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Review Article

Cellular, Molecular, and Pharmacological Characterization of a House Dust Mites (HDM)-Induced Murine Model of Dermatitis

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ABSTRACT

Background: House Dust Mites (HDM), common allergens that can induce Atopic Dermatitis (AD), are widely employed to generate mouse models of AD. In the current study, we compared the AD-like phenotypes between two mouse strains, NC/Nga, and BALB/c, in response to HDM, and performed cellular, molecular, and pharmacological characterization of HDM-induced dermatitis in NC/Nga mice.

Methods: In-life endpoints (skin clinical scores, ear thickness, Transepithelial Water Loss (TEWL), scratching bouts) and terminal endpoints (histopathology, total serum IgE and tissue cytokines) were measured. Further phenotyping of NC/Nga was performed by flow cytometry, gene expression analysis, and pharmacology.

Results: HDM applications resulted in a more robust AD-like dermatitis in NC/Nga than BALB/c mice as evidenced by greater changes in in-life endpoints (clinical scores, ear thickness, scratching bouts, and TEWL), histological markers (overall inflammation, acanthosis, and parakeratosis), and tissue inflammatory cytokines although serum total IgE level is higher in BALB/c than NC/Nga mice. Further flow cytometry analysis of skin immune cells in HDM-treated NC/Nga mice showed increased production of IL-4, IL-13, IL-17A and IFNγ, which was mainly from CD3- cells other than CD3+ cells. The immune/inflammatory responses in NC/Nga mice are supported by gene expression analysis, where multiple pathways are similar to human AD lesional skin. Treatment with JAK1 inhibitor or IL-4R antibody attenuated multiple AD-relevant endpoints in NC/Nga mice.

Conclusion: These data confirm NC/Nga mice are predisposed to HDM-induced dermatitis compared to BALB/c, and their immune profile is complex and shares several relevant pathways/pharmacological mechanisms with human AD.

Keywords: Atopic dermatitis; HDM; NC/Nga; BALB/C; Biostir AD ointment; JAK1 inhibitor; IL-4R antibody; Gene array

INTRODUCTION

Atopic Dermatitis (AD) is a highly prevalent and heterogeneous inflammatory skin disease with complex pathophysiology involving both innate and adaptive immune responses [1,2]. Despite advances in recently approved treatments of AD including dupilumab (a humanized anti-IL-4 receptor antibody) and several Janus Kinase (JAK) inhibitors, a substantial number of patients do not respond adequately or cannot tolerate these approved treatments. Therefore, an unmet need remains for safe and effective therapy for moderate-to-severe AD [1,3]. Because of the complex nature of human AD, there is no single animal

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model that recapitulates all aspects of this disease, although many animal models have been developed [4-6]. The incomplete understanding of model translatability continues to pose a challenge in selecting appropriate platforms to evaluate drug discovery candidates for AD treatment. In order to enhance clinical translatability, a paradigm shift is occurring towards heavier reliance on human models for drug discovery, including Complex *In vitro* Models (CIVM) which are in early phases of development [7,8]. Meanwhile, as the field is assessing translatability of these promising complex experimental human systems, there still remains a place for *in vivo* systems in AD research, for example to assess behavioral effects of a drug candidate on itch or establishing Pharmacokinetic-Pharmacodynamic (PK/PD) relationships in a multi-organ system to guide clinical dosing and biomarker strategies. The emphasis remains on deepening our understanding of mouse models to guide judicious use.

House Dust Mites (HDM) are common aeroallergens associated with human AD. HDM-induced dermatitis in NC/Nga mice is a model that has been studied more thoroughly than others. It captures relevant clinical and pathophysiological aspects of human AD. However, the utility of HDM-induced dermatitis model in NC/Nga mice as a pharmacology platform for drug discovery for AD is uncertain as the data to demonstrate translatability is lacking and immune profiling of this model is also not well understood.

When considering *in vivo* pharmacological platforms for AD drug discovery, factors such as model translatability, accessibility of agents (such as HDM preparation) and animals are crucial to consider. Ideal models should capture mechanisms and phenotypic features relevant to human disease and show reproducibility in the onset of these features. In the case of dermatitis, inducible models are preferred to spontaneous models as the latter are generally less predictable in skin lesion onset and severity [4]. Biostir AD ointment, a commercially available preparation of extracts from a major strain of HDM (*Dermatophagoides farinae*, DF), has been used to model dermatitis in NC/Nga mice under specific pathogen-free conditions. To our current knowledge this new formulation of HDM has not been examined in BALB/c mice, although HDM studies in this strain have been conducted using DF Extract (DFE), showing variable results. One group showed remarkable dermatitis can develop in BALB/c mice exposed to DFE when tape-stripping was used to disrupt the skin barrier before DFE application. In contrast, other studies showed no typical skin lesions in BALB/c mice. As NC/Nga mice are only bred in Japan, BALB/c mice may offer a more accessible model. The current study first compared induced AD-like phenotypes between the two mouse strains, NC/Nga and BALB/c, in response to repeated applications of Biostir AD ointment. Further characterization was performed in NC/Nga mice based on the robust induction of AD-like phenotypes and included flow cytometry, skin gene expression analysis, and pharmacology validation. For pharmacology validation, we tested a selective JAK1 inhibitor, ABT-317 and a surrogate anti-IL-4 receptor antibody, which are tools for clinically proven pathophysiological mechanisms for AD.

LITERATURE REVIEW

Animals

All animal studies were reviewed and approved by AbbVie's Institutional Animal Care and Use Committee (IACUC) and thus performed in accordance with the IACUC guidelines. The animal program at AbbVie is accredited by the American Association of Accreditation of Laboratory Animal Care. Female NC/Nga mice (6-8 weeks old) were purchased from Charles River Laboratories Japan (Tokyo, Japan). Age-matched female BALB/c mice were purchased from Charles River Laboratories (Horsham, PA, USA). Animals are quarantined/acclimated for 1-2 weeks before study initiation.

Induction of dermatitis

The model of AD-like dermatitis was generated as described previously. In brief, hair in the rostral and upper back area (~4 cm × 4 cm) is removed by shaving and then treatment with a depilatory cream on day 0. To induce dermatitis, 100 mg of Biostir AD ointment (Biostir Inc, Osaka, Japan; referred to as "HDM" herein) was applied topically to the shaved area and surfaces of both ears. To facilitate skin penetration of HDM antigens, the shaved area and ears were pretreated with 4% SDS to temporarily disrupt the skin barrier (10 µL of SDS on both surfaces of each ear and 150 µL on shaved back area of 2 × 3 cm) 1-2 hours before each HDM application. Six applications of HDM were performed twice weekly with the first starting on day 1. Re-shaving is performed as needed to remove the hair regrowth during the study. Sham animals were also treated with 4% SDS. All procedures were executed under isoflurane anesthesia. Animals were sacrificed on day 19, one day after the final HDM application, to collect tissues and blood for terminal endpoints. A general study scheme is shown in Figure 1s.

In-life observations

Ear thickness was measured with a digital caliper (Mitutoyo, Japan) before each application of HDM and 24 hours after the final HDM application. Ear thickness measurements were not significantly different between the right and left ears for both sham and HDM treated mice; data was expressed as the mean of both ears.

AD-like skin lesions were evaluated macroscopically by scoring 4 components as previously described: 1) Erythema/hemorrhage, 2) Excoriation/erosion; 3) Scarring/xerosis; 4) Edema. Each component was scored on a scale of 0-3 (no sign: 0; mild: 1; moderate: 2; severe: 3) with clinical score presented as the sum of the individual scores, ranging from 0 to 12. Clinical scores were taken at 5 time points (days 11, 12, 15, 18, and 19), with example images and clinical scores provided from day 19.

Scratching behavior was recorded with high-speed digital cameras (model: HD-Q7, CCTV Camera Pros, FL, USA) at 6 hours and 24 hours after the final HDM application. Recording duration was 30 min for each group of mice after an acclimation period of at least 30 min. Scratching bouts were then counted

manually by replaying the videos. A scratching bout is defined as one or a series of rapid movements (back and forth) of the hind-paw directed to the site of skin lesions, ending with licking, or biting of the toes and/or placement of the hind-paw on the floor.

Trans-Epidermal Water Loss (TEWL), an indicator of skin barrier function, was measured under sevoflurane anesthesia using a Tewameter® TM nano (Courage and Khazawa, Cologne, Germany) by following the manufacturer's instructions. TEWL measurement was obtained from dorsal skin of ears.

Tissue cytokine analysis and serum total IgE detection

For cytokine protein detection in ear tissue, a 5 mm section from the distal ear was collected and flash-frozen. Tissues were homogenized using a Bead Ruptor in a 2 mL tube with ceramic beads (1.4 mm in diameter) and 500 µL of lysis buffer containing 5% of 1 mol/L Tris (pH 7.4), 3% of 5 mol/L NaCl, 1% of Triton 100-X, and 1% of 100x protease inhibitor. Tissue lysates were centrifuged at 12,000 g for 10 min at 4°C. Supernatant was stored at -80°C until analysis. Cytokine content was analyzed with MSD multiplex kits (Meso Scale Discovery, Boston, MA) according to manufacturer's instructions. The cytokine protein level was then normalized to total protein and expressed as pg/mg total protein. Tissue total protein was determined using pierce BCA protein assay kit (ThermoFisher Scientific, USA).

Mouse serum was collected using 0.5 mL serum separator tubes and stored at -80°C until analysis. Total serum IgE was measured using Abcam mouse IgE ELISA kit (Ab157718) by following the manufacturer's instruction (Abcam, UK).

Histopathology

At study termination, ears and dorsal skin were collected and placed in 10% Neutral Buffered Formalin (NBF). Following fixation, tissues were subjected to routine histology processing, embedded in paraffin, cut at 5 µm and stained with Hematoxylin and Eosin (H and E) on a Leica ST5010 autostainer. A board-certified veterinary pathologist blindly evaluated H and E slides both qualitatively and semi-quantitatively for skin pathology attributed to AD-like disease. Semi-quantitative scores (0-3) were assessed based on the severity/degree of overall inflammatory infiltrate, epidermal thickness (acanthosis), eosinophil infiltrate, spongiosis and parakeratotic hyperkeratosis and were adopted from Murray et al. Skin findings that were consistent with normal tissue were given a score of 0. Mild histological findings were assessed with a score of 1, moderate findings were scored a 2 while severe findings were scored as a 3.

Gene array and gene expression analysis

For gene expression analysis, an 8 mm punch from back skin and an 5 × 10 mm ear tissue was placed in 1 mL RNAlater (ThermoFisher Scientific) at 4°C overnight and then transferred to -80°C for storage. Tissues were processed in Qiagen RLT lysis buffer utilizing the Qiagen TissueRuptor II (Germantown, Maryland). RNA was isolated from the homogenates *via* the automated QIAcube Workstation, utilizing the Qiagen RNeasy Fibrous Tissue Mini Kit with DNAse I Digest. Gene array was conducted using Mouse Clariom S Assay HT with 100 ng RNA added to the target preparation protocol, which followed the manufacture's instruction through completion of array plate hybridization. Data was collected on the GeneTitan Multi-Channel Instrument (ThermoFisher), and then analyzed using the SST-RMA preprocessing method within Transcriptome Analysis Console (TAC version 4.0.1) to generate summarized gene expression matrix and technical Quality Control (QC) metrics. All samples passed QC. Given that RIN values pose a confounding factor in Principal Component Analysis (PCA) plot interpretation, RIN was included in the Differential Gene Expression (DEG) analysis, which was conducted utilizing Limma Bioconductor package.

Pathway analysis was performed using Ingenuity Pathway Analysis (IPA) using standard cutoffs of logFC ≥ 1.0 and adjusted p value<0.05 for ear and back skin separately. IPA was also run on a public atopic dermatitis dataset GSE121212 comparing AD lesional to non-lesional skin, using the same cutoffs. The 10 most significant pathways with non-negative z-scores from each analysis are included. Results of the pathway analysis are displayed as -log10 p value. HDM gene expression data is available as GSE261542.

FACS analysis

Flow cytometry analysis on murine skin was performed as previously described. Briefly, ears and back skin from HDM or vehicle-treated mice were finely minced and digested with RPMI 1640 supplemented with 1% HEPES and 1% penicillin-streptomycin (ThermoFisher), 2 mg/mL Collagenase XI (Sigma Aldrich), 0.1 mg/mL DNase (Sigma), and 0.5 mg/mL hyaluronidase (Sigma) for 45 min at 37°C. The reaction was stopped by adding ice cold RPMI 1640 supplemented with 10% HI-FBS (ThermoFisher), 1% each penicillin-streptomycin, sodium pyruvate, HEPES, and non-essential amino acids (all from ThermoFisher). Cell mixture was vigorously vortexed for 15 seconds and filtered through a 40 µm cell strainer to yield single cell suspensions. The samples were acquired using a BD LSRFortessa (BD Biosciences). The FACS files were analyzed using FlowJo v10.9 software (FlowJo, LLC).

Standard cellular surface staining procedures were followed as previously described. Briefly, cells were first labeled with Zombie UV™ fixable viability kit (BioLegend) followed by block of Fc receptors (clone 2.4G2, BD Biosciences) for 15 minutes at room temperature. Cells were then incubated with fluorochrome-conjugated antibodies against cell surface antigens for 30 minutes at 4°C and subsequently washed. Single cell suspensions were stimulated with cell activation cocktail (with Brefeldin A, BioLegend) and incubated at 37°C for 6 hours. Cells were then fixed using fixation/permeabilization buffer (BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit, BD Biosciences) for 15 minutes at 4°C then subsequently washed with BD Perm/Wash™ buffer. Cells were then incubated with fluorochrome-conjugated antibodies against cytokines (in Perm/Wash™ buffer) at 4°C for 30 minutes prior to washing twice

with Perm/Wash™ buffer (Table 1s for listing of flow cytometry antibodies). Cells were resuspended in FACS buffer prior to acquisition.

Pharmacology studies

For pharmacology characterization of HDM-induced AD-like dermatitis in NC/Nga mice, a selective JAK1 inhibitor, ABT-317 was tested. ABT-317 has a similar selectivity profile as Upadacitinib (Rinvoq) which is an FDA-approved treatment for AD. The drug was orally dosed at 10, 30, and 60 mg/kg (BID) starting on day 1. To further provide pharmacological validation of the model, a mouse dupilumab surrogate, an anti-mouse IL-4 receptor alpha antibody was intraperitoneally dosed at 0.3, 3, and 30 mg/kg (twice/week) starting on day 1. Dupilumab is the first biologic product approved for treatment of AD. The anti-mouse IL-4R antibody showed similar potency as dupilumab in blocking IL-4-induced eotaxin release in huma KC cell line and mouse fibroblast cell line (NIH 3T3), respectively (Figure 1s).

Data analysis

Gene expression analysis is provided in the section of Gene array. All other data are expressed as mean ± SEM (standard error of the mean). Statistical analysis and graph preparation was performed using GraphPad Prism version 9 or 10 (GraphPad Software, CA, USA). Comparisons among study groups were conducted by either one or two-way ANOVA (analysis of variance) followed by Dunnett's or Bonferroni's multiple comparisons. A p-value of <0.05 was considered as statistically significant.

RESULTS

NC/Nga mice show stronger AD-like phenotype, skin pathology features, and increase of proinflammatory mediators than BALB/c mice after exposure to HDM

Since both NC/Nga and BALB/c mice have been reported to exhibit dermatitis-like phenotypes after exposed to HDM of various preparations, we have conducted a side-by-side comparison of both strains in response to a relatively new formulation of HDM (Biostir AD ointment) in our pilot study to select a more appropriate strain for further characterization and model development. By following a 19-day protocol with 6 applications of HDM, a more robust AD-like phenotype including skin histopathological changes and increases of a panel of AD-relevant proinflammatory cytokines has been observed in NC/Nga mice than BALB/c mice in both ears and back skin (Figures 2s–5s for data from ears, and back skin data not shown). Overall inflammation, acanthosis, and parakeratosis in both ear and back skin were more severe in NC/Nga mice compared to BALB/c mice. Increases of inflammatory cytokines IL-4, IL-13, IL-31, TNF-α, IL-1β, IL-17A, and TARC in skin tissues are significantly greater in NC/Nga mice. It should be noted that HDM exposure induced significant elevation of serum total IgE in both strains with the terminal level of serum total IgE significantly higher in HDM treated BALB/c than NC/Nga mice (87.5 ± 8.4 *vs.* 41.5 ± 4.6 ng/mL, $P<0.0001$).

FACS analysis of HDM NC/Nga skin infiltrate reveals complex immune response with both innate and adaptive components

Since changes in in-life endpoints, pathology, and pro-inflammatory cytokines were more robust in NC/Nga than BALB/c mice, subsequent analyses focused only on the NC/Nga model. Flow cytometric analysis was then applied to immune cells in ear and back skin. As histopathology assessment indicated presence of mixed HDM-induced inflammatory infiltrate, and pro-inflammatory cytokines could come from a variety of cell sources, flow cytometry gating strategies were applied to first characterize T-cell infiltrate, then assess T-cell and non-T-cell cytokine sources (Figure 6s). $CD3^+$ cellular count and frequency was significantly increased in both ear tissue and back skin from HDM-treated mice. Under control (sham) conditions, the count and frequency of $CD4^+$ T-cells is already much higher than $CD8^+$ T-cells in both ear and back skin, and neither was further increased upon HDM exposure, with the frequency of $CD4^+$ T-cells significantly reduced in back skin after HDM. In contrast, the count and frequency of $CD8^+$ T-cells was significantly increased in both ear and back skin after HDM treatment, elucidating this subpopulation likely responsible for the overall increase in $CD3^+$ T-cells as seen in Figure 7s.

To determine the major source of HDM-induced cytokines, intracellular staining of type 1 (IFNγ), type 2 (IL-4 and IL-13), and type 3 (IL-17A) cytokines was analyzed in $CD45^+CD3^+$ or $CD3^-$ cells in ear and back skin. There are significant increases in both $CD3^+$ and $CD3^-$ immune cells secreting these cytokines upon HDM stimulation, with the majority of cytokine-producing cells within the $CD3^-$ cell population. Further characterization of $CD3^+$ population showed that both $CD4^+$ and $CD8^+$ cells contributed to the production of these cytokines. In contrast to ears, cellular count and frequency of $CD3^+IL\text{-}13^+$ T-cells and $CD3^+IFN\gamma^+$ T-cells were not significantly increased in back skin. Overall, this data highlights a possible divergence of the HDM mouse model from human AD, where in humans the T-helper cells are widely recognized as key sources of these cytokines [2].

Pathway analysis of HDM-treated NC/Nga skin gene expression shows similarities and differences to human AD

Both skin cytokine and infiltrating immune profiles revealed a mixed inflammatory response, suggesting both innate and adaptive pathways, as well as type 1/2/3 immune mechanisms are evoked during HDM dermatitis in NC/Nga mice. To get a broader perspective of pathways stimulated by HDM treatment, we assessed global gene expression changes, performed pathway analysis, and compared pathways induced in NC/Nga HDM model to those in human AD lesions. Results show that compared to sham tissues, HDM-treated ear and back skin show significant activation in multiple biological pathways, with ear

skin showing a stronger profile. Furthermore, HDM-treated ear skin showed striking similarities to pathways induced in human AD lesional skin including granulocyte adhesion and diapedesis and agranulocyte adhesion and diapedesis, indicating leukocyte adherence to blood vessel walls and migration into the skin tissues. This complements the histological and FACS data, suggesting both innate and adaptive cells are recruited to the skin and likely play a role in the HDM NC/Nga model pathogenesis. Several other pathways are also shared between NC/Nga ear skin and human AD, such as atherosclerosis signaling, pathogen induced cytokine storm signaling pathway, and interleukin-4 and interleukin-13 signaling, highlighting examples where similar immune pathways are active in both the HDM model and human AD. Integrating this knowledge together with the previous FACS findings, our data indicates that although the main cellular sources of proinflammatory cytokines such as IL-4 and IL-13 may diverge between HDM model and human AD, the resulting gene expression patterns and induced downstream pathways of these cytokines may be similar [2].

In addition, this analysis illustrates several instances where NC-Nga HDM model gene expression changes do not align with human AD. For example, several pathways appear uniquely augmented only in the HDM model but not in human AD, such as neutrophil degranulation, cell cycle checkpoints, and acute phase response signaling. In addition, several pathways prevalent in human AD lesional skin appear under-represented in the HDM model, for example S100 family signaling pathway, keratinization, and role of osteoblasts in rheumatoid arthritis signaling pathway. In contrast to HDM treated ear skin, HDM treated back skin shared fewer pathways to human AD, and also demonstrated a unique pattern of pathways related to cellular injury and metabolism, suggesting that the underlying biology is different in back skin [3].

HDM NC/Nga model responds to clinically relevant AD treatments

Dupilumab, an anti-IL4Rα antibody, is the first biologic approved for treatment of AD and blocks IL-4/IL-13 signaling. Since our NC/Nga HDM model exhibits increased IL-4 and IL-13 protein, and IPA of gene signatures showed comparable IL-4 and IL-13 induced downstream pathway trends to human AD, we therefore hypothesized blocking signaling of these cytokines may prevent disease pathogenesis in our mice and tested the mouse surrogate antibody of dupilumab in this model (anti-mouse IL-4Rantibody). As shown in Figure 8s and Table 2s, clinical scores, terminal ear thickness, and serum total IgE was reduced by anti-mouse IL-4Rantibody in a dose-dependent manner. However, effects on TEWL, scratching bouts, and TARC level were not significant although there was a trend of decrease for those endpoints. It should be noted that the elevation of total serum IgE concentration was completely abrogated at the highest 30 mg/kg dose [4].

As antagonism of IL-4α yielded only partial prevention of AD-like symptoms in our mouse model, this suggested other factors outside IL-4 and IL-13 signaling were responsible for the phenotype we observed. Several JAK inhibitors including JAK1-selective upadacitinib have also been approved for treatment of AD. Upadacitinib inhibits wider net of cytokine signaling pathways than dupilumab, including not only IL-4 and IL-13, but also IL-2, IL-6, IL-7, IL-9, IL-15, and type 1 and type 2 interferons, and has proven more efficacious than dupilumab in AD patients. In this model, therefore, we tested ABT-317 which has a similar selectivity profile as upadacitinib. As illustrated in Table 3s, ABT-317 reduced clinical scores, terminal ear thickness, ear skin TEWL, and TARC level in ear skin, in a dose-dependent manner. There was a trend of decrease in scratch bouts that was not dose dependent. Serum total IgE was reduced only by the highest dose (60 mg/kg) tested. Elevation of TARC level was completely abrogated by ABT-317 at 30 and 60 mg/kg. This data suggests that a selective JAK1 inhibitor can improve several AD-relevant features in HDM-induced dermatitis in NC/Nga mice. Further, this data suggests that similar to human AD, targeting the JAK1 signaling pathway may overall lead to a more robust effect on AD-like endpoints as compared to targeting IL-4R, although a head-to-head NC/Nga HDM study would need to be conducted to confirm [5].

DISCUSSION

In the current study, it has been demonstrated that Biostir AD ointment induced a much stronger phenotype of skin inflammation, itch, and barrier impairment in NC/Nga mice compared to BALB/c mice. Drilling deeper into the HDM effects on skin tissues of NC/Nga mice using FACS analysis revealed a mixed immune response with increased production of type 1, type 2, and type 3 cytokines from $CD3^+$ and $CD3^-$ cells. Ingenuity pathway analysis of gene expression showed both similarities and differences between HDM model and human AD, with mouse ear showing greater similarities to human AD signatures than mouse back skin. Notably, anti-mouse IL4Rα antibody and JAK1 inhibitor effectively reduced multiple endpoints in NC/Nga mice with HDM-induced dermatitis [6].

Our observation that NC/Nga mice showed a much stronger AD-like phenotype than BALB/c mice in response to HDM treatment is consistent with previous reports. It is interesting to note that total serum IgE and/or DFE-specific IgE was significantly elevated in BALB/c mice in the current and previous studies even with less robust skin inflammation when compared to NC/Nga mice. In fact, total serum IgE was higher in BALB/c than NC/Nga mice in the current study. These data suggest that serum IgE is likely not closely related to the severity of HDM-induced dermatitis in mice. In addition, lack of correlation between the effects on total serum IgE level and the effects on other endpoints from two pharmacology studies also suggests that IgE may not be essential in the pathophysiology of HDM-induced dermatitis in mice, which is consistent with the current understanding regarding the relevance of IgE in human AD [7].

Previous studies and the current study have shown that HDM-induced dermatitis in NC/Nga mice and human AD share similarities in several aspects such as clinical manifestations (macroscopic skin lesions and itch), histological changes, and impairment of barrier function. However, the immune mechanisms responsible for HDM-induced dermatitis are not

well understood although Ewald et al have shown that NC/Nga mice with spontaneous dermatitis show robust Th1, Th2 and Th17 activation with 18% homology to human meta-analysis-derived AD transcriptome [8]. Increased expression/production of type 1, type 2, and type 3 cytokines has been reported in HDM-treated NC/Nga mice, which is also observed in HDM-treated NC/Nga mice in the current study. Nevertheless, it remained to be elucidated which cells were the main sources of these inflammatory cytokines and whether type 2 cytokines are the major drivers of HDM-induced skin inflammation. Our flow cytometry analysis of skin tissues showed that numbers of $CD4^+IL\text{-}4^+$ cells (Th2), $CD4^+IL\text{-}17A^+$ cells (Th17), and $CD4^+IFN\text{-}\gamma^+$ cells (Th1) were increased, which is consistent with the observation in the draining lymph nodes and $CD4^+$ T cells in skin. In the HDM NC/Nga model, Tc1, Tc2, Tc17 T-cells are also activated contributing to the production of type 1, type 2, and type 3 cytokines, respectively, since the number of $CD8^+IFN\gamma^+$ cells (Tc1), $CD8^+IL\text{-}4^+$ cells (Tc2), and $CD8^+IL\text{-}17A^+$ cells (Tc17) are increased (Figure 9s-12s). Surprisingly, we found that $CD3^-$ cells were the predominant population that stained positive for IL-4, IL-13, IL-17A and IFN-γ. This data indicate that HDM-induced immune response is complex, likely includes innate and adaptive components, and suggest the main cellular sources of inflammatory mediators may differ from those in human AD [9].

As summarized in gene expression pathway analysis shows strong similarity of upregulated pathways between the ear skin of HDM mouse model and human AD. Both show enrichment of granulocyte and agranulocyte adhesion and diapedesis pathway, indicating an influx of both innate and adaptive immune cells into tissue, as well as show enrichment of the Interleukin-4 and Interleukn-13 signaling pathway, a key cytokine pathway known to drive pathogenesis in human AD. In contrast, HDM-treated mouse back skin gene expression diverges considerably from human AD, showing a distinct pattern of upregulated pathways, many of which are not present in either ear skin or human AD, and under-representation of several pathways prominently augmented in human AD. These differences between ear and back skin may at least partially be explained by both the increased hair follicle density and depilatory treatment on the back skin, both of which have been known to alter immune responses. Furthermore, some pathways are more strongly represented in the mouse model and are less dominant in human AD, or conversely not prominent in mouse but indeed induced in human AD, providing deeper insights into potential mechanistic differences in the underlying biology in this mouse model and human disease. This is to be expected as several differences exist between our mouse model and human AD, for example chronicity, genetics, environment and contact with external pathogens, skin architecture including hair density and immune/lymphatic differences, and sensory nerve properties. Despite these differences, the AD-like features and response to pharmacology observed in our model are encouraging and corroborate this mouse model possesses several important similarities to human disease.

Pharmacology validationis an important part in the characterization of animal models used for drug discovery. IL-4 receptor alpha-mediated signaling and JAK/STAT pathways are both clinically proven mechanisms in the pathophysiology of AD, and it was encouraging to observe protection from AD-relevant endpoints with both of these mechanisms in our model. To the best of our knowledge, this is the first report showing efficacy of a selective JAK1 inhibitor administered orally in NC/Nga mice with HDM-induced AD-like dermatitis. It appeared that JAK1 inhibitor is more efficacious than anti-IL4Rα mAb in this model, which is consistent with the fact that JAK1 inhibitor upadacitinib demonstrated superior efficacy *vs.* dupilumab in patients with moderate-to-severe AD. The effects of anti-IL4Rα mAb in the model described in the current study are also consistent with that observed in a slightly different AD model in C57BL/6J, which was induced with HDM plus staphylococcal enterotoxins B. Overall, these data demonstrate that blockade of both IL-4Rα and JAK/STAT pathways are efficacious, suggesting that relevant pathophysiological pathways in human AD are also operative in HDM-induced dermatitis in NC/Nga mice, which is supported by the similarities in IPA pathway analysis and upstream regulator analysis between HDM model and human AD. To further enhance translational understanding of this model, one could consider also testing pharmacological blockade of clinical AD failures. As the model described herein appears to share several features of human AD, it holds promise for future studies assessing novel MOA for AD-based drug discovery.

Although results described in the current study significantly increased our understanding of HDM-induced dermatitis in mice, several limitations should be mentioned. For example, although it is a highly heterogeneous disease, all AD endotypes exhibit commonality in featuring increase in type 2 cytokines and Th2 cell expansion in the skin. While we showed an increased production of IL-4 and IL-13 in the mouse skin tissue, we were not able to demonstrate an expanded population of Th2 cells in the skin. The main cellular source of type 2 cytokines was from $CD3^-$ cells, and as a deeper FACS characterization of these $CD3^-$ cells was not performed in these experiments, it is currently not known which cell(s) was the main producer of type 2 cytokines. TARC has been reported to be the most reliable AD biomarker for disease severity. It was significantly increased in ear tissues. In contrast, the level of TARC in back skin (data not shown) is extremely low compared to that in ear skin although pathological changes are similar in ear and back skin in NC/Nga mice. It is unknown if this difference between ear and back skin is related to the increased hair follicle density and depilatory treatment on the back skin.

CONCLUSION

In summary, these data confirm NC/Nga mice are predisposed to the development of HDM-induced dermatitis compared to BALB/c mice. The immune profile in the HDM-induced phenotype is complex, including innate and adaptive responses and evidence of type 1, type 2, and type 3 pathway engagement. These findings are supported by gene expression analysis, which also highlights both similarities and differences comparing the NC/Nga HDM model to human AD. Prophylactic intervention with clinically proven mechanisms led to protection from several AD-relevant endpoints in the HDM-induced dermatitis model.

Taken together, these data elucidate commonalities and divergences to human AD, and suggest that the HDM model has utility in drug discovery for AD although further studies are needed to enhance our understanding of the model.

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DISCLOSURES

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CONFLICT OF INTEREST

Authors have no conflict of interest.

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