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MyD88 Contributes to Staphylococcal Enterotoxin B-Triggered Atopic Dermatitis-Like Skin Inflammation in Mice

Journal of Investigative Dermatology (2017) 137, 1802–1804; doi:10.1016/j.jid.2017.04.015

TO THE EDITOR

Atopic dermatitis (AD) is a chronic pruritic skin inflammation, caused by skin barrier defects and by a dysregulated immune system (Bieber, 2012). Patients with AD often suffer from infections with *Staphylococcus aureus*, mostly superantigen-producing strains (Boguniewicz and Leung, 2011). Superantigens, including staphylococcal enterotoxin B (SEB), directly crosslink major histocompatibility complex class II on professional antigen-presenting cells with the T-cell receptor to induce T-cell activation independent of antigens (Spertini et al., 1991). SEB-activated human dendritic cells enhance the T helper type 2 (Th2) response in AD (Mandron et al., 2006) and induce homing of T cells to the skin (Strickland et al., 1999). Furthermore, superantigens act as antigens, as patients with AD possess superantigen-specific immunoglobulins (Kotzin et al., 1993). Epicutaneous sensitization of mice with SEB induces skin inflammation, Th2 polarization, and SEB-specific humoral responses (Laouini et al., 2003) and was shown to mediate the itching response via IL-31 (Cevikbas et al., 2014). The combined application of SEB with other allergens to skin or lung enhanced the allergic response to the antigen (Forbes-Blom

et al., 2012; Huvenne et al., 2010; Savinko et al., 2005).

Previously, we showed that myeloid differentiation primary response gene 88 (MyD88)-deficient mice display reduced inflammation in a model of ovalbumin (OVA)-driven AD-like inflammation (Didovic et al., 2016). Here, we analyzed the contribution of MyD88 signaling to SEB- and OVA+SEB-induced allergic sensitization in mice. All animal experiments were performed with permission of the government of North Rhine-Westphalia, Germany. Epidermal thickness was higher after SEB treatment compared with solvent controls and strongly enhanced after OVA+SEB treatment in wild-type (WT) mice but barely in MyD88-deficient mice (Figure 1a, Supplementary Figure S1a online). Skin draining lymph node (LN) cell numbers were particularly high after OVA treatment of WT mice, but not further enhanced after OVA+SEB treatment, whereas cell numbers of MyD88-deficient mice were lower (Figure 1b). Dermal F4/80⁺ macrophage numbers were slightly enhanced after SEB or OVA treatment, and even more on combined OVA+SEB treatment. This enhancement was partly dependent on MyD88 (Figure 1c, Supplementary Figure S1c). Of note,

dermal Th cell numbers were higher in MyD88-deficient mice than in WT littermates. The highest numbers of dermal Th cells, however, were detected in WT mice after combined OVA+SEB treatment, whereas numbers in MyD88-deficient mice remained largely unchanged compared with solvent control (Figure 1d, Supplementary Figure S1d). Numbers of dermal CD8⁺ T cells as well as monocytes and dermal dendritic cells were only marginally altered (data not shown). Epidermal Langerhans cell numbers were significantly reduced in WT mice after OVA and after SEB treatment compared with solvent controls, and also compared with MyD88-deficient mice. Intriguingly, after combined OVA+SEB application, numbers of epidermal Langerhans cells of WT mice were higher than on treatment with one of the antigens alone and almost as high as in MyD88-deficient mice (Figure 1e, Supplementary Figure S1b), indicating impaired emigration compared with treatment with either antigen alone. Combined OVA+SEB exposure, but not OVA or SEB alone, also led to epidermal T-cell accumulation, which probably were CD8⁺ T cells (Savinko et al., 2005) (Figure 1f–h, Supplementary Figure S1b). Furthermore, a large proportion of epidermal T cells were T-cell receptor beta⁺ T cells, indicating that MyD88 mediates the accumulation of these T cells in the epidermis after OVA+SEB treatment. Thus, the combined application of OVA and SEB

Abbreviations: AD, atopic dermatitis; LN, lymph node; MyD88, myeloid differentiation primary response gene 88; OVA, ovalbumin; SEB, staphylococcal enterotoxin B; Th, T helper cell; WT, wild-type

Accepted manuscript published online 28 April 2017; corrected proof published online 23 June 2017

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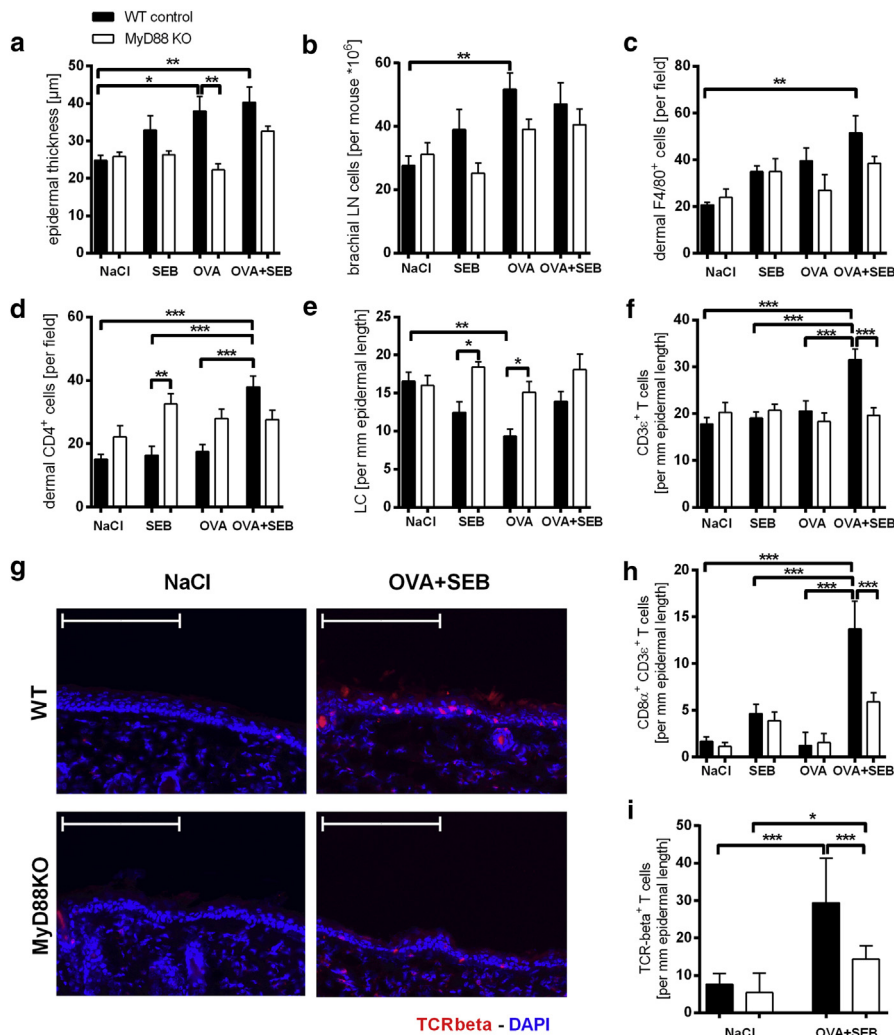


Figure 1. SEB-induced skin inflammation and progression of OVA-induced eczema in mice is in part dependent on MyD88. (a) Epidermal thickness in NaCl-, SEB-, OVA-, and OVA+SEB-treated MyD88-deficient and WT control mice (+SEM, n = 4–8 mice of 2 independent experiments). (b) Absolute cell numbers of skin-draining brachial LN of NaCl-, SEB-, OVA-, and OVA+SEB-treated MyD88-deficient and WT mice (+SEM, n = 8–15 mice of 4 independent experiments). (c) Mean numbers of dermal F4/80⁺ cells and (d) dermal CD4⁺ cells of NaCl-, SEB-, OVA-, and OVA+SEB-treated MyD88-deficient and WT mice. Dermal cells were counted per field (+SEM, n = 6–12 mice of 3–4 independent experiments). (e) Mean numbers of epidermal LC. Cells were counted per epidermal length. (f) Mean numbers of epidermal CD3⁺ T cells. (g) Representative immunohistology (×200) of TCRβ⁺ T cells and quantification of epidermal TCRβ⁺ T cells. (h) Epidermal CD3⁺ CD8α⁺ T cells in NaCl-, SEB-, OVA-, and OVA+SEB-treated MyD88-deficient and WT control mice. (i) Mean TCRβ⁺ T cells in NaCl- and OVA+SEB-treated MyD88-deficient and MyD88-proficient mice. Cells were counted per epidermal length (+SEM, n = 8–15 mice of 3–4 independent experiments). **P* < 0.05, ***P* < 0.01, ****P* < 0.001. LC, Langerhans cell; LN, lymph node; MyD88, myeloid differentiation primary response gene 88; OVA, ovalbumin; SEB, staphylococcal enterotoxin B; SEM, standard error of mean; TCR, T-cell receptor; WT, wild-type.

influences the epidermal immune response in a different way than single OVA or SEB application and MyD88 contributes to SEB- and OVA+SEB-induced skin inflammation. In line, emigration of Langerhans cells as well as dermal dendritic cells was reflected by enhanced frequencies of skin-derived dendritic cells in draining LN in WT mice on OVA or SEB treatment

compared with MyD88-deficient mice (Supplementary Table S1 online). AD is characterized by high expression of the Th2 polarizing chemokines CCL17 and CCL22 (Horikawa et al., 2002). In addition, CCL17 facilitates Langerhans cell migration (Stutte et al., 2010). Expression levels of CCL17 and CCL22 were higher in LN cultures of MyD88-deficient mice after OVA

restimulation, indicating that MyD88 deficiency may favor CCR4-dependent recruitment of T cells into the skin (Figure 2). Production of IFN-γ and IL-17A was dependent on MyD88, whereas IL-10 and IL-13 were produced MyD88-independently. Thus, SEB augments OVA-specific Th cell activation in WT mice and MyD88 is necessary for induction of Th1/Th17 responses as well as for CCL17/CCL22-induced T-cell recruitment. Restimulation of LN cells of solvent- and OVA-treated mice that were not exposed to SEB in vivo with SEB led to a vast MyD88-independent cytokine production as expected (Supplementary Figure S2 online). Interestingly, only SEB-induced IL-17A was strictly dependent on MyD88, whereas IFN-γ was only partly affected by MyD88 deficiency. In LN cells of mice treated with either SEB or OVA+SEB in vivo, levels of all cytokines were strongly reduced after SEB restimulation, indicating that repeated epicutaneous exposure to SEB leads to T-cell anergy as described for immunization with SEB (Eroukhanoff et al., 2009). Interestingly, a short-term 1-week in vivo treatment was not sufficient to induce this anergic state (Supplementary Figure S3 online), indicating that repeated or prolonged SEB treatment is responsible for this effect. CCL22, however, was not reduced in SEB-treated mice, indicating that CCL22 might be produced by different cell types. OVA+SEB treatment also boosted the OVA-specific IgG1 and IgG2c response, which was partly dependent on MyD88. OVA-specific IgE was independent of MyD88. SEB-specific production of IgE and IgG1 was higher in MyD88-deficient mice, whereas IgG2c production was MyD88-dependent. Importantly, SEB-specific immunoglobulin levels were lower on OVA+SEB sensitization than on single SEB treatment (Supplementary Figure S4 online).

In conclusion, we showed that SEB-induced skin inflammation and modulation of the allergic response to OVA are in part dependent on MyD88. During combined OVA+SEB treatment, adaptive responses to either antigen may interfere with each other. Thus, MyD88 signaling is involved in SEB-induced aggravation of AD-like

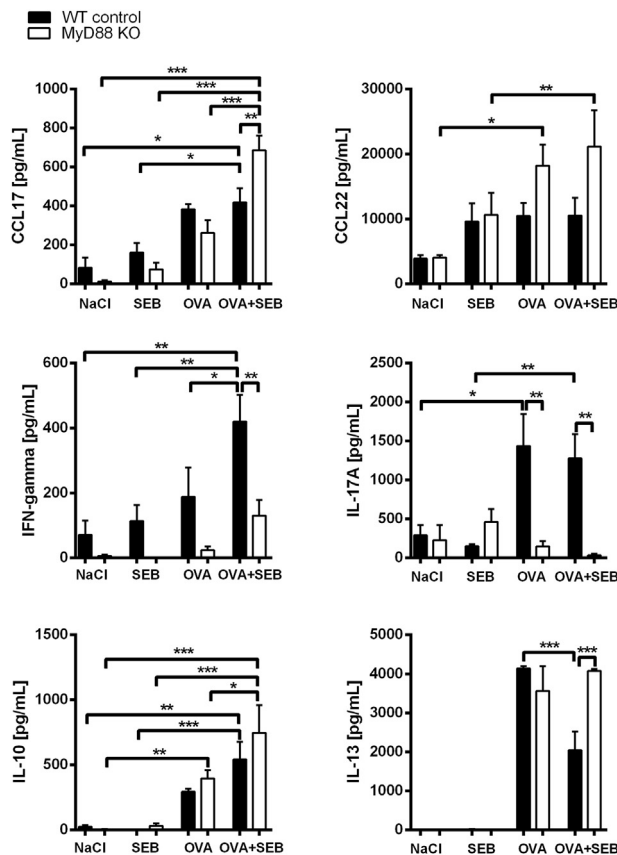


Figure 2. Combined OVA+SEB exposure boosts Th2 chemokine secretion in response to OVA and enhances MyD88-dependent Th1 and Th17 responses. Production of CCL17, CCL22, IFN- γ , IL-17A, IL-10, and IL-13 by LN cells of NaCl-, SEB-, OVA-, and OVA+SEB-treated MyD88-deficient and WT control mice after OVA restimulation (+SEM, n = 8–15 mice of 3–4 independent experiments)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. LN, lymph node; OVA, ovalbumin; SEB, staphylococcal enterotoxin B; SEM, standard error of mean; Th, T helper cell; WT, wild-type.

inflammation in mice by modulation of T-cell responses.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We thank Björn Zapke and Gabriele Schoder for expert technical assistance. This work was supported by grants of the Medical Faculty of the Heinrich-Heine University Düsseldorf (to HW), the Deutsche Forschungsgemeinschaft (WE 2625/2-1 to HW, SFB 704 to IF). IF is a member of the DFG-funded Cluster of Excellence ImmunoSensation.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at <http://dx.doi.org/10.1016/j.jid.2017.04.015>.

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