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TSLP expression in the skin is mediated via RARγ-RXR pathways

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ABSTRACT

TSLP is an important trigger and initiator for various atopic diseases mainly atopic dermatitis (AD). Activators of nuclear hormone receptors like bioactive vitamin A and D derivatives are known to induce TSLP up-regulation in the skin. In this study, various combinations of synthetic specific agonists and antagonists of the retinoic acid receptors (RARs), retinoid X receptors (RXRs) and vitamin D receptor (VDR) were topically administered to mice. The aim of the study was to elucidate via which nuclear hormone receptor pathways TSLP is regulated and how this regulation is connected to the development and phenotype of atopic dermatitis. TSLP expression was monitored using QRT-PCR and serum TSLP levels using ELISA. Synthetic agonists of the VDR and RARγ as well as the natural agonist all-*trans* retinoic acid (ATRA) increased TSLP expression in the skin, while an RXR agonist was not active. Treatments with antagonists of RXRs and RARs in addition to RARα-agonists reduced skin TSLP expression. Strong activation was found after a combination of a VDR and an RXR agonist (ca. 5 times induction) and even stronger by an RARγ and an RXR agonist treatment (ca. 48 times induction). We conclude that besides VDR-mediated signaling mainly RARγ-RXR mediated pathways in the skin are important patho-physiological triggers for increased skin TSLP expression. We conclude that topical synthesized retinoids stimulated by internal or external triggers or topically applied induce TSLP production and are thereby important triggers for atopic dermatitis prevalence.

1. Introduction

Atopic dermatitis (AD) is a highly pruritic, chronic and common inflammatory disease of the skin being often associated with strong hereditary background (Leung et al., 2004; Kang and Stevens, 2003). AD develops in early infancy and childhood and can persist till adulthood (Leung et al., 2003) and mainly Th2 pathways play a critical role during pathogenesis (Leung et al., 2004). Thymic stromal lymphopoietin (TSLP) is an IL-7 like cytokine and was shown to be a master switch of allergic inflammation at the epithelial cell—dendritic cell interface leading to allergic sensitization (Soumelis et al., 2002). TSLP expression is highly expressed in keratinocytes and myeloid dendritic cells in acute and chronic AD skin (Soumelis and Liu, 2004; Liu, 2006; Ziegler and Liu, 2006). It plays a critical role during initiation of allergic diseases in mice and humans (Liu, 2006; Huston and Liu, 2006; Miyata et al., 2008) and elevated TSLP levels were associated with a Th2 polarisation in numerous inflammatory diseases (Ying et al., 2005).

Topical and systemic application of lipid ligands of the nuclear hormone receptors vitamin D receptor (VDR), retinoid X receptor (RXR) and retinoic acid receptor (RAR) trigger TSLP expression (Li et al., 2006; Jessup et al., 2008; Sheu et al., 2002; Staumont-Salle et al., 2008). Previously, it was reported that even selective ablation of the retinoid X receptor α (RXRα), which is predominant in skin, triggers AD and results in the development of a chronic dermatitis in mice with similarities to human AD (Li et al., 2001). A Th2-like inflammatory reaction after increased TSLP expression was observed, suggesting that TSLP is involved in the AD-like skin syndrome.

The aim of our study was to find out how selective agonists and antagonists for the nuclear hormone receptors VDR, RARs and RXRs influence skin TSLP expression as well as serum TSLP levels

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and what is the consequence of this regulation on atopic dermatitis phenotype and development.

2. Materials and methods

2.1. Sensitization of mice

8–12 weeks old female C57BL6 and BALBc mice were obtained from and housed within the animal facility of the University of Debrecen, Hungary. Animals were maintained in single cages on standard animal chow and water ad libitum. All experimental procedures were approved by the Committee of Animal Research of the University of Debrecen, Hungary (Approval number: 25/2006 DEMÁB).

C57BL6 mice were anesthetized and subsequently shaved on dorsal skin sites using an electric razor. Retinoid receptor-specific agonists and antagonists were applied topically each other day in 25 μl acetone (vehicle/control; Merck, Darmstadt, D) per treatment for two weeks. According to previous studies by other groups (Chapellier et al., 2002; Calleja et al., 2006) agonists and antagonists were applied in the following concentrations: ATRA 40 nmol; LG268 (RXR-agonist) 100 nmol; BMS753 (RARα agonist) 40 nmol; BMS189961 (RARγ agonist) 40 nmol; BMS493 (RAR pan-antagonist) 100 nmol; UVI3003 (RXR pan-antagonist) 100 nmol; MC903 (VDR agonist) 1 nmol. On day 14, four hours after the last treatment, mice were sacrificed, sera and full thickness skin biopsies were collected, skin specimens were shock frozen in liquid nitrogen and all samples were kept at −80 °C until analyses. Skin samples were obtained from equal body sites by means of the same procedure for each mouse in order to control for variability among specimen. Samples were visibly controlled to ensure no excessive adipose tissue remained, though some contamination with remaining adipose tissue cannot be excluded.

Sensitization of BALBc mice was performed by repetitive systemic application of OVA. Briefly, mice were sensitized at days 47, 60 and 67 with 10 μg OVA intraperitoneally (i.p.) adsorbed to 1.5 mg aluminium hydroxide ($Al(OH)_3$) or with phosphate-buffered saline (PBS, control). For combined treatment mice were sensitized i.p. on days 1, 14 and 21 with 10 μg OVA adsorbed to 1.5 mg $Al(OH)_3$. This was followed by topical application of 100 μg OVA adsorbed to 1.5 mg $Al(OH)_3$ in 100 μl PBS onto shaved back skin, divided into four applications of 25 μl every other day of one week. Epicutaneous treatment was repeated for a total exposure of three weeks separated by two-week intervals. Three days after the last treatment mice were euthanized; serum samples were collected and kept at −80 °C until analyses.

2.2. Study using human atopic dermatitis volunteers

After informed consent and the approval of the local Ethics Committee of the University of Debrecen, Hungary, Medical and Health Science Center, peripheral blood was collected from 20 AD-patients (8 males, 12 females; mean age 20 years, range 15–32 years). A group of 20 healthy age-matched volunteers (6 males, 14 females, mean age 21 years, range 19–24 years) served as controls in this study (Mihaly et al., 2013). All AD-patients fulfilled the diagnostic criteria established by Hanifin and Rajka (1980).

2.3. RNA preparation and reverse transcription

Total RNA was isolated from frozen skin using Tri® reagent (Molecular Research Center Inc., Cincinnati, OH) following the manufacturer's instructions. 750 ng of total RNA was reverse transcribed into cDNA in a 30 μl reaction using the High Capacity cDNA Reverse Transcription Kit (Life Technologies, Budapest, H) according to the manufacturer's protocol.

2.4. Analysis of mRNA expression

mRNA expression in skin was determined by means of quantitative real time-PCR (qRT-PCR) and TaqMan® Low Density Array (TLDA) on an ABI Prism 7900. qRT-PCR measurements were performed in triplicate using pre-designed TaqMan® Gene Expression Assays and reagents; TaqMan® Low Density Array cards were used for duplicate determinations using TaqMan® Gene Expression Master Mix (all Applied Biosystems Applera Hungary, Budapest, H). Relative quantification of mRNA expression was achieved using the comparative C_T method and values were normalized to cyclophilin A mRNA. Gene expression values below detection limit were assumed to be zero for the purpose of statistical analysis.

2.5. ELISA

Quantikine human TSLP and mouse TSLP kits (RnD-Sytems, Budapest, H) were used for the quantitative determination of human and mouse TSLP in serum. All samples and standards were assayed in triplicate. 100 μl of Assay Diluent RD1X was added to each well and 50 μl standard, control or sample was added per well. The plate was incubated for 2 h at RT and covered securely with a foil plate sealer. Each well was aspirated and washed 4 times and 200 μl of conjugate was added to each well and incubated for 2 h at RT. The aspirating and washing step was repeated 4 times and 200 μl of substrate solution was added to each well and incubated for 30 min being protected from light. The reaction was stopped by adding 50 μl of stop solution to each well. The results were read within 30 min at 450 nm, with a λ correction 540 or 570 nm. The average of the duplicate readings has been calculated for each standard and sample.

2.6. Statistics

Data are indicated as mean and standard error mean. Statistical analysis of QRT-PCR and ELISA data was performed using student *t*-test and differences were considered significant at $p < 0.05$.

3. Results

3.1. Increased TSLP expression upon synthetic RARγ-agonist (BMS189961), combinative RARγ-agonist-RXR-agonist and ATRA treatment in mouse skin (Fig. 1)

Topical application of the RXR-agonist (LGD268) resulted in no significant change of TSLP expression in mouse skin, while the application of an RXR-antagonist (UVI3003) decreased TSLP expression ($p < 0.01$) (Fig. 1A). An RARα-agonist (BMS753) reduced, while an RARγ-agonist increased dermal TSLP expression. Topical application of ATRA also significantly increased TSLP expression, while an RAR-pan antagonist (BMS493) significantly reduced it (Fig. 1B). Combinative application of an RARγ-agonist and an RXR-agonist significantly increased TSLP expression up to ca. 50 times (Fig. 1C).

3.2. Increased TSLP expression upon combinative VDR-agonist-RXR-agonist treatment in mouse skin (Fig. 2)

Topical application of a VDR-agonist (calcitriol, MC903) showed a tendency of increased TSLP expression ($p = 0.08$), while the combination with an RXR-agonist significantly increased TSLP expression ca. 5 times . Respectively, an RXR-agonist alone did not display significant influence on TSLP expression.