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Transgenic rescue of desmoglein 3 null mice with desmoglein 1 to develop a syngeneic mouse model for pemphigus vulgaris

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ABSTRACT

Background: An active disease mouse model of pemphigus vulgaris (PV) was developed using the adoptive transfer of splenocytes from Dsg3$^{-/-}$ mice with a mixed C57BL/6J (B6) and 129/Sv genetic background into B6-Rag2$^{-/-}$ mice. Further immunological investigation is needed to resolve the genetic mismatch between host and recipient mice. The B6-Dsg3$^{-/-}$ mice did not grow old enough to provide splenocytes, probably due to severe oral erosions, with resulting inhibition of food intake.
Objective: To rescue the B6-Dsg3$^{-/-}$ mice and to produce syngeneic PV model mice.
Methods: Transgenic expression of mouse Dsg1 was attempted to compensate for the genetic loss of Dsg3 using the keratin 5 promoter. We evaluated the compensatory ability of Dsg1 *in vivo* by comparing Dsg1$^{wt/wt}$, Dsg1$^{tg/wt}$, and Dsg1$^{tg/tg}$ mice. We generated a PV model via the adoptive transfer of B6-Dsg1$^{tg/tg}$Dsg3$^{-/-}$ splenocytes to B6-Rag2$^{-/-}$ mice.
Results: Dsg1$^{tg/tg}$ and Dsg1$^{tg/wt}$ mice expressed ectopic Dsg1 on keratinocyte cell surfaces in the lower layers of the epidermis, oral epithelium, and telogen hair follicles. Ectopic Dsg1 blocked the pathogenic effects of AK23 anti-Dsg3 mAb, and improved the body weight loss, telogen hair loss, and survival rate dose-dependently. While the B6-Dsg1$^{wt/wt}$Dsg3$^{-/-}$ mice died by week 2, over 80% of the B6-Dsg1$^{tg/tg}$Dsg3$^{-/-}$ mice survived at week 6. Furthermore, the syngeneic PV model mice showed the characteristic phenotype, including stable anti-Dsg3 antibody production and suprabasilar acantholysis on histology.
Conclusion: Transgenic expression of Dsg1 rescued the severe B6-Dsg3$^{-/-}$ phenotype and provided a syngeneic mouse model of PV, which may be a valuable tool for clarifying immunological mechanisms in autoimmunity and tolerance of Dsg3.

1. Introduction

Pemphigus vulgaris (PV) is a fatal autoimmune blistering disease of the skin and mucous membranes, characterized clinically by flaccid blisters and erosions and histopathologically by the loss of cell–cell adhesion in the deep epidermis, just above the basal layer, resulting in acantholysis [1]. The target antigen of PV is desmoglein (Dsg) 3, a desmosomal transmembrane glycoprotein that belongs to the cadherin superfamily of cell adhesion molecules [2,3], and which is expressed in the lower epidermis and mucous membrane. Compelling evidence indicates that IgG autoantibodies against Dsg3 in PV play a primary pathogenic role in blister formation [4,5]. Dsg1 is another Dsg isotype and is the antigen of pemphigus foliaceus, and is also expressed in the epidermis and mucous membranes. The localization of Dsg1 and Dsg3 in the skin and mucous membranes was studied by immunofluorescent staining with isotype-specific antibodies [2,6]. In the mucous membranes, Dsg3 is highly expressed throughout the epithelia, while expression of Dsg1 is much lower, and there is no apparent expression in the basal layers. Although three isoforms of mouse Dsg1, namely that Dsg1α, Dsg1β and Dsg1γ, were identified previously [7], Dsg1α is described as Dsg1 in this article as long

Abbreviations: PV, pemphigus vulgaris; Dsg, desmoglein; Ab, antibody; K5, keratin 5.

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as there is not a necessity to distinguish, because Dsg1α means original Dsg1.

Previously, we developed an active disease mouse model of PV via the adoptive transfer of lymphocytes from Dsg3 knockout ($Dsg3^{-/-}$) mice, which have a mixed 129/Sv ($H\text{-}2^b$) and C57BL/6J (B6) ($H\text{-}2^b$) genetic background [8], into B6-$Rag2^{-/-}$ recipient mice that express Dsg3 [9]. The transferred lymphocytes produce anti-Dsg3 IgG stably and develop the PV phenotype in recipient mice, including oral erosions with suprabasilar acantholysis and telogen hair loss. In this model, although the major histocompatibility complex (MHC) was matched between donor and recipient, there is still concern that a nonspecific immune reaction can occur, such as graft-versus-host (GvH) reactions [10,11]. If the recipient's polymorphic protein differs from that of the donor, the protein can potentially be recognized as foreign (minor histocompatibility antigen) [12]. If such non-specific reactions were even slightly induced in the PV model, we would not be able to investigate defined autoimmune mechanisms further. To match the genetic background between donor and recipient, we attempted to backcross the $Dsg3^{-/-}$ mice to B6. However the $Dsg3^{-/-}$ mice with B6 (B6-$Dsg3^{-/-}$) died within 2 weeks of birth at the weaning stage, with oral erosions that probably inhibited their food intake (unpublished observation). Thus, we could not obtain lymphocytes to be transferred from adult mice.

To obtain surviving B6-$Dsg3^{-/-}$ mice, we postulated that Dsg1 was made to be expressed in the areas where only Dsg3 is expressed, based on the Dsg compensation hypothesis [13,14]. This hypothesis, that one desmoglein can compensate for the loss of function of another, was proposed to explain the tissue specificity of the autoantibody-induced loss of cell adhesion in pemphigus. In fact, transgenic mice that express ectopic Dsg3 in the areas that normally express only Dsg1 were protected from the blistering caused by anti-Dsg1 antibodies (Ab) [15]. Furthermore, transgenic expression of Dsg1, controlled by the keratin 14 (K14) promoter in areas that normally express only Dsg3, could partially correct the defective cell–cell adhesion in $Dsg3^{-/-}$ mice [16]. In these mice, telogen hair loss was delayed markedly and decreased due to the ectopic expression of Dsg1 in the telogen hair club. However, ectopic Dsg1 could not reverse the poor weight gain due to weak expression in the oral mucosa.

Thus, to rescue the B6-$Dsg3^{-/-}$ mice, we attempted to compensate for the genetic loss of Dsg3 with ectopic expression of Dsg1 in the lower layer of the epidermis and oral mucous membrane using the keratin 5 (K5) promoter. We also attempted to produce syngeneic PV model mice, which essentially do not show a non-specific immune reaction, using rescued B6-$Dsg3^{-/-}$ mice and B6-$Rag2^{-/-}$ mice. Because the K5 promoter has already been reported to work in the esophagus and stomach [17], the K5 promoter should express Dsg1 in the mucous membrane and suppress the oral erosions, resulting in survival of the B6-$Dsg3^{-/-}$ mice. If the B6-$Dsg3^{-/-}$ mice were rescued, we would obtain a potentially valuable tool for investigating the pathogenesis of autoimmunity and tolerance mechanisms by analyzing syngeneic PV model mice.

2. Materials and methods

2.1. Mice

A $Dsg3^{+/-}$ mouse line with a B6 background was obtained by backcrossing 129/Sv (Jackson Laboratory, Bar Harbor, ME) to B6 for at least ten generations. $Rag2^{-/-}$ mice with a B6 background (Central Institute for Experimental Animals, Tokyo, Japan) were maintained and used as recipients for PV model mice under specific-pathogen-free (SPF) conditions at Keio University.

All of the mice studies were approved by the Animal Ethics Review Board of Keio University.

2.2. Generation of $Dsg1^{tg/wt}$ mice and breeding into the B6-$Dsg3^{+/-}$ mouse line

To construct a transgene vector, we modified the K14pNotIp-GEM3Z vector (gift from Dr. Stanley), which contained the K14 promoter and β-globin exon–introns cassette [16]. First, the bovine growth hormone polyadenylation (BGHpA) site (pcDNA3.1(+); Invitrogen, Carlsbad, CA) was subcloned downstream from the β-globin cassette. Then, the full-length mouse Dsg1α cDNA with 39 nucleotides attached, encoding the Etag epitope, was subcloned between the β-globin cassette and BGHpA. Finally, the K14 promoter was exchanged for the K5 promoter, which was excised from pBSK5 [17] (gift from Drs. Tarutani and Takeda). The region from the K5 promoter to BGHpA containing the Dsg1α-Etag nucleotide sequence (Fig. 1A) was excised from the resulting vector (pGEM-K5Dsg1), and microinjected into the pronuclei of C57BL/6J mice zygotes before implantation into pseudopregnant foster C57BL/6J mice. One of the $Dsg1^{tg/wt}$ mouse lines obtained was bred to the B6-$Dsg3^{+/-}$ mouse line and mated with littermates to obtain B6-$Dsg1^{tg/tg}$ $Dsg3^{-/-}$ mice.

2.3. PCR and immunoblotting to characterize the transgenic mice generated

For genotyping and RT-PCR, a PCR primer pair to amplify the end of the Dsg1α cDNA and Etag containing site was designed as follows (Fig. 1A): DN1114 (5′-GGGTAATAGCACCAGGCTCAAG-3′) and DN1615 (5′-TTCCAGCGGATCCGGATACGGCACCGGCGCA-3′). DNA and RNA were extracted using the DNeasy Blood & Tissue Kit and RNeasy Mini Kit (QIAGEN, Germantown, MD), respectively. PCR was performed on DNA extracted from mice tails and cDNA was synthesized from total RNA using the SuperScript® III First Strand Synthesis System (Invitrogen, Carlsbad, CA). Copy number of transgene was determined by real-time PCR using genomic DNA of $Dsg1^{tg/tg}$ and $Dsg1^{wt/wt}$ mice. Dsg1α specific primers were designed as follows: mDsg1a-e11-132F (5′-CAAGGCACTTCTTCCACTGAG-3′) and mDsg1a-e11-233R (5′-ATCCCCTTGGAAAGGGTTAGT-3′).

To genotype the $Dsg3^{-/-}$ mouse, neomycin-specific primers M11 (5′-CTTGGGTGGAGAGGCTATTCGGCT-3′) and M12 (5′-AGGTGAGATGACAGGAGATCCTGCCC-3′) and Dsg3-specific primers M13 (5′-CAGACACACCAGCAACAATGACCT-3′) and M14 (5′-CCTGGAGAGCTGATGTGCTGGTA-3′) were used. Further, to determine the three genotypes of transgenic mice (*i.e.*, $Dsg1^{tg/tg}$, $Dsg1^{tg/wt}$, and $Dsg1^{wt/wt}$), immunoblotting with anti-Etag antibody and semi-quantitative densitometric analysis (LAS-1000; Fujifilm, Tokyo, Japan) were performed, using actin as an internal control (Fig. 2A).

2.4. Evaluating the compensatory effect of the transgene with PV pathogenic Ab

To evaluate the ability of ectopic Dsg1 to compensate for Dsg3 in transgenic mice *in vivo*, mouse monoclonal Ab AK23 [18], which induces the PV phenotype in adult mice, was inoculated into the peritoneal cavity. In detail, 100 μg of AK23 was diluted in phosphate-buffered saline (PBS), adjusting the total volume to 500 μL, for each mouse and inoculated into peritoneal cavities of mice of each of the three genotypes ($Dsg1^{tg/tg}$, $Dsg1^{tg/wt}$, and $Dsg1^{wt/wt}$). After inoculation, the weight changes and appearance of PV phenotypes were monitored temporally.

2.5. Generation of PV model mice

Pemphigus vulgaris model mice were produced, as described previously [9]. Briefly, 6- to 10-week-old B6-$Dsg1^{tg/tg}Dsg3^{-/-}$ mice were immunized with 10 μg of recombinant mouse Dsg3 (rDsg3) in complete Freund's adjuvant (SIGMA-ALDRICH, Inc., St. Louis,