



Benzopyrene, a major polyaromatic hydrocarbon in smoke fume, mobilizes Langerhans cells and polarizes Th2/17 responses in epicutaneous protein sensitization through the aryl hydrocarbon receptor

Chien-Hui Hong^{a,b,1}, Chih-Hung Lee^{c,d,1}, Hsin-Su Yu^{e,f}, Shau-Ku Huang^{f,*}

^a Department of Dermatology, National Yang Ming University, Taipei, Taiwan

^b Department of Dermatology, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan

^c Department of Dermatology, Kaohsiung, Chang Gung Memorial Hospital, Kaohsiung, Taiwan

^d Department of Dermatology, Chang Gung University, Taoyuan, Taiwan

^e Department of Dermatology, Kaohsiung Medical University, Kaohsiung, Taiwan

^f National Health Research Institute, Miaoli, Taiwan

ARTICLE INFO

Article history:

Received 30 December 2015

Received in revised form 6 April 2016

Accepted 7 April 2016

Available online xxxx

Keywords:

Benzopyrene

Epicutaneous protein sensitization

Langerhans cells

Aryl hydrocarbon receptor

ABSTRACT

Background: Atopic dermatitis (AD) is a common disease with genetic and environmental interactions. We previously reported lifetime exposure to cigarette smoke is associated with adult-onset AD. Aryl hydrocarbon receptor (AhR) is important in regulating environmental exposure to xenobiotics, including benzopyrenes (BP), a major polycyclic aromatic hydrocarbon (PAH) present in cigarette smoke. However, how AhR regulates immune responses in sensitization phase of AD remained elusive.

Methods: We investigated how BP affects epicutaneous sensitization response through AhR axis. We compared AhR expression in skin from AD patients and healthy controls. We measured immune responses (Langerhans cell migration and T cell polarization in epicutaneous Ova sensitization in mice with or without AhR defect.

Results: We found AhR and ARNT (AhR nuclear translocator) are upregulated in AD skin. BP exposure increases Langerhans cell migration, and increases IL-5, IL-13, and IL-17 levels when lymph node cells were re-challenged with Ova. The increased cytokine levels were attenuated in AhR defected mice. AhR agonists (BP and ITE) decreased E-cadherin expression, while AhR antagonist (CH223191) increased it in human primary keratinocytes.

Conclusions: These results suggested AhR interacts with BP to polarize T cell responses, along with Langerhans cell migration. This study revealed a regulatory mechanism how cigarette smoking affects atopic sensitization through the benzopyrene-AhR interaction.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Atopic dermatitis (AD) is a common chronic relapsing inflammatory skin disease which usually heralds other types of atopic diseases [1], including bronchial asthma, allergic rhinitis, and allergic conjunctivitis. AD affects about 10% of the general population [2]. Studying early events of the “atopic march” contributes to the development of targeting therapy for atopic diseases [3]. The molecular pathogenesis of AD may involve abnormal epidermal differentiation and aberrant immune responses of inducible skin-associated lymphoid tissue (iSALT) [4]. For example, genetic mutations of filaggrin, an important epidermal differentiation molecule, has been found in a significant

proportion of patients with AD [5]. The aberrant immune responses in AD involve the regulation of T cells, antigen presentation cells, and cells in innate immunity among the iSALT [6].

In addition to genetic factors, environmental factors and immune adaptations to these factors may contribute importantly to the occurrence of AD as well as disease severity and disease course [2]. Little research has been undertaken to elucidate the regulatory mechanisms of skin immunity adaptation to the environmental stress [7]. However, aryl hydrocarbon receptor (AhR) and the aryl hydrocarbon receptor nuclear translocator (ARNT) axis are known to lie at the intersection of several crucial adaptive pathways in cells facing environmental stresses [8]. For example, when ligand binds to environmental chemicals such as dioxin (TCDD), AhR translocates into the nucleus and dimerizes with ARNT (AhR nuclear translocator), an important transcriptional factor [9]. AhR and ARNT are known to recognize many small xenobiotic and natural molecules and to play an role in the pathogenesis of many human diseases [10].

* Corresponding author at: National Environmental Health Research Center, No. 35, Keyan Road, Zhunan Town, Miaoli County 35053, Taiwan.

E-mail address: skhuang@nhri.org.tw (S.-K. Huang).

¹ Equal contribution.

ARNT is required for tumor initiation by benzo[*a*]pyrene (BP) in mouse epidermis [11]. It might be possible that BP could bind to AhR to activate ARNT, which would lead to tumor formation. Our previous study reported an association between a lifetime accumulated risk to cigarette smoking and the development of AD [12]. The tobacco smoke contains many chemicals and polycyclic aromatic hydrocarbons (PAH) [13], a major portion being BP [14]. BP may induce aberrant immune responses [15] and the subsequent development of cancers [16]. Aside from its role in carcinogenesis, BP is able to bind with high specificity to the aryl hydrocarbon receptor (AhR), activating ARNT, modifying the expression of CYP1A1, and regulating inflammation [17]. Vigorous efforts have been made to study the role of AhR/ARNT axis in cancers initiated by BP [11,18]. However, little research attention has been paid to understand the mechanisms underlying apparent inflammatory and erythematous responses following epicutaneous exposure to BP. Because targeted K14-driven deletion of ARNT in the mouse epidermis has been reported to cause failure of epidermal barrier function [19], ARNT may be critical to homeostasis of skin barrier function, making it a potential candidate in AD. Roberston et al., using in vitro skin equivalents, showed that ARNT controls the expression of epidermal differentiation genes through HDAC- and EGFR-dependent pathways, possibly impairing barrier function leading to the development of AD [20]. However, it is unknown how AhR/ARNT axis regulates the epicutaneous sensitization process of AD. This study aims to determine the role of AhR/ARNT in the sensitization process of AD by animal studies, cell models, and human atopic skin examinations.

2. Materials and methods

2.1. Immunofluorescence (IF)

The specimens of skin from AD and controls were obtained from 8 patients (4 men and 4 women aged 21–48 years) and 6 controls (3 men and 3 women aged 25–57 years). All patients and control subjects provided informed consent before receiving biopsies. This study was approved by the Institutional Review Board of the affiliated hospital. For the animal study, mouse skin samples were obtained by 2-mm punch biopsy. The tissue sections from both humans or mice were fresh frozen, fixed in acetone (Sigma, St Louis, MO) at 20 °C for 5 min, air dried for 5 min, and washed in PBS for 15 min. They were incubated overnight at room temperature in a humidifier with or without the addition of a rabbit anti-AhR IgG (1:50, Santa Cruz Biotechnology, Dallas, TX), a rabbit anti-ARNT IgG (1:100, Cell Signaling, Danvers, MA), or a rabbit anti-CYP1A1 IgG (Novus Biologicals, Littleton, CO). The slides were washed in PBS, and then incubated with either Alexa Fluor® 488 -goat anti-rabbit IgG or Alexa Fluor® 568-goat anti-rabbit IgG (Invitrogen, Carlsbad, CA) for 1 h at room temperature. Nuclei were counterstained with DAPI. Image analysis was performed by NIH Image J, an open image analysis software program (<http://rsbweb.nih.gov/ij/>) with a plugin that measures the fluorescent intensity score from 0 to 255. Numbers of positive-stained cells were measured and averaged in the epidermis from five random high power fields. Based on an artificial cut point in the intensity score, the percentages of AhR-, ARNT-, or CYP1A1-positive-staining epidermal DAPI-stained nucleated cells in the epidermis were then calculated. Similarly, the fluorescent intensity (relative unit from 0 to 255) of E-cadherin per keratinocytes from five random high power fields was also acquired for further statistical analysis.

2.2. Mice

Female C57BL mice (8–12 weeks old) were obtained from the National Laboratory Animal Center-Tainan Facility (Tainan, Taiwan). AhRd congenic mice, which have been described previously [21]

and which are characterized as having a low affinity form of cytosolic Ah receptor, were obtained from Jackson Laboratory (Bar Harbor, ME). AhRd congenic mice (B6.D2N-Ahrd/J) carry a low-responder allele (AhRd), whereas C57BL/6 mice carry high-responder allele (AhRb-1) [21]. It has been demonstrated that the affinity of AhRd for ligand is 10–100 times lower than that of AhRb alleles [22]. All mice were housed in a specific pathogen-free animal facility and treated following experimental protocols approved by the Animal Care and Use Committee of the Kaohsiung Medical University (Approval Number 96019). OVA (Grade V) was purchased from Sigma-Aldrich (St Louis, MO).

2.3. Ova-epicutaneous sensitization with patched BP

Mice were immunized as previously described [23]. Briefly, 20 µl of OVA (100 mg/ml) was placed on the disc of a Finn chamber (Epitest, Tuusula, Finland). This disk was applied to an area of shaved skin on the back of a mouse. For each course of immunization, freshly prepared OVA patches were applied on five consecutive days. The BP-treated group was patched with BP at 0.5 ppm/mice/day immediately prior to receiving the patch on days 1–5. Control mice received a similar treatment but patched with acetone.

2.4. Flow cytometry

Skin-draining LNs (axillary, subscapular, and inguinal) were excised 96 h after the start of the immunization course. LN cells were prepared by digestion of isolated LNs with 2.5 mg/ml collagenase for 30 min at 37 °C, and the CD11c⁺ cells were isolated by anti-CD11c microbeads (Miltenyi, Bergisch Gladbach, Germany). Cells were stained with combinations of antibodies (CD11c-APC, CD207-PE) (both from e-Bioscience, San Diego, CA) and their isotype controls.

2.5. Cell counting and ELISA

Ten days after the start of the immunization course, mice were sacrificed, and axillary, subscapular, and inguinal LNs were harvested. Pooled LN cells (1×10^6) were cultured in the presence or absence of 100 mg/ml OVA. Cell proliferation was measured using an automatic counter (Countess, Invitrogen, Carlsbad, CA) according to manufacturer's directions. Supernatants were harvested 48 h later and stored at minus 80 °C. The levels of IL-5, IL-13, IL-10, TGF-β, and IL-17 were measured by standard sandwich ELISAs (R&D, Minneapolis, MN) with the limit of detection at 10 pg/ml.

2.6. Measurement of E-cadherin in human primary skin keratinocytes treated with AhR agonists and antagonists

Human primary cultured keratinocytes were obtained from adult foreskins as previously described [12]. Keratinocytes at early passage (typically <5) were then grown in keratinocyte-SFM medium (Gibco, Grand Island, NY) free of supplements 24 h before the experiments. Keratinocytes reaching 80–90% of confluence were treated with AhR ligands (ITE 5 µM or BP 10 µM) or AhR antagonists (CH223191 10 µM) for 24 h. The expression of E-cadherin was measured by immunofluorescence as described above. The keratinocytes were incubated overnight at room temperature in a humidifier with or without the addition of a rabbit anti-E-cadherin IgG (1:100, Abcam, Cambridge, MA). The slides were washed in PBS, and then incubated with Alexa Fluor® 568-goat anti-rabbit IgG (Invitrogen, Carlsbad, CA) for 1 h at room temperature. Nuclei were counterstained with DAPI.

2.7. Statistical analyses

The numeric variables between two groups were compared by non-parametric Mann-Whitney *U* test. These numeric variables include the

cell numbers of lymph nodes after Ova re-challenge, the cytokine concentrations of the supernatant collected from the lymph node cells after Ova re-challenge, and the fluorescent intensity (relative unit per cell) of *E*-cadherin expressions in keratinocytes. The ratio variables (for example, the percentages of AhR-, ARNT-, or CYP1A1-positive-stained cells among epidermal DAPI-stained nucleated cells in the epidermis, and the percentage of CD207 and CD11c double positive cells) were compared by Chi-square test using SPSS version 14 (Chicago, IL). A *p*-value <0.05 was considered significant with two tails.

3. Results

3.1. Expressions of ARNT and AhR were increased in atopic human skin

To find out whether the expressions of AhR, CYP1A1, and ARNT were increased in skin from patients with AD, we performed immunofluorescent exams of skin tissue sampled from patients with AD and normal controls (*n* = 8 and 6, respectively). In the control skin, the expressions of AhR, CYP1A1, and ARNT in epidermal keratinocytes were minimal (Fig. 1). While the expressions of CYP1A1 were similar in the

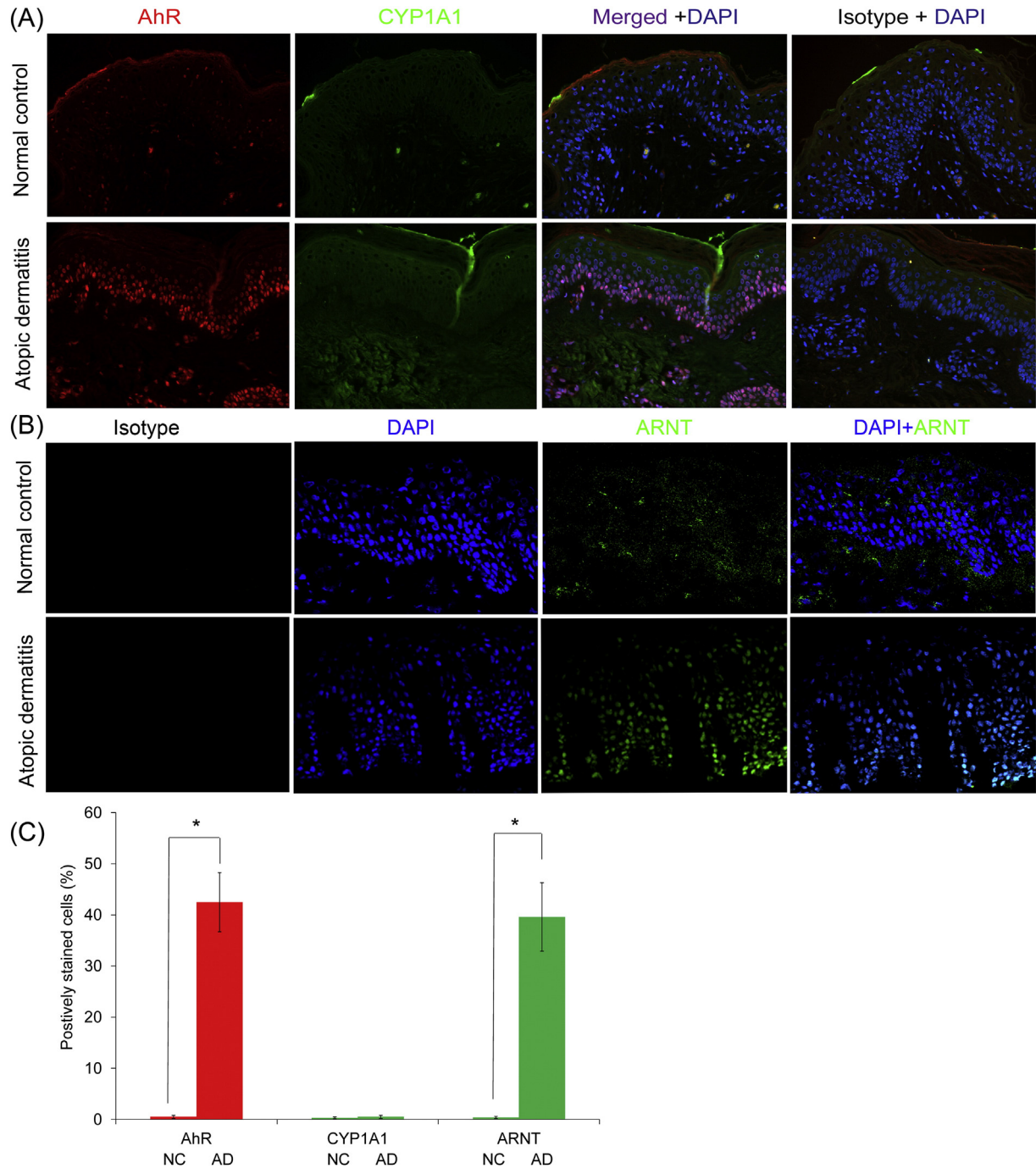


Fig. 1. Expressions of AhR and ARNT were increased in skin from atopic dermatitis (AD). Skin specimens were obtained from AD and controls. The expressions of (A) AhR, CYP1A1, and (B) ARNT were measured by immunofluorescence. (C) The percentages of cells expressing AhR, ARNT, or CYP1A1 among DAPI positive cells in epidermis are shown (3 repeated experiments for skin samples from 8 AD and 6 control skin samples). Representative data are shown in (A) and (B). * denotes *p* < 0.05.

epidermis from AD and in that from control skin (percentage of CYP1A1 positive stained cells among DAPI positive nucleated cells averaged from five randomly sampled high power fields), we found a significant increase in expressions of AhR in AD epidermal skin as compared to those in control epidermal skin (Fig. 1A, C). Similarly, we found a significant increase in expressions of ARNT in AD epidermal skin as compared to those in control epidermal skin (Fig. 1B, C). In addition to the increased expressions of AhR in basal keratinocytes in epidermis, interestingly, there was also a moderate increase in AhR expression in the dermal cells of AD skin (data not shown).

3.2. Epicutaneous protein sensitization of BP enhanced Langerhans cell migration and cell proliferation with Th2 and Th17 responses

A typical initial phase of AD is the sensitization stage caused by exogenous proteins. To investigate whether AhR activation would

modulate the epicutaneous protein sensitization stage of AD, we used an Ova-epicutaneous sensitization model, together with simultaneous painting of the AhR ligand BP, to study the effect of AhR/ARNT axis in T cell polarization and Langerhans cell migration in the sensitization phase. BP painting induced significant epidermal thickening in Ova-patched mice (Fig. 2A). It also increased the migration of Langerhans cells (CD207/CD11c) to regional lymph nodes (Fig. 2B). When the lymph node cells were challenged with Ova, cell proliferation increased (Fig. 2C). Furthermore, while PBS patch (without Ova) with either acetone or BP painting in mice led to no significant in vitro Ova-specific induction of IL-5, IL-13, IL-10, IL-17, and TGF- β in Ova-challenged lymph node cells, BP painting significantly increased the IL-5, IL-13, and IL-17 production in Ova-challenged lymph node cells from mice treated with Ova (Fig. 2D). In contrast, this increase was not observed for IL-10 and TGF- β .

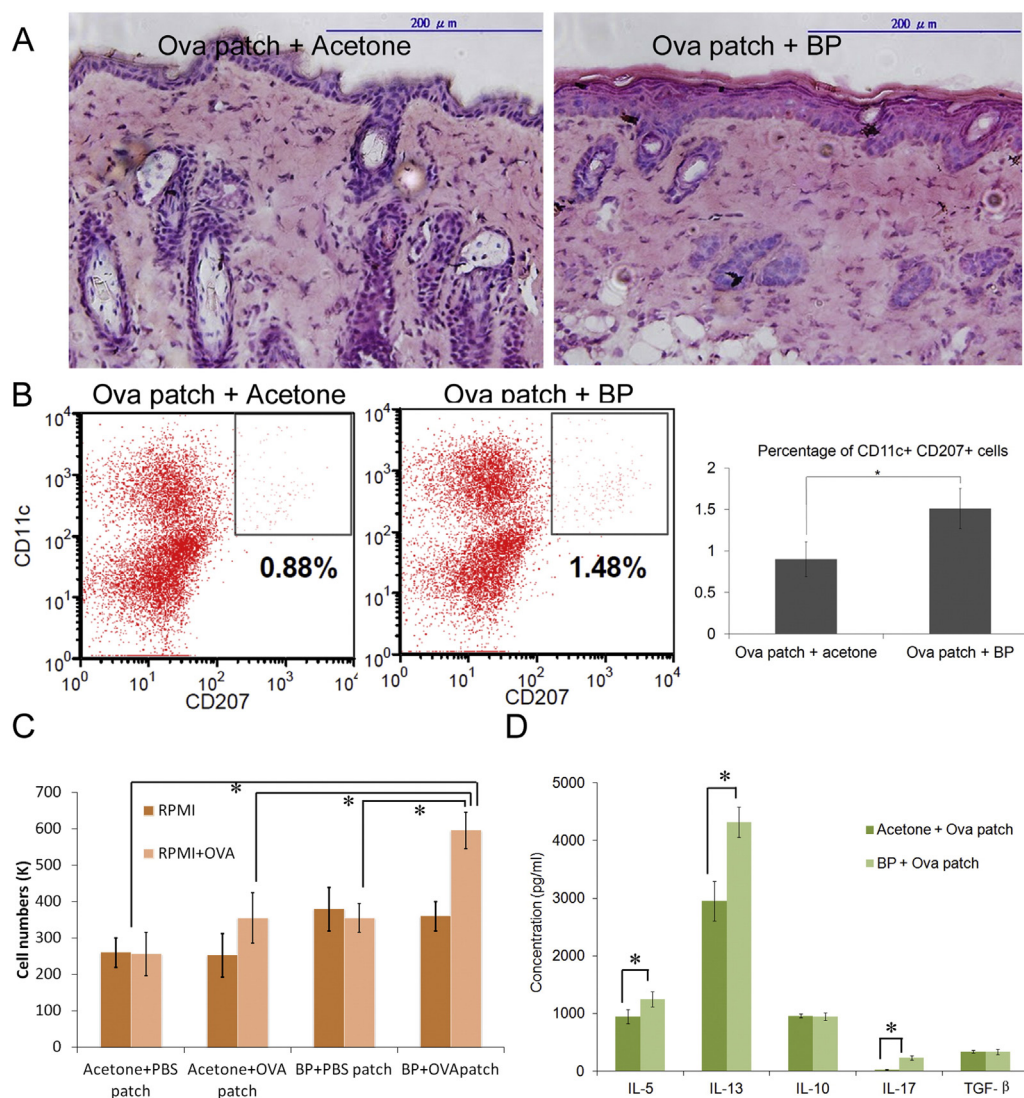


Fig. 2. Langerhans cell migration and Th2/Th17 polarization were enhanced in Ova epicutaneous sensitization. (A) The backs of mice were patched with Ova either with acetone control or benzopyrene (BP) consecutively for 5 days. Skin samples were obtained by punch biopsy and processed with regular H&E stain. (B) Skin-draining lymph nodes were isolated and the cells from these lymph nodes were pooled for flow cytometry (5 mice in each group; two repeated experiments). The lymph node cells were stained with CD207-PE and CD11c-FITC. The percentages of CD207 and CD11c positive cells in lymph nodes (duplicates) from 5 mice in each group in one of the two repeated experiment are summarized in the bar graph. (C) Cells from lymph nodes were challenged with Ova 100 mg/ml for 24 h in vitro. Cell numbers were calculated by an automatic counter. (D) Supernatants were harvested 48 h later and stored at -80°C . The levels of IL-5, IL-13, IL-10, TGF- β , and IL-17 were measured by standard sandwich ELISAs. The increases in cytokine levels are Ova-specific since cytokine levels from lymph nodes treated with RPMI in vitro (but not Ova) were undetectable. There were 5 mice in each group; 2 repeated experiments with triplicates. The bar graph was based on the data from one of the two repeated experiments.* denotes $p < 0.05$.

3.3. Th2 and Th17 response was diminished in lymph node cells, when rechallenged with Ova, from Ova-patched AhR defected mice

To find out if the AhR/ARNT axis played an important role in the enhanced epicutaneous sensitization response, we repeated the above Ova epicutaneous sensitization model in AhR defected mice instead of wild type mice. While the RPMI treatment resulted in no antigen-specific induction of cytokines (IL-5, IL-13, IL-10, IL-17, and TGF- β) in lymph nodes cells, it turns out that the enhanced Ova-specific Th2 and Th17 responses by BP were attenuated in AhR defected mice (decreases in IL-5, IL-13, and IL-17 levels), suggesting that AhR/ARNT axis plays a role in the enhancement of Th2/Th17 responses by BP in the protein epicutaneous sensitization (Fig. 3). Together, these results suggest that BP epicutaneous exposure regulates the polarization of Th2/Th17 responses in protein epicutaneous sensitization response.

3.4. AhR activation by BP in keratinocytes increased the expression of E-cadherin

It has been reported that AhR-deficient mice exhibited a substantial exacerbation imiquimoid-induced psoriasis model as compared to AhR-sufficient controls, attributable mostly to epidermal keratinocytes [24]. Since we found an enhanced migration of LC and Th2/Th17 polarization in BP/Ova-patched mice, we asked whether the AhR activation in keratinocytes, the major cell population in epidermis, decreased expression of E-cadherin in keratinocytes, allowing the detachment and migration of the adjacent LC from keratinocytes. We treated keratinocytes with ligands (ITE and BP) and antagonists (CH223191) of AhR and measured the expressions of E-cadherin by immuno-fluorescence (Fig. 4) as average fluorescent intensity from five randomly sampled high power fields. E-cadherin expression was clearly present in the cell membrane of culture keratinocytes (the DMSO control group). Treatment of keratinocytes with the AhR antagonist CH223191 induced a mild increase in the expression of E-cadherin. Treatment of the keratinocytes with AhR ligands, ITE or BP, resulted in a loss of E-cadherin. This finding could be considered a possible explanation for how BP induces LC migration in the protein epicutaneous sensitization model.

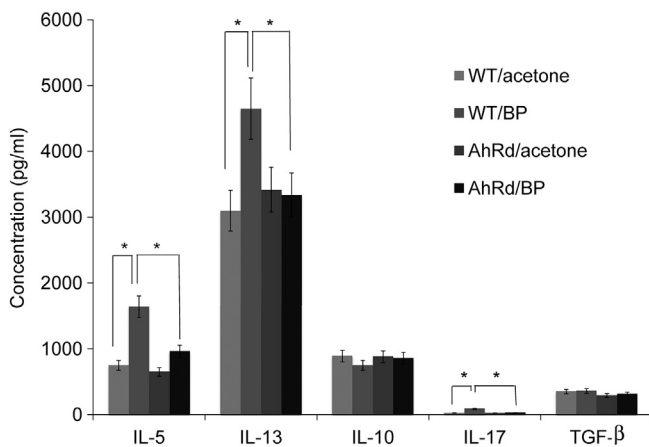


Fig. 3. Th2/Th17 polarization was diminished in BP-patched AhR defected mice. The same experiment procedures used to performed the experiment described in Fig. 3D were repeated with AhR defected mice instead of wild type mice. Supernatants were harvested 48 h later and stored at minus 80° until assayed. The levels of IL-5, IL-13, IL-10, TGF- β , and IL-17 were measured by standard sandwich ELISAs (5 mice in each group; 2 repeated experiments with triplicates, * $p < 0.05$). The increases in cytokine levels are Ova-specific since cytokine levels from lymph nodes treated with RPMI in vitro (but not Ova) were undetectable. The bar graph was based on the data from one of the two repeated experiments.

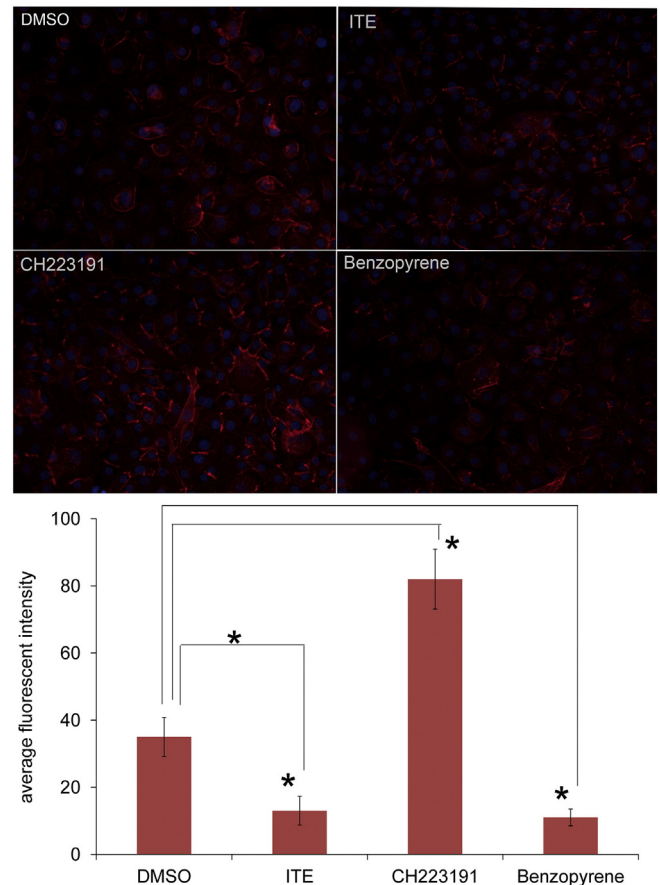


Fig. 4. AhR activation by BP in keratinocytes decreased E-cadherin expression. Human primary cultured keratinocytes were obtained from adult foreskin. Early passage of keratinocytes reaching 80–90% of confluence was treated with AhR ligands (ITE 5 μ M and BP 10 μ M) or AhR antagonists (CH223191 10 μ M) for 24 h. From two repeated experiments, the expressions of E-cadherin were measured by immunofluorescence exam. In each experiment, there were three sets of keratinocytes used for the four groups (DMSO, ITE, BP, and CH223191). Representative immunofluorescent images are shown. The average fluorescent intensity (from five high power fields) is summarized in the bar graph based on the data from one of the two repeated experiments. * $p < 0.05$.

4. Discussion

In this study of the role AhR/ARNT might play in the sensitization process of AD, we first found AhR and ARNT to be increased in the epidermis from patients with AD. Our mouse experiment showed that exposure to BP, an AhR ligand, resulted in increased Langerhans cell migration as well as increased IL-5 and IL-13 levels when rechallenged with Ova. The enhanced Th2 and Th17 responses were attenuated in AhR defected mice. These results suggest that AhR/ARNT axis might interact with smoke fumes to induce Th2 17 polarization in epicutaneous protein sensitization.

AD is characterized by dysregulated immune responses, abnormal epidermal differentiations, and impaired skin barriers. For dysregulated immune responses, our study first found both AhR and ARNT to be increased in human AD skin. Cutaneous exposure to BP causes a polarized Th2 responses in mice [25], an effect which may result from the activation of AhR/ARNT axis. The fact that regulations by AhR/ARNT in epidermal keratinocytes are important in AD is evidenced by the fact that constitutive activation of the AhR pathway in keratinocytes causes atopic-like skin lesions in transgenic mice after exposure to occupational and environmental xenobiotics [26]. In addition, one recent study found that keratinocytes were responsible in an imiquimoid-induced proinflammatory psoriasis model through upregulation of AP-1 transcription factors [24]. Regarding to the abnormal epidermal differentiations and impaired skin barrier, treatment with emollient, which helps

restore the skin barrier, is the cornerstone in the treatment of AD. Paradoxically, coal tar, the classic emollient, has been shown to activate AhR, resulting in the induction of epidermal differentiation and the repair of skin barrier functions in an organotypic skin model [27]. Coal tar has been shown to interfere with Th2 cytokine signaling via dephosphorylation of STAT6, most likely due to AhR-regulated activation of the NRF2 antioxidative stress pathway in the same model [27]. This discrepancy of the altered expressions and activation of AhR in AD of that study from the current study may result from (A) the differences in the heterogeneous components of coal tar used in that study and more pure BP used in the current study; (B) the differences of the organotypic model in that study and the animal study with human AD samples in the current study. It is likely that the increased AhR expressions in basal keratinocytes of AD may facilitate the treatment responses to coal tar emollient in AD skin.

AhR and ARNT genetic polymorphisms have been associated with a variety of human cancers, including colon cancers [28], lymphomas [29], lung cancers [30], and breast cancers [31]. In DMBA-induced inflammation and carcinogenesis mouse model, AhR polymorphism was found to modulate the inflammatory phenotype in those mice [32]. Thus, AhR may regulate inflammation-assisted carcinogenesis. In addition to the polarized Th2 response in BP-patched epicutaneous sensitization model, a mild elevation of Th17 response was observed, a finding similar to a recent study showing selective AhR expression on Th17 cells in the induction of the cytokine IL-22 [33].

In this study, the AhRd mice were used. The affinity of AhRd for its ligand is 10–100 times lower than that of AhRb alleles [22]. An apparent T to C transition replaces the opal termination codon in the C57BL/6J allele with an arginine codon in the AhRd allele, resulting in a bigger transcript. A second T to C transition changes a leucine codon in the C57BL/6J allele to a proline codon in the AhRD allele, resulting in different ligand affinity. One limitation that the AhR-defected mice used in the current study are not condition knockouts, which obscures the role of AhR in specific skin cells. Either the differences in the AhR activation and processing other than ligand binding affinity or the expression of AhR in cells other than epidermal keratinocytes might changed the in vivo results we observed in this study. However, this study found the increased expressions of AhR and ARNT in the epidermis of human AD skin. In transgenic mice, constitutive activation of the AhR pathway in keratinocytes is reported to cause atopic-like skin lesions [26], indicating the role of AhR/ARNT is likely regulated in epidermal keratinocytes.

In summary, this study provides evidence that AhR/ARNT axis polarizes a predominant Th2 and Th17 responses in epicutaneous protein sensitization, a finding that might help explain how tobacco smoking induces atopic dermatitis during the sensitization phase.

Conflict of interests

The authors declare no conflict of interests.

Acknowledgement

This study is supported by research funding provided by the Ministry of Science and Technology (NSC102-2314-B-010-005-MY2), Kaohsiung Veterans General Hospital (KSC104-050), and Kaohsiung Chang Gung Memorial Hospital (CMRPG8C0821).

References

- [1] C.H. Lee, H.Y. Chuang, C.C. Shih, S.B. Jong, C.H. Chang, H.S. Yu, Transepidermal water loss, serum IgE and beta-endorphin as important and independent biological markers for development of itch intensity in atopic dermatitis, *Br. J. Dermatol.* 154 (6) (2006) 1100–1107.
- [2] C.H. Lee, H.Y. Chuang, C.C. Shih, S.H. Jee, L.F. Wang, H.C. Chiu, C.H. Chang, C.S. Wu, H.S. Yu, Correlation of serum total IgE, eosinophil granule cationic proteins, sensitized allergens and family aggregation in atopic dermatitis patients with or without rhinitis, *J. Dermatol.* 31 (10) (2004) 784–793.
- [3] C.H. Lee, H.S. Yu, Biomarkers for itch and disease severity in atopic dermatitis, in: T. Shiohara (Ed.), *Current Problems in Dermatology*, Karger, Basel 2011, pp. 136–148.
- [4] K. Kabashima, New concept of the pathogenesis of atopic dermatitis: interplay among the barrier, allergy, and pruritus as a trinity, *J. Dermatol. Sci.* 70 (1) (2013) 3–11.
- [5] C.N. Palmer, A.D. Irvine, A. Terron-Kwiatkowski, Y. Zhao, H. Liao, S.P. Lee, D.R. Goudie, A. Sandilands, L.E. Campbell, F.J. Smith, G.M. O'Regan, R.M. Watson, J.E. Cecil, S.J. Bale, J.G. Compton, J.J. DiGiovanna, P. Fleckman, S. Lewis-Jones, G. Arseculeratne, A. Sergeant, C.S. Munro, B. El Houate, K. McElreavey, L.B. Halkjaer, H. Bisgaard, S. Mukhopadhyay, W.H. McLean, Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis, *Nat. Genet.* 38 (4) (2006) 441–446.
- [6] K.M. Leiferman, Eosinophils and itch: partners in crime or strange bedfellows? *J. Allergy Clin. Immunol.* 135 (2) (2015) 488–490.
- [7] C. Esser, A. Rannug, B. Stockinger, The aryl hydrocarbon receptor in immunity, *Trends Immunol.* 30 (9) (2009) 447–454.
- [8] J. Abel, T. Haarmann-Stemmann, An introduction to the molecular basics of aryl hydrocarbon receptor biology, *Biol. Chem.* 391 (11) (2010) 1235–1248.
- [9] J. Mimura, Y. Fujii-Kuriyama, Functional role of AhR in the expression of toxic effects by TCDD, *Biochim. Biophys. Acta* 1619 (3) (2003) 263–268.
- [10] D.W. Nebert, A. Puga, V. Vasilou, Role of the Ah receptor and the dioxin-inducible [Ah] gene battery in toxicity, cancer, and signal transduction, *Ann. N. Y. Acad. Sci.* 685 (1993) 624–640.
- [11] S. Shi, D.Y. Yoon, K.C. Hodge-Bell, I.G. Bebenek, M.J. Whitekus, R. Zhang, A.J. Cochran, S. Huerta-Yepez, S.H. Yim, F.J. Gonzalez, A.K. Jaiswal, O. Hankinson, The aryl hydrocarbon receptor nuclear translocator (Arnt) is required for tumor initiation by benzo[a]pyrene, *Carcinogenesis* 30 (11) (2009) 1957–1961.
- [12] C.H. Lee, C.H. Hong, W.T. Yu, H.Y. Chuang, S.K. Huang, G.S. Chen, T. Yoshioka, M. Sakata, W.T. Liao, Y.C. Ko, H.S. Yu, Mechanistic correlations between two itch biomarkers, cytokine interleukin-31 and neuropeptide beta-endorphin, via STAT3/calcium axis in atopic dermatitis, *Br. J. Dermatol.* 167 (4) (2012) 794–803.
- [13] H. Rubin, Synergistic mechanisms in carcinogenesis by polycyclic aromatic hydrocarbons and by tobacco smoke: a bio-historical perspective with updates, *Carcinogenesis* 22 (12) (2001) 1903–1930.
- [14] D.J. Fitzgerald, N.I. Robinson, B.A. Pester, Application of benzo(a)pyrene and coal tar tumor dose-response data to a modified benchmark dose method of guideline development, *Environ. Health Perspect.* 112 (14) (2004) 1341–1346.
- [15] R.E. Albert, M.L. Miller, T.E. Cody, G. Talaska, P. Underwood, A. Andringa, Epidermal cytokines, DNA adducts, and dermal inflammation in the mouse skin in response to repeated benzo[a]pyrene exposures, *Toxicol. Appl. Pharmacol.* 136 (1) (1996) 67–74.
- [16] C. Costa, S. Catania, R. De Pasquale, R. Stancanelli, G.M. Scribano, A. Melchini, Exposure of human skin to benzo[a]pyrene: role of CYP1A1 and aryl hydrocarbon receptor in oxidative stress generation, *Toxicology* 271 (3) (2010) 83–86.
- [17] G. Tsuji, M. Takahara, H. Uchi, S. Takeuchi, C. Mitoma, Y. Moroi, M. Furue, An environmental contaminant, benzo(a)pyrene, induces oxidative stress-mediated interleukin-8 production in human keratinocytes via the aryl hydrocarbon receptor signaling pathway, *J. Dermatol. Sci.* 62 (1) (2011) 42–49.
- [18] S.R. Kondraganti, P. Fernandez-Salguero, F.J. Gonzalez, K.S. Ramos, W. Jiang, B. Moorthy, Polycyclic aromatic hydrocarbon-inducible DNA adducts: evidence by 32P-postlabeling and use of knockout mice for Ah receptor-independent mechanisms of metabolic activation in vivo, *Int. J. Cancer* 103 (1) (2003) 5–11.
- [19] S. Geng, A. Mezentsev, S. Kalachikov, K. Raith, D.R. Roop, A.A. Panteleyev, Targeted ablation of Arnt in mouse epidermis results in profound defects in desquamation and epidermal barrier function, *J. Cell Sci.* 119 (Pt 23) (2006) 4901–4912.
- [20] E.D. Robertson, L. Weir, M. Romanowska, I.M. Leigh, A.A. Panteleyev, ARNT controls the expression of epidermal differentiation genes through HDAC- and EGFR-dependent pathways, *J. Cell Sci.* 125 (Pt 14) (2012) 3320–3332.
- [21] A.B. Okey, L.M. Vella, P.A. Harper, Detection and characterization of a low affinity form of cytosolic Ah receptor in livers of mice nonresponsive to induction of cytochrome P1-450 by 3-methylcholanthrene, *Mol. Pharmacol.* 35 (6) (1989) 823–830.
- [22] C. Esser, The immune phenotype of AhR null mouse mutants: not a simple mirror of xenobiotic receptor over-activation, *Biochem. Pharmacol.* 77 (4) (2009) 597–607.
- [23] C.H. Lee, C.H. Hong, C.L. Yu, L.F. Wang, B.E. Clausen, W.T. Liao, S.K. Huang, G.S. Chen, H.S. Yu, Arsenic mobilizes Langerhans cell migration and induces Th1 response in epicutaneous protein sensitization via CCL21: a plausible cause of decreased Langerhans cells in arsenic-induced intraepithelial carcinoma, *Biochem. Pharmacol.* 83 (9) (2012) 1290–1299.
- [24] P. Di Meglio, J.H. Duarte, H. Ahlfors, N.D. Owens, Y. Li, F. Villanova, I. Tosi, K. Hirota, F.O. Nestle, U. Mrowietz, M.J. Gilchrist, B. Stockinger, Activation of the aryl hydrocarbon receptor dampens the severity of inflammatory skin conditions, *Immunity* 40 (6) (2014) 989–1001.
- [25] K. Kadkhoda, A.A. Pourfathollah, Z. Pourpak, A. Kazemnejad, The cumulative activity of benzo(a)pyrene on systemic immune responses with mite allergen extract after intranasal instillation and ex vivo response to ovalbumin in mice, *Toxicol. Lett.* 157 (1) (2005) 31–39.
- [26] M. Tauchi, A. Hida, T. Negishi, F. Katsuoka, S. Noda, J. Mimura, T. Hosoya, A. Yanaka, H. Aburatani, Y. Fujii-Kuriyama, H. Motohashi, M. Yamamoto, Constitutive expression of aryl hydrocarbon receptor in keratinocytes causes inflammatory skin lesions, *Mol. Cell. Biol.* 25 (21) (2005) 9360–9368.
- [27] E.H. van den Bogaard, J.G. Bergboer, M. Vonk-Bergers, I.M. van Vlijmen-Willems, S.V. Hato, P.G. van der Valk, J.M. Schroder, I. Joosten, P.L. Zeeuwen, J. Schalkwijk, Coal tar induces AHR-dependent skin barrier repair in atopic dermatitis, *J. Clin. Invest.* (2013).
- [28] H. Wang, J.F. Yamamoto, C. Caberto, B. Saltzman, R. Decker, T.M. Vogt, L. Yokochi, S. Chanock, L.R. Wilkens, L. Le Marchand, Genetic variation in the bioactivation

- pathway for polycyclic hydrocarbons and heterocyclic amines in relation to risk of colorectal neoplasia, *Carcinogenesis* 32 (2) (2011) 203–209.
- [29] C.H. Ng, R. Janoo-Gilani, P. Sipahimalani, R.P. Gallagher, R.D. Gascoyne, J.M. Connors, J.P. Weber, A.S. Lai, S. Leach, N.D. Le, A.R. Brooks-Wilson, J.J. Spinelli, Interaction between organochlorines and the AHR gene, and risk of non-Hodgkin lymphoma, *Cancer Causes Control* 21 (1) (2010) 11–22.
- [30] J.H. Kim, H. Kim, K.Y. Lee, J.W. Kang, K.H. Lee, S.Y. Park, H.I. Yoon, S.H. Jheon, S.W. Sung, Y.C. Hong, Aryl hydrocarbon receptor gene polymorphisms affect lung cancer risk, *Lung Cancer* 56 (1) (2007) 9–15.
- [31] J.R. Long, K.M. Egan, L. Dunning, X.O. Shu, Q. Cai, H. Cai, Q. Dai, J. Holtzman, Y.T. Gao, W. Zheng, Population-based case-control study of AhR (aryl hydrocarbon receptor) and CYP1A2 polymorphisms and breast cancer risk, *Pharmacogenet. Genomics* 16 (4) (2006) 237–243.
- [32] V.R. De Souza, W.K. Cabrera, A. Galvan, O.G. Ribeiro, M. De Franco, F. Vorraro, N. Starobinas, S. Massa, T.A. Dragani, O.M. Ibanez, Aryl hydrocarbon receptor polymorphism modulates DMBA-induced inflammation and carcinogenesis in phenotypically selected mice, *Int. J. Cancer* 124 (6) (2009) 1478–1482.
- [33] B. Stockinger, K. Hirota, J. Duarte, M. Veldhoen, External influences on the immune system via activation of the aryl hydrocarbon receptor, *Semin. Immunol.* 23 (2) (2011) 99–105.