Establishment and characterization of patient-oriented, individualized mouse model in atopic dermatitis: AVATAR mouse

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Directed by Professor Chang Ook Park

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This certifies that the Master's Thesis of
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Hye Li Kim

TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

Establishment and characterization of patient-oriented, individualized mouse model in atopic dermatitis: AVATAR mouse

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Atopic dermatitis(AD) is a chronic, relapsing inflammatory skin disease with increasing incidence worldwide. AD shows a variety of immune responses with heterogeneous disease phenotypes. Thus many AD mouse models have been developed, yet the limitation occurs when the findings from mouse model is interpreted into human AD subjects. Therefore, development of humanized mouse model for AD is essential to reduce this gap between mouse model and human subjects, which will lead to more homologous pre-clinical tools for drug development in AD. By establishing the new, customized mouse model (AVATAR mouse) which represents the immune response pattern of individual AD patients, the cornerstone of personalized medicine might be set up in AD. AVATAR mouse was made by using NOD-scid IL2Rγnull (NSG) mouse engrafted with CD3+ T cells and peripheral blood mononuclear cells (PBMCs) derived from AD patients who were sensitized to house dust mite (HDM). The AVATAR mice were treated with HDM ointment for 4 weeks. After 4 weeks, we observed that the inflammatory response occurred in AD AVATAR mice treated with HDM ointment, whereas healthy controls (HC) AVATAR mice with HDM ointment did

not. Skin engraftment of human CD3+ T cells increased gradually and maintained its number even after 4 weeks, we also confirmed the presence of human CD1c+CD11c+HLADR+FcεRI+ antigen presenting cells together with human CD3+ T cells, which suggests HDM-specific T-cell responses in our AVATAR mouse. Interestingly, clinical and laboratory correlation between individual AD patients and matched AVATAR mice were also observed. Taken together, our results suggest that AVATAR mouse represents the immune response of individual AD patients, which might enable the individualized, patient-oriented approach to treat AD patients in the near future.

Key words : Atopic dermatitis, avatar mouse, humanized mouse model, personalized medicine, house dust mite

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I. INTRODUCTION

Atopic dermatitis(AD) is a chronic, relapsing inflammatory skin disease with increasing incidence worldwide. Also, AD is a heterogeneous disease showing various prevalence depending on region, age, sex, social and cultural differences[1-5]. Recently, specific immune responses of various AD patient groups have been actively studied and differences in immune responses between age group and race have been reported. AD is characterized by its heterogeneous etiologies, such as the genetic predispositions, environmental factors, patients' immunological abnormalities. Since the triggering factors or exacerbating factors are slightly different for each patient, it is important to select a treatment method suitable for each patient in individualized manner.[6-8]

Therefore, many researches are being actively conducted to effectively treat AD depending on the specific etiologies. Human-based researches may be accompanied by ethical issues, but a well-controlled clinical trial is required. So, animal models have been used as a way to overcome those problems. There have been three major animal models made for in vivo studies that simulate

human atopic dermatitis. One of the AD mouse models is an inbred model reflecting the natural course of AD in human. Representative examples are NC/NgA mice and Flaky tail mice. These models show as close as possible to the actual dermatitis lesion through immunological changes and barrier damage of AD.[9,10] Transgenic mouse model helps understanding the biological, molecular, and functional aspects of critical genes relating AD in vivo.[11] Allergen induced mouse model shows the phenotype of AD through various external stimuli. These models use the various sensitization protocols by using specific antigens to induce allergic contact dermatitis, resulting in a condition similar to AD.[12] These mouse models are valuable tools to deepen the current understanding of pathophysiology which is underlying AD and to develop novel therapeutic agent in AD because of their ease of maintenance and handling, short reproductive cycles, sharing of physiological characteristics with human.[13-16] Despite of these advantages, limitations exist in that it is difficult to apply the experimental results from animal models directly to the human disease in clinical settings, possibly due to mismatch between immune systems of human and that of animals.[14-16]

To overcome these limitations, the humanized mouse models have been used in various studies It is believed that the use of humanized mice can address the risks and ethical issues that may arise in studies requiring clinical trials.[14,15] Humanized mouse models are usually developed by not only transplanting human cells or tissue into animals but also introducing genes derived from the individual human patients into immunodeficient mice.[15] In normal mice, when human cells or tissues are transplanted, they are regarded as foreign antigens, which causes side effects such as xenogenic graft-versus host disease

(GVHD).[14,15,17] So, immunodeficient mice are used to create humanized mouse which can avoid the confounding effects due to host immune systems. Currently, there are several types of immunodeficient mice in immunologic research fields. Among them, NOD-SCID IL2Rγnull (NSG) mice do not have T cells, B cells and NK cells, and their cytokine pathways are artificially regulated to reduce the immunity of mice, so GVHD reactions can be minimized.[14,18,19] These humanized mouse models are used in various studies, such as cancer, infectious diseases and allergic diseases,[20] but a reliable mouse model which is injected with patient-derived T cells representing the immune response of individual AD patients has not been established.

To date, the only humanized mouse model derived from AD patients is the oxazolone-induced AD model.[21,22] However, it is not known whether oxazolone actually causes atopic dermatitis in clinical cases. Most of the patients with AD are sensitized to HDM, the most common indoor allergen, and T lymphocytes recognizing HDM allergens are found in AD skin biopsy specimens.[23-25] Therefore, we decided to observe a specific immune response with sensitizing HDM. And we also tried to develop the new, customized mouse model (e.g. AVATAR mouse) which represents the immune response pattern of individual AD patients, so that the cornerstone of personalized medicine could be set up in AD.

II. MATERIALS AND METHODS

1. Recruitment of AD patients and house dust mite (HDM) microneedle patch

Blood and skin samples were obtained from AD patients as defined by the criteria of Hanifin and Rajka.[1] In order to select AD patients who are sensitized by HDM, we observed an immune response after applying a HDM microneedle patch to AD patients for 24 hours. All the clinical experimental procedures were approved by the institutional Review Board (IRB) of Yonsei university and the HDM microneedle patch was produced at 3000PAU, 2000PAU concentrations, which were provided by Rapas.

2. Mice

Female NOD-SCID IL2Rγnull (NSG) mice, 6-8 weeks old, were purchased form the Jackson Laboratory (USA). The mice were kept under conventional SPF conditions in individually ventilated cages and raised in an air-conditioned room maintained at 24±2℃ and 55±5% humidity. All the animal experimental procedures were in accordance with the international welfare guidelines taking in consideration the 3Rs (Refinement, Reduction, Replacement).

3. Isolation of peripheral blood mononuclear cells (PBMCs) and Pan T cells.

In order to obtain PBMC of atopic dermatitis patient, using Ficoll-Paque reagent, put the blood and reagent 1:1, put it in a centrifuge, and separate for 15 minutes at 2,500 rpm. Red blood cells are collected in the bottom layer and separated into a buffy coat layer and a plasma layer, and the plasma layer is

discarded and a buffy coat layer is taken to obtain PBMC.

CD3+ T cells are isolated from the PBMC to obtain pathogenic cells causing atopic dermatitis. To separate CD3+T cells, use the Pan T Cell Isolation kit of Miltenyi Biotec, put MACS buffer in the PBMC according to the experimental method of the kit above, and then add Pan T Cell Biotin Antibody Cocktail and Pan T Cell MicroBead Cocktail, and then LS Magnetic seperation is performed using a column.

4. Flow cytometry

Cells were isolated from avatar mouse spleen, lymph node and skin. After crusing the spleen and lymph node tissue, it is filtered through a strainer and centrifuged to obtain cells. In the case of skin lesion tissue, it is cut into very small pieces and filter it through a strainer after digestion. Then, the cells were stained with anti-human CD45, CD3, CD4, CD8, HLA-DR, FcεRI, CD1a, CD1c, CD11c antibody conjugated with fluorescent dye (eBioscience). Labeled cells were quantified using a BD LSR Fortessa™ flow cytometer, and then the data was analyzed using FlowJo Software (BD Bioscience, San Jose, CA, USA).

5. Immunofluorescence staining

For the paraffin section staining, deparaffinization and antigen retrieval were done. Next, the tissue was blocked with 5% BSA for 1hour and treated with a primary antibody overnight at 4℃. After washing with 1% PBST, the tissue was treated with a fluorescent labelled secondary antibody. Finally, the tissue was mounted with a VECTASHIELDTM Mounting Medium (Vector Laboratories,

Burlingame, CA, USA) and investigated with a fluorescent microscope. The primary antibodies were the anti-human FcεRI antibody (Biolegend) and the anti-human CD3 antibody (Bioss Inc.), and the secondary antibody was the anti-mouse FITC (Life Technologies, Carlsbad, CA, USA) and anti-rabbit Alexa-555 (Abcam).

6. TCR sequencing

A. sample prep

For samples extracted by Adaptive Biotechnologies: Genomic DNA was prepared from human and mouse skin using Qiagen kit method according to the manufacturer's instructions. Samples were quantified using Dropsense96 and diluted for library preparation in AE buffer normalized concentration.

B. Library Prep and Sequencing

Sample data was generated using the immunoSEQ assay (Adaptive Biotechnologies, Seattle, WA). The somatically rearranged locus CDR3 region was amplified from genomic DNA using a two-step, amplification bias-controlled multiplex PCR approach.[26,27] Specifically, the first PCR consists of forward and reverse amplification primers specific for every V and J gene segment, and amplifies the hypervariable complementarity-determining region 3 (CDR3) of the immune receptor locus. The second PCR adds a proprietary barcode sequence and Illumina adapter sequences. CDR3 libraries were sequenced on an Illumina instrument according to the manufacturer's instructions.

C. Data analysis

Raw Illumina sequence reads were demultiplexed according to Adaptive's proprietary barcode sequences. Demultiplexed reads were then further processed to: remove adapter and primer sequences; identify and correct for technical errors introduced through PCR and sequencing; and remove primer dimer, germline and other contaminant sequences. The data is filtered and clustered using both the relative frequency ratio between similar clones and a modified nearest-neighbor algorithm, to merge closely related sequences. The resulting sequences were sufficient to allow annotation of the V(N)D(N)J genes constituting each unique CDR3 and the translation of the encoded CDR3 amino acid sequence. V, D and J gene definitions were based on annotation in accordance with the IMGT database (www.imgt.org). The set of observed biological CDR3 sequences were normalized to correct for residual multiplex PCR amplification bias and quantified against a set of synthetic CDR3 sequence analogues [27]. Data was analyzed using the immunoSEQ Analyzer toolset.

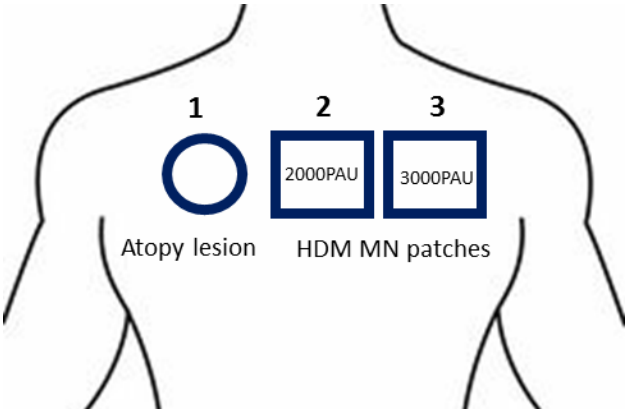
III. RESULTS

1. Selection of donor to establish AVATAR mouse

Currently, it is known that pathogenic T cells play an important role in AD. So we performed PBMCs isolation and skin biopsy from AD patients in order to confirm patient-specific immune responses by targeting T cells. In addition, since sensitization to house dust mite(HDM) is one of the important mechanism of pathogenesis of AD, patients sensitized to HDM were selected.

We confirmed whether a reaction to the skin appeared after attaching a microneedle patch with a different concentration of HDM as shown in Figure 1A among patients who were diagnosed with atopic dermatitis. Figure 1B showed that the immune response appears when HDM microneedle patch is attached to AD patients, whereas it does not appear when attached to a normal person. In addition, when 3000PAU was attached, it was confirmed that a higher positive reaction than 2000PAU was attached (Figure 1C). In this way, we selected AD patients who exhibited immune response to HDM, and most of them showed severity.

A



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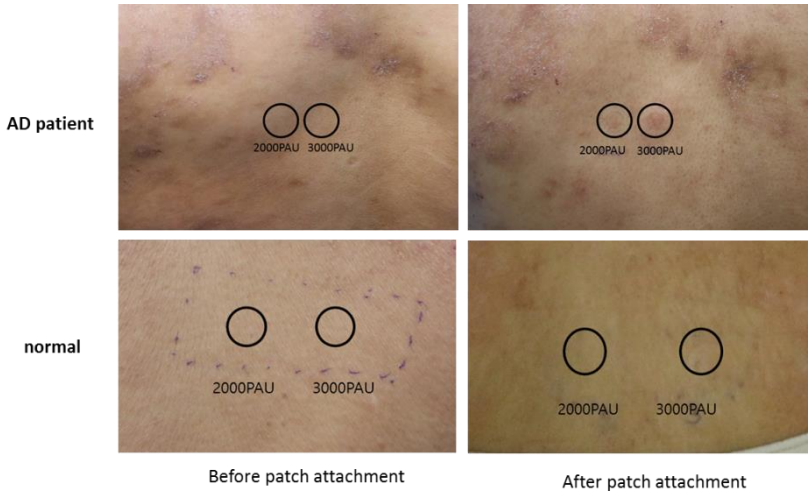

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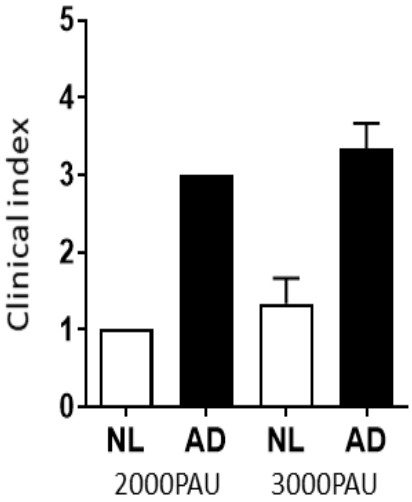


Figure 1. Confirmation of HDM sensitization in AD patients. (A) Scheme of HDM microneedle patch attachment. (B) Reaction when HDM microneedle patch is attached to AD patients and normal person. AD patients are positive reaction. (C) Quantitative graph of clinical immune response in normal(n=3) and AD patients(n=18).

2. AD patient PBMC engraftment in NSG mice and induction of immune response

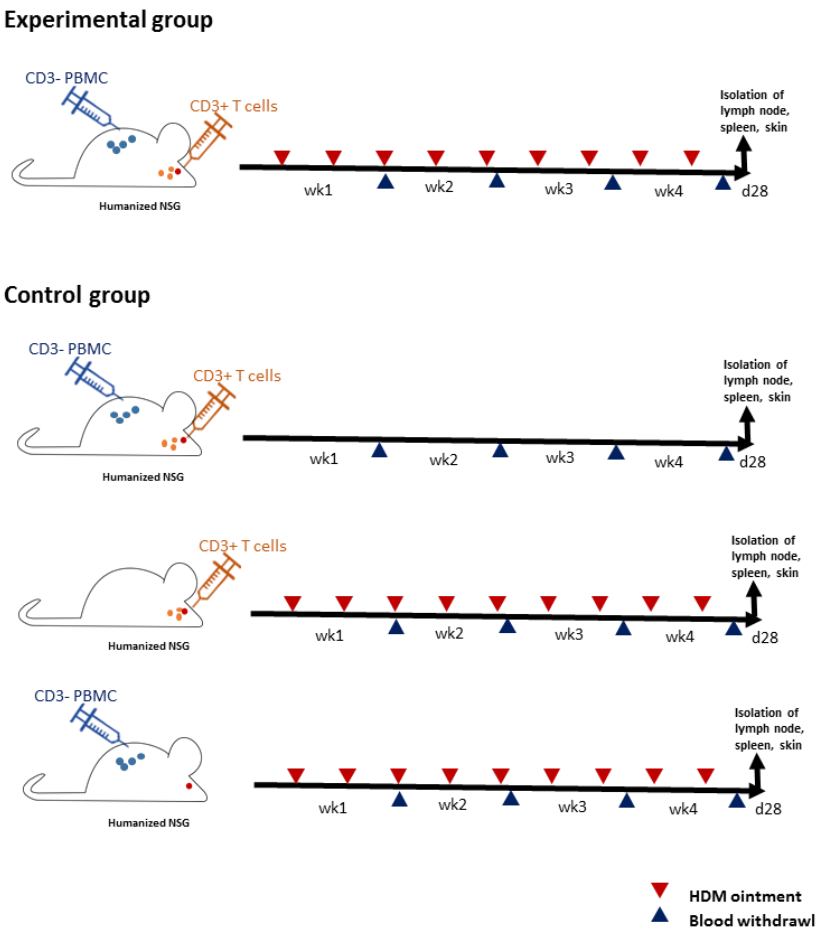
There are three main methods of making humanized mice using human cells. Among them, we decided to use the method of injecting PBMC into immunodeficient NOD-scid IL2Rγnull (NSG) mice, known as Hu-PBL-SCID.[14,15,28] This model is good for studying human T cell function and can be engrafted quickly, but only has a short experimental window due to the development of lethal xenogeneic graft-versus-host disease(GVHD), usually within 4-8 weeks.[14,15,20] So we limited these experiments to 28 days, which was before onset of GVHD thus avoiding convoluting effects on our studies.

We engrafted AD patient PBMCs to NSG mice by intravenous injection to allow CD3+ T cells to act as pathogenic cells, and intradermal injection to allow CD3 depleted PBMCs to act as antigen-presenting cells. Also, we injected only one of the two to confirm whether the immune response occurred only with CD3+ T cells or CD3 depleted PBMCs. As shown in Figure 2A, these three group were treated with HDM antigen for 4 weeks. Then, inflammatory response occurred in the group receiving both injections, whereas no response occurred in the group receiving only one of them. Therefore, we could confirm that CD3 depleted PBMCs act as antigen-presenting cells and activate CD3+ T cells.

Next, to verify that inflammation is induced by HDM antigen, the HDM-inducing group and the non-inducing group were compared (Figure 2A). After 4 weeks, we observed that the inflammatory response occurred in HDM-inducing AVATAR mice, whereas non-inducing avatar mice did not. So it is confirmed that this is an antigen-specific response (Figure 2B).

In order to clarify whether HDM had an effect on the inflammatory response, skin sections from AVATAR mice were analyzed. As shown in Figure 2C, the epidermis of the HDM-treated AVATAR mice was thicker than that of the HDM-non treated AVATAR mice.

A



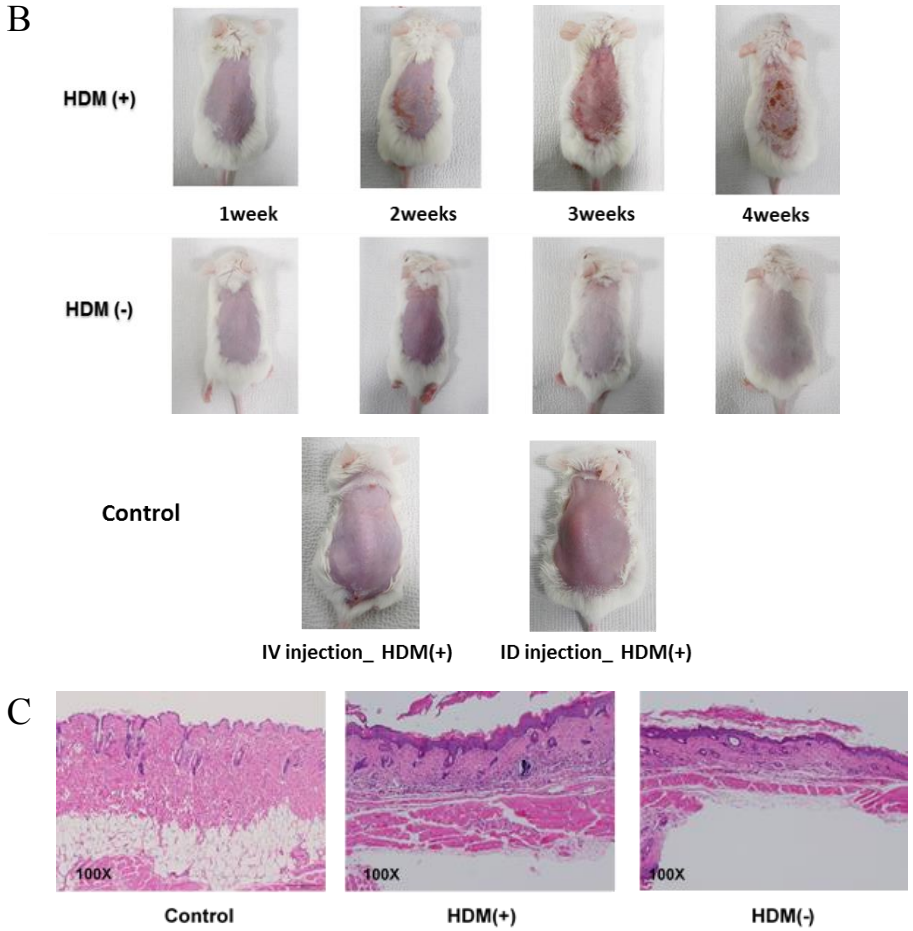


Figure 2. AD patient derived human PBMC engraft in NSG mice and confirm inflammatory response. (A) Schema of 4weeks engraftment protocol. In the experimental group, CD3 T cells were engrafted by intravenous injection and CD3 depleted PBMCs were engrafted by intradermal injection, followed by HDM treatment. Negative control groups were not treated with HDM, CD3 T cells were not added, and CD3 depleted PBMCs were not added. (B) Inflammatory responses were observed in the experimental group compare to the control group. (C) H&E stained photomicrograph of skin sections from NSG normal and AD AVATAR mice.

3. FACS analysis of human lymphocytes in AVATAR mouse

After confirming that the inflammatory response occurred visually in AVATAR mice, flow cytometry was used to verify whether the response was actually caused by human T cells.

First, human T cells were identified in blood of AVATAR mice and focused our analysis on quantification of human CD45+CD3+T cells. Weekly analysis of showed human T cells engraftment and increased gradually in HDM treated AVATAR mice more than HDM non-treated AVATAR mice (Figure 3A).

In addition, as a result of confirming human T cells in the lymph nodes, spleen and skin of AVATAR mice after 4 weeks, human CD45+CD3+ T cells were observed and the percentage of human CD3+CD4+ T cells is higher than human CD3+CD8+ T cells and it also observed that more human T cells were found in the HDM-treated group.

In comparison with healthy AVATAR mice, AD AVATAR mice skin and lymph node showed significant expression of human T cells, it could be seen that the increased proliferation and activation of human CD3+ T cells in response to HDM antigen.

Taken together, these data illustrate that human T cells functioned normally in mice and caused immune response. So we found that human T cells isolated from patient oriented AVATAR mouse are suitable to study human T cell function.

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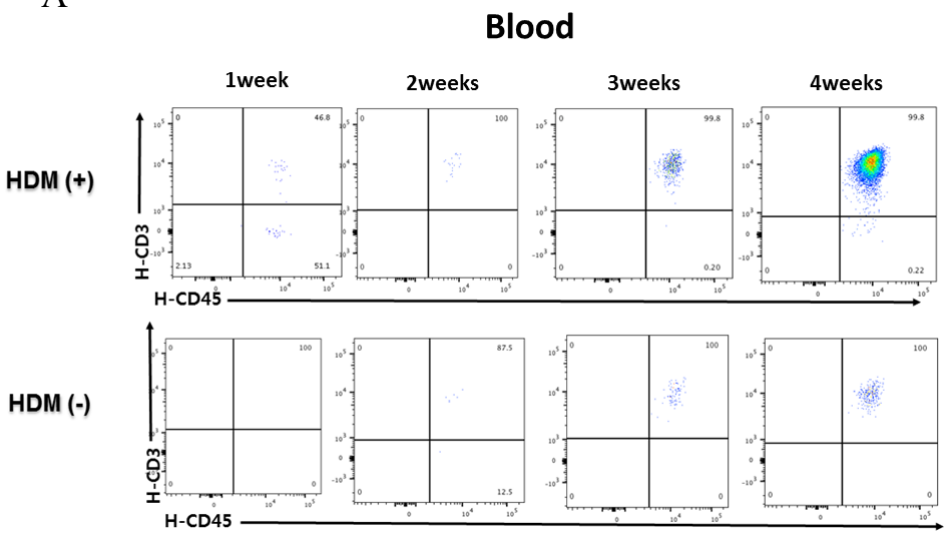

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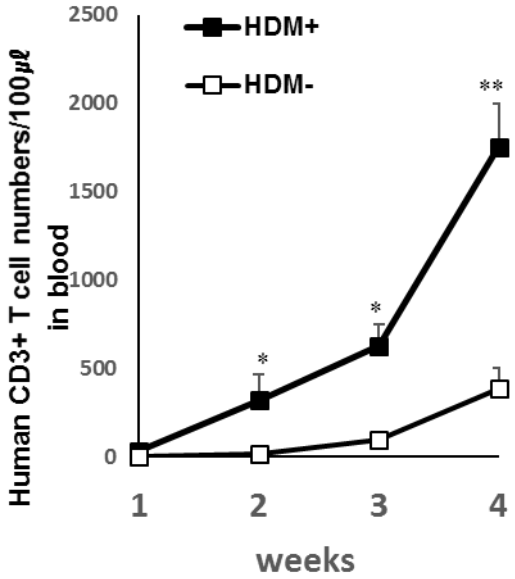

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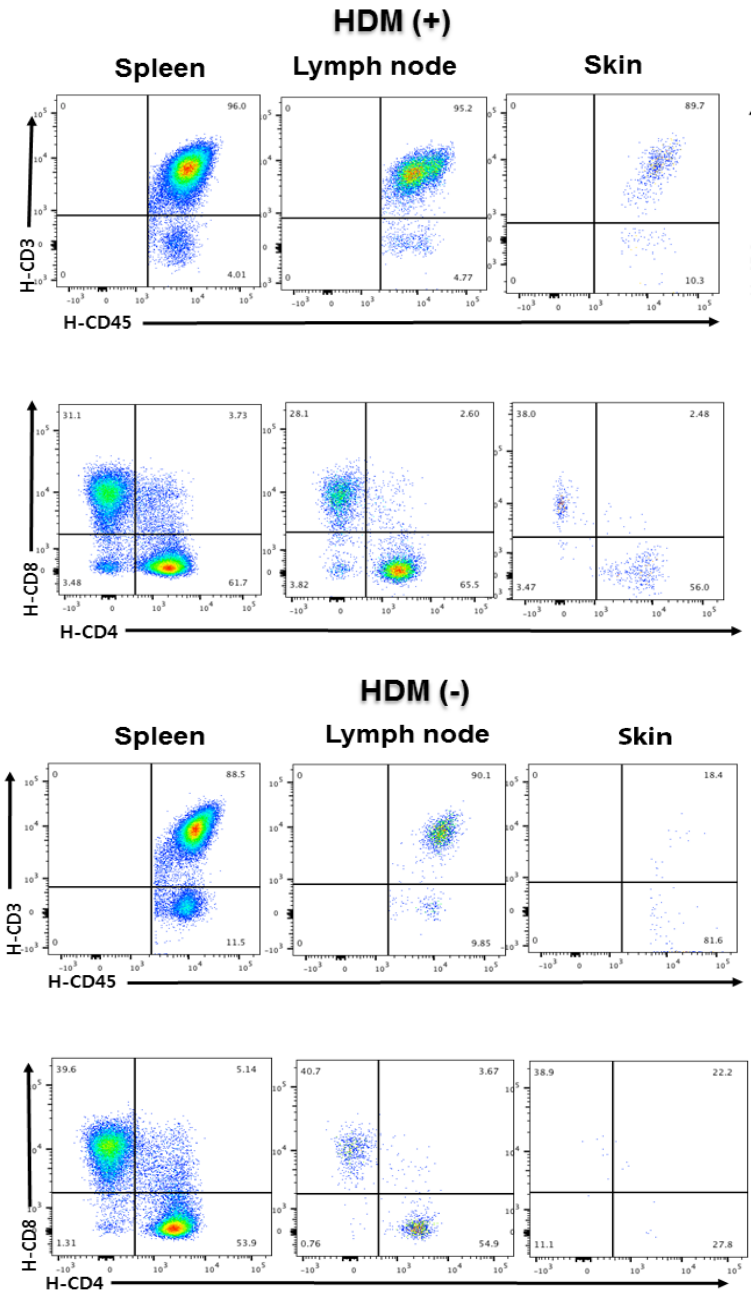

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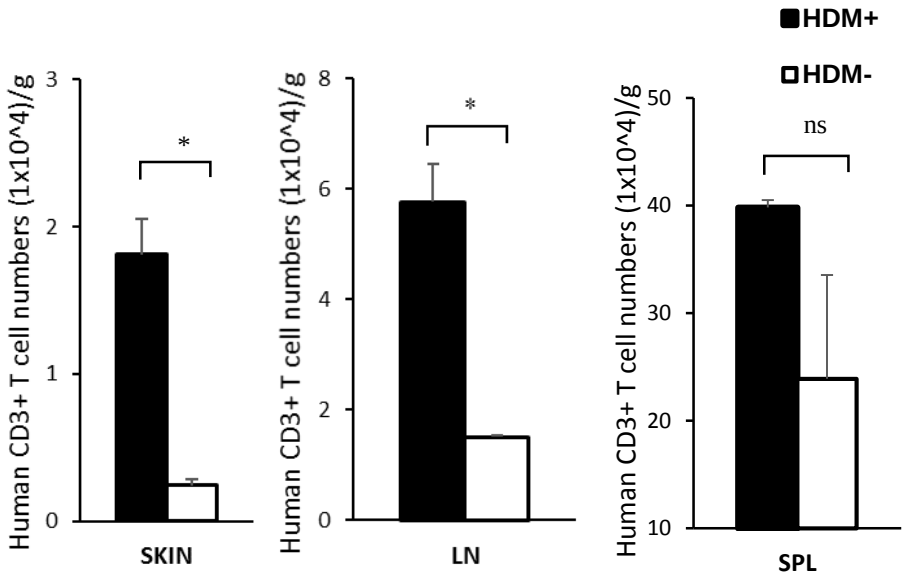


Figure 3. AD patients derived human T cells were confirmed in spleen, lymph node and skin of AVATAR mice. (A, B) Representative flow cytometry analysis of human CD3+ T cells in blood. (C, D) After 4 weeks, flow cytometry resulted in human CD3+ T cells (gated on CD45+ cells) from spleen, lymph node and skin of both experimental and control groups, higher number of human T cells detected in experimental group with inflammatory response.

4. Confirmation of antigen presenting cell

Since dendritic cells play an essential role in the generation and regulation of T cell immune response, we investigated which cells captured HDM antigens in the engrafted CD3 depleted PBMCs and verified that it actually plays the role of antigen presenting cell (APC).

CD1a and FcεRI expression which are associated with Langerhans cells and inflammatory dendritic epidermal cells, showed mainly in patient with AD. [29-31] So we hypothesized that these cells would act as APC in AD AVATAR mice and we first checked whether these cells were expressed in the human PBMCs engrafted.

Based on FSC-SSC gating strategy, we defined two morphologically distinct populations; presumable lymphocytes and non-lymphoid cells (Figure 4A). In the human PBMC when we analyzed from monocyte populations at the FSC-SSC, we detected CD45+CD3-HLADR+ FcεRI + cells but CD1a cells are not presenced. Also, we observed that in the FSC-SSC, lymphocyte and monocyte populations were classified, and as a result of analyzing them separately, it was confirmed that CD45+CD3-HLADR+ FcεRI+ cells appeared in the monocyte population and CD3+ T cells appeared in the lymphocyte population (Figure 4A).

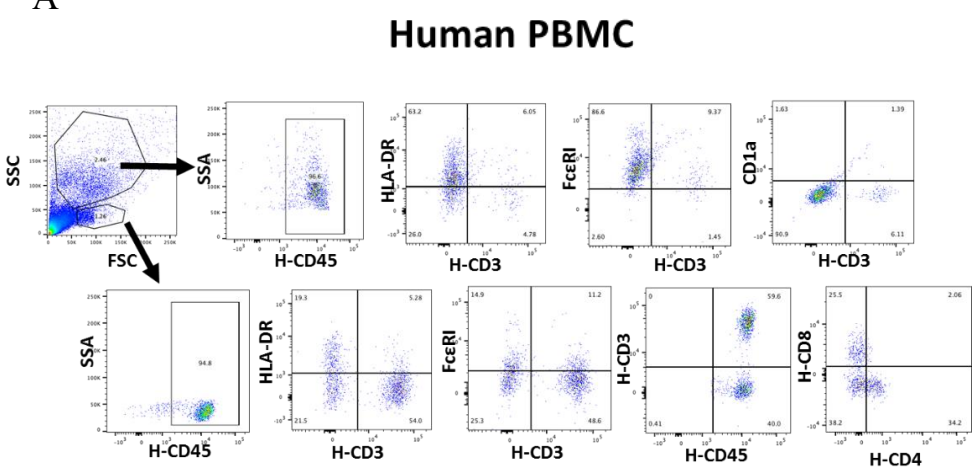
Next, when we analyzed the lymph node and skin of AVATAR mouse, we confirmed that FSC-SSC population is divided into two populations like human PBMCs and same cells were detected. So we confirmed that human APCs activate human T cells by acting as dendritic cells in AVATAR mice and it was observed that more human T cells were activated in lymph nodes and skin when HDM was treated (Figure 4B). Also, when we compared the lymph node and

skin of avatar mice at 1week and 4weeks, CD3-HLADR+FcεRI+ cells increased in the lymph nodes at 4weeks, and activation T cells increased in the skin and lymph node when HDM was treated (Figure 4C). By confirming the intradermally injected APCs in the lymph nodes at week 1, it could be seen that human APCs present in the skin had migrated to the draining lymph nodes (dLN) of AVATAR mice. Therefore, we suggest that human CD45+CD3-HLADR+ FcεRI+ cells, which act as APCs in avatar mice, migrate to the lymph nodes to activate human T cells, and the activated human T cells migrate to the skin, causing an inflammatory response. (Figure 4D, E)

Finally, we investigated which dendritic cells play the role of APC in human PBMC. In human PBMC, plasmacytoid DC is predominant, 0.6% myeloid cDC2 is present, and myeloid cDC1 is almost absent.[32-35] However, whether plamacytoid DC plays a role APC in AD is not yet known. So, we hypothesized that myeloid cDC2 would play an APC role in AVATAR mice.

Major markers expressed by myeloid cDC2 include CD1c and CD11c, and inflammatory epidermal dendritic cell (IDEC) markers that make up a major population in atopic dermatitis include HLADR, CD11c, CD1a, and FcεRI.[30,32,36,37] Therefore, as a result of analyzing CD1c, CD11c, CD1a, HLADR, and FcεRI markers in human PBMC through FACS, we identified CD1c+CD11c+CD1a-HLADR+ FcεRI + cells (Figure 4F). Through this, it was confirmed that myeloid cDC2, not myeloid cDC1, constitutes the main population in our model, and this dendritic cell population is almost identical to that of inflammatory epidermal dendritic cells.

A



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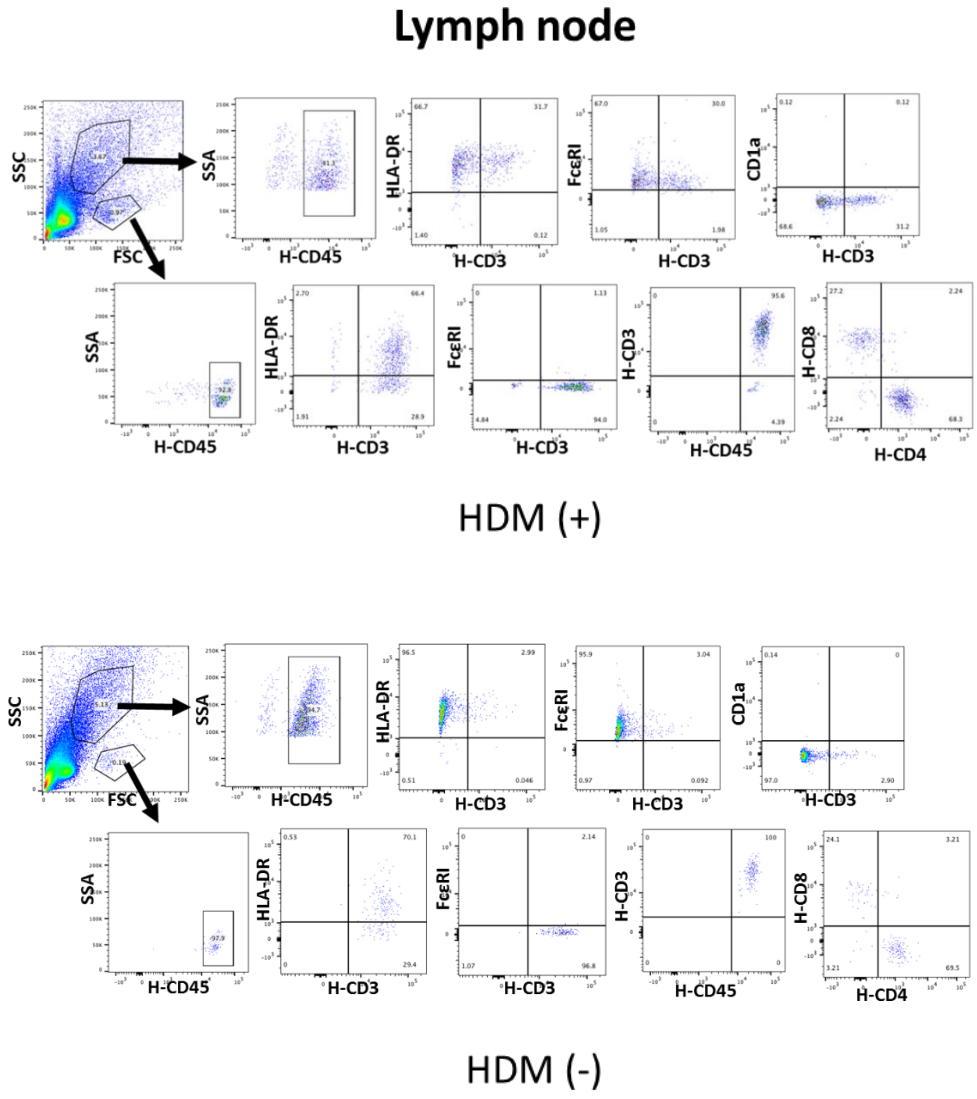

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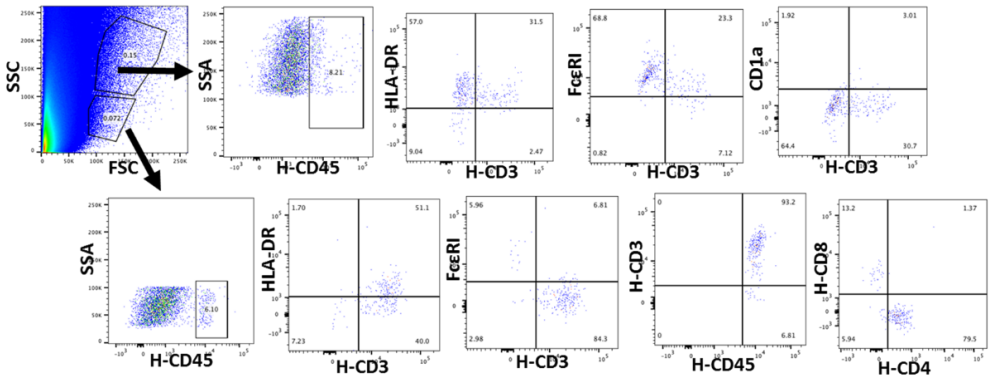
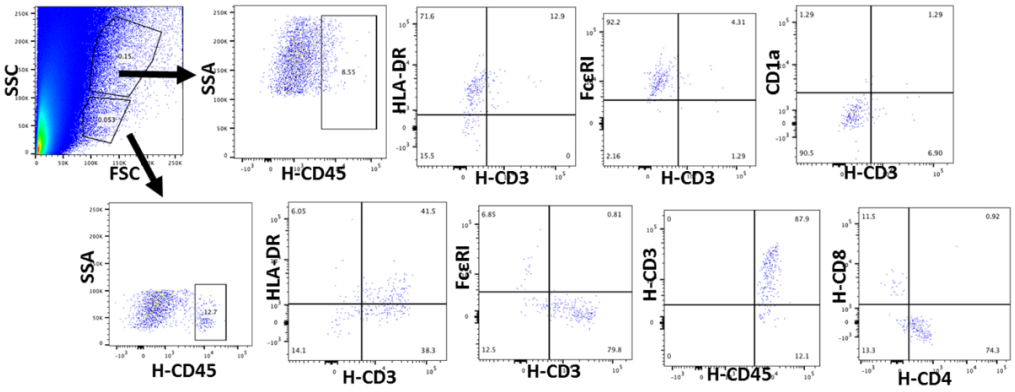


HDM (+)



HDM (-)

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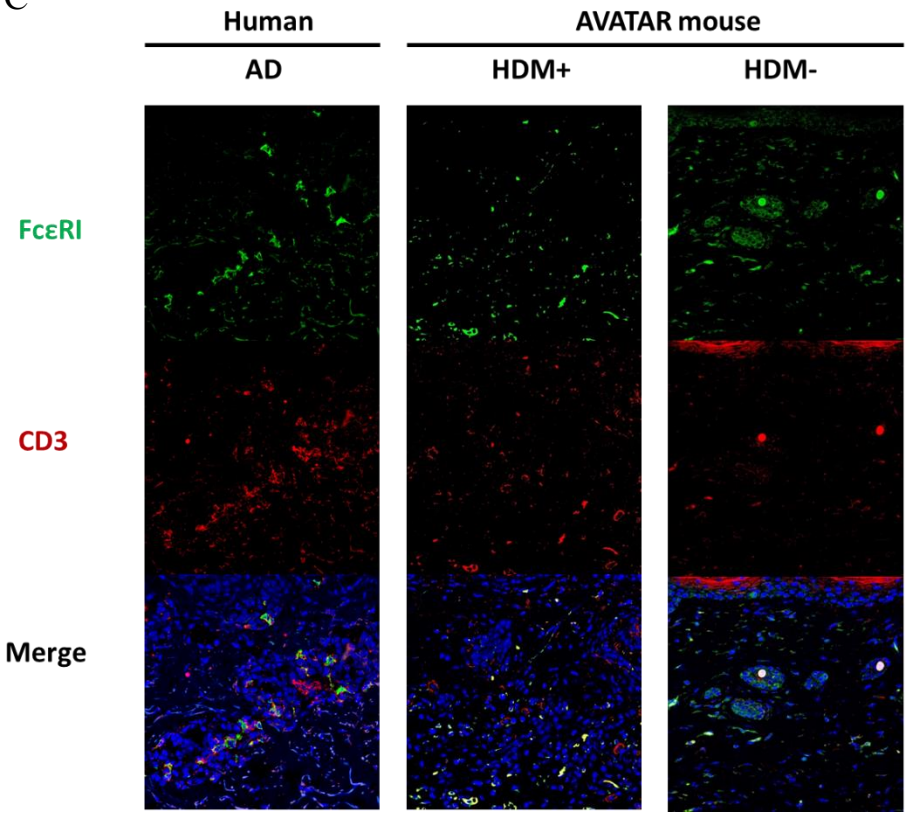

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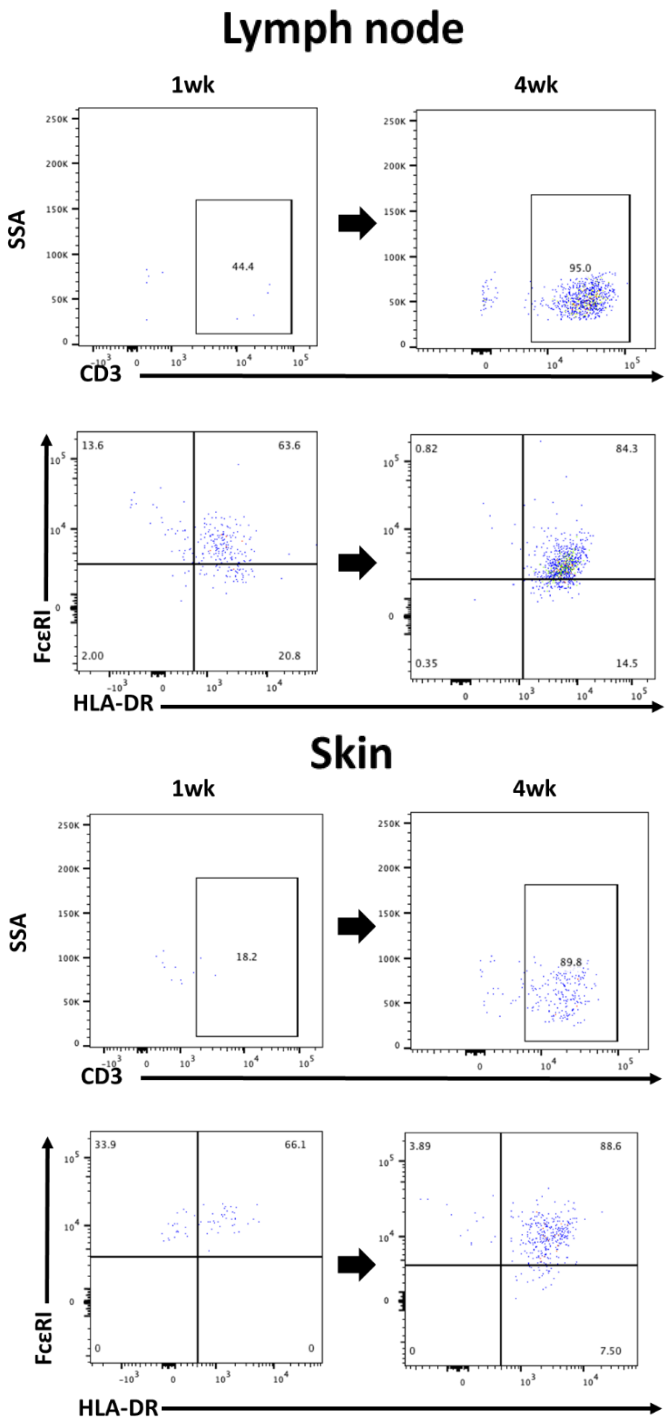

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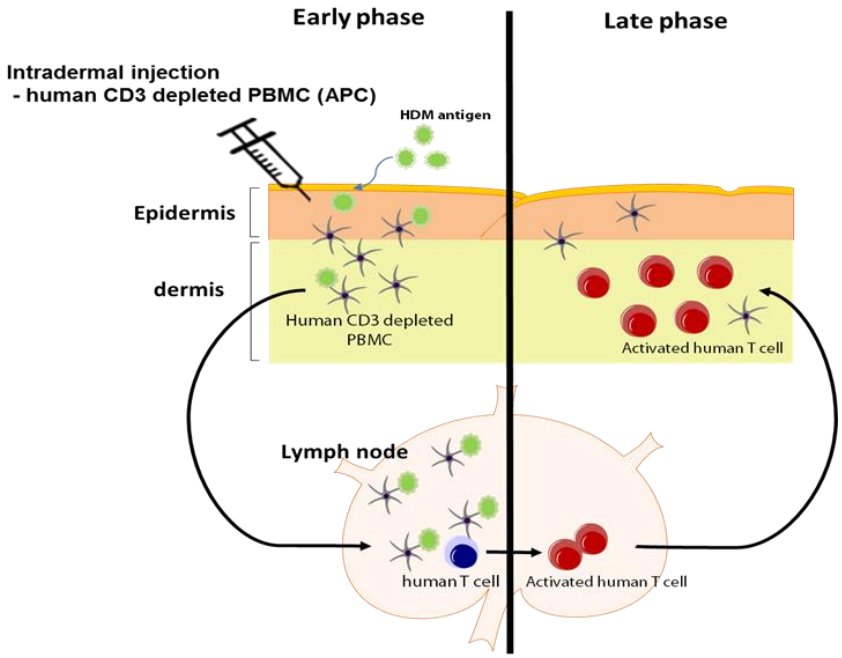

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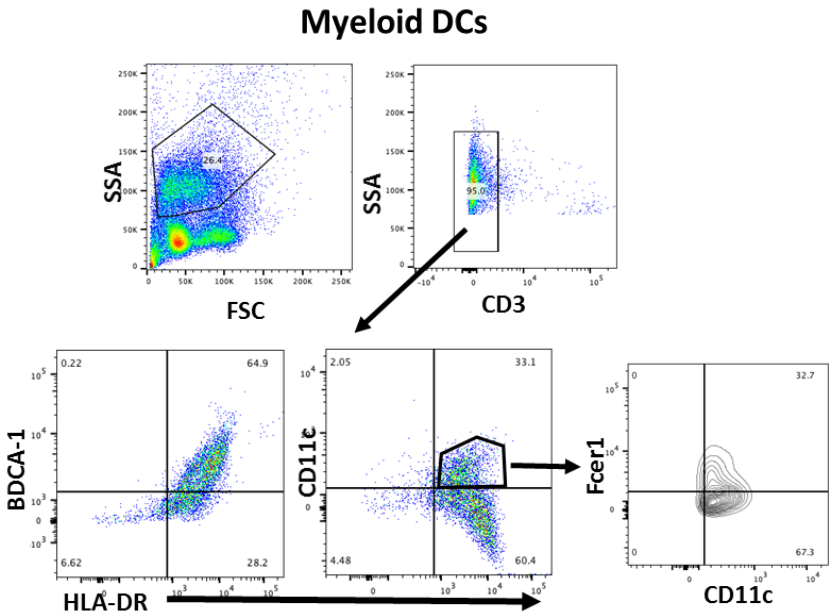


Figure 4. Human CD1c+CD1d1c+HLA-DR+ FcεRI + antigen presenting cells together with human CD3+ T cells suggesting HDM-specific T-cell responses in our AVATAR mouse. (A, B) Confirmation and comparison of APCs expression in human PBMC and AVATAR mice. (C) Confirming CD3-HLADR+ FcεRI + cell expression of the lymph nodes and skin at week 1 and week4. It was observed to increase in week 4. (D) Representative immunofluorescence FcεRI staining of avatar mouse skins on day 28 with HDM treatment. (E) Schematic diagram of the possible pathomechanism of HDM-specific T cell response in avatar mouse. (F) Confirming which human dendritic cells play a role in avatar mouse.

5. Establishment of evaluation standard between AD patients and AVATAR mouse

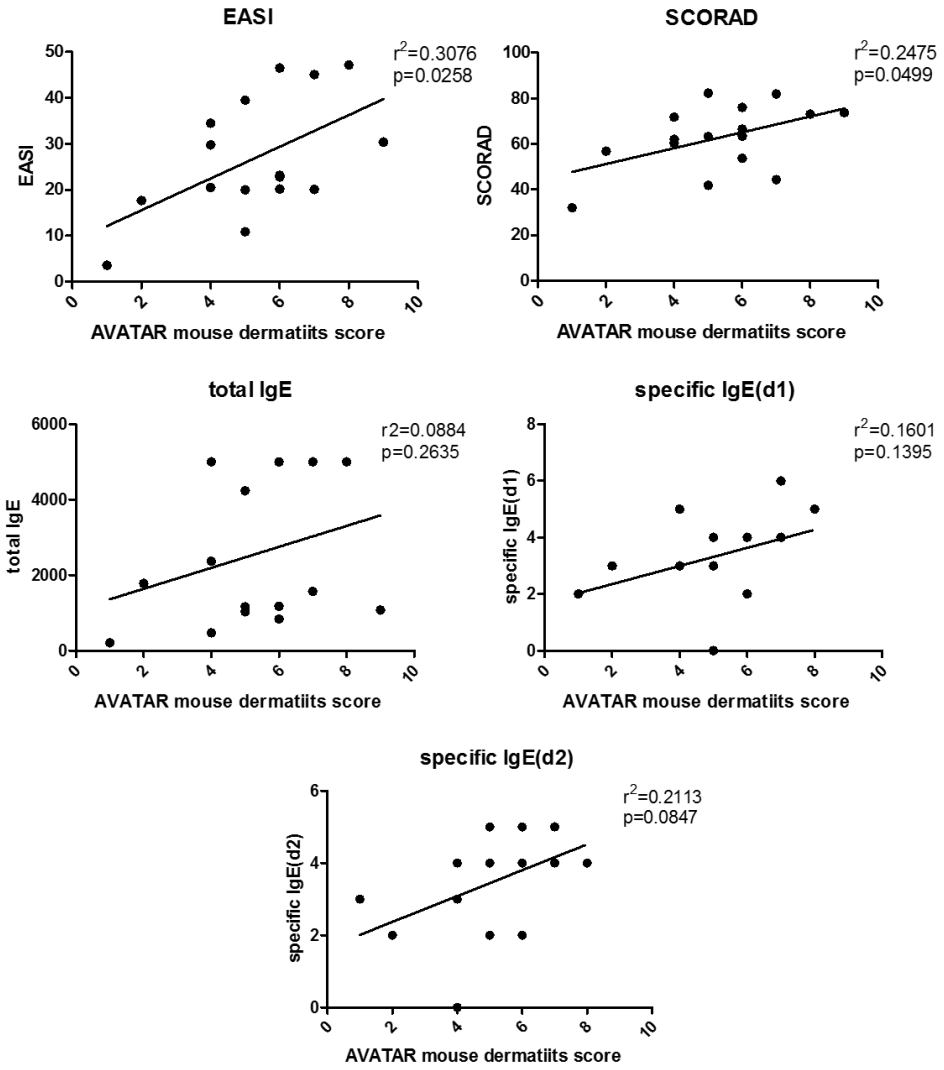
Next, we compared the patient's clinical value and the severity of the AVATAR mouse to see how much correlation there was.

First of all, among several clinical index of the patient, the severity of atopic dermatitis was best indicated and the values related to HDM allergy were selected and compared.

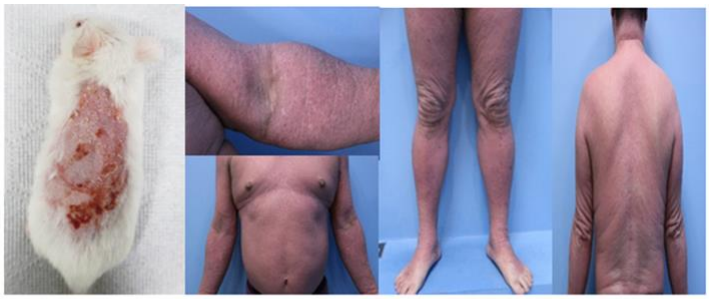
The severity of AVATAR mice dermatitis was evaluated by the development of 1) erythema/hemorrhage, 2) scarring/dryness, 3) edema, 4) excoriation/erosion was scored as 0 (none), 1 (mild), 2 (moderate) and 3 (severe).[38] The sum of the individual scores was taken as the dermatitis score.

As a result of comparing AD patients with the matching AVATAR mice, it was confirmed that both EASI and SCORAD were significantly correlated with dermatitis score.

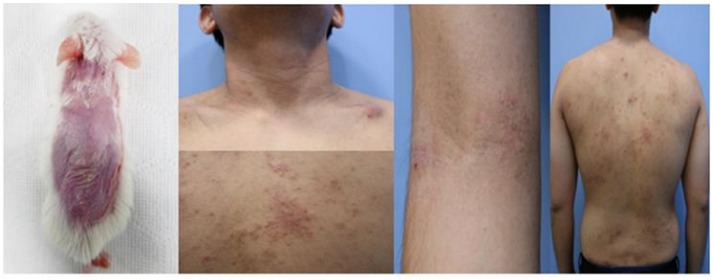
A



B



Mouse dermatitis score	EASI	SCORD	Total IgE	Specific IgE (or MAST)
8	47.2	73.14	>5000	d1: 5/d2: 4



Mouse dermatitis score	EASI	SCORD	Total IgE	Specific IgE (or MAST)
2	17.7	56.9	1781	d1: 3/d2: 2

Figure 5. Clinical and laboratory correlation between individual AD patients and matched AVATAR mice were observed. (A) AD patients clinical severity and matched AVATAR mice atopic dermatitis scores were compared. The EASI and SCORAD index were correlated. (B) Representative images of AD patients and matched AVATAR mice

6. Comparison of TCR repertoire diversity between human and AVATAR mouse

Next, TCR sequencing was performed to compare the TCR beta usage of AD patients responding to HDM and AVATAR mice exhibiting inflammatory reactions after sensitization to HDM.

Figure 6A shows the diversity of TCR repertoire through the productive frequency ratio of the top 10 clones in each group. The higher the productive frequency ratio of the top 10 clones, the lower the diversity of TCR repertoire. When comparing the productive frequency of human skin, it was confirmed that the ratio of top 10 clones in the skin with the 3000PAU and 2000PAU HDM microneedle patch was relatively higher than that of the AD skin. And when comparing the AVATAR mice of the HDM-treated group and the non-treated group, it was confirmed that the ratio of the top 10 clones was higher in the HDM-treated group.

This can be interpreted as a low diversity of TCR repertoire due to the large number of HDM-specific TCRs in the HDM microneedle patch attached skin and HDM-treated AVATAR mice. It can also be interpreted that an antigen-specific reaction occurred in AVATAR mice.

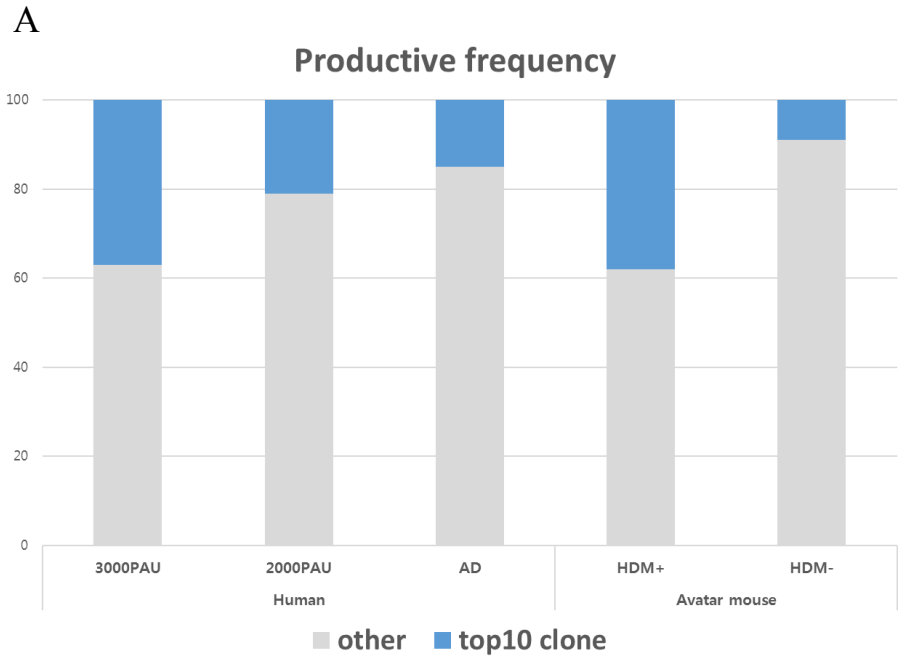


Figure 6. Productive frequency ratio comparison between human and AVATAR mice. (A) Diversity of TCR repertoire types was confirmed by comparing the productive frequency ratio of the top 10 clones in human skin and AVATAR mouse skin.

IV. DISCUSSION

Current therapeutic strategies in AD have several challenges particularly adverse events by common immunosuppressants such as cyclosporine and methothrexate, which induced non-specific and extensive immune suppression.[39] Those conventional immunosuppressants are not based on the individual immune responses caused by the patient's triggering factors or exacerbating factors. Biologics and small molecules that have been recently developed are in the spotlight because they are found to have better efficacy while minimizing side effects due to non-specific immunosuppression.[39,40]

However, AD is a highly heterogenous condition based on various clinical, immunological, and epidemiological factors. Thus, it is essential to build a customized treatment system that predicts a specific immune response for each patient. Therefore, we thought it was necessary to construct a humanized mouse model that similarly expresses the immunologic molecules with the patient with AD.

In this study, to study patient-specific immune responses with an emphasis on the T cell, the core immune cell type of atopic dermatitis, we injected PBMCs derived from atopic dermatitis patients into immunodeficient NSG mice. We named this humanized mouse an AVATAR mouse. We used NSG mice to minimize the occurrence of GVHD reactions, and the total number of cells transplanted into these mice was limited to $5X10^6$. When the number of cells to be transplanted is large, the probability of GVHD is increased due to excessive immune response by human cells in the mouse.[41,42] Therefore, the average number of cells injected was referenced in the humanized mouse model injected with PBMC. Specifically, to study the in vivo function of human pathogenic T

cells, CD3 + T cells were sorted from PBMCs of AD patients who are sensitized to house dust mites and injected intravenously into mice. CD3 depleted PBMCs were intradermally injected as antigen-presenting cells. This has the advantage of further increasing the engraftment rate by using a different route of injection than the existing hu-PBL-SCID mouse model.[14,15,20] 24 hours after PBMC injection, the mice were sensitized to the HDM allergen via an injection schedule of every 3 days for 27 days (total 9 times) to induce an inflammatory reaction. No inflammatory response was observed in entire control groups. Therefore, it can be inferred that in order for the inflammatory reaction to occur in AVATAR mice, the HDM allergen must be applied to the injected APC from human AD patients, and subsequently the HDM-sensitized APC have to contact with the injected CD3 T cell from human AD patients.

We performed FACS analysis to verify that the inflammatory response in AVATAR mice was actually caused by human cells from AD patients. First, by analyzing human T cells in the blood of AVATAR mice for every week, it was confirmed that when HDM was treated, human T cells proliferate compared to the non-treated group. After 4 weeks, the lymph node, spleen and skin were also found to have more T cells when HDM was treated. From this, it could be inferred that the transplanted human cells are functioning normally in the AVATAR mouse. Also, the CD4:CD8 ratio of engrafted T cells isolated from avatar mouse of lymph node and skin was higher in CD4, indicating that the actual patient's disease state was reflected in AVATAR mice.

Next, we confirmed that myeloid cDC2 and inflammatory dendritic cells showing CD1c+CD11c+CD1a-HLADR+ FcεRI + among human CD3 depleted PBMCs given intradermal injection play a role in APC in avatar mice. In AD,

inflammatory dendritic epidermal cells act as APCs in the skin, and these cells are defined by the following: HLA-DR+lin-CD11c+CD1a+ and these DCs co-express FcεRI, etc.[29,32,34,35] Cells expressing the above markers are different from the DC population pattern in PBMCs because they form the main population in the skin. Therefore, it is expected that the patient's immune response can be implemented more specifically if the skin cells of an AD patient is implanted into an avatar mouse for the role of antigen-presenting cells (APCs) in the future.

In addition, it was confirmed that APC, which recognized HDM antigens in the skin of avatar mice, entered the lymph node to activate T cells, and the activated T cells contributed to the inflammatory response. Through this, it was found that avatar mice can implement an antigen-specific immune response that appears to the AD patient. Next, TCR sequencing was performed on the skin tissues of human and avatar mice to confirm that the inflammatory response that appeared after HDM treatment in avatar mice was an actual HDM antigen-specific immune response. As a result, it was confirmed that the TCR repertoire diversity of HDM sensitized human skin and HDM-treated AVATAR mice was low, and this was interpreted as being due to the large proportion of TCR repertoire specific to HDM. The TCR sequencing analysis in this study was approached broadly. In the future, if a sequence that is estimated as HDM-specific TCR is selected from human skin and a comparative analysis is performed to see if these sequences are present in HDM-treated avatar mice and to what extent. If human HDM-specific clones were found in AVATAR mice, it could be concluded that AVATAR mice more fully embody the immune response of AD patients.

Also, we thought that it was important for avatar mice to reflect the actual patient's disease state, so we compared the clinical severity and clinical values of patients with the clinical values of avatar mice. When the EASI, SCORAD and IgE levels, which represent the patient's severity well, were compared with the clinical index of AVATAR mouse, it was found that the more severe the patients, the higher inflammatory response of the avatar mouse. As a result, it is expected that the immune response of the avatar mouse will appear similar to that of the actual patient.

In the case of the previously introduced AD humanized mouse model, since all human PBMCs were transplanted only by intravenous injection, it is difficult to confirm that it is an antigen-specific reaction, and it is thought that the cause of the T cell action is not clear. Unlike the previous model, our model has an advantage in that it can clearly simulate the immune response of each patient's antigen-specific T cells. However, if the immunodeficient mice used for making avatar mice come down through breeding for a long period of time, the health status of each mouse may be difficult to trust, making it difficult to say that the patient's disease state is reflected. Also, the protocol for transplanting PBMCs is not suitable for studies longer than 4 weeks, because GVHD can occur in avatar mice.[14,15,20] Therefore, for long-term studies such as drug treatment evaluation studies, it is necessary to develop a stable protocol that prevents GVHD.

IV. CONCLUSION

In summary, we created an avatar mouse to overcome the limitations of animal models used in atopic dermatitis research and to confirm the specific immune response of individuals with atopic dermatitis at the animal model level. PBMC derived from atopic dermatitis patients were transplanted into NSG mice, and then the antigen-specific immune response by human cells was confirmed in mice, confirming that avatar mice are suitable for studying patient specific immune responses. Therefore, since our avatar mouse model can identify patient-specific immune responses and predict the prognosis of newly developed therapeutic agents, it is expected[43]that personalized treatment for atopic dermatitis patients will be possible.

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ABSTRACT (IN KOREAN)

아토피피부염 환자로부터 유래된 인간화 마우스 모델 구축 및 특성 규명: 아바타 마우스

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김 혜 리

아토피피부염은 만성적으로 재발하는 염증성 피부질환으로, 최근 30년간 전세계적으로 발생률이 증가하고 있으며 지역, 연령, 성별, 사회문화적 특성에 따라 다양한 유병률을 보이는 heterogeneous한 질병이다. 따라서 환자 개개인에게 적합한 치료 방법을 선택하는 것이 무엇보다 중요하다. 따라서 아토피피부염을 발생 원인에 따라 보다 효과적으로 예방 또는 치료하기 위한 연구가 활발하게 이루어지고 있다. 이러한 연구를 위해 다양한 마우스 모델이 보고되고 있는데 마우스 모델에서의 결과를 아토피피부염 환자에게 적용하는 데에는 한계가 발생한다. 따라서 아토피피부염에 대한 인간화 마우스 모델의 개발은 마우스 모델과 환자간의 차이를 줄이기 위해 필수적이며, 이는 치료 약물 개발을 위해 보다 나은 전임상 도구로 이어질 것이라 생각된다. 따라서 개인 맞춤형 의료의 초석을 AD에 설정할 수 있도록 환자 개개인의 면역 반응 패턴을 구현하는 새로운 맞춤형 마우스 모델 (AVATAR mouse)을 구축하고자 한다.

아바타 마우스는 집먼지진드기에 감작 된 AD 환자에서 유래 한 CD3 + T 세포와 CD3 depleted PBMC를 NOD-scid IL2Rγnull(NSG) 마우스에 이식하여 제작하였다. 아바타 마우스를 4주 동안 집먼지진드기를 감작시켜주고, 4주 후

집먼지진드기를 감작시킨 아바타 마우스에서는 염증 반응이 발생하는 반면 집먼지진드기를 감작시키지 않은 대조군에서는 염증 반응이 발생하지 않는 것을 관찰하였다. 또한 집먼지진드기를 감작시킨 아바타 마우스에서 환자의 CD3 + T 세포가 대조군에 비해 증가하고 4주 후에 집먼지 진드기 항원을 인식한 환자의 CD1c + CD11c + HLADR + FcεRI + 항원 제시 세포가 CD3 + T 세포를 활성화시켜 염증반응을 일으키는 것을 확인함으로써 우리의 아바타 마우스 모델이 항원 특이적 T 세포 반응을 유발한다는 것을 확인하였다. 또한 개개인 아토피피부염 환자와 일치하는 아바타 마우스 간의 임상 지표 상관성을 확인함으로써 아바타 마우스가 실제 환자의 질병 상태를 잘 반영해 줄 것이라 생각된다.

이러한 결과를 통해 아바타 마우스를 통해 환자 개개인별 특이적 면역 반응을 확인하고 실제 새로 개발된 약제에 대한 치료 반응을 미리 확인 및 예측할 수 있기에 가까운 미래에 아토피피부염 환자의 개인맞춤형 치료 가능성을 제시하고자 한다.

핵심되는 말 : 아토피피부염, 아바타마우스, 인간화 마우스 모델, 개인맞춤형 치료, 집먼지진드기