Aggravated TSLP-Induced Atopic Dermatitis in Mice Lacking Dicer in Adult Skin Keratinocytes

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TO THE EDITOR

Thymic stromal lymphopoietin (TSLP) is a key keratinocyte (KC)-derived cytokine involved in the pathogenesis of atopic diseases, including atopic dermatitis (AD) (Liu et al., 2007; Ziegler and Artis, 2010). Its expression was found to be increased in skin KCs from AD patients (Soumelis et al., 2002). We (Li et al., 2005), and others (Yoo et al., 2005) have shown that transgenic mice overexpressing TSLP in KCs develop an AD-like syndrome, indicating that TSLP expression is sufficient to initiate AD-like inflammatory responses. More
recently, we reported that topical application of the low-calcemic analog of vitamin D3 (MC903; calcipotriol) triggers a mouse AD (Li et al., 2006), which is mediated by induced keratinocytic TSLP (Li et al., 2009), as TSLP is downregulated in Dicer−/− mice in which TSLP is ablated selectively in KCs failed to develop AD. These studies have highlighted a critical role for keratinocytic TSLP in AD pathogenesis, yet, how TSLP expression is regulated remains to be investigated. Studies have suggested that TSLP could be regulated at the transcriptional level by nuclear receptor signaling (i.e., VD3, retinoid acids, glucocorticoids, and their receptors) as well as by nuclear factor-κB signaling (Li et al., 2005, 2006; Lee and Ziegler, 2007; Surjit et al., 2011), and our unpublished data). However, post-transcriptional regulation of TSLP expression remains unexplored.

The RNase III enzyme Dicer is essential for processing pre-miRNAs into mature, functional miRNAs, which are capable of post-transcriptional gene regulation by binding to their target mRNAs, leading to mRNA degradation or suppression of translation. A critical role for Dicer has been shown during mammalian skin development (Andl et al., 2006; Yi et al., 2006). However, evidence implicating miRNAs expressed in adult KCs in the pathogenesis of inflammatory skin diseases (e.g., AD) is still lacking.

To investigate the possible involvement of keratinocytic miRNAs in AD pathogenesis, we generated Dicer−/− mutant mice with induced ablation of Dicer in epidermal KCs (called hereafter MT), by tamoxifen injection to K14-CreERT2ΔE0/DicerL3/L3 mice obtained by breeding of Dicer1/3/L3 mice (Cobb et al., 2006) with K14-CreERT2ΔE0 transgenic mice (Li et al., 2000, 2005) (Supplementary Figure S1 online). Tamoxifen-injected K14-CreERT2ΔE0/Dicer1/3/L3 littermate mice were used as control (CT). MT mice did not exhibit apparent skin abnormalities up to 9 months after Dicer ablation. Histological analyses performed at 4–8 weeks and at 9 months after Dicer ablation did not reveal obvious modification in skin morphology (Figure 2a and data not shown). Therefore, adult skin homeostasis (in an unchallenged situation) does not seem to be obviously impaired in Dicer−/− mice, which may reflect a different requirement of keratinocytic Dicer in developmental (Andl et al., 2006; Yi et al., 2006) and adult stages.

We then subjected CT and MT mice (4 weeks after tamoxifen injection) to topical MC903 treatment on ears (Li et al., 2006, 2009), to investigate (i) MC903-induced TSLP production in the skin; and (ii) TSLP-mediated AD development in these mice. Analyses of ears at day 2 (D2) (with MC903 application at D1) or at D4 (with MC903 application at D1 and D2) showed that both TSLP transcript (Figure 1a) and protein (Figure 1b) levels were higher in MC903-treated MT compared with MC903-treated CT skin. Immunohistochemical analyses confirmed an enhanced TSLP production in MC903-treated MT epidermal KCs (Figure 1c).

We next treated CT and MT mice continuously with MC903 (every other day, starting at D1), to induce a TSLP-mediated AD phenotype. At D8, MC903-treated MT ears were more red, scaly, and swollen compared with MC903-treated CT ears. At D12, a time when AD appearance was clearly seen in MC903-treated CT skin, the MC903-treated MT ears exhibited a much more heavily inflamed skin with lesions (data not shown). Histological analyses at D8 and D12 showed that upon MC903 application, MT mice developed a more severe skin inflammation (Figure 2a), with heavily infiltrated eosinophils (Figure 2b), CD4+ T cell and mast cells (data not shown) in the dermis, as well as enhanced epidermal hyperplasia (Figure 2a). RT-PCR analyses at D12 revealed that MC903-induced cytokines were further increased in MC903-treated MT skin (Figure 2c): (a) Th2 cytokines (IL4 and IL13), eosinophil-attractant chemokine (eotaxin-2), and chemokine receptor (CCR3), which have been previously demonstrated to be fully

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**Figure 1. Increased thymic stromal lymphopoietin (TSLP) expression in MC903-treated Dicer−/− skin.** (a) Thymic stromal lymphopoietin (TSLP) RNA levels in ears, and (b) TSLP protein levels in ear extract at day 2 (D2) and D4 from control (CT) and Dicer−/− mutant (MT) mice, topically treated with ethanol (EtOH, as vehicle control) or MC903 (1 nmol per day and per ear). Error bars indicate s.e.m. (n ≥ 3). (c) Immunohistochemical (IHC) staining of TSLP on ear sections from MC903 topically treated CT (left panel) and MT (right panel) mice at D4 (with MC903 application at D1 and D2, 1 nmol per day and per ear). Methods are described in Supplementary Material online. White dashed lines indicate the dermal/epidermal junction. Yellow corresponds to staining of antibody, whereas blue corresponds to DAPI staining of nuclei. Bar = 50 μm.
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Figure 2. Aggravated MC903-induced skin inflammation in Dicer<sup>imp−/−</sup> mice. (a) Hematoxylin/eosin-stained sections of ears at day 8 (D8) or D12, from control (CT) and MC903-treated (MT) mice. (b) Immunohistochemical (IHC) staining of major basic protein (MBP, specific for eosinophils) on ear sections from MC903-treated CT and MT at day 12 (D12). Bar = 50 μm. (c) Quantitative RT-PCR analyses of cytokine and chemokine mRNA levels in ears at D12. Error bars indicate s.e.m. (n=3). Methods are described in Supplementary Material online.

TSLP-dependent (Li et al., 2009), were all significantly increased; (b) IL6, IL10, and MCP2, which are partially TSLP-dependent (Li et al., 2009), showed a tendency to increase (the statistic analyses did not reach significance). The Th1 cytokine IFN-γ, which is TSLP-independent (Li et al., 2009), was also elevated in MC903-treated MT skin. Similar results were obtained by RT-PCR analyses of skin samples at D8 (data not shown). Of note, RT-PCR analyses of these cytokines and chemokines at D2 showed no difference between MC903-treated CT and MT mice (Supplementary Figure S2 online), whereas TSLP expression was significantly elevated in MT at this stage (Figure 1a), suggesting that the enhanced TSLP production in MT mice was indeed an early event. Furthermore, total serum IgE was higher in MT than in CT mice upon MC903 treatment (Figure 2d). Taken together, these results indicate that MC903 treatment induces aggravated AD skin inflammation in Dicer<sup>imp−/−</sup> mice.

In conclusion, we show here that tamoxifen-induced ablation of Dicer from mouse epidermal KCs results in an aggravated MC903-induced AD, accompanied by an elevation of TSLP production by KCs. We also observed that, upon topical treatment of dibutyl phthalate, which was shown to induce keratinocytic TSLP (Larson et al., 2010), Dicer<sup>imp−/−</sup> MT skin had increased TSLP production (Supplementary Figure S3 online). These results highlight the implication of keratinocytic miRNA in the pathogenesis of AD, through modulating the production of key cytokines such as TSLP. Interestingly, evidence for the involvement of miRNAs in regulating TSLP expression was recently shown in gut epithelium (Biton et al., 2011). Identifying miRNA(s) implicated in regulating TSLP expression in the skin and other epithelia will be of great interest for elucidating the regulation of this key cytokine, and for developing new therapeutic approaches for AD and other atopic diseases. The MC903-induced AD mouse model should provide an easy method by which to screen for miRNA factors in AD pathogenesis.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES


Serum Levels of Inhibitors of Apoptotic Proteins (IAPs) Change with IVIg Therapy in Pemphigus

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TO THE EDITOR

Pemphigus is a rare autoimmune blistering disease characterized by high levels of antibodies against the epidermal attachment components, desmoglein1 (Dsg-1) and desmoglein3 (Dsg-3; Amagai, 1999). Intravenous Ig (IVIg) reduces the levels of these antibodies, as well as clinical manifestations of pemphigus, but its mechanism of action is unknown (Czernik et al., 2008). There is emerging evidence that pemphigus antibodies can cause keratinocyte apoptosis and contribute to acantholysis (Arredondo et al., 2005; Li et al., 2009; Schmidt and Waschke, 2009). Inhibitors of apoptotic proteins (IAPs) can target the caspasas, and increases in their levels lead to resistance to apoptosis (Schimmer, 2004). Antiapoptotic effect of IVIg on keratinocytes is well known in disorders such as toxic epidermal necrolysis (Viard et al., 1998). Our goal in this study was to determine whether IAP levels change in sera during IVIg therapy as a possible mechanism of suppression of acantholysis.

We measured three IAPs (survivin, livin, and X-linked IAP (XIAP)) in sera of seven patients with active pemphigus (five pemphigus vulgaris and two pemphigus foliaceus) before and after treatment with 10% IVIg (Gamunex, Talecris Biotherapeutics, Durham, NC). Average age of the patients was 59 (45–74) years. Each course of IVIg consisted of four cycles, administered every 2 weeks. Each cycle consisted of 400 mg kg⁻¹ per day infused slowly for 5 days. In four patients, IVIg was administered together with 50-200 mg per day of oral cyclophosphamide. All patients were under treatment with prednisone (20-80 mg per day) before IVIg therapy. Sera were collected before treatment, 1 and 2 weeks after the first and 1 and 4 weeks after the fourth IVIg cycle. All patients gave written informed consent according to the Declaration of Helsinki Principles, and the study was approved by the New York University Institutional Review Board. Serum levels of intercellular IgG antibodies were measured by indirect immunofluorescence using epithagus of rhesus monkey as the substrate. Skin cryosections were incubated with serial serum dilutions of 1:10 to 1:1,280. Goat anti-human IgG-FITC was used as the secondary antibody and slides were analyzed with an epiluminescence microscope. Anti-Dsg-1 and anti-Dsg-3 antibodies were measured by ELISA, using a kit from MBL International, Woburn, MA, and monoclonal mouse anti-human IgG4 antibodies (GeneTex, Irvine, CA) with the technique described previously (Green and Bystryn, 2008). Disease severity was measured using a score described earlier (Lolis et al., 2011). For quantitative measurement of IAPs, ELISA kits from R&D Systems, Minneapolis, MN were used according to the manufacturer’s instructions.

Serum survivin, livin, and XIAP were measured in 31 healthy individuals, and the average levels were 40.1 pg ml⁻¹ (SD = ±8.4), 26.2 pg ml⁻¹ (±14.4), and 0.9 μg ml⁻¹ (±0.29), respectively. Pretreatment levels of XIAP and livin in pemphigus patients (Table 1) were not significantly different from those of healthy individuals. Survivin

Abbreviations: Dsg-1, desmoglein1; IAP, inhibitors of apoptotic proteins; IVIg, intravenous Ig; XIAP, X-linked inhibitor of apoptotic proteins