Calcitriol Treatment Ameliorates Inflammation and Blistering in Mouse Models of Epidermolysis Bullosa Acquisita

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A link between hypovitaminosis D and development of autoimmune bullous disorders has been suggested recently, but this association has not been elaborated experimentally. Here, the role of vitamin D was investigated in epidermolysis bullosa acquisita (EBA), an anti–type VII collagen autoantibody-induced blistering skin disease. Oral administration of the hormonally active vitamin D metabolite calcitriol ameliorated clinical disease severity and dermal neutrophil infiltration in both an antibody transfer- and immunization-induced EBA mouse model. Mechanistically, calcitriol hindered immune effector cell activation as evidenced by increased L-selectin expression on Gr-1$^+$ cells in calcitriol-treated mice with antibody transfer-induced EBA, as well as suppressed in vitro immune complex–induced reactive oxygen species production in calcitriol-treated murine neutrophils. Additionally, calcitriol administration was associated with an increase of regulatory T (CD4$^+$FoxP3$^+$) and B (CD19$^+$IL10$^+$) cells as well as reduction of pro-inflammatory T helper 17 (CD4$^+$IL-17$^+$) cells in mice with immunization-induced EBA. In line, levels of circulating anti–type VII collagen autoantibodies were lower in mice that received calcitriol compared to solvent-treated animals. Together with the observed state of hypovitaminosis D in most cases of an analyzed EBA patient cohort, the results of this study support the use of vitamin D derivatives or analogs for patients with EBA and related diseases.

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INTRODUCTION

In addition to its role in calcium absorption and maintenance of bone health, vitamin D exerts suppressive functions on cells of both innate and adaptive immune responses through not yet fully elucidated mechanisms. Vitamin D is a hormone precursor present in two basic natural forms, namely ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3), with the latter being synthesized in the skin upon sunlight exposure and subsequently converted to 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D (calcitriol) in the liver and kidney, respectively. 25-Hydroxyvitamin D is the major inactive circulating form of vitamin D commonly used as a serological biomarker to evaluate vitamin D status, whereas calcitriol represents the biologically active vitamin D metabolite (Mazzaferro et al., 2014).

Several immune cells express a vitamin D receptor and can also produce calcitriol. In keeping with the vitamin D regulation of the immune system, previous studies demonstrated that vitamin D deficiency can impair immune function, resulting in an increased prevalence of autoimmune diseases, such as multiple sclerosis, type 1 diabetes mellitus, and systemic lupus erythematosus (Yang et al., 2013).

More recently, the role of vitamin D has also been investigated in autoimmune bullous diseases. Accumulating evidence suggests an increased frequency of hypovitaminosis D in patients with pemphigus and pemphigoid (El-Komy et al., 2014; Joshi et al., 2014; Marzano et al., 2012, 2015; Moravvej et al., 2016; Sarre et al., 2016; Tukaj et al., 2013; Zarei et al., 2014), although some studies found no difference in the vitamin D status compared to healthy subjects due to concomitantly observed suboptimal 25-hydroxyvitamin D serum levels also in the corresponding control cohorts (Joshi et al., 2014; Moravvej et al., 2016; Sarre et al., 2016; Tukaj et al., 2013). Importantly, serum concentrations of 25-hydroxyvitamin D have been reported to be inversely associated with clinical disease severity in these patients, pointing toward a possible causative role of hypovitaminosis D in the disease process (Marzano et al., 2015; Moravvej et al., 2016; Zarei et al., 2014). In addition, we recently reported that calcitriol exerts anti-inflammatory effects in cultured keratinocytes treated with pemphigoid autoantibodies (Tukaj et al., 2016).

Despite this evidence, and the well-established use of vitamin D agents in different dermatologic diseases (Wadhwa et al., 2015), information on clinical effects of this vitamin and the underlying immunomodulatory mode of action in autoimmune bullous diseases is generally lacking. Therefore, the aim of this study was to investigate the role of vitamin D in epidermolysis bullosa acquisita (EBA), a subepidermal

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Abbreviations: COL7, type VII collagen; EBA, epidermolysis bullosa acquisita; ROS, reactive oxygen species; Th, T helper

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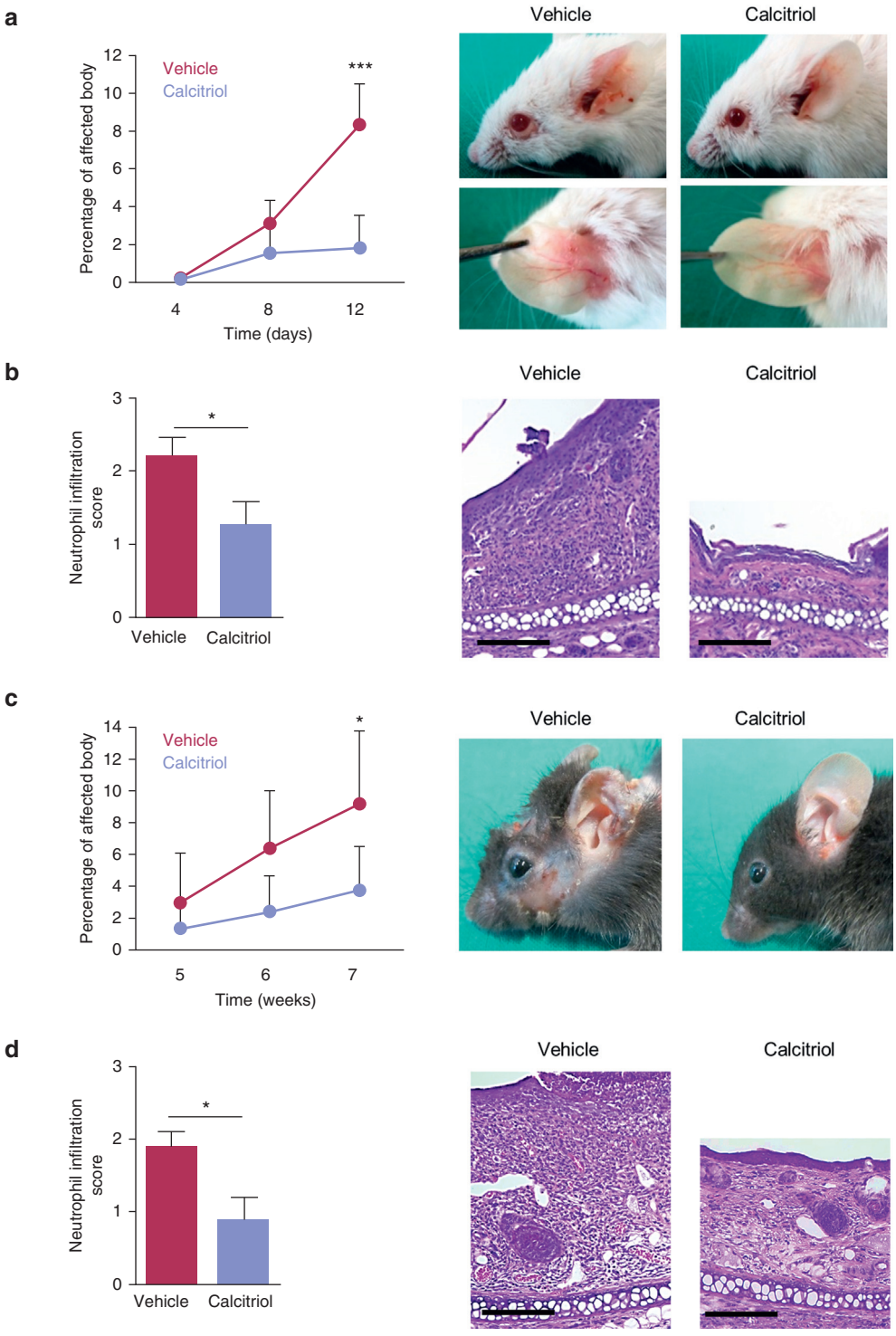

Figure 1. Calcitriol leads to clinical and histological disease amelioration in mice with experimental EBA. (**a**) In mice with antibody transfer–induced EBA, clinical scores, calculated as the percentage of the body surface area covered by EBA lesions, were significantly lower in calcitriol (5 μg/kg per os) compared with vehicle (methylcellulose)-treated mice during a daily treatment over 12 days; corresponding representative clinical presentations of vehicle- and calcitriol-treated mice at the end of the 12-day treatment period are shown on the right. (**b**) Semiquantitative evaluation of dermal neutrophil infiltration in hematoxylin and eosin–stained skin sections revealed significantly lower scores (0–3: no, mild, moderate, and severe infiltration, respectively) in calcitriol-treated mice at the end of the 12-day treatment period; corresponding representative histological presentations of vehicle- and calcitriol-treated mice at the end of the 12-day treatment period are shown on the right. (**c**) In mice with immunization-induced EBA, clinical scores were significantly lower in calcitriol (10 μg/kg per os) compared with vehicle (methylcellulose)-treated mice during an alternate-day treatment over 7 weeks; corresponding representative clinical presentations of vehicle- and calcitriol-treated mice at the end of the 7-week treatment period are shown on the right. (**d**) Semiquantitative evaluation of dermal neutrophil infiltration in hematoxylin and eosin–stained skin sections revealed significantly lower scores in calcitriol-treated mice at the end of the 7-week treatment period; corresponding representative histological presentations of vehicle- and calcitriol-treated mice at the end of the 7-week treatment