

(Cross)-presentation of Ovalbumin (OVA) by Langerhans Cells and dermal Dendritic Cells

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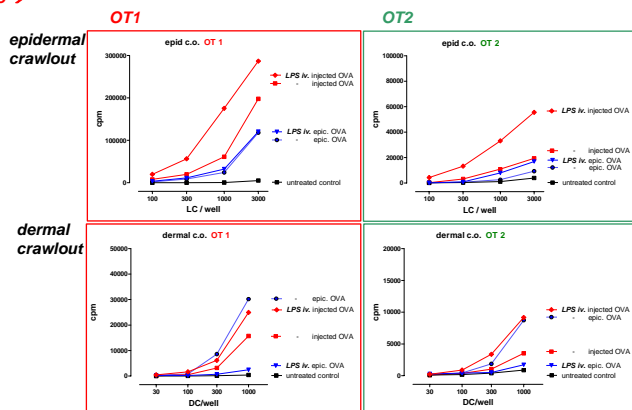
RATIONALE

We recently demonstrated that murine Langerhans Cells (LC) are capable of cross-presenting soluble protein antigen. In this study we tried to augment those findings in terms of the mode of antigen application. The influence of systemic (à la Wilson / Villadangos) vs. local LPS / Endotoxin on the antigen presenting capacity of skin DC is the second issue.

While it is not definite that LC themselves present antigen *in vivo* in the lymph node, epicutaneous immunisation may provide benefits by induction of locally restricted T cell responses.

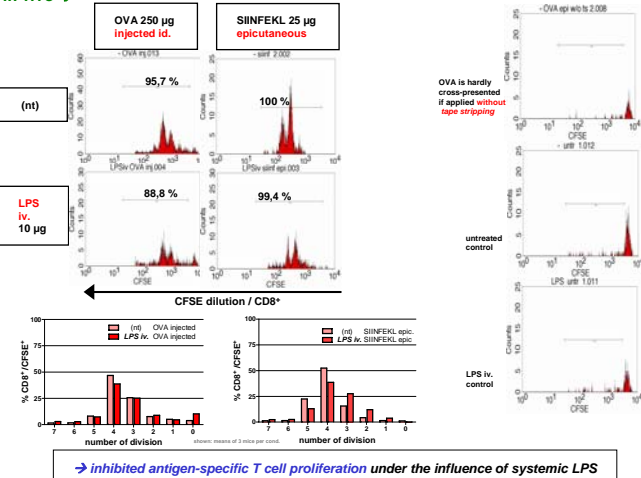
1. LC (cross)-present OVA protein more efficiently when applied intradermally - systemic LPS adjusts this function !

in vitro →



2. Antigen presentation of injected OVA protein and MHC-I presentation of SIINFEKL peptide *in vivo* is marginally suppressed by systemic LPS.

in vivo →



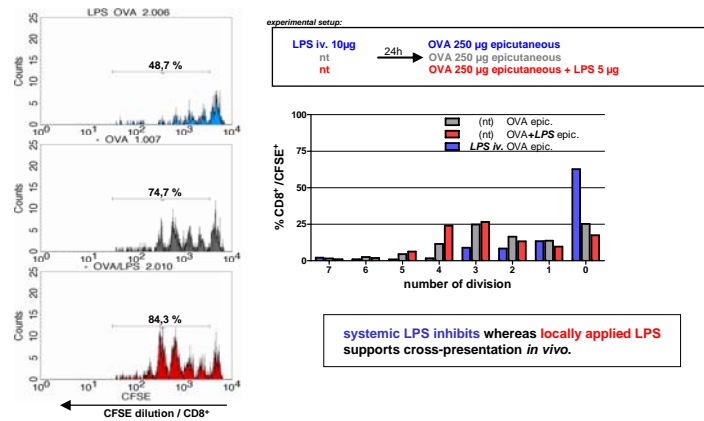
→ inhibited antigen-specific T cell proliferation under the influence of systemic LPS

METHODS

Endotoxin-free OVA protein was applied epicutaneously or intracutaneously on mouse skin. To dissect the influence of endotoxin, LPS was given systemically prior to OVA immunisation or applied locally together with OVA. Epidermal and dermal explants were placed in culture 6h after immunisation. Subsequently, migratory DC were co-cultured with antigen-spec. T cells (OT1/2) *in vitro*.

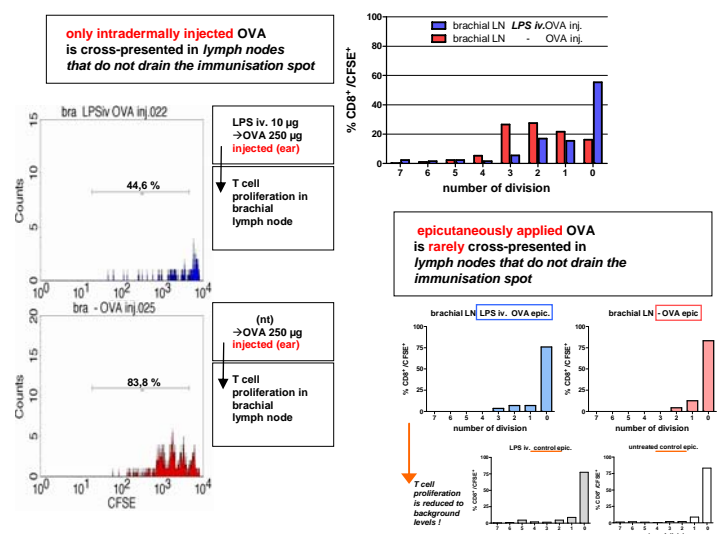
To test for *in vivo* relevance, CFSE labelled OT1 T cells were injected i.v. 24h before immunisation. T cell responses induced by cross-presentation were detected by CFSE proliferation assay. In addition, the systemic influence of LPS was measured.

3. Systemic LPS inhibits cross-presentation *in vivo* after epicutaneous application of Ovalbumin protein



systemic LPS inhibits whereas locally applied LPS supports cross-presentation *in vivo*.

4. Antigen specific T cell responses are locally restricted if Ovalbumin is applied epicutaneously



epicutaneously applied OVA is rarely cross-presented in lymph nodes that do not drain the immunisation spot

T cell proliferation is reduced to background levels!

SUMMARY-CONCLUSIONS

- # systemic LPS enhances the cross-presenting capacity of LC *in vitro*, similar to LPS applied topically...or with endotoxin traces in Ovalbumin protein
- # epicutaneous immunisation with Ovalbumin protein results in poor CD4+ T cell responses *in vitro*.
- # MHC-I presentation of OVA peptide and cross-presentation is suppressed under systemic LPS *in vivo*
- # cross-presentation of epicutaneously applied Ovalbumin is locally restricted *in vivo*
- ? do epicutaneous immunisations 'overrule' systemic inhibition of T cell responses ?

