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Cite this article as: M Carretero, S Guerrero-Aspizua, N Illera, V Galvez, M
Navarro, F García-García, J Dopazo, J L Jorcano, F Larcher, M del Rio,
Differential Features Between Chronic Skin Inflammatory Diseases Revealed in
Skin-Humanized Psoriasis and Atopic Dermatitis Mouse Models, Journal of
Investigative Dermatology accepted article preview 23 September 2015; doi:
10.1038/jid.2015.362.

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Received 13 November 2014; revised 4 August 2015; accepted 17 August 2015;
Accepted article preview online 23 September 2015

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Differential features between chronic skin inflammatory diseases revealed in skin-humanized psoriasis and atopic dermatitis mouse models

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Short title: Skin-humanized model for inflammatory diseases

Abbreviations:
AD, atopic dermatitis; AMP, antimicrobial peptide; PS, psoriasis; EDC, epidermal differentiation complex; EMBP, eosinophil major basic protein; hBD, human beta defensing; HPF, high-power field; ICAM-1, intercellular adhesion molecule 1; IHC, immunohistochemistry; IL, interleukin; IVL, involucrin; KRT, keratin; LFA-1, leukocyte function-associated antigen 1; LOR, loricrin; PI, proliferative index; PMA, phorbol myristate acetate; SC, stratum corneum; Th1, T helper type 1; Th2, T helper type 2; Th17, T helper type 17; TSLP, Thymic stromal lymphopoietin

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Abstract

Psoriasis (PS) and atopic dermatitis (AD) are chronic and relapsing inflammatory diseases of the skin affecting a large number of patients worldwide. Psoriasis is characterized by a Th1/Th17 immunological response whereas acute AD lesions exhibit Th2-dominant inflammation. Current single gene and signaling pathways-based models of inflammatory skin diseases are incomplete. Previous work allowed us to model psoriasis in skin-humanized mice through proper combinations of inflammatory cell components and disruption of barrier function. Herein we describe and characterize an animal model for AD using similar bioengineered-based approaches, by intradermal injection of human Th2 lymphocytes in regenerated human skin after partial removal of stratum corneum. In the present work we have extensively compared this model with the previous and an improved version of the PS model, in which Th17/Th1 lymphocytes replace exogenous cytokines. Comparative expression analyses revealed marked differences in specific epidermal proliferation and differentiation markers and immune-related molecules including antimicrobial peptides. Likewise, the composition of the dermal inflammatory infiltrate presented important differences. Availability of accurate and reliable animal models for these diseases will contribute to the understanding of the pathogenesis and provide valuable tools for drug development and testing.

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Introduction

Atopic dermatitis (AD) and Psoriasis (PS) are the most common chronic inflammatory diseases of the skin. Both pathologies are associated with a skin barrier defect that correlates in degree with the severity of the lesions although they present unique characteristics regarding immune cell involvement and gene expression patterns (Segre, 2006). AD is characterized by an immunological imbalance leading to the predominance of Th2 cells in acute skin lesions whereas Th1 and Th17 constitute the main lymphocyte subpopulations found in psoriatic plaques. During chronification of atopic skin disease, the immunologic response switches towards the Th1 type. The involvement of Th17 and/or Th22 lymphocytes subpopulations in AD disease has also been discussed (Koga et al., 2008; Nograles et al., 2009). For both inflammatory skin diseases, several animal models have been developed, most of them based on transgenic and knockout mice (Danilenko, 2008; Gudjonsson et al., 2007; Jin et al., 2009). However, differences between mouse and human skin architecture and immune function reveal major limitations in the use of these animal models for drug discovery and pathophysiological studies. Moreover, manipulating a single gene usually does not recapitulate the full phenotype of such complex inflammatory skin diseases. In this regard, the xenotransplantation model recreates more accurately the microenvironment found in human skin diseases (Boehncke et al., 1996; Wrone-Smith and Nickoloff, 1996). Nonetheless, a major drawback to the use of xenotransplantation models is the limited number of animals that can be obtained harboring large and homogeneous skin samples. In our laboratory we have developed a skin-humanized mouse model for psoriasis based on a bioengineered skin approach that overcomes the main limitations of the above mentioned animal models, such as heterogeneity and availability of human biopsy samples (Guerrero-Aspizua et al., 2010). Using this technology, we have demonstrated that a healthy normal human skin regenerated in immunodeficient mice might develop a psoriasiform phenotype in the presence of adequate signals provided by a wounding stimulus and the appropriate cytokines. These cytokines were provided either by in vitro polarized specific lymphocyte subpopulations (Th1) obtained from unrelated healthy donors or produced in their recombinant form in the case of IL17 and IL22 Th17 cytokines. The versatility of the system prompted us to investigate the possibility of generating a skin-humanized mouse model for a typical Th2-mediated skin disease, such as AD. In the present work we have demonstrated by histological,
immunohistochemical and qPCR analyses that the injection of in vitro differentiated Th2 lymphocytes in regenerated human skin elicited an AD phenotype that closely resemble the acute human AD condition. Moreover, a comparative analysis with PS biopsies as well as with the skin-humanized PS model, revealed the major differential traits between these two prevalent cutaneous inflammatory diseases.

Results

Generation of PS and AD skin-humanized mouse models

We took advantage of the previously characterized skin-humanized mouse model for PS (Carretero et al., 2013; Guerrero-Aspizua et al., 2010). In those studies we demonstrated that normal human skin regenerated by bioengineered based-approaches onto the back of immunodeficient mice developed a psoriasiform phenotype after the injection of in vitro differentiated Th1 lymphocytes together with recombinant IL22 and IL17 cytokines and moderate stratum corneum (SC) removal through tape-stripping. In the present study, we present an improved animal model for PS in which differentiated Th17/Th1 lymphocytes produce the cytokines (IFNγ, IL22 and IL17) that have been shown to be relevant for the cutaneous phenotype. In addition, and following the same experimental approach, a skin-humanized mouse model for AD has been developed by introducing in vitro differentiated Th2 lymphocytes (Figure 1A). We used established protocols for in vitro activation and cytokine-directed polarization of human naive CD4+ T cells isolated from cord blood in order to obtain the specific Th1, Th2 and Th17 lymphocyte subpopulations (de la Fuente et al., 2014; Ghoreschi et al., 2010) (see Materials and Methods and Supplementary Material). The expression of specific transcription factors and cytokines was evaluated by flow cytometry in each case (Supplementary Figure 1). The transcription factor T-bet is preferentially expressed by Th1 cells (Szabo et al., 2000). By day 7 of Th1 differentiation a large percentage of cells expressed both IFNγ and the T-bet transcription factor (20-40 % of positive cells depending on donor) reaching a maximum by day 14 of differentiation (60-80 %) (Supplementary Figure 1, left panels). Most of them also expressed TNFα (data not shown). A high percentage of Th2 cells (40%-50%) expressed IL4 together with the specific GATA-3 transcription factor (Zheng and Flavell, 1997) by day 7 of
differentiation. By day 14, GATA-3 and IL4 expression is up regulated and then maintained until day 21 of differentiation (50-60%) (Supplementary Figure 1, central panels). IL5 and IL13 expression was also detected at days 14 and 21 of Th2 differentiation (data not shown). Differentiation of human Th17 cells yielded a high percentage of cells (~70 %) expressing the RORγt transcription factor (Ivanov et al., 2006) between days 14 and 21 of differentiation. Most cells also expressed IL22, IFNγ and TNFα. IL17 expression was also detected at day 14 of differentiation (Supplementary Figure 1, right panels and data not shown). This cell population showed similar characteristics to a previously described in vitro derived Th17/Th1 cell subpopulation (Wilson et al., 2007) which was demonstrated to secrete both IL17 and IFNγ among other factors. The presence of these cells has also been demonstrated in elevated numbers in inflamed tissues (Boniface et al., 2010). For this reason we have referred to this in vitro-derived lymphocyte subpopulation as Th17/Th1 all along the manuscript.

Histological characterization of the PS and AD-like phenotype in the humanized mouse models

Histological analyses showed that injection of either Th1 lymphocytes together with recombinant IL17 and IL22 cytokines or in vitro differentiated Th17/Th1 cells (PS models) induced the typical epidermal changes associated with PS when tape-stripping (TS) was applied, including epidermal hyperplasia, elongation and fusion of rete ridges, parakeratosis and partial loss of the granular layer ((Guerrero-Aspizua et al., 2010) and Figure 1B). The dermis was characterized by an influx of inflammatory cells and increased vascularity with the presence of dilated capillaries. Histological examination of regenerated human skin in which human Th2 lymphocytes were injected together with the application of TS (AD model), revealed moderate thickening of the epidermis as compared to control samples, with no obvious changes in the differentiation process. At the dermal level, the AD model also showed increased numbers of inflammatory cells and enhanced angiogenic response (Figure 1B). All the histological features closely resemble those found in human skin biopsies from PS and AD patients (Supplementary Figure 2).
Differential epidermal hallmarks between the PS and AD skin-humanized mouse models

We evaluated the expression of keratinocyte proteins that are present in normal human skin but whose expression was shown to be altered in PS and AD. The IHC analysis of involucrin (IVL) showed similar increased expression in samples of both humanized AD (Th2+TS) and PS (Th17/Th1+TS and Th1+IL17+IL22+TS) models, as compared to control samples (PBS1x+TS) (Figure 2). In contrast, focal down regulation of KRT1 was observed in all samples corresponding to the humanized model for AD and PS (Figure 2), while patchy expression of loricrin (LOR) was observed exclusively in the PS model (Figure 2), corresponding to areas where a lack of a well-developed granular layer was evident (Figure 1B). In contrast, no discontinuity of loricrin expression was observed in any of the samples in which Th2 cells were included (Figure 1B and Figure 2, AD model). The expression of other markers associated with hyperproliferation, such as Ki-67 and KRT17, was also evaluated. Increased Ki-67-positive keratinocyte nuclei as compared to controls were found in both PS and AD models. However, a high proportion of positive cells showed suprabasal localization in the PS model while mainly confined to the basal/parabasal compartment in AD. Suprabasal KRT17 expression was specifically induced in hyperproliferative samples corresponding to the humanized model for PS (Figure 3). Another characteristic of atopic dermatitis lesions is the presence of apoptotic cells in epidermis (Trautmann et al., 2001). Increased numbers of TUNEL positive cells were also identified in the skin-humanized mouse model for AD as compared to the PS model and control samples (Supplementary Figure 3).

In order to validate the changes observed in the PS and AD models, a comparative marker expression analysis was performed including PS and AD patient biopsies. The comparative IHC analyses for the epidermal markers revealed similar staining patterns to those observed in human plaque psoriasis and AD lesions corresponding to the acute/subacute stages. A significant alteration in the epidermal differentiation pattern (involucrin, KRT1 and loricrin staining) was preferentially observed in PS skin biopsies as compared to normal human skin. KRT17 and psoriasin (S100A7) were highly expressed in PS samples as compared to normal human skin and AD (Supplementary Figure 2A).
Thymic stromal lymphopoietin (TSLP) is a type I cytokine that has been shown to be elevated in the lesional skin of human AD patients and promotes Th2 responses (Soumelis et al., 2002). It has been previously shown that skin-specific overexpression of TSLP resulted in an AD-like phenotype in mice (Yoo et al., 2005). We have explored the expression of this cytokine in our skin-humanized mouse models. IHC analysis revealed an increased expression of TSLP in tissue sections of the AD model as compared to PS model and control samples (Supplementary Figure 4A). It has been previously shown that IL-1β, TNFα, IL-4 and IL-13 cytokines, found at high levels in lesional skin of atopic dermatitis patients, can synergize to induce TSLP expression in keratinocytes (Bogiatzi et al., 2007). Quantitative mRNA expression analysis confirmed these results. We found high levels of expression of this cytokine exclusively in those samples with detectable human IL4 mRNA expression (Supplementary Figure 4B). TSLP expression was also found to be increased in human biopsy samples corresponding to AD patients as compared to PS or normal human skin (Supplementary Figure 2C).

The immune defense barrier function of the skin is enhanced by the inducible expression of antimicrobial peptides and proteins (AMPs). This barrier is rapidly activated when skin integrity is compromised after injury or infection. Difference in AMP expression between PS and AD appears to be an important factor in the susceptibility of both disorders to secondary infection (Ong et al. 2002; de Jongh et al. 2005). In the present work, we have observed a clear upregulation of psoriasin (S100A7) expression in samples corresponding to both skin-humanized mouse models, although higher levels of expression were observed in the PS model (Figure 4). The induction of psoriasin expression correlated with the observed increase in epidermal proliferation (Figure 3). We have also observed a clear up-regulation of calprotectin (S100A8/9) expression exclusively in PS skin-humanized mouse model samples involving all layers of epidermis, except for the basal cell layer (Figure 4). Both Th2 and Th17/Th1 appeared to slightly induce elafin (P13) expression in the most upper layers of the epidermis. In the presence of a mild barrier disruption by tape-stripping, Th17/Th1 but not Th2 cells, increased elafin expression that is localized in spinous layers of the epidermis. Th1+IL17+IL22+TS was the condition that yielded the highest levels of elafin expression with more epidermal layers affected (Figure 4). The epidermal protease inhibitor SLPI was detected by IHC exclusively in the upper layers of epidermis of the PS model (Figure 4). We have also observed higher expression...
levels of hBD-2 and LL-37 AMP expression in samples corresponding to the PS as compared to the AD skin-humanized mouse model (Supplementary Figure 5). As observed with the epidermal differentiation markers, our models recapitulate the patient conditions since, epidermal expression of calprotectin (S100A8/9), elafin (PI3) and SLPI were also highly induced in human skin biopsies from PS patients as compared to AD (Supplementary Figure 2).

Dermal characterization of PS and AD skin-humanized mouse models

In order to demonstrate the persistence of the injected human Th1, Th17 and Th2 cells in samples obtained from our skin-humanized mouse model, we used a human-specific anti-hCD3ε antibody. T cells were localized by IHC near the dermo-epidermal junction and even infiltrating the epidermis in some sample sections (Figure 5). Mast cells have been shown to be important players in the pathogenesis of PS and AD (Fischer et al., 2006). In the present work, we have observed increased number of IL8+ mast cells by toluidine blue staining and IHC in samples corresponding to the skin-humanized mouse model for PS and AD (Figure 5). Increased numbers of myeloperoxidase (MPO) positive cells were detected in samples corresponding to the PS model as compared to the AD model. In contrast, higher numbers of eosinophils were detected in samples corresponding to the AD model by using a specific anti-EMBP antibody which reacts with the eosinophil major basic protein (EMBP), a constituent of the crystalline core of the eosinophil granule (Figure 5). Augmented angiogenic reaction and increased vascular permeability are also key features of many chronic inflammatory diseases, such as PS and AD. The expression of adhesion molecules on activated blood vascular endothelial cells enables extravasation of inflammatory cells (Zgraggen et al., 2013). In this context, ICAM-1 marker expression for endothelial activation has also been investigated. The presence of dilated capillaries in the dermis of both PS and AD skin-humanized mouse models was assessed by CD31 staining, that correlated with an enhanced ICAM-1 expression in dermal vessels in both models (Supplementary Figure 6).
Discussion

An intact barrier function is required in order to protect the body from a wide variety of external insults. A defective skin barrier is a key feature of chronic inflammatory skin diseases. Several reports have demonstrated an altered expression of genes belonging to the epidermal differentiation complex (EDC) in samples obtained from lesional skin of PS and AD patients compared to healthy donors or uninvolved skin. Consistently, we have observed increased involucrin expression in the experimental humanized mouse model for PS that is in agreement with results obtained by different laboratories using microarray analyses on mRNA from patients with chronic plaque PS and AD (de Jongh et al., 2005; Suarez-Farinas et al., 2010). Altered patterns of loricrin expression were detected in both the skin-humanized mouse models for PS and AD. We have previously reported the presence of focal areas of inhibition of loricrin expression in the skin-humanized mouse model for PS corresponding to areas where granular layer was absent, and that this inhibition is mainly due to the presence of the Th17-derived IL22 cytokine (Guerrero-Aspizua et al., 2010). In the present work, we have observed that loricrin expression was induced in the skin-humanized mouse model for AD, concomitantly with an increased thickness in the granular layer found in these samples. With respect to epidermal proliferation markers, it has been shown that induction of KRT17 is specific for the inflammatory reactions associated with high levels of STAT1-activating cytokines, such as IFN\(\gamma\), IL6 and leukemia inhibitory factor (Komine et al., 1996). It has also been described that IL22, IL17A and IFN\(\gamma\), can synergistically induce KRT17 expression in keratinocytes (Zhang et al., 2012). Other cytokines working through STAT1-independent pathways, such as IL3, IL4, IL10, and granulocyte macrophage colony stimulating factor (GMSF) were shown to have no effect on KRT17 expression (Komine et al., 1996). In agreement with these reports, we have observed a clear induction in KRT17 expression exclusively in the skin-humanized mouse model for PS. We have also observed increased numbers of Ki67-positive nuclei by IHC in the skin-humanized mouse model for PS as compared to AD, especially in suprabasal layers of the epidermis.

In addition to structural proteins, multiple antimicrobial peptides and proteins (AMPs) contribute to the skin barrier function playing a crucial role in defense. Among them, cathelicidins, \(\beta\)-defensins, and S100 proteins are part of this antimicrobial skin barrier.
It has been demonstrated that IL22, together with IL17, synergistically increases expression of skin AMPs, including S100A7 (psoriasin), S100A8/9 (calprotectin) and β-defensin-2 (hBD-2) (Liang et al., 2006). The increased susceptibility of AD skin to microorganisms has been explained as the result of local up-regulation of Th2 cytokines, such as IL4 and IL13 that have been shown to inhibit AMP expression (Ong et al., 2002). In the present work we have demonstrated that a large number of AMPs are highly induced in the skin-humanized mouse model for PS as compared with the AD model, thus confirming previous studies (de Jongh et al., 2005).

Accumulating evidence supports the notion that skin barrier dysfunction plays a prominent role in the development and perpetuation of PS and AD inflammatory diseases of the skin. In fact, the use of emollients to replace ceramide content and to achieve normalized AMP expression has been shown to be effective in restoring epidermal function (Elias et al., 2008; Rodriguez-Martin et al., 2011). In this regard, we have previously demonstrated that normalization in hBD-2 expression in the epidermis of the skin-humanized mouse model for PS by topical application of DEFB4-siRNA-containing SECosomes, resulted in the recovery of TGM activity, filaggrin expression and SC appearance, as well as a reduction in the number and size of blood vessels in the dermal compartment (Bracke et al., 2014).

Modeling a complex disease such as atopic dermatitis is a challenge as it cannot be envisioned as a unique clinical entity. Lesions of AD may be classified in acute, subacute or chronic according to the clinical findings and differential histological hallmarks of each stage of the disease can be distinguished in skin lesions. Chronic lichenified lesions of AD share some of the histological features of psoriasis with marked epidermal hyperplasia, elongation of the rete ridges, and prominent hyperkeratosis. Additional variability is found depending on patient’s age, severity of inflammation, chronic scratching and the presence of secondary infections. Lesions in various stages often exist in the same individual. While a Th2 signal predominates the acute phase, chronic lesions are characterized by a Th2 to Th1 switch response with elevated expression of IFNγ, IL12 and GM-CSF. The skin-humanized mouse model for AD presented herein, more likely reflects the acute condition, as no Th1 lymphocytes are present in these samples. The comparative histological and immunohistochemical analyses in human skin biopsies corresponding to the acute/subacute phenotype supports this assumption (Supplementary Figure 2). In addition, two distinct forms of AD can be distinguished clinically. The intrinsic variant (15–45% of patients) is
characterized by a lack of IgE-mediated sensitization while the extrinsic type of AD occurs in the majority of affected children and is associated with the presence of allergen specific IgE against inhalant and/or nutritive allergens. Early onset of this type of AD constitutes a known risk factor for the subsequent development of other atopic diseases. Our present skin-humanized mouse model for AD, does not include specific antigen-sensitized T-lymphocytes. However, it has also been demonstrated that a high total serum IgE level is also a strong risk factor for AD in children and is associated with persistent eczema in adults (Zheng et al., 2011). Although we were able to detect human T lymphocytes in lymph nodes from animals that were intradermally injected with Th2 cells (AD model), it seems that the low percentage of human T cells detected (0.186-0.375 %), although significant, is not sufficient to drive a B cell response with detectable serum IgE production (data not shown).

Although a limitation exists, as the skin-humanized mouse model for AD presented here do not cover all the clinical manifestations of the disease, it offers unique possibilities to dissect the contribution of the different lymphocyte subpopulations/cytokines to the cutaneous phenotype. Accepted mouse models for AD include those induced by epicutaneous application of sensitizers, transgenic and knockout mice and spontaneous mouse models of AD (Jin et al., 2009). The present AD animal model developed in a humanized context is to our knowledge previously unreported. Skin-humanized models for inflammatory diseases emerge as powerful tools to study the mechanisms underlying the pathogenesis of these diseases. These models offer unique possibilities to study the specific phenotypic alterations of the epithelium that occur in the presence of either Th17/Th1 or Th2 lymphocyte subsets in a humanized context. These studies will be of interest to unravel the mechanisms involved in different stages of both pathologies. Although generation of skin-humanized mouse models for inflammatory diseases is rather technically complex, the possibility of obtaining a large number of animals with the disease phenotype starting from healthy donor cellular components represents a main advantage over other current animal models.
Materials and Methods

Lymphocyte Isolation and T Cell Expansion
Human umbilical cord blood samples from normal full-term deliveries were obtained from the Centro de Transfusión de la Comunidad de Madrid and in all cases written informed consent was obtained. Primary human T cells were isolated from umbilical cord blood by Ficoll-Hypaque (Pharmacia, Piscataway, NJ) gradient centrifugation and negative immunomagnetic selection using the Naive CD4+ T Cell Isolation Kit II (Miltenyi Biotec Inc., Auburn, CA, USA). For Th1 differentiation, cells (2 x 10^6/ ml) were activated in anti-CD3 plus anti-CD28 (clones HIT3a and CD28.2; BD PharMingen, Franklin Lakes, NJ, USA) coated plates. Then, cells were cultured for 24 h in the presence of 40 U/ml hIL2 (Roche Applied Science, Indianapolis, IN, USA) and for additional 6–20 days in the presence of 5 μg/ml blocking anti-IL4 mAb and 2.5 ng/ml hIL12 (R&D Systems Inc., Minneapolis, MN, USA). For Th17/Th1 cell differentiation isolated naïve CD4+ T cells were cultured for 5 days in anti-CD3 plus anti-CD28-coated plates in the presence of 5 μg/ml blocking anti-IL4 and anti-IFNγ antibodies (R&D Systems Inc.), 50 ng/ml hIL-6 and 10 ng/ml IL1β (Miltenyi Biotec Inc.). Then, cells were washed and incubated in media supplemented with 20 U/ml hIL-2 and 20 ng/ml IL23 (R&D Systems Inc.) for additional 2-16 days. For Th2 differentiation cells were cultured for 7-21 days in anti-CD3 plus anti-CD28-coated plates in the presence of 10 U/ml IL2, 10 ng/ml IL4 (Miltenyi Biotec Inc.), 2 μg/ml blocking anti-IL12 (R&D Systems Inc.) and 5 μg/ml blocking anti-IFNγ antibodies.

Bioengineered Human Skin Equivalents and Grafting
Primary human keratinocytes and dermal fibroblasts were isolated by enzymatic digestion from donor skin biopsies that were obtained from Spanish Blood and Tissue Bank (Centro Comunitario de Sangre y Tejidos, Oviedo, Spain) and in all cases written informed consent has been obtained. The study was conducted according to the Declaration of Helsinki Principles. Primary keratinocytes were cultured on a feeder layer of lethally irradiated (X-ray; 50 Gy) 3T3-J2 cells as previously described (Guerrero-Aspizua et al., 2010). Primary fibroblasts were cultured in DMEM containing 10% FBS at 37°C in a humid atmosphere containing 5% CO₂. The bioengineered human skin equivalent is based on the use of fibrin containing live fibroblasts as a
dermal component and was prepared as previously described (Guerrero-Aspizua et al., 2010).

For the grafting protocol immunodeficient Rj: NMRI—Foxn1nu (NMRI nu) mice (6–8 weeks old) were used (Elevage Janvier, Le Genest Saint Isle, France). Mice were aseptically cleansed and grafted as previously described (Carretero et al., 2013). Mice were housed for the duration of the experiment at the CIEMAT Laboratory Animal Facility (European Registration number ES280790000183) in pathogen free conditions using microisolators, individually ventilated cages type IIL, at a maximum of six mice per cage, with 25 air cage changes per hour and heat-treated soft wood pellets as bedding. All experimental procedures were carried out according to the European and Spanish laws and were in keeping with regulations on the protection and use of animals in scientific research. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) according to all legal regulations, biosafety and bioethics guidelines.

**Intradermal Injection of T Lymphocyte Subpopulations and Recombinant Cytokines**

Recombinant cytokines (200 ng) and/or *in vitro* derived T lymphocyte subpopulations (5x10⁵) were inoculated by intradermal injection into the stable engrafted human skin every other day for two weeks as previously described (Guerrero-Aspizua et al., 2010). Where indicated, the same skin area was tape stripped 15 times before injection. Mice were sacrificed by carbon dioxide asphyxiation two days after the last intradermal injection and skin biopsies were taken and processed for histological and IHC analysis.

**Histology and IHC**

Formalin-fixed paraffin sections (4–6 μm) were used for standard histological and IHC analyses. To determine tissue architecture, sections were stained with hematoxylin–eosin (Gill 2 Hematoxylin and Eosin Y alcoholic; Thermo Sandon, Cheshire, UK) following a standard procedure. For immunoperoxidase staining, the following primary antibodies were used: anti-Elafin/Skalp (Abcam, Cambridge, UK), anti-IL8 (LifeSpan Biosciences Inc., Seattle, WA, USA), anti-involucrin (clone SY5; Sigma-Aldrich), anti–Ki-67 (clone SP6, Neomarkers), anti–KRT1 and anti–KRT17 (Sigma-Aldrich), antiloricrin (Babco, Richmond, CA), anti-macrophage L1 protein calprotectin (Abcam), anti-myeloperoxidase (MPO) (HyCult bio-technology b.v., Uden, The Netherlands),
anti-psoriasin/HID5/S100A7 (Imagenex, San Diego, CA), anti-SLPI (Sigma-Aldrich) and anti-EMBP (S-16, Santa Cruz Biotechnology, Inc., Santa Clara, CA, USA). For alkaline phosphatase detection rabbit anti-human anti-TSLP (LifeSpan BioSciences, Inc.) and anti-hCD3ε antibody (Dako, Glostrup, Denmark) were used. Mast cells were detected by toluidine blue staining of formalin-fixed samples.

Conflict of interest

The authors state no conflict of interest.

Acknowledgements

We especially thank our technician L. Retamosa and F. Sánchez-Sierra for histology assistance, R. Sanchez for help with flow cytometry data analysis and J. Martinez and E. Almeida for animal care. This work was partially supported by grants from the Science and Innovation Ministry of Spain (SAF2013-43475), Comunidad de Madrid (S2010/BMD-2420 and S2010/BMD-2359), Instituto de Salud Carlos III (PI11/01225 and PI14/00931) and Spanish Ministry of Economy and Competitiveness (MINECO) [BIO2011-27069].
References


Figure legends

Figure 1. **PS and AD skin-humanized mouse models.** a) Schematic diagram of the experimental design for the generation of a skin-humanized mouse models for PS and AD. b) H&E staining was performed on formalin-fixed paraffin-embedded sections of human skin grafts treated with PBS1x or those injected intradermally with *in vitro*-differentiated Th17/Th1 or Th2 lymphocyte subpopulations. Tape-stripping (TS) was applied where indicated. Scale bar = 100 μm. Images are representative of two independent experiments with two to three mice per group.

Figure 2. **Immunohistochemical analysis of epidermal differentiation markers in the PS and AD skin-humanized mouse models.** Consecutive formalin-fixed paraffin-embedded sections used in Figure 1b were stained for involucrin, KRT1, and loricrin differentiation markers. Scale bar = 50 μm. Images are representative of two independent experiments with two to three mice per group.

Figure 3. **Immunohistochemical analysis of epidermal proliferation markers in the PS and AD skin-humanized mouse models.** a) Consecutive formalin-fixed paraffin-embedded sections used in Figures 1b and 2 were stained for Ki67 and KRT17. Arrowheads show Ki-67-positive proliferating cells. Scale bar = 50 μm. Images are representative of two independent experiments with two to three mice per group. b) The proliferation index (PI) was determined as the percentage Ki67 staining of the epidermis either in the basal layer (basal PI) or suprabasal layers (suprabasal PI), and both are referred as number of positive stained cells per 100 total basal cells. At least two non-overlapping high power fields (HPF) were quantified (1 field = 0.379 mm²), and 3-5 samples per experimental group of animals were included. Data represent mean ± SEM (*P<0.05 when compared with the PBS1x + TS control group according to a t-test).

Figure 4. **Immunohistochemical analysis of epidermal AMP expression in the PS and AD skin-humanized mouse models.** Consecutive formalin-fixed paraffin-embedded sections used in Figures 1b, 2 and 3 were stained for psoriasin (S100A7),
calprotectin (S100A8/9), elafin (PI3) and SLPI. Scale bar = 50 μm. Images are representative of two independent experiments with two to three mice per group.

**Figure 5. Composition of dermal inflammatory infiltrate in the PS and AD skin-humanized mouse models.**

a) The injected human T cells in the tissue were localized by using an antibody against CD3-ε T cell specific antigen. The composition of the inflammatory infiltrate was analyzed by using specific stains and IHC of consecutive formalin-fixed paraffin-embedded tissue sections. Mast cells were detected using toluidine blue staining. Anti-IL8 was used to detect dermal positive mast cells. Cells of the granulocyte series were detected using specific antibodies against MPO and EMBP. The arrowheads indicate positive cell staining. Scale bar = 50 μm. Images are representative of two independent experiments with two to three mice per group.

b) Quantification of inflammatory infiltrate in the PS and AD skin-humanized mouse models. The average number of positive-stained inflammatory cells were quantified by examining two-eight randomly selected high power fields (HPF) per sample (1 field = 0.093 mm²) from three to five tissue sections for each experimental group of animals. The number of positive cells was expressed as cells per mm² (mean ±SEM). *P<0.05, **P<0.01.
a

Peripheral blood

Skin biopsy (enzymatic digestion)

Healthy donors

In vitro isolation and cytokine-directed polarization

Fibroblasts

Keratinocytes

Bioengineered skin equivalents

Intradermal injection and tape-stripping

Graft onto nu/nu mice

AD model

Th2

Th17/Th1

Th2 + TS (AD model)

Th17/Th1 + TS (PS model)

b

PBS 1x

PBS1x + TS