Epicutaneous and Oral Low-Zone Tolerance Protects from Colitis in Mice

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Tolerance to environmental antigens that encounter the organism at interfaces like skin or gut prevents deleterious systemic immune responses. The aim of this study was to analyze whether and how low doses of haptens, by entry through the skin or gastrointestinal tract, affect the outcome of the predominantly Th1/Th17-mediated 2,4,6-trinitro-benzenesulfonic acid-induced colitis, which mimics an autoimmune bowl disease in man. Epicutaneous and oral applications of low doses of the allergen resulted in the induction of low-zone tolerance (LZT) and protected from colitis development, demonstrated by a significantly reduced inflammatory response of the gut in vivo. In line with this observation, we found a significantly diminished Th1/Th17-mediated T cell response and reduced T cell proliferation after both tolerance regimes, indicating that epicutaneous LZT is just as well efficient as oral tolerance in prevention of a gut-associated inflammatory immune response. Use of a second, unrelated hapten for LZT induction revealed an antigen-specific tolerance mechanism. Intriguingly, in the absence of hapten-activated $CD4^+CD25^+Foxp3^+$ regulatory T cells and IL-10, epicutaneous and oral LZT failed to abrogate the development of the intestinal inflammation. In conclusion, this study highlights in particular epicutaneous immunotherapies in the form of LZT through activation of $CD4^+CD25^+Foxp3^+$ regulatory T cells as treatment strategies for inflammatory, allergic, or autoimmune diseases.

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INTRODUCTION

Antigens elicit qualitatively distinct immune responses based on their portal of entry, their dose, and their specific properties, thereby either inducing activated immune responses or leading to tolerance. Exposure of the skin to high doses of contact allergens (haptens) results in the development of an allergic contact dermatitis, a $CD8^+$ Tc1-mediated cutaneous inflammation and one of the most frequent occupational skin diseases in man (Fyhrquist et al., 2014). Cutaneous antigen-presenting cells are activated by contact allergens and migrate to the skin-draining lymph nodes where they stimulate antigen-specific $CD8^+$ effector T cells (Kaplan et al., 2012; Martin, 2015). In contrast, we have shown previously that epicutaneous applications of small amounts of haptens did not induce the generation of an allergic contact dermatitis but resulted in a hapten-specific tolerance reaction that is termed low-zone tolerance (LZT). LZT significantly inhibited the development of a contact hypersensitivity (CHS), the murine model of the allergic contact dermatitis in man (Luckey et al., 2011, 2012; Steinbrink et al., 1996). Epicutaneous LZT was mediated by $CD8^+$ suppressor T cells that activated TNF-producing killer dendritic cells leading to apoptosis of $CD8^+$ effector T cells of CHS, and thereby, to prevention of the allergic contact dermatitis (Luckey et al., 2011, 2012).

One well-known physiological mechanism of peripheral tolerance is the induction of oral tolerance that prevents the development of local and systemic T-cell-mediated inflammatory responses to self and exogenous dietary and environmental antigens (Cassani et al., 2011; Mowat, 2003). Low doses favor the activation of regulatory $CD4^+$ T cells, whereas high doses result in anergy and deletion of antigen-specific T cells (Cassani et al., 2011; Mowat, 2003). Numerous studies in rodents have documented that oral tolerance to protein antigens is an efficient mean to inhibit autoimmune diseases in experimental models such as experimental arthritis, uveitis, or diabetes (Higgins and Weiner, 1988; Nussenblatt, 1990; Thompson and Staines, 1986; Zhang et al., 1991).

Understanding of the immune system's ability to balance between tolerance and immunity remains one of the key challenges in immunology and may result in the development of innovative therapeutic strategies. Therefore, in the context of LZT, we have addressed two questions: first, whether low doses of antigens in the form of allergens/haptens may affect the outcome of a predominantly $CD4^+$ Th1/Th17-mediated intestinal inflammation by use of the 2,4,6-trinitro-benzenesulfonic acid (TNBS)-induced model of colitis that resembles Crohn's disease as an inflammatory

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Abbreviations: CHS, contact hypersensitivity; DNBS, 2,4-dinitrobenzenesulfonic acid; DNFB, 1-fluro-2,4-dinitrobenzene; LZT, low-zone tolerance; TNCB, 2,4,6-trinitro-1-chlorobenzene; TNBS, picrylsulfonic acid, 2,4,6-trinitro-benzenesulfonic acid; Tregs, regulatory T cells

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autoimmune disorder in man (Wirtz et al., 2007; Wirtz and Neurath, 2007); second, whether in particular the epicutaneous (as well as the oral) route of LZT induction may be efficient in the control of systemic T-cell-mediated immune responses. Our data demonstrate that irrespective of the route of application, LZT significantly inhibited the development of colitis by activation of $CD4^+CD25^+Foxp3^+$ Treg- and IL-10-controlled immune reactions. Thus, this study indicates that epicutaneously and orally applied low doses of haptens induce a tolerance reaction that protects from a systemic inflammatory disease and supported the concept of epicutaneous immunotherapies for allergic and autoimmune diseases.

RESULTS

Epicutaneous and oral induction of LZT to contact allergens prevents colitis development

As previously demonstrated (Luckey et al., 2011, 2012; Seidel-Guyenot et al., 2006; Steinbrink et al., 1996), LZT to haptens abolished the CHS reaction, a cutaneous $CD8^+$ Tc1-mediated immune response. In this study, we addressed the question whether low allergen doses do affect the course of a predominantly Th1/Th17-mediated immune response mimicking an autoimmune disease in man (Crohn's disease) and whether epicutaneous and oral LZT to haptens can inhibit an inflammatory immune response of an organ system that is related (gut) or unrelated (skin) to the site of tolerance induction.

Repeated epicutaneous painting or oral feeding of low, subimmunogenic doses of the hapten 2,4,6-trinitro-1-chlorobenzene (TNCB) (or the water-soluble form TNBS for oral application) (Figure 1a) resulted in LZT induction and in a significantly impaired colitis as observed by a markedly reduced inflammation of the gut compared with control animals (Figure 1b–f). The reduced intestinal inflammation was determined by a decreased immune response in the intestine according to the endoscopic score by high-resolution mini-endoscopy (Supplementary Figure S1a and b online) (Figure 1b, total score [left panel], single parameters [right panel]). A significant reduction of stool consistency, granularity of the mucosal surface, changes of the vascular pattern, visibility of fibrin, and translucency of the colon was demonstrated, indicating that oral as well as epicutaneous LZT induction prevented colitis development (Figure 1b and c).

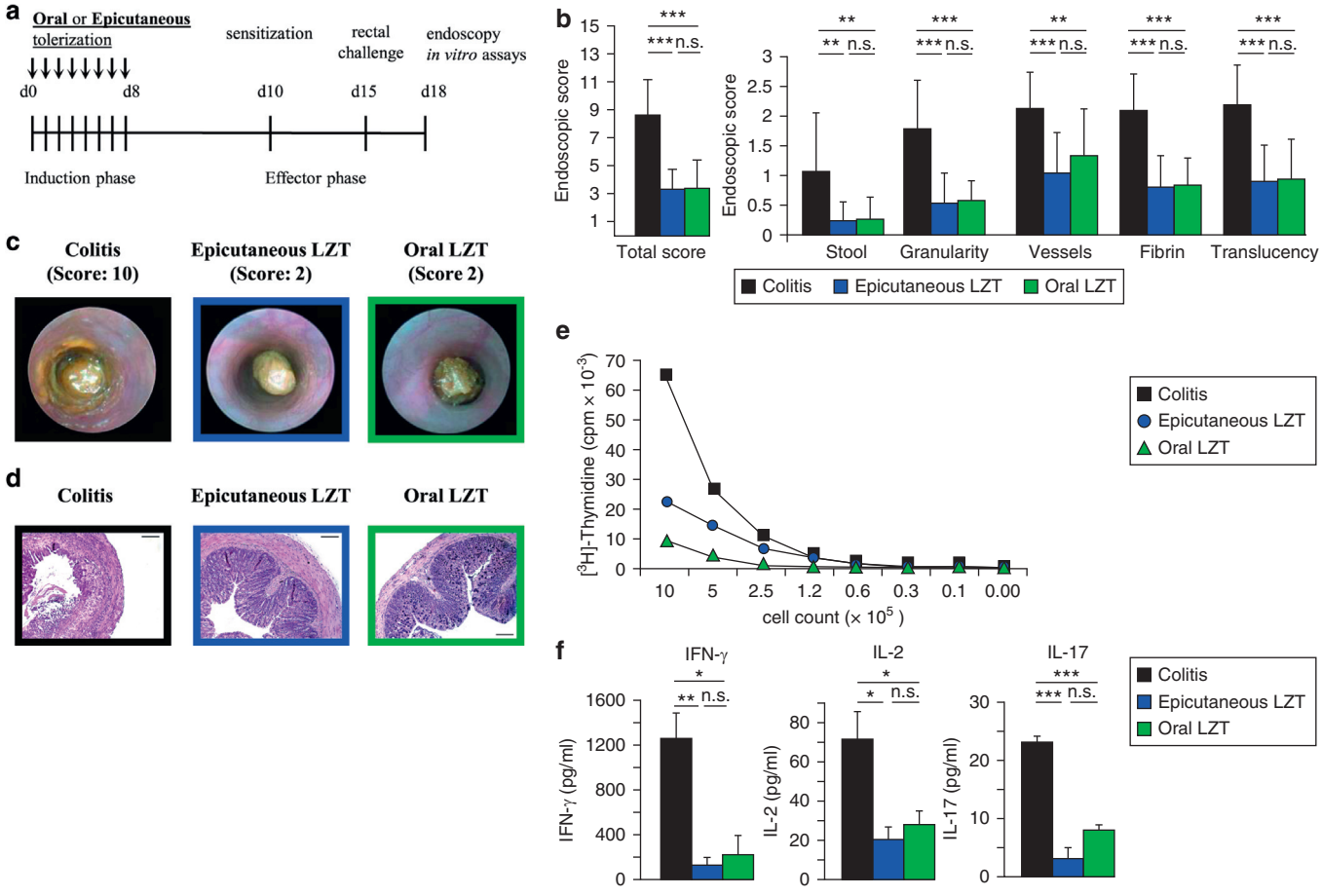

Figure 1. Epicutaneous and oral low-zone tolerance (LZT) protects from colitis. (**a**) Protocol of LZT and colitis induction. (**b**) Results were presented as pooled data (mean ± SD) of three independent experiments of the total endoscopic score (left panel) and of single parameters (right panel). Representative images of the mini-endoscopy (**c**) and of histology (**d**) of the rectum tissue are displayed. Scale bar = 150 μm. (**e**, **f**) Three days after TNBS enema, lymph nodes were obtained for T cell analysis. (**e**) After hapten-specific restimulation, T-cell proliferation was depicted (mean value of triplicates in cpm). One representative out of five independent experiments is shown. (**f**) Pooled data of T cell cytokine production (IFN-γ, IL-2, and IL-17) (mean ± SEM, n = 3–5) are demonstrated. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, n.s. not significant. cpm, counts per minute, TNBS, picrylsulfonic acid, 2,4,6-trinitro-benzenesulfonic acid; SD, standard deviation; SEM, standard error of the mean.