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

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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 plakins, 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 observed in the lungs of PNP mice, mirroring the observation that squamous metaplasia is often found in the lungs of PNP

Among tissue-specific autoimmune diseases, pemphigus comprises a group of IgG autoantibody-mediated blistering diseases of the skin and mucous membranes, characterized histologically by intraepidermal blisters due to the loss of keratinocyte cell–cell adhesion, and immunopathologically by the in vivo finding of bound and circulating IgG autoantibodies directed against the surface of keratinocytes. Pemphigus has three major forms: pemphigus foliaceus (PF), pemphigus vulgaris (PV), and paraneoplastic pemphigus (PNP) (1). The classic forms of pemphigus are PF and PV, which are mediated solely by humoral autoimmunity involving IgG autoantibodies against desmoglein (Dsg)1 and Dsg3, respectively, both of which are cadherin-type cell–cell adhesion molecules in desmosomes (2, 3).

PNP shares the basic feature of pemphigus in that IgG autoantibodies against Dsg3 or Dsg1 play a pathogenic role by mediating blister formation in the mucous membranes and skin (4), but its phenotype is more complex than those of PV and PF. PNP occurs in association with underlying neoplasms, mainly of lymphoid origin, and has characteristic IgG autoantibodies against the plakin family (plectin, desmplakin I and II, BP230, envoplakin, and perilplakin) (5, 6). Additionally, PNP patients exhibit a cellular immune response in the skin and mucous membranes. Histological examination shows not only blister formation caused by IgG autoantibodies against Dsg3 and/or Dsg1, but also interface dermatitis showing keratinocyte apoptosis and T cell infiltration in the epidermis and mucosal epithelia. Furthermore, some patients with PNP develop pulmonary involvement, such as bronchiolitis obliterans, which can cause fatal respiratory failure (7–11). Further investigation is required to determine the target Ag(s) of T cells that infiltrate the skin and mucous membranes as well as the reason that such T cells also attack the lungs. The technical hurdles to identification of these T cell Ags and the rarity of the disease hamper progress in solving these questions.

An active disease mouse model for PV has been generated by adoptive transfer of T and B lymphocytes from Dsg3−/− mice immunized with recombinant mouse Dsg3 plus Freund’s adjuvant to Dsg3-expressing Rag2−/− mice (12, 13). PV model mice persistently produce anti-Dsg3 IgG and develop blisters on the skin and mucous membranes, as found in PV patients. Subsequently, Dsg3-specific CD4+ T cell clones were isolated, and Dsg3-specific TCR transgenic (Dsg3H1) mice were generated (14, 15). Interestingly, Dsg3-specific transgenic CD4+ T cells, which developed in the absence of Dsg3 or in Dsg3−/− mice, induced not only the blister formation caused by anti-Dsg3 IgG, but also interface dermatitis with CD4+ T cell infiltration in the epidermis and keratinocyte necrosis upon adoptive transfer with Dsg3−/− B cells (15). This demonstrates that the cellular autoimmune response against Dsg3 can lead to the formation of interface dermatitis, suggesting that Dsg3 may be a target autoantigen of the cellular immune reaction seen in PNP.

In this study, by adoptive transfer of lymphocytes from Dsg3−/− mice immunized with wild-type (WT) skin grafts, we generated a PNP model mouse that showed both humoral and cellular immune reactions against Dsg3. PNP model mice showed supra-
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 longstanding 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

Dsg1+/+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 “Dsg1+/+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 Dsg3+/+ skin grafts

Dorsal skin from Dsg3+/+ 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 adaptively 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 (βα8 and ββ6) 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...
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-nonspecific 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 (RMH-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+/− littersmates, the skin grafts on Dsg3−/− mice (n = 8), but not on Dsg3+/− littersmates (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+/− littersmates, 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 titers 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 grafted of Dsg3-expressing WT skin onto Dsg3−/− mice induces both cellular and humoral Dsg3-specific immune responses.
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+ and 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.
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 × 106) 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

FIGURE 4. PNP model mice showed higher mortality and CD4+ and CD8+ T cell infiltration in the lungs. (A) Survival rates of PNP model mice (○) and PV model mice (●) throughout the course after adoptive transfer. (B) Histology of the lungs of PV model mice (left panel) and PNP model mice (right panel) under various magnifications. Scale bars, 100 μm. (C) Staining of CD4+ and CD8+ T cells in the intraepithelial and peribronchial areas in the lungs of PV model mice (left) and PNP model mice (right). Scale bar, 20 μm. (D) The numbers (± SD) of intraepithelial and peribronchial CD4+ and CD8+ T cells in PV model mice (filled bars) and PNP model mice (open bars) per 100 μm basement membrane (BM). (E) RT-PCR of lung tissues for Dsg3 expression (arrow) in PV model mice and PNP model mice on days 12–14 after adoptive transfer (negative control [NC] using WT lung).
Dsg3 in Freund’s adjuvant or naive 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 CD4+ T cells transduced using a retrovirus did not infiltrate in the lungs in the absence of ectopic Dsg3 expression; however, they preferentially infiltrated into the pulmonary epithelia with squamous metaplasia, in which Dsg3 was expressed ectopically, in naphthalene-treated mice (Fig. 5D, 5E). These findings indicate that the ectopic expression of Dsg3 in the pulmonary epithelial cells was sufficient to recruit Dsg3-specific T cells.

It is unclear how the ectopic Dsg3 expression is induced in the first place in the lung of PNP mice. One speculation is that bronchial epithelia may be constantly remodeled after minor physiological insults, followed by transient ectopic expression of epidermal Ags, including Dsg3 (19). Another speculation is that the inflammatory processes in the skin may produce certain cytokines, such as IL-22, which are able to affect epithelial proliferation (38, 39). These humoral factors may get into circulation and remotely affect bronchial epithelia to induce ectopic Dsg3 expression. Epidermal Ags, including Dsg3, keratin 14, and involucrin, are able to be upregulated by TGF-β in human bron-
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 response.

**FIGURE 6.** Naphthalene-induced ectopic Dsg3 expression in the lungs is sufficient to recruit Dsg3-specific CD4+ T cells. (A) Histological changes in the bronchial epithelia after a single dose of naphthalene (200 mg/kg) or control corn oil. Scale bar, 50 μm. (B) RT-PCR for Dsg3 in lung tissue after a single dose of naphthalene (200 mg/kg) or control corn oil. (C) RT-PCR for Dsg3, keratin 5 (K5), keratin 14 (K14), and α-fibrinogen (α-Fib, liver-specific Ag) in lung tissue of mice that received weekly naphthalene injections (100 mg/kg) for 4 wk. As positive controls (P), skin was subjected to RT-PCR for Dsg3, K5, and K15, and liver tissue for α-Fib. (D) Experimental protocol for determination of recruitment of Dsg3-specific CD4+ T cells in the lungs. CD4+ T cells from CD45.1+ mice and CD45.2+ mice were retrovirally transduced with Dsg3-specific TCR (Dsg3H1) and mock, respectively, and equal numbers (1 × 10^6) of CD45.1+ and CD45.2+ T cells were adoptively transferred into Rag2-/- mice, followed by weekly naphthalene administration (100 mg/kg). (E) Lung tissue was stained with anti-CD4 (red), anti-CD45.1 (green) Abs, and Hoechst (blue). CD45.1+CD4+ T cells (arrowheads) were found in the intraepithelial area in naphthalene-treated mice, but not in control corn oil-treated mice. Scale bar, 50 μm. (F) The numbers (±SD) of CD45.1+CD4+ T cells and CD45.2+CD4+ T cells infiltrating the intraepithelial area in the lungs of mice treated with naphthalene or corn oil were counted per 200 μm basement membrane (BM).
setting, although further studies are needed to clarify the mechanism(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.

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Disclosures
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