Ectopic Expression of Epidermal Antigens Renders the Lung a Target Organ in Paraneoplastic Pemphigus

Tsuyoshi Hata, Shuhei Nishimoto, Keisuke Nagao, Hayato Takahashi, Kazue Yoshida, Manabu Ohyama, Taketo Yamada, Koichiro Asano and Masayuki Amagai

*J Immunol* published online 31 May 2013
http://www.jimmunol.org/content/early/2013/05/31/jimmunol.1203536
Ectopic Expression of Epidermal Antigens Renders the Lung a Target Organ in Paraneoplastic Pemphigus

Tsuyoshi Hata,*† Shuhei Nishimoto,* Keisuke Nagao,* Hayato Takahashi,* Kazue Yoshida,* Manabu Ohyama,* Taketo Yamada,‡ Koichiro Asano,§∥ and Masayuki Amagai*

Paraneoplastic pemphigus (PNP) is an autoimmune disease of the skin and mucous membranes that can involve fatal lung complications. IgG autoantibodies target the cell adhesion molecules desmoglein (Dsg)3 and plakin, but the nature and targets of infiltrating T cells are poorly characterized. Moreover, the lung involvement in this skin Ag-specific autoimmune condition represents a paradox. To mimic autoimmunity in PNP, we grafted wild-type skin onto Dsg3−/− mice, which resulted in graft rejection and generation of anti-Dsg3 IgG and Dsg3-specific T cells. Transfer of splenocytes from these mice into Rag2−/− mice induced a combination of suprabasilar acantholysis and interface dermatitis, a histology unique to PNP. Furthermore, the recipient mice showed prominent bronchial inflammation of CD4+ and CD8+ T cells with high mortality. Intriguingly, ectopic Dsg3 expression was observed in the lungs of PNP mice, mirroring the observation that squamous metaplasia is often found in the lungs of PNP patients. Dsg3 and other epidermal Ags were ectopically expressed in the lungs after pulmonary injuries by naphthalene, which was sufficient for recruitment of Dsg3-specific CD4+ T cells. These findings demonstrate that squamous metaplasia after pulmonary epithelial injury may play a crucial role in redirecting the skin-specific autoimmune reaction to the lungs in PNP. The Journal of Immunology, 2013, 191: 000–000.

Received for publication December 27, 2012. Accepted for publication April 30, 2013.

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, Health and Labor Sciences Research Grants for Research on Measures for Intractable Diseases from the Ministry of Health, Labor, and Welfare of Japan, and Keio Gijuku Academic Development Funds.

Address correspondence and reprint requests to Prof. Masayuki Amagai, Department of Dermatology, Keio University School of Medicine, 35 Shinkanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. E-mail address: amagai@med.keio.ac.jp

The online version of this article contains supplemental material.

Abbreviations used in this article: B6, C57BL/6J; Dsg, desmoglein; PE, pemphigus foliaceus; PNP, paraneoplastic pemphigus; PV, pemphigus vulgaris.

Copyright © 2013 by The American Association of Immunologists, Inc. 0022-1767/13/$16.00
basilar acantholysis caused by anti-Dsg3 IgG Abs as well as interface dermatitis with CD4⁺ as well as CD8⁺ T cell infiltration and keratinocyte apoptosis. Furthermore, these mice demonstrated bronchial inflammation with a high mortality rate. Surprisingly, Dsg3 was ectopically expressed in the lungs of the PNP model mice, but not in the lungs of WT or PV model mice. Ectopic expression of Dsg3 and other epidermal Ags in the lungs was detected during epithelial remodeling after naphthalene-induced pulmonary injury. CD4⁺ T cells containing retrovirally transduced Dsg3-specific TCR (15) preferentially infiltrated into bronchial epithelia in naphthalene-injected mice, but not in those that received the control corn oil injection. Squamous metaplasia in the lungs, which showed ectopic expression of various epidermal Ags, was indeed frequently identified in patients with PNP. These findings using PNP model mice provide valuable clues to solving a long-standing mystery in PNP: why the lungs, which were thought to exhibit no epidermal Ag expression, are involved in this skin-specific autoimmune condition.

Materials and Methods

Mice

Dsg1¹⁰⁰/Dsg3⁻/⁻ mice with the C57BL/6J (B6) background, in which Dsg3-deficient phenotypes are rescued by ectopic Dsg1 expression (16), were used as Dsg3⁻/⁻ mice. In this study, “Dsg3⁻/⁻ mice” refers to “Dsg1¹⁰⁰/Dsg3⁻/⁻ mice” unless noted otherwise. These mice and Rag2⁻/⁻ mice in a B6 background (Central Institute for Experimental Animals, Tokyo, Japan) were maintained under specific pathogen-free conditions at Keio University. All mouse studies were approved by the Animal Ethics Review Board of Keio University.

Immunization of Dsg3⁻/⁻ mice via Dsg3³/⁴ skin grafts

Dorsal skin from Dsg3³/⁴ WT mice (B6) was grafted onto the backs of sex-matched Dsg3⁻/⁻ or Dsg3⁺/⁺ mice, as described previously (17), and the grafted skin was biopsied at various times after transplantation. A second skin graft was performed 28 d after the first graft for adoptive transfer studies. Graft survival was determined in accordance with a method described previously (18). For adoptive transfer studies, splenocytes from mice that received a second skin graft for 14 d or more were transferred adoptively into Rag2⁻/⁻ mice via the tail vein. In control PV model mice, Dsg3⁻/⁻ mice were immunized with recombinant mouse Dsg3 in CFA, as described previously (12).

Induction of acute lung injury by naphthalene

To examine whether a skin-specific protein could be expressed ectopically in bronchial epithelium under certain conditions, we injected naphthalene (Sigma-Aldrich, St. Louis, MO), which selectively injures Clara cells, and observed bronchial epithelium during the repair process. Naphthalene was dissolved in corn oil at 10 or 20 mg/ml and administered to mice (100 or 200 mg/kg) by i.p. injection weekly for 4 wk (19). Control mice received an identical volume of corn oil (Sigma-Aldrich).

Retroviral transduction

Retroviral transduction of a Dsg3-specific TCR (Dsg3H1) to CD4⁺ T cells from B6 mice was performed using a set of TCRs (Vα8 and β6B) of a CD4⁺ T cell clone that recognizes the peptide Dsg3301–315, as described previously (14, 15, 20).

Histology and immunostaining in mice and humans

To quantify the induction of cellular immune responses, infiltrating CD4⁺ and CD8⁺ cells were counted in frozen sections. The numbers of lymphocytes and TUNEL⁺ cells in the oral epithelia (hard palate) and the peribronchial area (cells in the epithelia and within 100-μm depth from the basement

---

**FIGURE 1.** WT skin grafted onto Dsg3⁻/⁻ mice induced both humoral and cellular immune responses with resultant graft rejection. (A) Experimental protocol for skin graft immunization. WT skin (Dsg3⁺/⁺) was grafted onto Dsg3⁻/⁻ or Dsg3⁺/⁺ mice and was biopsied (yellow circles) weekly. (B) Histological changes on Dsg3⁻/⁻ mice (top) and Dsg3⁺/⁺ littermates (bottom) on days 14, 21, and 28 after WT skin graft. Scale bar, 20 μm. (C) Skin graft survival rates after first (filled symbols) and second (open symbols) WT skin grafts on Dsg3⁻/⁻ mice (diamonds) and Dsg3⁺/⁺ littermates (squares). On day 28 after the first skin graft, the second skin graft was applied. (D) Anti-Dsg3 IgG titers as measured by ELISA after first (day 0) and second (day 28) WT skin grafts on Dsg3⁻/⁻ mice (○) and Dsg3⁺/⁺ littermates (●). (E) CD4⁺ (red) and CD8⁺ (red) T cell infiltrations, apoptotic cells as determined by TUNEL assay, and in vivo IgG deposition on keratinocyte surfaces in WT skin grafts on Dsg3⁻/⁻ mice (top) and Dsg3⁺/⁺ littermates (bottom) on day 21. Scale bars, 20 μm.
membrane) were calculated per 100-μm width of basement membrane in PNP model mice. The numbers of CD45.1+CD4+ T cells and CD45.1+CD8+ T cells infiltrating into bronchial epithelia (cells in the epithelia) were counted per 200-μm width of basement membrane as Dsg3-specific cells and Dsg3-non-specific T cells, respectively. Anti-Dsg3 IgG production was monitored using a mouse Dsg3 ELISA (21), and immunoprecipitation and immunoblotting were performed to detect the production of anti-plakin family Abs (22). Statistical analyses were performed using an unpaired t test with Welch’s correction.

Histological analyses and immunostaining of lungs were performed in a representative PNP patient, a 61-y-old female with diffuse large B cell lymphoma and positive for anti-Dsg3, anti-envoplakin, and anti-periplakin IgG autoantibodies. Deparaffinized sections were subjected to immunostaining after peroxidase deactivation by methanol containing 0.3% H2O2. Experiments with human samples were approved by Keio University Research Ethics Committee according to the Declaration of Helsinki.

Abs

The following Abs were used: anti–E-tag mAb (GE Healthcare Biosciences) conjugated with Alexa Fluor 488; anti-fluorescein/Oregon Green goat IgG fraction conjugated with Alexa Fluor 488; goat anti-rat IgG conjugated with Alexa Fluor 568 (Invitrogen); AK18 mouse anti-Dsg3 mAb (23); anti-mouse CD4 (RM4-5, GK1.5), CD8 (53-6.7), and CD45.1 conjugated with FITC (A20; BioLegend, San Diego, CA); anti-human CD4 (1F6) and CD8 (4B11; Novocastra Laboratories, Newcastle, U.K.); anti-Dsg3 (3G133; Abcam, Cambridge, UK); HRP-conjugated anti-mouse IgG Ab (Medical and Biological Laboratories, Nagoya, Japan); and anti-envoplakin Ab (Santa Cruz Biotechnology). TUNEL+ cells were detected using the In Situ Cell Death Detection kit and TMR red (Roche, Mannheim, Germany).

Results

Grafting Dsg3-expressing skin onto Dsg3−/− mice elicits Dsg3-specific cellular and humoral immune responses

To generate both humoral and cellular immune responses against Dsg3, we sought to use the skin graft technique as an immunization procedure instead of immunizing Dsg3−/− mice with recombinant Dsg3 protein, because skin allografting is known to induce immune responses mediated by CD4+ and CD8+ T cells, as well as Abs (24–26). When Dsg3+/− WT skin was grafted onto Dsg3−/− mice or Dsg3+/− littermates, the skin grafts on Dsg3−/− mice (n = 8), but not on Dsg3+/− littermates (n = 4), were rejected on days 14–28 (Fig. 1A, 1C). Second, Dsg3+/− skin grafts onto the same mice resulted in even more rapid rejection (days 7–10, n = 7). Histologically, the first skin grafts on Dsg3−/− mice showed acanthosis with mild lymphocytic infiltration on day 14, interface dermatitis with significant lymphocytic infiltration and occasional keratinocyte apoptosis on day 21, and complete necrosis of the grafted skin on day 28, in contrast to the grafts on Dsg3+/− littermates, which showed no apparent change (Fig. 1B). The infiltrating lymphocytes included both CD4+ and CD8+ T cells, and the presence of apoptotic basal keratinocytes was confirmed via in situ TUNEL assay (Fig. 1E). In addition to the cellular immune response, Dsg3−/− mice with WT skin grafts demonstrated circulating anti-Dsg3 IgG on day 14 with continuous increases in their titer thereafter (Fig. 1D), as well as in vivo IgG deposition on keratinocyte cell surfaces of grafted skin (Fig. 1E, day 21).

To determine whether the T cell infiltration in the grafted skin was Dsg3-specific, WT skin and Dsg3−/− skin were grafted simultaneously onto Dsg3−/− mice that had been primed with WT skin 28 d prior (Fig. 2A). The WT-grafted skin showed interface dermatitis with marked CD4+ and CD8+ T cell infiltration and liquefaction degeneration, whereas the Dsg3−/− grafted skin showed no apparent histological changes and no T cell infiltration (Fig. 2B, n = 3). These findings indicate that grafting of Dsg3-expressing WT skin onto Dsg3−/− mice induces both cellular and humoral Dsg3-specific immune responses.

FIGURE 2. T cells infiltrating WT skin grafts are Dsg3-specific. (A) Experimental protocol for determination of Dsg3-specific immune reactions caused by skin graft immunization. Dsg3−−/− mice (n = 3) were first primed with WT (Dsg3+/+) skin grafts and challenged with second skin grafts side-by-side with WT skin and Dsg3−−/− skin. Biopsies were taken from the grafted skin for histology and CD4/CD8 staining. (B) Histology, CD4+ and CD8+ T cells (red) of WT skin grafts (left panel) and Dsg3−−/− skin grafts (right panel) on Dsg3−−/− mice after priming with WT skin grafts. Scale bar, 20 μm.

Splenocytes from Dsg3−−/− skin-grafted Dsg3−−/− mice induce characteristic features of PNP in recipient mice

Patients with PV and PNP present with mucocutaneous blisters and erosions that histologically show suprabasilar acantholysis or the loss of keratinocyte cell–cell adhesion just above the basal cell layer, which results from the binding of anti-Dsg3 IgG autoantibodies to keratinocyte surfaces (Fig. 3A, 3B, Table I) (2, 4, 27). However, the oral lesions of PNP clinically appear more inflamed with necrotic and lichenoid changes than do those of PV, and histological examination shows suprabasilar acantholysis with CD4+ and CD8+ T cell infiltration and keratinocyte apoptosis (6, 28).

When lymphocytes from naive Dsg3−−/− mice or recombinant Dsg3-immunized Dsg3−−/− mice were adoptively transferred to Rag2−−/− mice, the recipient mice began to produce anti-Dsg3 IgG, which mediates suprabasilar acantholysis. However, no obvious T cell infiltration was identified in the skin or mucous membranes (Fig. 3C, Supplemental Fig. 1) (12, 29). In contrast, when splenocytes from Dsg3−−/− mice immunized with Dsg3−/− skin grafts were transferred adoptively, the recipient mice developed oral lesions with combined suprabasilar acantholysis caused by anti-Dsg3 IgG and interface dermatitis with CD4+ and CD8+ T cell infiltration and keratinocyte apoptosis (Fig. 3D). The numbers of infiltrating CD4+ and CD8+ T cells and TUNEL+ apoptotic cells...
in the oral epithelia were significantly higher in these mice than in PV model mice (Fig. 3E). Furthermore, by day 30, 4 of 12 mice produced anti-envoplakin IgG, a characteristic autoantibody in PNP, whereas none of the 40 PV model mice did so (Fig. 3F). Thus, mice receiving splenocytes from Dsg3−/− skin graft–immunized Dsg3−/− mice developed characteristic histological and immunological features seen in PNP. Consequently, these mice constitute a model for analyzing the immunological aspects of PNP without neoplastic complications, and are referred to as PNP mice in this study.

High mortality rate and lung involvement in PNP mice

PNP mice had significantly higher mortality than did PV mice (Fig. 4A, survival rate 46% [n = 41] versus 81% [n = 16] at day 49, p = 0.02 [Wilcoxon test]). To investigate the cause of this difference, we performed whole-body histological analyses and found intense mononuclear cell infiltrates in the peribronchial and perivascular spaces in the lungs of PNP mice, but not in those of PV mice (Fig. 4B). Most of the infiltrating cells were CD4+ or CD8+ T cells, which were significantly more plentiful in PNP mice than in PV mice (Fig. 4C, 4D). The degree of inflammation was profound in some animals, and robust infiltration of neutrophils and mononuclear cells was observed in the alveolar spaces; other organs, including the liver, kidney, heart, and small intestine, showed no such findings (Supplemental Fig. 2).

To determine why skin Ag-specific T cells were found in lungs, we examined the lungs of PNP and PV mice for Dsg3 expression. Surprisingly, the PNP mice expressed various levels of Dsg3, as shown by RT-PCR, whereas no Dsg3 was detected in PV mice (Fig.

---

**Table I. Summary of the phenotypes of PV and PNP in humans and mice**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Human PV</th>
<th>Human PNP</th>
<th>Mouse PV Model</th>
<th>Mouse PNP Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical phenotype</td>
<td></td>
<td>+**</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Blister formation</td>
<td>+</td>
<td>+**</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Histology</td>
<td>+</td>
<td>+**</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Interface dermatitis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Humoral autoimmunity</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anti-Dsg3 IgG</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anti-plakin IgG</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cellular autoimmunity</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>T cell-mediated cytotoxicity</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dsg3-specific T cell attack</td>
<td>-</td>
<td>N.D.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lung involvement</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Note:**
- Oral PNP lesions showed more necrosis and lichenoid changes than did PV lesions.
- Human PNP shows bronchiolitis obliterans.

---

*Figure 3.* Generation and characterization of a mouse model with immunological features of PNP. Clinical and histological images stained for CD4+ and CD8+ T cells in a representative PV patient (A) and PNP patient (B). (C) PV model mice were generated by adoptive transfer of splenocytes from Dsg3−/− mice immunized with recombinant Dsg3 into Rag2−/− mice. The recipient mice stably produced anti-Dsg3 IgG and developed the characteristic PV phenotype, including suprabasilar acantholysis in the hard palate and in vivo IgG deposition (green) on keratinocyte surfaces with no obvious infiltration of CD4+ and CD8+ T cells (red). Scale bars, 40 μm. (D) PNP model mice were generated by adoptive transfer of splenocytes from Dsg3−/− mice immunized twice with WT skin grafts into Rag2−/− mice. The recipient mice showed marked CD4+ and CD8+ T cell infiltration (red) with keratinocyte apoptosis (TUNEL+ in red) in addition to anti-Dsg3 IgG production (green) and suprabasilar acantholysis. (E) Infiltrating CD4+ T cells, CD8+ T cells, and apoptotic cells were counted per 100 μm basement membrane (BM) in the oral mucosa. Error bars indicate SD. (F) Anti-envoplakin (top panel, arrow; molecular mass, 210 kDa) or anti-Dsg3 (bottom panel, arrowhead; molecular mass, 130 kDa) IgG production was determined by immunoprecipitation and immunoblotting.
4E). Dsg3 in the lungs of PNP mice could not be detected at protein level using either various mAbs (23) or sera from patients with PV, probably due to its low expression or poor stability on cell membranes that lacked proper formation of desmosomes at the time points examined (up to day 49).

These findings suggest that ectopic pulmonary Dsg3 expression might redirect Dsg3-specific CD4+ and CD8+ T cells to the lungs and there induce inflammation.

**Squamous metaplasia is frequently found in the lungs of patients with PNP**

It is well established that Dsg3 is not expressed in the normal lungs of mice and humans (8, 30). However, the lungs of patients with PNP frequently show squamous metaplasia at autopsy or in bronchial biopsy specimens, which is often accompanied by CD4+ and CD8+ T cell infiltration (Fig. 5A) (8, 10, 31). Squamous metaplasia develops in response to acute or chronic inflammation caused by smoking (32) or viral infection (33–35), and it shows striking similarities to the skin and mucosa not only in terms of morphology, but also gene expression (36). Immunohistochemical analysis of human lungs with squamous metaplasia confirmed ectopic Dsg3 expression in 18 of 19 samples (representative data in Fig. 5B).

**Ectopic Dsg3 expression during the tissue repair process in the lungs is sufficient to recruit Dsg3-specific CD4+ T cells**

The above findings indicate that the stratified squamous epithelial-specific adhesion molecule Dsg3 may be ectopically expressed under certain conditions. To further explore a setting in which ectopic expression of Dsg3 is induced in bronchial epithelium, a single dose of naphthalene (200 mg/kg), which selectively injures non-ciliated Clara respiratory epithelial cells, was administered i.p. Naphthalene is concentrated in Clara cells that are enriched in p450 enzymes, and its metabolism generates toxic metabolites that result in selective injury to Clara cells (37). On day 1, the bronchiolar surface appeared to be denuded, and exfoliated cells were evident in the bronchiolar lumen. On day 3, squamous cells were lining the injured bronchioles, which exhibited a relatively homogeneous cuboidal cell appearance (Fig. 6A), as described previously (19, 37). Ectopic expression of Dsg3 was transiently found on days 1–3, which declined after day 4, as determined by RT-PCR (Fig. 6B). To examine the effects of naphthalene during the chronic phase, a lower dose (100 mg/kg) was administered once per week for 4 wk and the expression of epidermal Ags was examined. Interestingly, not only Dsg3, but also other epidermal Ags such as keratin 5 and keratin 14 were detected by RT-PCR throughout the course (Fig. 6C); however, non-epidermal peripheral Ags, such as α-fibrinogen (liver-specific Ag), were not detected. Thus, these results demonstrate that Dsg3 and other epidermal Ags may be ectopically expressed during epithelial remodeling or squamous metaplasia as part of the wound healing process after tissue injury.

Next, we determined whether Dsg3-reactive CD4+ T cells are preferentially recruited to lung in which Dsg3 was ectopically expressed. CD4+ T cells from CD45.1+ congenic B6 mice and B6 mice (CD45.2+) were retrovirally transduced with Dsg3-specific TCR (Dsg3H1) and mock T cells, respectively. Equal numbers (1 × 10^6) of Dsg3H1 (CD45.1+) and mock T cells (CD45.2+) were adoptively transferred into Rag2^−/− mice (n = 5), followed by weekly naphthalene administration (100 mg/kg) (Fig. 6D). Dsg3H1 T cells infiltrated to the skin and induced interface dermatitis, with the gross phenotype becoming apparent ~4 wk after adoptive transfer (15). We therefore examined the lungs of naphthalene-treated mice at the time points after adoptive transfer of T cells
findings indicate that ectopic Dsg3 expression by bronchial epithelial cells, in the absence of Dsg3 or in Dsg3−/− mice, were capable of inducing not only humoral but also cellular responses to Dsg3 in the form of interface dermatitis (15). In contrast, Dsg3H1-transgenic CD4+ T cells, which developed in the presence of Dsg3 or in WT mice, induced only cellular responses (15). Taken together, these findings indicate that CD4+ T cells with the same TCR specificity for Dsg3 may be involved in induction of both pemphigus and interface dermatitis.

To induce polyclonal Dsg3-specific CD4+ and CD8+ T cells, we modified the immunization step and immunized Dsg3−/− mice with WT skin grafts and transferred splenocytes to Rag2−/− mice. The phenotype of the skin and oral mucosa in recipient mice showed both acantholysis and interface dermatitis with CD4+ and CD8+ T cell infiltration and keratinocyte apoptosis, which is a histological finding unique to PNP (Fig. 3, Table I). These findings suggest that Dsg3 is a target Ag of autoimmune T cells in PNP. The identification of target Ags is an important step in understanding the pathophysiology of autoimmune diseases. However, it is more difficult to identify a T cell–targeted Ag than the target of IgG, because TCRs recognize antigenic peptides presented in the context of MHC molecules. Additionally, direct evidence for autoimmune diseases requires transmissibility of the characteristic lesions from humans to animals, which is difficult to do with T cells, primarily because of MHC mismatches. Currently, it is not feasible to identify the target Ag of infiltrating individual T cells in the skin or mucosal lesions of PNP.

When PV and PNP model mice were compared, the latter showed significantly higher mortality, and intense CD4+ and CD8+ T cell infiltrations were found in the peribronchial area in the lungs of PNP model mice, but not in PV model mice (Fig. 4). It is not certain whether the lung involvement is the direct cause of mortality in PNP model mice, but it seems to be at least associated. It has been long considered that the lung expresses Dsg2, but not Dsg3 (8, 30). However, the expression profile had been examined only under normal conditions. Our unexpected finding of Dsg3 expression in the lungs of PNP mice suggests that Dsg3 and other epidermal Ags are expressed in the lungs of mice during squamous metaplasia after naphthalene-induced pulmonary epithelial injury (Fig. 5A, 5C). Dsg3H1-transgenic CD4+ T cells transduced using a retrovirus did not infiltrate naphthalene-treated mice (Fig. 5D, 5E). These findings indicate that CD4+ T cells with the same TCR specificity for Dsg3 may be involved in induction of both pemphigus and interface dermatitis.

At which the skin phenotype became apparent. Compared with corn oil–treated control mice, naphthalene-treated mice showed greater T cell infiltration in the lungs. The lungs of naphthalene-treated mice showed significantly higher numbers of infiltrating CD45.1+CD4+ T cells in bronchial epithelia with squamous metaplasia than did mock CD45.2+CD4+ T cells (Fig. 6E, 6F). These findings indicate that ectopic Dsg3 expression by bronchial epithelial cells is sufficient to recruit Dsg3-specific CD4+ T cells.

 Taken together, these findings indicate that Dsg3 and other epidermal Ags can be expressed ectopically in the lungs during tissue repair after acute inflammation, as well as in tissues showing squamous metaplasia, rendering the bronchial epithelium prone to Dsg3-specific or epidermal Ag-specific cellular autoimmune attack in mice and humans.

Discussion

The autoimmune reaction in PNP is unique among the pemphigus group in at least two immunological aspects (Table I): 1) patients with PNP show cellular autoimmune attack of stratified squamous epithelia in the skin and oral mucosa in addition to humoral autoimmune attack by anti-Dsg IgG autoantibodies; and 2) a subset of patients with PNP develops fatal pulmonary involvement as a form of bronchiolitis obliterans, although no epidermal Ags are known to be expressed in the lungs. In this study, we attempted to clarify the mechanisms underlying these unique features of PNP using an experimental pemphigus mouse model.

PV model mice were originally generated by adoptive transfer of lymphocytes from Dsg3−/− mice immunized with recombinant Dsg3 in Freund’s adjuvant or naïve Dsg3−/− mice into Rag2−/− mice (12, 29). These PV model mice showed only a humoral immune reaction as a form of anti-Dsg3 IgG production, but no apparent cellular immune reaction to Dsg3 (Table I). When Dsg3-specific T cell clones isolated from Dsg3−/− mice were adoptively transferred together with Dsg3−/− B cells, the recipient mice exhibited only humoral responses (14). However, Dsg3H1 transgenic CD4+ T cells, which developed in the absence of Dsg3 or in Dsg3−/− mice, were capable of inducing not only humoral but also cellular responses to Dsg3 in the form of interface dermatitis (15). In contrast, Dsg3H1-transgenic CD4+ T cells, which developed in the presence of Dsg3 or in WT mice, induced only cellular responses (15). Taken together, these findings indicate that CD4+ T cells with the same TCR specificity for Dsg3 may be involved in induction of both pemphigus and interface dermatitis.

To induce polyclonal Dsg3-specific CD4+ and CD8+ T cells, we modified the immunization step and immunized Dsg3−/− mice with WT skin grafts and transferred splenocytes to Rag2−/− mice. The phenotype of the skin and oral mucosa in recipient mice showed both acantholysis and interface dermatitis with CD4+ and CD8+ T cell infiltration and keratinocyte apoptosis, which is a histological finding unique to PNP (Fig. 3, Table I). These findings suggest that Dsg3 is a target Ag of autoimmune T cells in PNP. The identification of target Ags is an important step in understanding the pathophysiology of autoimmune diseases. However, it is more difficult to identify a T cell–targeted Ag than the target of IgG, because TCRs recognize antigenic peptides presented in the context of MHC molecules. Additionally, direct evidence for autoimmune diseases requires transmissibility of the characteristic lesions from humans to animals, which is difficult to do with T cells, primarily because of MHC mismatches. Currently, it is not feasible to identify the target Ag of infiltrating individual T cells in the skin or mucosal lesions of PNP.

When PV and PNP model mice were compared, the latter showed significantly higher mortality, and intense CD4+ and CD8+ T cell infiltrations were found in the peribronchial area in the lungs of PNP model mice, but not in PV model mice (Fig. 4). It is not certain whether the lung involvement is the direct cause of mortality in PNP model mice, but it seems to be at least associated. It has been long considered that the lung expresses Dsg2, but not Dsg3 (8, 30). However, the expression profile had been examined only under normal conditions. Our unexpected finding of Dsg3 expression in the lungs of PNP mice suggests that Dsg3 and other epidermal Ags are expressed in the lungs of mice during squamous metaplasia after naphthalene-induced pulmonary epithelial injury (Fig. 5A, 5C). Dsg3H1-transgenic CD4+ T cells transduced using a retrovirus did not infiltrate naphthalene-treated mice (Fig. 5D, 5E). These findings indicate that CD4+ T cells with the same TCR specificity for Dsg3 may be involved in induction of both pemphigus and interface dermatitis.
chial epithelial cells in vitro (40). Further intensive investigations are necessary to clarify these issues.

T cell infiltration observed in the lungs of PNP model mice was a mixture of CD4+ and CD8+ cells, and it remains to be elucidated whether both populations are necessary or whether one population is more important than the other for the lung injury. Based on the observation that Dsg3H1 CD4+ T cells alone caused interface dermatitis (15), it is speculated that CD4+ T cells are sufficient to induce tissue injury. However, mice receiving Dsg3H1 CD4+ T cells from Dsg3H1 TCR transgenic mice by adoptive transfer did not necessarily show increased mortality rates (H. Takahashi and M. Amagai, unpublished observations). Additionally, it was previously reported that CD4+ T cells were required for efficient CD8+ T cell function in general (41, 42). These findings together suggest that the combination of CD4+ and CD8+ T cells is more efficient than each population alone to induce the lung injury. Further investigations will elucidate the exact roles of CD4+ and CD8+ T cells in PNP mice model.

Squamous metaplasia is often found in the lungs of patients with PNP (8, 10, 31, 43). Linear IgG deposition on epithelial cell surfaces and acantholysis of epithelial cells were found in the lungs of PNP patients (8). However, at the time of the previous study, no target Ag was known. It is tempting to speculate that the autoantibodies deposited in pulmonary lesions with squamous metaplasia are anti-Dsg3 IgG that recognize ectopic Dsg3, thereby inducing acantholysis in bronchial epithelia.

Respiratory epithelia are constantly exposed to hazardous conditions, including viral or bacterial infection, chemical inhalation, and smoking. Repair and maintenance of lung functions under such hazardous conditions requires rapid proliferation and differentiation into the appropriate epithelial cell subsets that constitute the normal lungs (19). To achieve this, pulmonary epithelial cells undergo squamous metaplasia with dynamic changes in gene expression and ectopic expression of various epidermal Ags. In the presence of Dsg3- or other epidermal Ag-specific IgG and/or T cells, pulmonary epithelia with squamous metaplasia might represent an unexpected target. Thus, the ectopic expression of Dsg3 and other epidermal Ags provides a missing link in pulmonary involvement in PNP in an Ag-specific autoimmune
setting, although further studies are needed to clarify the mecha-
nism(s) underlying the occurrence of bronchiolitis obliterans,
a form of pulmonary inflammation, in a subset of PNP patients.
Ectopic expression of tissue-specific Ags at different anatomical sites under inflammatory conditions also provides important clues as to the connections among the organs involved in tissue-specific autoimmune diseases.

Acknowledgments

We thank H. Ito, S. Kagawa, and M. Suzuki for excellent technical support.

Disclosures

The authors have no financial conflicts of interest.

References


