MATERIALS AND METHODS

Atopic dermatitis-like inflammation in mice

Female 7-10 week old MyD88-deficient mice (Akira et al., 2000) and WT littermates (C57BL/6J) were bred under specific pathogen-free conditions at the LIMES GRC (Bonn, Germany). All animal experiments were performed with permission of the government of North Rhine-Westphalia. AD-like inflammation was induced as described (Didovic et al., 2016, Stutte et al., 2010, Wang et al., 2007). Briefly, back skin of mice was shaved and tape-stripped 3-4 times with medical tape Tegaderm 1624W (3M, Neuss, Germany). 100µg OVA (grade V; Sigma-Aldrich, Taufkirchen, Germany), 5µg SEB (Toxin Technologies, Sarasota), or OVA+SEB in 0.9 % NaCl, or 0.9% NaCl alone were applied onto a 1cm² patch of sterile gauze and attached to the irritated skin on day 0. Antigen solutions were tested for endotoxin contamination (Hyglos, Bernried, Germany): Endotoxin content of SEB solution was below 1 Endotoxin Unit (<1EU) per mouse or cell sample, OVA solution was <3EU per mouse or cell sample. Treatment was repeated on day 3. On day 7 patches were removed followed by recovery of mice for 2 weeks. This treatment regime was repeated thrice. In the third treatment cycle, tape-stripping and patch application was also done on day 6. Mice were analyzed 18h after this last treatment.

Skin histology

Cryosections were prepared from biopsies of patched skin area. To measure epidermal thickness, toluidin blue staining was performed and evaluated via 6 measurement points per micrograph and 3 micrographs per mouse. Sections were stained with fluorophore-coupled antibodies against F4/80 (CI:A3-1, eBioscience, San Diego), CD4 (RM4-5), CD3e (145-2C11), CD8a (53-6.7), MHCII (M5/114.15.2) TCRbeta (H57-597, all Biolegend, San Diego) and Langerin (923B7, Acris, Herford, Germany). Slides were mounted in DAPI mounting medium (Vector Laboratories, Burlingame). Dermal immune cells were quantified as the number of cells per 0.25 mm² field. LC and epidermal T cells were stained with anti-Langerin and anti-CD3epsilon,
respectively, and were counted as the number of cells per mm epidermal length. Quantification was performed for 5 randomly chosen areas per individual at 200x magnification, using a Keyence BZ-9000 microscope.

**Cell preparation**

Skin-draining brachial LN were collected and single cell suspensions were generated. Cells were (re)stimulated for 3 days with 100µg/ml endotoxin-free OVA (Hyglos) or 5µg/ml SEB (Toxin Technologies).

**ELISA**

Total and antigen-specific immunoglobulins were determined by ELISA (BD Biosciences and AbD Serotec, Oxford, UK). OVA-specific IgG1 and IgG2c and SEB-specific IgE, IgG1 and IgG2c were measured by coating ELISA plates with 10µg/ml endotoxin-free OVA or SEB and were detected according to the manufacturers protocols for IgE (SEB only, BD Biosciences) IgG1 (BD Biosciences) and IgG2c (Bethyl, Montgomery). Results were calculated as units relative to a reference serum of OVA-Alum-immunized or SEB-Alum-immunized mice, respectively. OVA-IgE ELISA was measured by coating with anti-IgE (BD Biosciences) and detection via OVA-HRP compared to mouse-anti-OVA-standard (AbD Serotec). Cell supernatants were measured by sandwich ELISA for IFNgamma, IL-10, CCL17, CCL22 (all R&D Systems, Wiesbaden, Germany), or IL-13 and IL-17A (both Biolegend).

**Statistics**

Data are presented as mean ± SEM. Data were analyzed with Graphpad Prism software using Student's two-tailed t test or One Way ANOVA with Sidak’s correction for multiple comparisons. Significance level for p<0.05 was denoted as (*), for p< 0.01 as (**) and for p<0.001 as (***)
Supplementary References


### Table S1: Diminished skin-derived dendritic cell frequencies in draining lymph nodes upon OVA+SEB exposure compared to single antigen treatment

<table>
<thead>
<tr>
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<th>NaCl</th>
<th>OVA</th>
<th>SEB</th>
<th>OVA+SEB</th>
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<tr>
<td><strong>WT control</strong></td>
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<tr>
<td>% MHCII++</td>
<td>34.9% ± 6.3</td>
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<td>37.4% ± 8.5</td>
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<tr>
<td><strong>MyD88 KO</strong></td>
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<tr>
<td>% MHCII++</td>
<td>39.6% ± 1.1</td>
<td>28.6% ± 1.4</td>
<td>28.6% ± 13.4</td>
<td>14.1% ± 4.9</td>
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<tr>
<td>relative to % all DC</td>
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<td>6.6% ± 0.4</td>
<td>6.2% ± 1.2</td>
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Representative data shown as mean/SD of one of four experiments with n=3-4 mice.

* Diverging proportions of skin-derived MHCII++ skin-derived dendritic cells between experimental groups within the relatively stable total CD11c⁺ DC population in the draining lymph nodes.
Figure S1: Matching representative micrographs for histologic evaluation of the skin of NaCl- and OVA+SEB treated mice

(a) Toluidin staining to evaluate epidermal thickness (b) Langerin (green), CD8a (red), CD3e (blue) epidermal immune cells, note upregulation of CD8a in the WT OVA+SEB sample (c) F4/80+ macrophages (green). (d) CD4+ T helper cells (red). All micrographs 200x magnification, scale bars represent 200µm.
Figure S2: SEB (re-)stimulation reveals anergy of LN T cells upon repeated SEB encounter in vivo

Production of CCL17, CCL22, IFNγ, IL-17A, IL-10 and IL-13 by LN cells of NaCl-, SEB-, OVA- and OVA+SEB-treated MyD88-deficient and WT control mice after SEB (re-)stimulation (+ SEM, n=8-15 mice of 3-4 independent experiments) * p < 0.05, ** p < 0.01, *** p < 0.001
Figure S3: One-week SEB exposure is not sufficient to induce anergy in LN T cells

Production of IFNγ and IL-10 by draining LN cells of naïve WT mice and WT mice after 1 week tape-stripping and NaCl-, SEB-, OVA- and OVA+SEB-treatment to Concanavalin (Con) A, SEB, and OVA (re-)stimulation (+ SD, n=2-3 mice of 1 representative experiment).
Figure S4: SEB boosts OVA-specific immunoglobulin production whereas SEB-specific immunoglobulin production is suppressed during combined treatment

(a) Production of OVA-specific immunoglobulins and (b) Production of SEB-specific immunoglobulins of NaCl-, SEB-, OVA- and OVA+SEB-treated MyD88-deficient and WT control mice (+ SEM, n=8-12 mice of 3-4 independent experiments) * p < 0.05, ** p < 0.01, *** p < 0.001