more responsive to IL-33 than were IL-33–elicited BMBas. The types of basophils that develop in the murine models of prurigo and AD are uncertain. However, the immunological environment in these models appeared to be ideal for AREG generation irrespective of the types of basophil, because both of these models showed increased local production of IL-33 and TSLP, along with elevated serum IgE (Hashimoto et al., 2015; Li et al., 2006, 2017).

The relationship between AREG and immunological processes in chronic skin inflammation is still uncertain. Epidermal proliferation induced by AREG (Pastore et al., 2008) may contribute to increased generation of IL-33 and TSLP from epidermal cells. Nonetheless, our results indicate that basophil-derived AREG may be a therapeutic target for chronic skin inflammation, including that occurring in prurigo and AD.

Data availability statement
Data sets related to this article can be found at https://doi.org/10.17632/y5nkxkfr.1, an open-source online data repository hosted at Mendeley Data.

ORCIDs
Takashi Hashimoto, https://orcid.org/0000-0001-6779-5598
Takahiro Satoh, https://orcid.org/0000-0002-2244-6332
Hajime Karasuyama, https://orcid.org/0000-0003-0689-0836
Hiroyo Yokozeki, https://orcid.org/0000-0002-5773-9485

CONFLICT OF INTEREST
The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS
TH: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, validation, visualization, and writing (the draft). TS: methodology, project administration, supervision, and writing (review and editing). HK: methodology, resources, supervision, and writing (review and editing). HY: funding acquisition, project administration, supervision, and writing (review and editing). All authors have read the manuscript and approved this submission.

Takashi Hashimoto1,2,*
Takahiro Satoh2
Hajime Karasuyama3
and Hiroyo Yokozeki1
1Department of Dermatology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan; 2Department of Dermatology, National Defense Medical College, Saitama, Japan; and 3Department of Immunology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan
*Corresponding author e-mail: hashderm@tmd.ac.jp

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2019.02.023.

REFERENCES


Differential Hairless Mouse Strain-Specific Susceptibility to Skin Cancer and Sunburn


TO THE EDITOR
Although there is an ever-growing variety of hairless allelic mutations on a diverse number of inbred and outbred strains (Benavides et al., 2009), albino hairless mice, particularly outbred (largely uncharacterized/nonpedigree) Crl:SKH1–Hhr (hereafter, SKH1) mice have been used extensively, almost exclusively, in UV light-induced skin cancer studies for many decades. These mice lack hair (but not hair follicles) and grow long curved nails as adults. They have essentially an intact immune system, and some experts claim that the skin resembles human skin. These mice develop the equivalent of the human genetic disease papular atrichia (Sundberg et al., 1989) and develop skin cancers that resemble UV-induced squamous cell carcinoma (SCC) at both the molecular and morphological levels (Benavides et al., 2009). When these outbred mice are crossed with inbred or insipient congenic strains to study the effect of a mutated gene, investigators

Abbreviation: SCC, squamous cell carcinoma

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often disregard the fact that they have essentially no controls. Because the vast majority of genetically engineered mice are created on the C57BL/6J or 6N inbred backgrounds (or made congenic on these and other closely related substrains (e.g., Aylor et al., 2011), having the same Hrhr allele on one of these strains would potentially provide a better model to study the genetics underlying UV light-induced skin cancer. Such a congenic line was created, the C57BL/6(B6).Cg-Tyr<sup>C</sup>-<sup>−</sup> Hrhr/hr/J strain (hereafter, B6.Cg). In initial acute UV light studies, these B6.Cg mice exhibited a more pronounced sunburn response to UVB irradiation than SKH1 mice (Konger et al., 2016).

To determine the comparative effects of prolonged exposure to UV light between these strains, congenic B6.Cg (stock no. 017840, The Jackson Laboratory, Bar Harbor, ME) (n = 7 females, 2 males) and outbred albino SKH1 (strain code 477, Charles River Laboratories, Wilmington, MA) (n = 7 females, 7 males), both homozygous for the hairless mutation, were chronically irradiated with 180 mJ/cm<sup>2</sup> of UVB three times per week. After the mice were euthanized, representative lesions of each size were processed for histologic evaluation. Two or more skin sections of 1 cm or longer containing two or more neoplasms or ulcers were evaluated. Lesions were scored based on criteria previously described (Benavides et al., 2009). Briefly, tumors were graded as premalignant papillomas (grades 1–3), microinvasive SCC (grades 1–3), or fully invasive SCCs. Spindle cell neoplasms were labeled by immunohistochemistry with antibodies against S100, KRT5, KRT14, pankeratin, and vimentin. All work was approved by the institutional animal care and use committees. Detailed materials and methods are provided in the Supplementary Materials online.

The B6.Cg strain developed wounds or ulcers that attempted to heal by pseudoepitheliomatous hyperplasia (Figure 1). These wounds often developed in the neck area and became progressively worse by scratching. Ulceration was observed as early as 4 weeks after UV light exposure and became pronounced at 13–19 weeks into the study, requiring that mice be euthanized (see Supplementary Table S1 online). All B6.Cg mice (9/9) developed cutaneous ulcers. Four of the nine B6.Cg mice developed papillomas, three with grade 1 and one with grade 2. Three of the nine B6.Cg mice developed grade 2 malignant microinvasive SCC, all of which were very

### Table 1. Summary of histology-based diagnoses of representative lesions

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>Ulcer, n (%)</th>
<th>Premalignant Papilloma Grade, n (%)</th>
<th>Malignant Microinvasive SCC Grade, n (%)</th>
<th>Fully Invasive SCC, n (%)</th>
<th>Total Malignant SCC Lesions Summary, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6.Cg (n = 9)</td>
<td>9 (100)</td>
<td>3 (33.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (33.3)</td>
</tr>
<tr>
<td>SKH1 (n = 14)</td>
<td>6 (42.9)</td>
<td>3 (21.4)</td>
<td>2 (14.3)</td>
<td>4 (28.6)</td>
<td>13 (92.9)</td>
</tr>
</tbody>
</table>

Abbreviations: SCC, squamous cell carcinoma.

*Lesion diagnosis and staging followed the criteria described by Benavides et al. (2009).*
small. None of the B6.Cg mice developed fully invasive SCC (Table 1).

By contrast, fewer SKH1 mice developed cutaneous ulcers (one of which was histologically confirmed); however, none of the ulcers progressed to the same level of severity as those in the B6.Cg mice. Ulcers observed in SKH1 mice developed significantly later compared with B6.Cg mice (mean = 28 vs. 14.8 weeks, \( P > 0.05 \)) (see Supplementary Table S1). Severe ulcer formation in B6.Cg was also observed at the Moffitt Cancer Center (by KYT) in three of four UV-irradiated B6.Cg mice (12.50 kJ/m² per week). In SKH1 mice, three of four UV-irradiated B6.Cg mice the Moffitt Cancer Center (by KYT) in three of four UV-irradiated B6.Cg mice (12.50 kJ/m² per week). In SKH1 mice, three of four UV-irradiated B6.Cg mice (12.50 kJ/m² per week). In SKH1 mice, three of four UV-irradiated B6.Cg mice (12.50 kJ/m² per week). In SKH1 mice, three of four UV-irradiated B6.Cg mice (12.50 kJ/m² per week). In SKH1 mice, three of four UV-irradiated B6.Cg mice (12.50 kJ/m² per week). In SKH1 mice, three of four UV-irradiated B6.Cg mice (12.50 kJ/m² per week). In SKH1 mice, three of four UV-irradiated B6.Cg mice (12.50 kJ/m² per week). In SKH1 mice, three of four UV-irradiated B6.Cg mice (12.50 kJ/m² per week).

Comparison of average total ulcer length resulted in a significant difference, with 32 ± 14 mm for B6.Cg (19 ulcers from nine mice) and 10 ± 5 mm for SKH1 mice (six ulcers from six mice) (\( P < 0.05 \)) (see Supplementary Figure S1). Other types of neoplasms observed included one of the 14 SKH1 mice with a spindle cell tumor (S100 weakly positive, KRT5 and KRT14 negative, pan-keratin negative, vimentin positive) (see Supplementary Figure S2 online); in addition, two of the nine B6.Cg mice had mammary ductal adenocarcinomas.

Because the B6.Cg is an inbred strain, Konger et al. (2016) suggested that the strain could be an improved model to assess the influence of the genetic background in cutaneous environmental or toxicological and photo-biology studies. However, the current study shows limitations of this strain for chronic UV irradiation studies. Although very small, premalignant papilloma and microinvasive SCC were diagnosed in a subset of B6.Cg mice. Larger groups of these mice might develop invasive SCCs over time in an adjusted experimental setup (e.g., reduced UVB dosage), because ulceration might be decreased under less UVB exposure; however, under the current experimental settings, this strain developed large ulcers, necessitating that mice be euthanized.

These observations support the critical role of genetics in the ability of skin to respond to UVB light by sunburn, with subsequent clinically severe ulceration or malignant transformation but not necessarily both together. Predilection to ulcer formation in the B6.Cg strain is known spontaneous dermatologic condition in B6 substrains (Sargent et al., 2015; Sundberg et al., 2014, 2011). Although chronic UV exposure could potentially exacerbate this strain predisposition to ulceration, the B6.Cg mice in this study lacked the characteristic follicular dystrophy of ulcerative dermatitis in B6 mice (Sundberg et al., 2011).

C57BL/6J mice are known to be resistant to UV-induced skin tumor formation (Kitajima et al., 1995; Naito and DiGiovanni, 1989). Dissecting the genetic differences between the outbred SKH1 and congenic B6.Cg albinohairless mice underlying the differential UV response is difficult due to the outbred and uncharacterized/non-pedigreed status of SKH1. However, extensive genomic and gene expression analysis could allow for identification of cross-strain genomic drivers, explaining the susceptibility to tumor or ulcer development (Chitassaadeh et al., 2016; Fujwara et al., 2018; Nagase et al., 1996). An inbred SKHIN/Sprd strain was created and described; however, these mice are no longer available (Perez et al., 2012). Alternatively, if similar or more extreme response differences can be shown among inbred strains, such as the collaborative cross-strains, or large populations of outbred strains, such as the diversity outcross, genetic susceptibility to sunburn versus SCCs can be easily defined and are attainable future goals.

CONFLICT OF INTEREST

JPS sponsors or has sponsored research with Takeda, Theravance, and Curadim and is a consultant for Bioniz, all of which have no relevance to this project. AYV, MM, KYT, and JO state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: AYV, JPS, KYT, and JO. Methodology: AYV and MM. Validation: KYT. Formal

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SUPPLEMENTARY MATERIALS AND METHODS

Animals

B6 congenic albino hairless mice (B6.Cg-Tyr-c21/c21 H3h/hr); stock no. 017840) were obtained from The Jackson Laboratory. The albino hairless SKH1 mice (Crl:SKH1-H3h/hr, strain code 477) were obtained from Charles River Laboratories. All mice were housed in the animal facility at the University of Connecticut Health Center (Farmington, CT). All experiments involving mice followed protocols that were approved by the institutional animal care and use committees from both the University of Connecticut Health Center and The Jackson Laboratory and were performed in accordance with National Institutes of Health regulations.

SKH1-H3h/hr mice were 9 weeks (pilot study: n = 2 female, 2 male) or 27 weeks of age (main study: n = 5 female, 5 male) at the initiation of UV light irradiation. B6.Cg-Tyr-c21/c21 H3h/hr mice were 13 weeks (pilot study: n = 2 female, 2 male) and 24 weeks (main study: n = 5 female) of age at the start of the UV treatment.

Mice in the pilot study were either housed singly or two mice co-housed. In the main study, five mice were co-housed as long as possible unless co-housing was not justifiable because of fighting (among males).

The mice were originally part of a microbiome-SCC related study (manuscript in preparation). Here, we report the data on the control mice that received a topical application of the vehicle control (20% glycerol in tryptic soy broth) twice per week during the course of the UV treatment until harvest. Tryptic soy broth (DB Bacto Tryptic Soy Broth) was manufactured by Becton, Dickinson and Company (Sparks, MD), and glycerol was purchased from Sigma-Aldrich (St. Louis, MO). Because these control mice were part of a larger data set, the sexes were not uniformly distributed. Therefore, sex differences reported by others (Thomas-Ahner et al., 2007) with regard to tumor development could not be statistically assessed.

After 20 weeks of UV treatment, mice were monitored for development of skin ulcers or cancers for up to 14 weeks, were euthanized by CO2 asphyxiation, and were necropsied. Skin was collected and processed routinely for histologic evaluation, and lesions were scored based on the criteria described by Benavides et al. (2009). The mice were euthanized, and tissues were harvested; additionally, a small number of mice developed nontumorous lesions from the UV treatment and were euthanized before their cancerous tumors reached the maximum size (1 cm2), as approved in the institutional animal care and use committee protocol. The animal study was conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

UVB treatment

Most mice were UV-irradiated for 2 weeks with 90 mJ/cm2 times per week and then with 180 mJ/cm2 times per week for 18 weeks. A small subset of mice (n = 2 female, 2 male from each strain) initially received an acute one-time dosage (72–630 mJ/cm2) to determine the optimal dosage, followed by 8 weeks of 100–200 mJ/cm2 and 12 weeks of 180 mJ/cm2 three times weekly (pilot study). Four Narrowband TL40W/01 RS (Ultraviolet-B) bulbs (Philips, Hamburg, Germany) connected to an adapter were used for accurate UV apparatus. Mice were transferred into custom-made acrylic glass cages (covered with metal mesh) and irradiated from above. The UVB radiation was regularly checked using a calibrated meter (UVB-500C; National Biological Corporation, Beachwood, OH).

Tumor counting

During the course of the experiment, diameters of all lesions were measured with a digital caliper, counted, and documented. In gross observation, lesions larger than 1 mm2 were included in this article as SCC-related lesions, similar to Thomas-Ahner et al. (2007). After mice were euthanized, the skin was harvested, and representative lesions of each type and size, including adjacent normal-appearing skin, were removed, fixed, and processed. Two or more skin sections of 1 cm or longer containing two or more neoplasms or ulcers were histologically evaluated. Because the focus of the original study was on tumor development, some ulcers (for SKH1) were not histologically evaluated or measured.

Measurement of lesions

With an Olympus BX50 microscope and a DP27 digital camera (Olympus, Tokyo, Japan), lesions were measured in microns by using the arbitrary line-measuring tool using 2× or 4× objectives. Ulcers were measured from one edge, where the epidermis ended, to the opposite side of the ulcer, where the epidermis was intact. Benign or malignant neoplasms were measured in two dimensions, as the linear distance from the normal epidermis to the normal epidermis on the opposite side. Normal refers to the moderate to severe acanthosis that was a diffuse feature of these hairless mice. The height of the neoplasms was measured from the lowest point of invasion into the dermis to the top of the Malpighian layer, excluding the orthokeratotic hyperkeratosis.

The average tumor area (mm2) and sum tumor area per mouse (mm2) were calculated based on the diameters of each tumor measured at the study endpoint (using digital calipers), assuming approximately circular tumor formation.

Total ulcer length was assessed using endpoint gross images of the mice in ImageJ, version 2.0.0 (National Institutes of Health, Bethesda, MD).

Histology

The tissue was fixed in Fekete's acid-alcohol-formalin solution (Silva and Sundberg, 2012) or fresh 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA) in Dulbecco's phosphate buffered saline (Gibco by Life Technologies, Grand Island, NY), processed routinely for histology, sectioned at 5 μm, and stained with hematoxylin and eosin. Lesions were scored based on the criteria previously described (Benavides et al., 2009). Additional photomicrographs are available in the Mouse Tumor Biology Database (http://tumor.informatics.jax.org/).

Immunohistochemistry

Spindle cell neoplasms were labeled by immunohistochemistry with anti-S100 (catalog no. ab11598, 1:200), anticytokeratin 14 (catalog no. ab181595, 1:10,000), anti-cytokeratin 5 (catalog no. ab24647, 1:2,000), anti-pankeratin (pk-26, catalog no. ab6401, 1:500) and anti-vimentin (catalog no. ab92547, 1:200) antibodies, all purchased from Abcam (Cambridge, MA) at the Histopathology Sciences Shared Service at The Jackson Laboratory. Protocols are available on the Mouse Tumor Biology

Statistical analysis
Statistical significance of lesion onset data and lesion measurements were assessed using the Mann-Whitney Wilcoxon test with $P < 0.05$ deemed as significant. R packages ggplot2 and reshape were used to plot data (Wickham, 2009, 2017).

**SUPPLEMENTARY REFERENCES**

Supplementary Figure S1. Tumor frequency, multiplicity, and lesion size for B6.Cg and SKH1 mice. UVB treatments were completed after 20 weeks, and mice were continuously monitored until tumor/lesion development. Tumor frequency (based on gross observation) in SKH1 reached a plateau of 100% at 23 weeks. One SKH1 mouse was euthanized in week 26, and the lesion biopsied was diagnosed as premalignant; hence, the tumor frequency decreased to 92.9%. (a) The tumor frequency in B6.Cg mice reached 55%, of which 33.3% were histologically confirmed as malignant at the endpoint. (b) Mean numbers of premalignant and malignant tumors (based on gross observation) that developed over time. Asterisks indicate incomplete data. For SKH1, the upper half and for B6.Cg, the lower half of the error bar (standard deviation) is shown for demonstration purposes. (c–e) Endpoint measurements of lesions show a significant size difference between the two strains ($P < 0.05$). Vertical lines indicate median lesion size.
Supplementary Figure S2. Spindle cell neoplasms in SKH1. (a, b) A representative large spindle cell sarcoma was locally invasive. (c–h) The surface epidermis and hair follicle remnants were positive for (c, d) pan-cytokeratin (multiple keratin proteins) and mouse-specific keratins that normally localize to the basal cells of stratified squamous epithelium, (e, f) KRT14 and (g, h) KRT5. (i–m) The spindle cells were (i, j) positive for vimentin and (k, l) weakly positive for S100. Scale bars = 500 μm in the upper panels (a, c, e, g, i, k) and 50 μm for the lower panels (b, d, f, h, j, l). H&E, hematoxylin and eosin.

Supplementary Table S1. Overview of gross observation of ulcer and SCC-related lesion development

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>Onset of Ulcers, weeks</th>
<th>Onset of SCC-Related Lesions, weeks</th>
<th>Strain</th>
<th>Sex</th>
<th>Onset of Ulcers, weeks</th>
<th>Onset of SCC-Related Lesions, weeks</th>
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<tbody>
<tr>
<td>B6.Cg</td>
<td>M</td>
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<td>SKH1</td>
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<td>19</td>
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</tbody>
</table>

Average 14.8 24.8 28 18.4

Abbreviations: F, female; M, male; SCC, squamous cell carcinoma.

1Gross observation of the onset of SCC-related lesions and the onset of ulcers between the B6.Cg and in SKH1 strains was significantly different (P < 0.05).

2Did not develop into SCC.

3Lesion not confirmed as ulcer.

4Small ulcer diagnosed at the endpoint.