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APRIL expression is upregulated in atopic dermatitis skin lesions and at sites of antigen driven allergic skin inflammation in mice

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Abstract

Atopic dermatitis (AD) is the most common inflammatory skin disease. It is characterized by a defective skin barrier and a Th2 dominated skin inflammation. The TNF family member a proliferation-inducing ligand (APRIL) and its receptors transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) and B cell maturation antigen (BCMA) are expressed by immune cells and epithelial cells including keratinocytes. We demonstrate that APRIL expression is upregulated in the epidermis of skin lesions from patients with AD as well as in mouse skin undergoing allergic inflammation elicited by epicutaneous (EC) sensitization with the antigen ovalbumin. We show that APRIL from OVA sensitized mouse skin causes keratinocytes to upregulate the expression of IL-6, an inflammatory cytokine implicated in AD pathogenesis. These results suggest a role for APRIL in allergic skin inflammation and a potential role for APRIL blockade in treating AD.

1. Introduction

Atopic dermatitis (AD) is the most common inflammatory skin disease. It is characterized by a defective skin barrier, aggravated by mechanical skin injury due intense scratching, a Th2 dominated skin inflammation, and predisposition to cutaneous infection [1]. Our laboratory has developed a mouse model of allergic skin inflammation induced by repeated epicutaneous sensitization (EC) of tape stripped skin with ovalbumin [2, 3]. Our model shares a number of characteristics with human AD. These include skin barrier disruption, local and systemic type 2 immune response, epidermal thickness, dermal infiltration by

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CD4^{+} T cells and eosinophils and susceptibility to cutaneous *S. aureus* and vaccinia virus infections [2–5].

The tumor necrosis ligand superfamily member a proliferation-inducing ligand (APRIL) is predominantly secreted as soluble molecule by monocytes, macrophages and dendritic cells [6]. APRIL has two receptors, B cell maturation antigen (BCMA) and TACI (transmembrane activator and calcium modulator and cyclophilin ligand interactor), both of which play an important role in B cell proliferation, survival and maturation [6]. APRIL and its receptors BCMA and TACI are also expressed by epidermal keratinocytes [7]. APRIL and BCMA, but not TACI, are upregulated in psoriasis, squamous cell carcinoma and cutaneous lupus erythematosus [7, 8].

APRIL drives IL-6 expression in human keratinocytes [7]. IL-6 is a ubiquitously expressed inflammatory cytokine produced by immune and non-immune cells including keratinocytes (KCs) [9]. IL-6 has been shown to drive collagen deposition in the skin [10]. A role for IL-6 in AD pathogenesis is suggested by the observation that interrupting IL-6-receptor (IL-6R) signaling is beneficial in AD [11] and by the finding that a functional IL-6R variant is a risk factor of persistent AD [12]. The role of APRIL and its receptors in the pathogenesis of AD remains unknown. Here we demonstrate that APRIL and BCMA expression is upregulated in the epidermis of skin lesions from patients with AD as well as in mouse epidermis at sites of antigen-driven allergic skin inflammation. Importantly, we show that APRIL activates *Il6* expression in keratinocytes in an autocrine manner.

2. Material and methods

2.1. Patients

Samples from healthy controls and from patients with AD were evaluated. All patients gave signed consent and the study fulfilled the Declaration of Helsinki Principles.

2.2. Immunohistochemistry

Immunohistochemical analyses were performed from 4mm skin-punch biopsies taken for diagnostic purposes from untreated lesional skin in an acute state of the disease. Biopsies were fixed in 5% formalin solution overnight. Sections were prepared from formalin-fixed, paraffin-embedded skin biopsies. For immunohistochemistry we used the REAL™ Detection Systems with Fast Red as chromogen, following the manufacture s protocol (DAKO™, Hamburg, Germany) as previously published [8]. The following monoclonal antibodies were used anti-APRIL (clone :Aprily-8 diluted 1:100), anti-BCMA (clone:Vicky-1 diluted 1:100) both from Enzo and anti-TACI (clone sc-80335 dilutred 1:150) from Santa Cruz.

2.3. Mice

BALB/c mice were purchased from Charles River Laboratories (Wilmington, MA). All mice were kept in a pathogen-free environment and fed an OVA-free diet. All procedures performed on the mice were in accordance with the Animal Care and Use Committee of the Children's Hospital Boston.

2.4. Epicutaneous (EC) sensitization with ovalbumin

Six- to eight-week-old female mice were EC sensitized as described previously [2]. Each mouse had a total of three one-week exposures to an OVA or saline impregnated patch separated by two-week intervals. Mice were euthanized at the end of the third cycle of sensitization (day 49).

2.5. RNA extraction and quantitative RT-PCR

Total skin RNA was extracted with Total RNA Isolation Kit (Ambion). cDNA was prepared with iscript cDNA synthesis kit (Biorad). PCR reactions were run on ABI Prism 7300 (Applied Biosystems) sequence detection system platform. Taqman primers and probes were obtained from Life technologies. The housekeeping gene β_2-microglobulin was used as an internal control. Relative mRNA expression was quantified using the $2^{-\quad Ct}$ method.

2.6. Skin explant culture and *in vitro* cytokine expression

EC sensitized skin explants (1 cm^2) were chopped with scissors and cultured in complete RPMI. The supernatants were harvested after 18 hrs and APRIL was measured by an ELISA kit according to the manufacturer's instructions (LS bio).

2.7. IgG1 production by B cells

Naïve B cells were sorted from mouse splenocytes, cultured with supernatants of EC sensitized or unmanipulated skin explants mice in presence or absence of rIL-4 (50 ng/ml) and mBCMA-Fc (5μg/ml). IgG1 secretion was evaluated after 5 days of culture by ELISA.

2.8. Epidermal layers preparation, culture and stimulation

Ventral and dorsal halves of ears were split. To separate the epidermis and dermis, both halves were floated dermal side down on 4 mg/ml dispase (Roche) in PBS for 30 min at 37°C. Epidermal sheets were floated in medium containing rAPRIL (100 ng/ml) or supernatants of EC sensitized skin in presence of mBCMA-Fc (200 ng/ml) or mCD8-Fc (200 ng/ml).

2.9. Statistical analysis

Results were analyzed using one-way ANOVA or non-parametric t tests. A p value of less than 0.05 was considered significant.

3. Results and Discussion

3.1. APRIL is upregulated in keratinocytes of AD lesional skin

We performed Immunohistochemical (IHC) staining of lesional skin from 5 adult patients with active AD and skin from 3 healthy controls. The patients had not used local steroids for 2 weeks or more prior to biopsy and were not on systemic immunosuppresants. Normal skin exhibited faint APRIL staining in the epidermis (Fig. 1A). In contrast, there was intense and uniform staining for APRIL in the epidermis of lesional skin from patients with AD (Fig. 1A). There were scattered APRIL staining cells in the dermis of AD lesional skin and normal skin (Fig. 1A). IHC staining for BCMA revealed faint staining in the epidermis of

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normal skin that was slightly more intense in the epidermis of AD lesional skin (Fig. 1B). Few scattered cells in the dermis exhibited BCMA staining in normal and AD lesional skin (Fig. 1B). There was negligible staining for TACI in both epidermis and dermis of AD lesional skin and normal skin (data not shown). These results demonstrate that APRIL expression by keratinocytes is strongly upregulated in AD skin lesions.

3.2. APRIL and its ligand BCMA are upregulated in keratinocytes at sites of allergic skin inflammation in mice

To understand the potential role of APRIL in allergic skin inflammation we examined a mouse model in which EC sensitization with antigen by repeated topical application on tape stripped skin results in a Th2 dominated skin inflammation that shares many characteristics with AD skin lesions [2]. OVA-sensitized, but not saline-sensitized skin demonstrated significantly increased mRNA levels of *Tnfsf13* (encoding APRIL) and *Tnfrsf17* (encoding BCMA) compared to untreated skin (Fig. 2A). Importantly, EC sensitization with OVA, but nor saline, resulted in significant upregulation of *Tnfsf13* and *Tnfrsf17* mRNA expression in the isolated epidermal layer, compared to untreated skin (Fig. 2B). We also examined APRIL levels in skin homogenates using ELISA. OVA-sensitized, but not saline-sensitized skin, demonstrated significantly increased levels of APRIL compared to untreated skin (Fig. 2C). These results demonstrate that keratinocyte expression of APRIL and its ligand BCMA is upregulated at sites of allergic skin inflammation in mice.

APRIL drives IgG1 isotype switching and synthesis in naïve B cells in the presence of IL-4 [13]. We used this observation to assess APRIL biologic activity in the skin. Supernatants of homogenates from OVA sensitized skin induced IgG1 secretion by naïve B cells in the presence of rIL-4 (Fig. 2D). No detectable IgG1 secretion was induced by the supernatants alone, or rIL-4 alone (Fig. 2D). APRIL blockade by addition of mBCMA-Fc, but not addition of control mCD8-Fc, significantly inhibited IgG1 secretion by naïve WT B cells stimulated by OVA-sensitized skin homogenates and IL-4 (Fig. 2D).These results demonstrate that APRIL at sites of allergic skin inflammation is biologically active.

3.3. APRIL produced by keratinocytes at sites of allergic skin inflammation acts in an autocrine manner to promote *Il6* expression in keratinocytes

Il6 mRNA was significantly upregulated in the epidermal layer of OVA sensitized mouse skin compared to saline-sensitized mouse skin (Fig. 2E). Given that APRIL drives *Il6* expression in human keratinocytes [7], we examined the potential role of APRIL present in skin undergoing allergic skin inflammation on *Il6* expression by mouse keratinocytes. Recombinant mouse APRIL upregulated *Il6* mRNA expression by mouse ear skin epidermal layers (Fig. 2F). This upregulation was completely inhibited by the addition of mBCMA-Fc (Fig. 2F). Importantly, homogenates from epidermal layers of OVA-sensitized, but not saline-sensitized skin of WT mice, upregulated *Il6* mRNA expression by mouse ear skin epidermal layers (Fig. 2G). This was significantly inhibited by addition of mBCMA-Fc, but not mCD8-Fc control. These results indicate that APRIL produced by keratinocytes at sites of allergic skin inflammation acts in an autocrine manner to activate *Il6* expression in keratinocytes.

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In summary, our findings suggest that APRIL drives IL-6 production by keratinocytes in autocrine manner at sites of allergic skin inflammation, and thereby could contribute to AD pathogenesis. APRIL blockade may be useful in treating AD.

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Abbreviations

AD	Atopic dermatitis
APRIL	a proliferation-inducing ligand
BCMA	B cell maturation antigen
TACI	transmembrane activator and calcium modulator and cyclophilin ligand interactor
KC	keratinocytes
EC	Epicutaneous
IHC	Immunohistochemical

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Highlights

- APRIL is upregulated in keratinocytes of AD lesional skin
- APRIL and its ligand BCMA is upregulated in keratinocytes at sites of allergic skin inflammation in mice
- APRIL produced by keratinocytes at sites of allergic skin inflammation acts in an autocrine manner to promote *Il6* expression in keratinocytes

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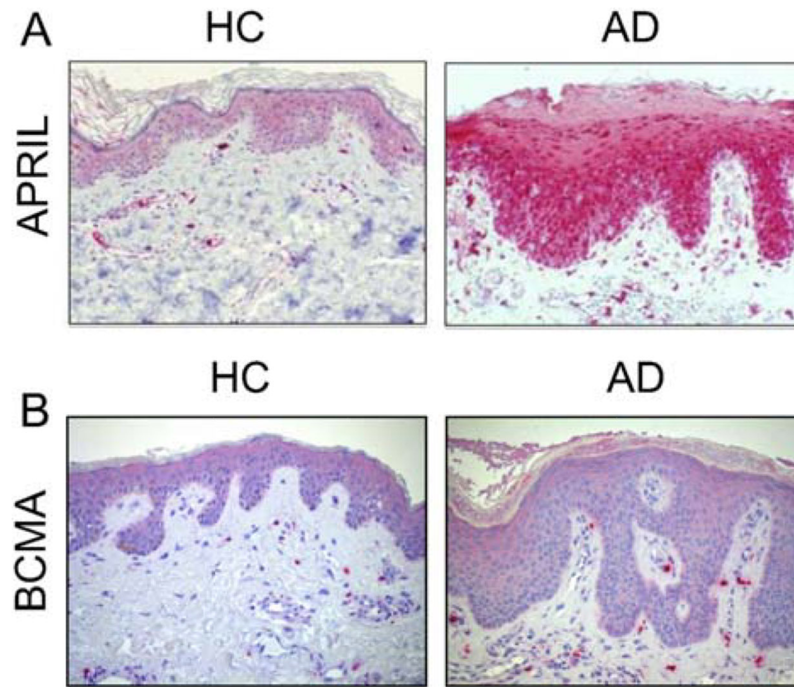

Figure 1. immunohistochemical staining for APRIL and BCMA in lesional skin from a patient with AD and skin from a healthy control.

A, B. Representative immunohistological micrographs of APRIL (A) and BCMA (B) in lesional skin from AD patients and skin healthy controls. Magnification 200 X. Similar results were obtained in 2 other patients and 2 healthy controls.

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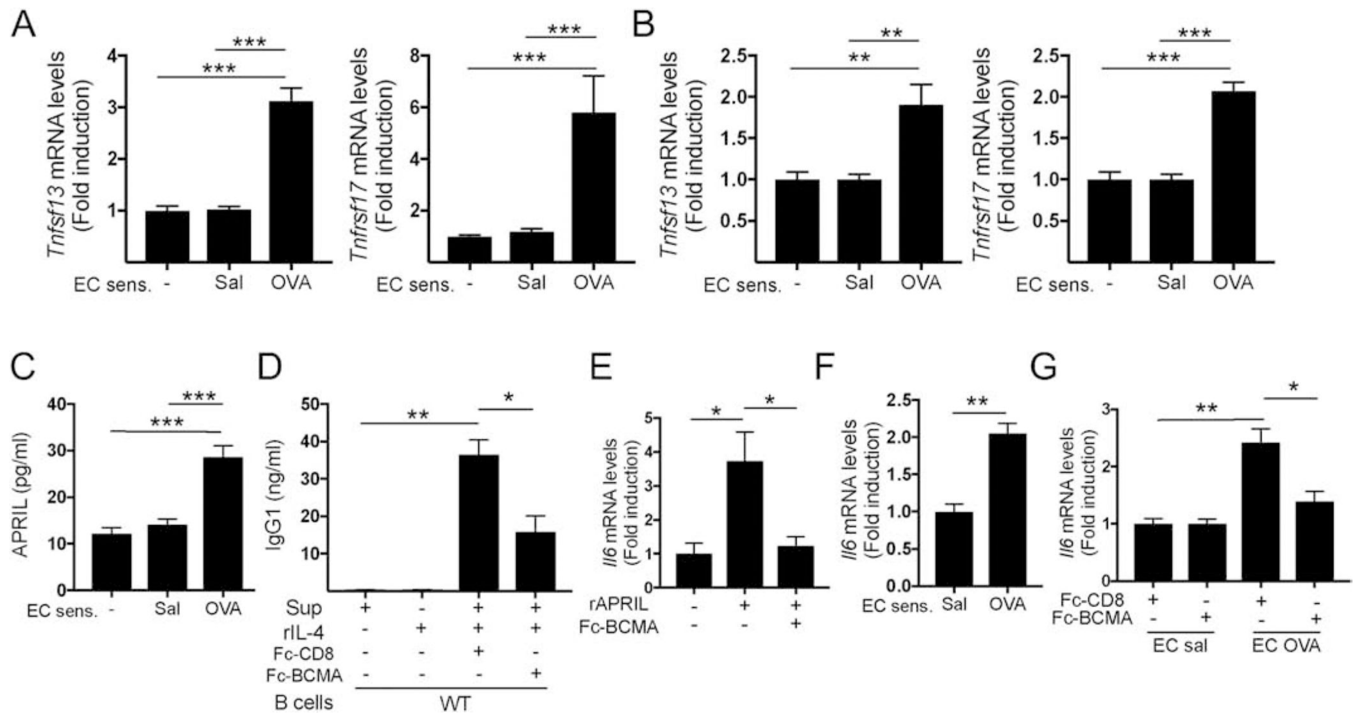

Figure 2. APRIL expression and biologic activity in murine skin sites of allergic skin inflammation.

A, B. mRNA expression of *Tnfsf13* (encoding APRIL) and *Tnfrsf17* (encoding BCMA) in skin (A) and epidermal layers (B) from unmanipulated mouse skin and mouse skin EC sensitized with saline (Sal) or OVA. Values are expressed a relative to unmanipulated mouse skin control **C.** APRIL levels in supernatants of skin homogenates from unmanipulated mouse skin and mouse skin EC sensitized with Sal or OVA. **D.** IgG1 synthesis by purified naïve B cells, from WT mice stimulated with supernatants of explants from OVA sensitized mouse skin and rIL-4. WT without or with the addition of mBCMA-Fc c or mCD8-Fc as control. **E.** *Il6* mRNA expression in epidermal layers of EC sensitized skin of WT mice. Values are expressed a relative to saline sensitized skin. **F.** *Il6* mRNA expression in epidermal layers of WT mice stimulated with rAPRIL in presence or absence of mBCMA-Fc. Values are expressed a relative to unstimulated cultures. **G.** *Il6* mRNA expression in epidermal layers of WT mice cultured with homogenates from skin EC sensitized with Sal or OVA and with the addition of mBCMA-Fc or mCD8-Fc as control. Values are expressed a relative to cultures stimulated with supernatants of explants from skin EC sensitized with Sal in the presence of mCD8:Fc. Results in A-G are representative of 2 independent experiments with 4–5 mice/group. Bars represent means±SEM. * = $p<0.05$, ** = $p<0.005$ and *** = $p<0.001$.