways in LCs and KCs

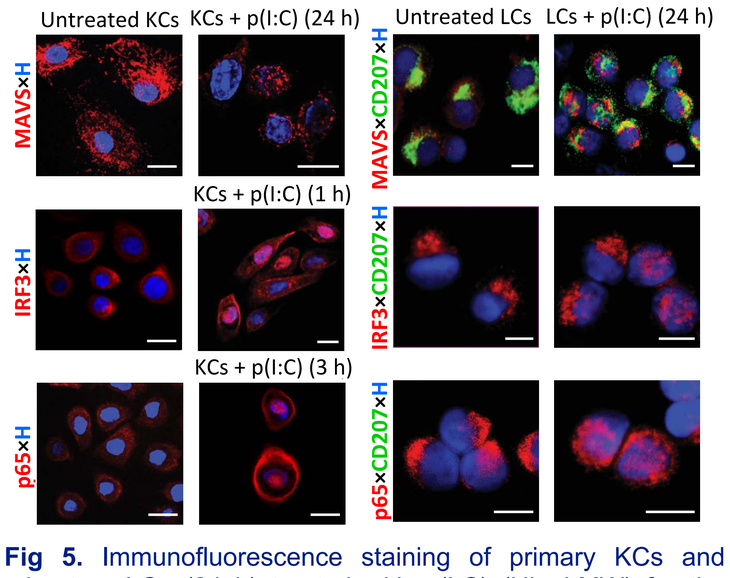


Fig 5. Immunofluorescence staining of primary KCs and migratory LCs (24 h) treated with p(I:C) (UL, LMW) for the indicated time points. Aggregation of MAVS, translocation of IRF3 and NF-κB (p65) from the cytoplasm into the nucleus was observed in KCs and in LCs (n=3). Nuclear counterstain was performed with Hoechst. Scale bar = 5 µm.

Induction of IFNs and proinflammatory cytokines in KCs and LCs

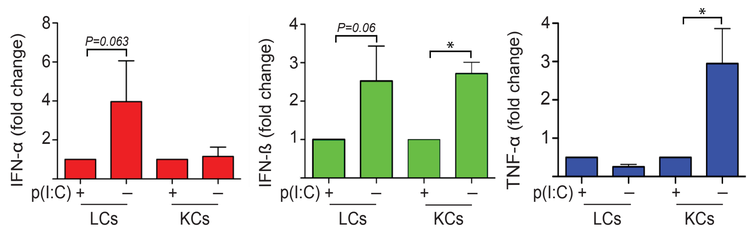


Fig 6. Fold change of IFN-α, IFN-β and TNF-α mRNA expression in FACS-sorted LCs and KCs cultured without or with p(I:C) is shown. Data are mean ± standard error of mean (n=3). T-test; *P<0.05, compared to unstimulated, cultured group.

LCs and KCs take up rhodamine-labeled p(I:C)

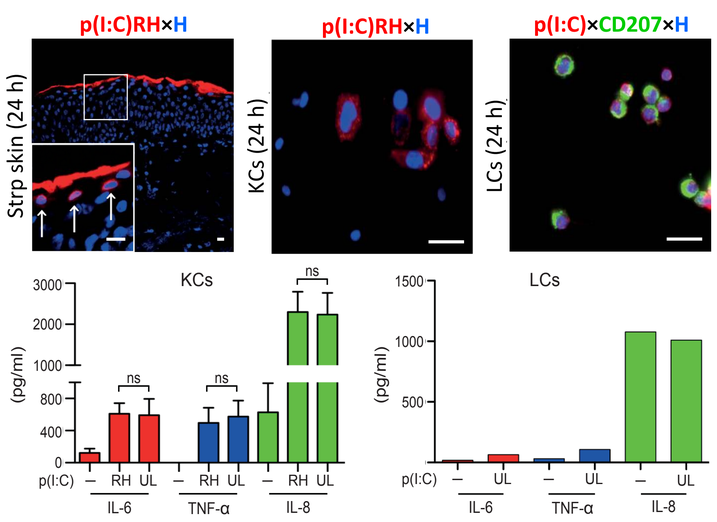


Fig 2. Uptake of topically applied rhodamine (RH)-labeled p(I:C) (red) in tape-stripped (strp) human skin explant cultures in vitro (upper panel, left). Primary KCs, cultured on cover slips, were incubated with RH-labeled LMW p(I:C) for 24 h, fixed with formaldehyde and analyzed (upper panel, middle). Emigrated LCs from 48 h cultured epidermal explants were incubated with RH-labeled p(I:C) for 2 h, placed onto adhesion slides and counterstained with an anti-CD207 mAb (upper panel, left). Nuclear staining was performed with Hoechst (H). Primary KCs were cultured for 24 h with either RH-labeled or unlabeled (UL) p(I:C) or left untreated (n=4) (lower panel, left). Freshly isolated and highly enriched (>86%) LCs were cultured without or with UL p(I:C) for 24 h (n=1) (lower panel, right). Indicated cytokine concentrations from supernatants were determined by ELISA. Statistics show t-test comparisons and were not significant (ns).

MDA5 is downregulated in LCs upon stripping and p(I:C) treatment

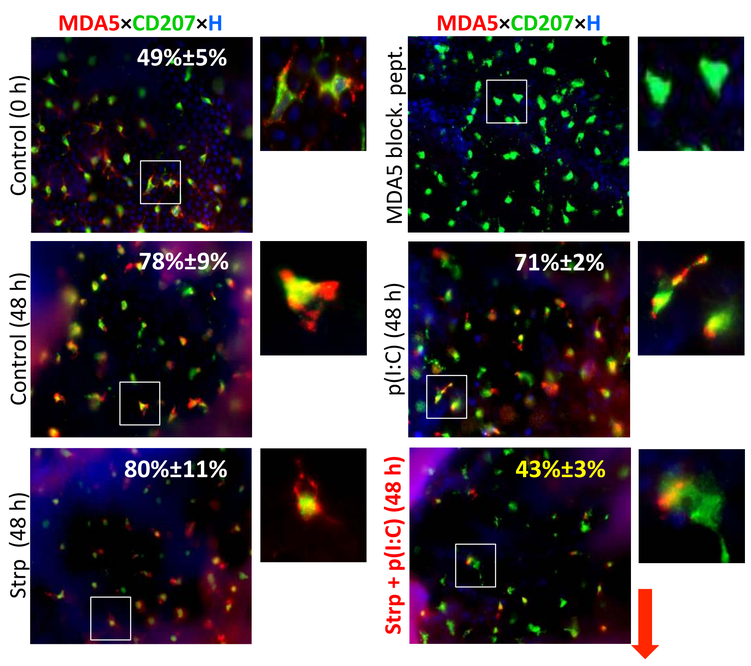
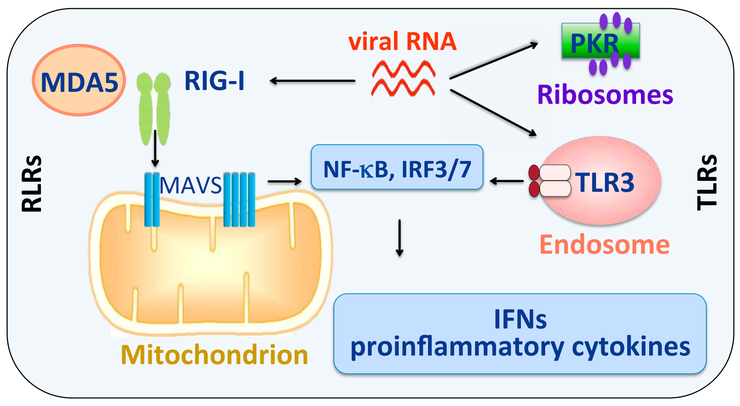


Fig 4. Immunofluorescence double labeling of epidermal sheets from untreated, freshly isolated and cultured skin (control), stripped cultured skin, barrier unperturbed but p(I:C)-treated (UL, LMW) cultured skin, as well as stripped, p(I:C)-treated (UL, LMW) cultured skin (n=3). Percentages and standard deviation are indicated for MDA5⁺ LCs of total CD207⁺ LCs. MDA5 specific blocking peptide was used for epidermal sheets to test antibody specificity. Nuclear counterstain was performed with Hoechst.

Conclusion and Outlook



This model will now allow us to further define the key signaling pathways involved in LCs as well as in KCs upon viral infection such as Herpes simplex which are particularly frequent in individuals with atopic dermatitis because of barrier dysfunction.

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