

Development of an enzyme-linked immunosorbent assay for detection of circulating IgG autoantibodies against canine desmoglein 3 in dogs with pemphigus

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Conflict of Interest

None declared.

Abstract

Pemphigus, a group of autoimmune blistering dermatoses recognized in humans and dogs, is divided into three subgroups: pemphigus vulgaris (PV), pemphigus foliaceus (PF) and paraneoplastic pemphigus (PNP). Previously, we have demonstrated that circulating anti-desmoglein 3 (Dsg3) IgG in dogs with PV and PNP disrupts Dsg3-mediated keratinocyte cell–cell adhesion. The aim of this study was to develop an enzyme-linked immunosorbent assay (ELISA) for the detection of circulating IgG against canine Dsg3 in dogs with pemphigus. A secreted form of recombinant protein representing the entire extracellular domain of canine Dsg3 (cDsg3) was produced by baculovirus expression and used as the coated antigen in the ELISA. The titre of anti-cDsg3 ELISA was significantly higher in canine PV ($n = 14$) than that in healthy dogs ($n = 44$) ($P < 0.05$). Of the canine PV and PNP sera, 11/14 (79%) canine PV sera and the canine PNP serum were positive in the anti-cDsg3 ELISA. On the other hand, 14/37 (38%) sera from canine PF and 9/18 (50%) sera from canine autoimmune subepidermal bullous dermatoses were positive in the ELISA. Circulating IgG against cDsg3 was not recognized in dogs with superficial pyoderma ($n = 5$), atopic dermatitis ($n = 8$), or in healthy dogs ($n = 44$). Moreover, IgG immunoreactivity against cDsg3 in all 21 sera was completely absorbed by preincubation with cDsg3. These findings alto-

gether suggest that the anti-cDsg3 ELISA can be used as a rapid screening test for the detection of circulating anti-Dsg3 IgG autoantibodies in canine pemphigus.

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Introduction

Pemphigus is a group of autoimmune blistering dermatoses of the skin and mucous membranes recognized in certain mammals including humans and dogs. Classically, there are two main subtypes of pemphigus described, pemphigus vulgaris (PV) and pemphigus foliaceus (PF).¹ In addition to these two classical types of pemphigus, paraneoplastic pemphigus (PNP), a variant of pemphigus that occurs in association with underlying neoplasms, has been reported in humans and dogs.^{2–4} Moreover, pemphigus vegetans (PVeg), a rare phenotype of pemphigus in which intraepidermal blisters are seen along with hyperplastic changes in the epidermis, has been described in humans and dogs.^{5–7}

Target molecules for circulating autoantibodies in human pemphigus have been characterized in the early 1990s. In human PV, circulating IgG autoantibodies target desmoglein (Dsg) 3, a desmosomal cadherin-type cell–cell adhesion molecule.⁸ Circulating IgG autoantibodies in human PF target Dsg1.⁹ In addition, approximately 2/3 of human patients with PV have circulating anti-Dsg1 IgG in conjunction with anti-Dsg3 IgG.¹⁰ Furthermore, human patients with PNP have circulating anti-Dsg3 IgG and/or anti-Dsg1 IgG along with antibodies against plakine family desmosomal cytoplasmic plaque proteins.^{2,11,12} The anti-Dsg IgG autoantibodies in human pemphigus are indeed pathogenic, as they are capable of inducing intraepidermal blisters when injected into neonatal mice.^{11, 13–15} Moreover, enzyme-linked immunosorbent assays (ELISAs) using baculovirus-expressed recombinant extracellular domains of human Dsg3 and Dsg1 have been developed and are widely used as rapid serological diagnostic tests for human pemphigus with high sensitivity and specificity.^{10,16}

In veterinary medicine, several attempts have been made to identify cell surface molecules of keratinocytes recognized by circulating antibodies in canine pemphigus. Immunoblot analyses revealed that circulating IgG antibodies in canine PV recognize a 130-kDa protein corresponding to Dsg3.^{17,18} In addition, it was revealed that

circulating IgG in dogs with PV and PNP recognizes the extracellular domains of canine Dsg3 produced by baculovirus expression.^{19,20} The canine PV and PNP sera were able to disrupt Dsg3-dominant cell–cell adhesion of cultured keratinocyte sheets in a dissociation assay, and removal of anti-Dsg3 IgG from the canine sera blocked the dissociation of keratinocytes.²⁰ Thus, the anti-Dsg3 IgG antibodies in canine PV and PNP sera appeared to be pathogenic, because they are able to disrupt the cell–cell adhesion of keratinocytes. In contrast, anti-Dsg1 IgG has been detected in canine sera obtained from a dog in which clinical and histopathological manifestations corresponded to those of human PVeg.⁷ A recent study reported that circulating anti-Dsg1 IgG are detectable at least in a minor subset of dogs with PF, as determined by immunofluorescence using cultured mammalian cells expressing canine Dsg1.²¹

In this study, we aimed to develop a novel ELISA using baculovirus expressed extracellular domains of canine Dsg3. We also wished to determine if the detection of anti-Dsg3 by ELISA was useful as a serological test for the diagnosis of canine pemphigus.

Materials and methods

Sera

Sera were obtained from 14 dogs whose clinical and histopathological findings were the characteristic of PV. The diagnostic criteria for canine PV included flaccid blisters and erosions on the oral mucosa and/or skin, and histopathological changes of intraepidermal blisters containing acantholytic cells just above the basal layer of the epidermis.^{6,22} In addition, sera were obtained from one dog diagnosed with PNP and splenic sarcoma,⁴ and one dog whose clinical and histopathological findings resembled human PVeg.⁷ Among those dogs, three dogs with PV and one dog with PNP were reported to have circulating IgG against canine Dsg3 in a previous study.²⁰ The dog with PVeg was reported to have circulating IgG against canine Dsg1, but not canine Dsg3.⁷

Sera were also obtained from dogs whose clinical and histopathological findings were consistent with the diagnosis of PF ($n = 37$). Diagnostic criteria for canine PF included crusted erosions and pustules, and histopathological findings of neutrophil-rich intraepidermal pustules with acantholytic cells at the superficial layer of the epidermis.^{6,22,23} In addition, sera were obtained from dogs with autoimmune subepidermal bullous dermatoses (AISBD), a group of bullous skin diseases in which the individuals exhibited tense blisters or erosions with subepidermal blisters present at the dermo–epidermal junction.^{6,22,24} The latter dogs included two cases of bullous pemphigoid (BP), seven cases of mucous membrane pemphigoid (MMP) and nine cases of epidermolysis bullosa acquisita (EBA) classified by circulating auto-antibody profiles.^{24–27} Additional sera were obtained from five dogs with superficial pyoderma, eight dogs with atopic dermatitis and 44 dogs that had no dermatologic disease. Three human sera obtained from patients with mucosal-dominant type of PV were used in this study. The human PV sera have been reported to recognize epitopes on the extracellular domains of Dsg3 including conformational ones.²⁸

Culturing of canine keratinocytes

The canine keratinocyte cell line MCA-B1²⁹ (a gift from S. Tateyama, Miyazaki University, Miyazaki, Japan) was cultured in Dulbecco's Modified Eagle's Medium with Ham's Nutrient Mixture F-12 (DME/F-12; Sigma-Aldrich, St Louis, MO, USA) supplemented with 10% fetal bovine serum (Invitrogen Corp., Carlsbad, CA, USA) and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin; Invitrogen).

Production and purification of recombinant proteins

The secreted form of the recombinant proteins representing the entire extracellular domain of canine Dsg3 (cDsg3) and Dsg1 (cDsg1), fused with the hinge region of human IgG₁, an E tag and a His tag at their carboxyl termini, were produced using a baculovirus-expression system, as previously described.^{19,30} The cDsg3 was reported to retain the conformation of its native form and is able to absorb heterogeneous anti-Dsg3 IgG from human PV sera.¹⁹ The cDsg1 was also reported to absorb heterogeneous anti-Dsg1 IgG from human PF sera.³⁰ High-Five insect cells cultured in serum-free EX Cell 405 medium (JRH Biosciences, Lenexa, KS, USA) were infected with the recombinant baculoviruses and incubated for 3 days at 27 °C. Culture supernatants were stored at –80 °C after the removal of cell debris by centrifugation. The cDsg3 protein was purified with TALON metal affinity resin (BD Biosciences Clontech, Mountain View, CA, USA), and then dialysed against phosphate-buffered saline containing 0.5 mM calcium (PBS-Ca).

cDNA encoding the hinge region of human IgG₁, an E tag and a 6 × His tag were amplified by PCR, and subcloned into pET32a(+) (Novagen, Madison, WI, USA) to generate pET32a-Trx-hlgG₁hinge-EHis. Transformation of pET32a-Trx-hlgG₁hinge-EHis into BL21 (DE3) competent cells (Novagen) resulted in the production of a recombinant protein of Trx-hlgG₁hinge-EHis, in which thioredoxin (Trx) is expressed at its amino terminus to increase the solubility of the recombinant protein. The Trx-hlgG₁hinge-EHis protein was harvested from the soluble fraction of the BL21 (DE3) cells in lysis buffer [20 mM Tris–HCl (pH 8.0), 0.2 M NaCl, 0.2% Triton X-100 and protease inhibitor cocktail (Complete Mini, EDTA-free; Roche Diagnostics)] and purified with the TALON metal affinity resin. Efficiency of production and purification of the recombinant proteins were analysed by SDS–PAGE followed by Coomassie brilliant blue (CBB) staining as well as by immunoblotting with anti-E tag monoclonal antibody (Amersham Biosciences Corp. Piscataway, NJ, USA).

Live keratinocyte staining

Live keratinocyte staining was performed using MCA-B1 cells as previously described.^{19,20,31} Briefly, MCA-B1 cells grown to confluence in eight-well chamber slides (Nalge Nunc International, Rochester, NY USA) were incubated with human and canine sera diluted serially in serum-free DME/F-12 medium, at 37 °C in a CO₂ incubator for 30 min. After washing with PBS, the cells were fixed with 100% methanol at –20 °C for 20 min, and incubated with fluorescein isothiocyanate (FITC)-conjugated anti-human IgG (Invitrogen Corp.) or anti-canine IgG (ICN Biomedicals Inc., Aurora, OH, USA). Specimens were examined under a fluorescence microscope (BX50; Olympus Corp., Tokyo, Japan). Images were captured and processed using a digital processor (DP71; Olympus Corp.) and DP controller (Olympus Corp.).

Immunoabsorption assay

Three human PV sera were diluted serially in 50 µg/mL of purified cDsg3 in DME/F-12. Human PV sera diluted in control medium were also prepared. After incubation at 4 °C overnight, the diluted sera were added to MCA-B1 cells, which were then subjected to live keratinocyte staining.

ELISA against cDsg3

Microtitre 96-well plates (Maxisorp; Nalge Nunc International) were coated with 50 µL of cDsg3 at 5 µg/mL (0.25 µg/well) in Tris-buffered saline with 1 mM CaCl₂ (TBS-Ca, pH 7.4) at 4 °C overnight. The plates were then blocked with 3% skim milk, 5% bovine serum albumin (BSA) and 0.1% Nonidet P-40 in TBS-Ca at room temperature for 2 h. Canine sera were diluted 1/100 and 1/200 in assay diluent (3% skim milk, 5% BSA, 10% High-Five insect culture supernatant and 0.1% Nonidet P-40 in TBS-Ca) and 50 µL were incubated in the cDsg3-coated wells at room temperature for 1 h. After washing four times with 300 µL of wash buffer (0.05% Tween 20 in TBS-Ca), the wells were incubated with 50 µL of horseradish peroxidase-conjugated goat anti-dog IgG (Bethyl Laboratories, Montgomery, TX, USA) diluted 1/1000 in conjugate diluent (1% BSA and 0.05% Tween 20 in TBS-Ca) at room temperature for 1 h. After

washing, colour development was achieved by incubation with 100 μ L of colour development reagent (from peroxidase colour development kit; Sumitomo Bakelite Co., Tokyo, Japan) at room temperature for 10 min. The colour development was stopped by adding 100 μ L of stop solution from the same kit. The absorbance at 492 nm (A_{492}) was measured using a microtitre plate reader (Multiscan JX; Thermo Fisher Scientific Ltd., Waltham, MA, USA).

A canine PNP serum and a normal canine serum were selected as positive and negative controls respectively for the anti-cDsg3 ELISA. The index value of anti-cDsg3 ELISA was calculated by the following formula: index = [optical density (OD) of tested serum – OD of negative control]/(OD of positive control – OD of negative control) $\times$ 100. A positive cutoff value of 25.1 was established as >5 standard deviations (SDs) above the mean of 44 normal canine sera.

Blocking ELISA

Insect culture supernatants containing either cDsg3 or cDsg1, or purified Trx-hlgG₁hinge-EHis were diluted serially, and added to the assay diluent of the anti-cDsg3 ELISA. As a control, a supernatant

containing no recombinant proteins was added to the assay diluent. Canine sera were diluted to 1/100 in assay diluent containing the various insect culture supernatants, incubated at 4 °C overnight, and then subjected to anti-cDsg3 ELISA.

Results

Purified cDsg3 retains the epitopes expressed in native form Dsg3 recognized by IgG in canine PV sera

cDsg3 secreted in the culture supernatant of High-Five insect cells was purified using TALON metal affinity resin. Immunoblot analysis with anti-E tag antibody revealed cDsg3 in the insect culture supernatant flow-through material, as well as in eluted fractions of the column, but not in the washing fraction; the material appeared as a doublet of higher and lower molecular weight bands (Figure 1a). The product with higher molecular weight was considered to represent the unprocessed molecule without proteolytic cleavage of signal peptide and prosequence.¹⁹ To determine the purity of cDsg3 in the eluates, the eluates were size-fractionated by SDS-PAGE and visualized by Coomassie brilliant blue staining. A major band with a molecular weight of approximately 75 kDa, likely corresponding to cDsg3, was recognized in the eluates, although two minor bands with molecular weights of approximately 27 and 23 kDa were also recognized (Figure 1b).

To determine whether the purified cDsg3 retained its native conformation, immunoadsorption analysis was performed with three representative human PV sera using MCA-B1 cells as the substrate. The IgG immunoreactivity of all three human PV sera was completely abolished when the sera were preincubated with purified cDsg3 (Figure 2).

These findings indicated that cDsg3 retaining its native conformation was purified successfully using TALON metal affinity resin.

Circulating IgG antibodies against cDsg3 are detected in dogs with PV and PNP by anti-cDsg3 ELISA

An ELISA was developed using purified cDsg3 as the coated antigen. Using the anti-cDsg3 ELISA, IgG immunoreactivity was first analysed with canine sera obtained from 14 dogs with PV, one dog with PNP and 44 healthy dogs. The titre of the anti-cDsg3 ELISA was significantly higher in canine PV than that of healthy dogs (Mann-Whitney *U*-test, $P < 0.05$). The titre in the canine PNP serum was also considerably higher than that of healthy dogs. When the cutoff value of 25.1 was established as 5

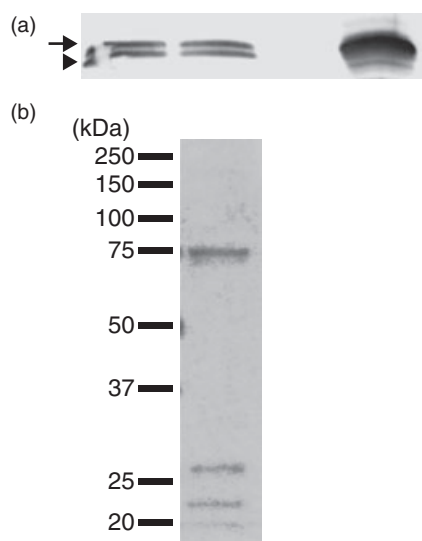


Figure 1. Production and purification of baculovirus-expressed recombinant cDsg3. (a) Immunoblot analysis of fractions from TALON metal affinity resin column, using anti-E tag monoclonal antibody. Insect culture supernatant infected with recombinant baculovirus of cDsg3 (lane 1), flow-through (lane 2), washing fraction (lane 3) and eluted fraction from column (lane 4). Note that cDsg3 was detected in the insect culture supernatant, flow-through, and eluted fractions as a doublet of higher (arrow) and lower (arrowhead) molecular weight bands. (b) The eluted fraction of the column was subjected to Coomassie brilliant blue staining. Note that there is a major band of approximately 75 kDa, although two minor bands of approximately 27 and 23 kDa were also recognized. Bars in the left panel indicate molecular weight standards.

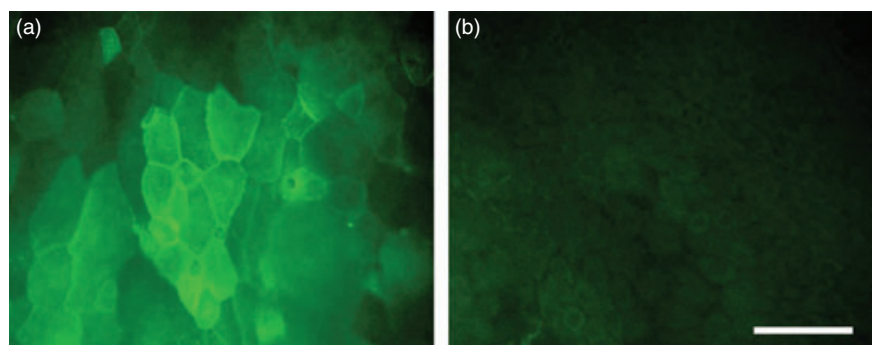


Figure 2. Purified cDsg3 absorbs the IgG immunoreactivity in human pemphigus vulgaris (PV) serum directed against the surface of canine keratinocytes. A human PV serum at 1 : 20 dilution was preincubated with either control solution without recombinant proteins (a) or purified cDsg3 (b), and subjected to live keratinocyte staining using MCA-B1 cells. The cDsg3 completely blocked the IgG immunoreactivity in the human PV serum. Bar = 50 μ m.

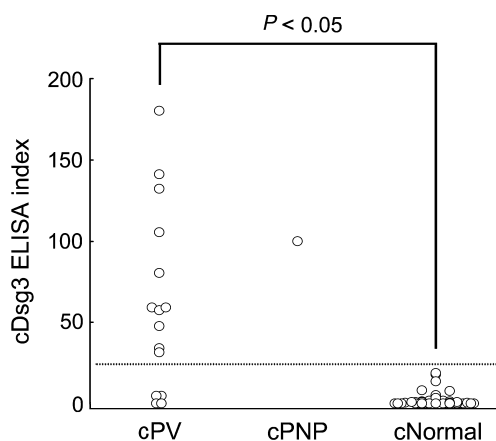


Figure 3. Anti-cDsg3 IgG antibodies are detected in canine pemphigus vulgaris (PV) and paraneoplastic pemphigus (PNP) sera by the anti-cDsg3 ELISA. The dashed line indicates a cutoff value defined as 5 SD above the mean of 44 sera from healthy dogs. Note that the IgG titres in 11 of 14 canine PV sera and the one canine PNP serum exceed the cutoff value, but titres of the normal canine sera do not. The index values of IgG against cDsg3 are significantly higher in a group of canine PV sera than in sera from a group of healthy dogs ($P < 0.05$, Mann–Whitney U -test).

SDs above the mean of 44 healthy dogs, 11 of 14 (79%) canine PV sera and the canine PNP serum were positive in the anti-cDsg3 ELISA, whereas none of 44 normal canine sera exceeded the cutoff value (Figure 3). Interestingly, two of three canine PV sera that were negative in the anti-cDsg3 ELISA stained the cell surface of MCA-B1 cells, although the immunoreactivity was not blocked by preincubation with either cDsg3 or cDsg1 or combination thereof (data not shown).²⁰

To test whether the canine PV and PNP sera react with cDsg3 in an antigen-specific manner, a blocking ELISA using cDsg3 as the absorbent was also performed using the anti-cDsg3 ELISA. Seven of eight canine PV sera and the canine PNP serum were used in this blocking ELISA. Index values of IgG against cDsg3 in all canine PV sera tested and the canine PNP serum were reduced by preincubation with serially diluted cDsg3 in a dose-dependent fashion, and significantly decreased at the maximum concentration of cDsg3 in the absorbents (Figure 4).

These findings indicate that circulating IgG against cDsg3 in dogs with PV and PNP can be detected by the anti-cDsg3 ELISA.

Circulating IgG against baculovirus cDsg3 are also detectable in some dogs with PF or AISBD

To further determine test specificity, the anti-cDsg3 ELISA was performed with canine sera obtained from one dog with PVeg, 38 dogs with PF, 19 dogs with AISBD, five dogs with superficial pyoderma and eight dogs with atopic dermatitis. The index values of the anti-cDsg3 ELISA exceeded the cutoff value of 25.1 in 14 of 37 (38%) canine PF and 9 of 18 (50%) canine AISBD sera (one serum from BP, four sera from MMP and four sera from EBA) (Table 1). Nine canine PF sera and six canine AISBD sera were used in the blocking ELISA using cDsg3, and their IgG immunoreactivity was significantly reduced when the sera were preincubated with serially

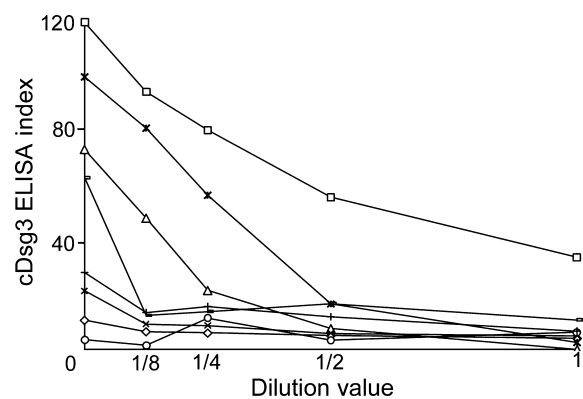


Figure 4. IgG immunoreactivity of canine pemphigus vulgaris (PV) and paraneoplastic pemphigus (PNP) sera in the anti-cDsg3 ELISA is blocked by cDsg3. Seven canine PV sera and one canine PNP serum were preincubated with serially diluted insect culture supernatant containing cDsg3, and subjected to anti-cDsg3 ELISA. Note that the index values of IgG against cDsg3 in all canine PV and PNP sera tested were reduced in a dose-dependent fashion.

Table 1. Summary of results of cDsg3 ELISA with canine sera obtained from dogs with pemphigus, autoimmune subepidermal bullous dermatoses (AISBD) and other skin diseases

Diagnosis	n	Index value of cDsg3 ELISA		
		>50	>25.1	<25.1
Pemphigus				
PV	14	8 (57.1)	3 (21.4)	3 (21.4)
PNP	1	1 (100)	0 (0.0)	0 (0.0)
PVeg	1	0 (0.0)	0 (0.0)	1 (100.0)
PF	37	9 (24.3)	5 (13.5)	23 (62.2)
AISBD				
BP	2	1 (50.0)	0 (0.0)	1 (50.0)
MMP	7	2 (28.6)	2 (28.6)	3 (42.9)
EBA	9	3 (33.3)	1 (11.1)	5 (55.6)
Others				
Pyoderma	5	0 (0.0)	0 (0.0)	5 (100)
AD	8	0 (0.0)	0 (0.0)	8 (100)

Values are given as the number of sera (%).

AD, atopic dermatitis; AISBD; autoimmune subepidermal bullous dermatoses; BP, bullous pemphigoid; EBA, epidermolysis bullosa acquisita; MMP, mucous membrane pemphigoid; PF, pemphigus foliaceus; PNP, paraneoplastic pemphigus; PV, pemphigus vulgaris; PVeg, pemphigus vegetans.

diluted cDsg3 (Figure 5a). On the other hand, one canine PVeg serum, in which circulating anti-Dsg3 IgG was not detected in a previous study,⁷ showed no reactivity in the anti-cDsg3 ELISA. As expected, none of canine sera obtained from five dogs with superficial pyoderma and eight dogs with atopic dermatitis showed positive IgG reactivity in the anti-cDsg3 ELISA (Table 1).

We speculated that the positive reactivity of the canine PF or AISBD sera could be due to reactivity against polypeptide tags in cDsg3 including the hinge region of human IgG₁, E tag and 6 × His tag (Figure 5b). To determine whether a recombinant protein representing only the peptide tags was able to block the reactivity, these sera were preincubated with purified Trx-hlgG₁hinge-EHis, and then subjected to anti-cDsg3 ELISA. IgG immunoreactivity in the ELISA was not altered by preincubation with purified Trx-hlgG₁hinge-EHis at the maximum

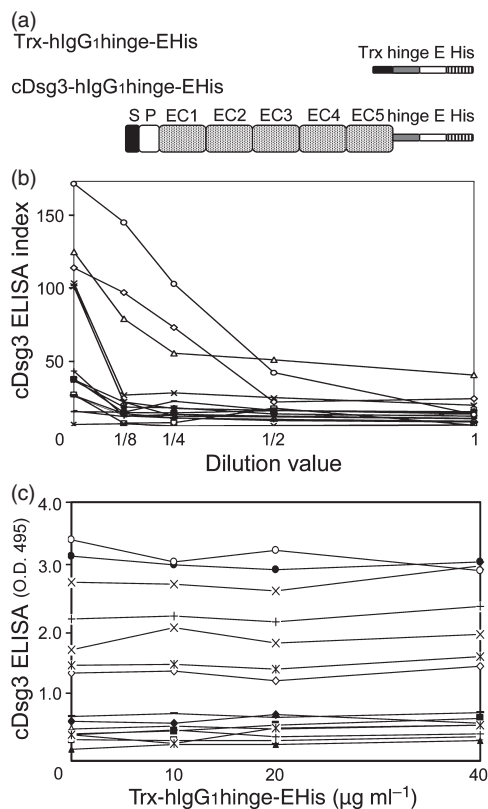


Figure 5. IgG immunoreactivity of canine PF and AISBD sera in the anti-cDsg3 ELISA is blocked by cDsg3, but not by peptide tags expressed in cDsg3. (a) IgG immunoreactivity of nine canine PF sera and six canine AISBD sera in the anti-cDsg3 ELISA is significantly reduced by preincubation with cDsg3. (b) A recombinant Trx-hlgG₁hinge-EHis is expressed in *E. coli* as a fusion protein in which thioredoxin (Trx), His tag, hinge region of human IgG₁, E tag and 6 × His tag are inserted in tandem. IgG immunoreactivity of the canine PF and AISBD sera in the anti-cDsg3 ELISA is not altered by preincubation with Trx-hlgG₁hinge-EHis.

concentration of 40 µg/mL (Figure 5b). Furthermore, none of the canine PF and AISBD sera with index values above 50 was found to stain the surface of MCA-B1 cells, which express canine Dsg3 on their cell surfaces (Figure 6).¹⁹ Thus, it is considered that this reactivity may be due to reactivity against neo-epitopes including signal peptide and prosequence expressed on cDsg3 during baculovirus expression, or some minor contaminants in the preparation of cDsg3 as was similarly reported in a human anti-Dsg ELISA.¹⁰

Discussion

In this study, we developed a novel ELISA using baculovirus recombinant cDsg3, and we assessed whether the ELISA could be a serological diagnostic tool for canine PV and PNP. Circulating IgG antibodies against cDsg3 were detected in the majority of dogs with PV tested, and one dog with PNP, whereas none of the normal canine sera tested showed reactivity. This suggests that the anti-cDsg3 ELISA can be a useful tool to detect circulating IgG autoantibodies in dogs with PV and PNP.

This study revealed that three of 14 canine PV sera were negative in the anti-cDsg3 ELISA. Two of these negative sera showed IgG immunoreactivity to the surface of MCA-B1 cells, while the immunoreactivity was not blocked by preincubation with either cDsg3 or cDsg1 or combination thereof (data not shown).²⁰ In the third negative serum, IgG immunoreactivity against MCA-B1 cells was not recognized.²⁰ Reasons why we could not detect anti-cDsg3 IgG in these sera include the possibility that the canine sera were lacking IgG directed against the extracellular domains of Dsg3, or that they had titres of anti-Dsg3 IgG too low to be detectable by our ELISA. It is also possible that the two canine sera positive on MCA-B1 cell staining but negative in the anti-cDsg3 ELISA might

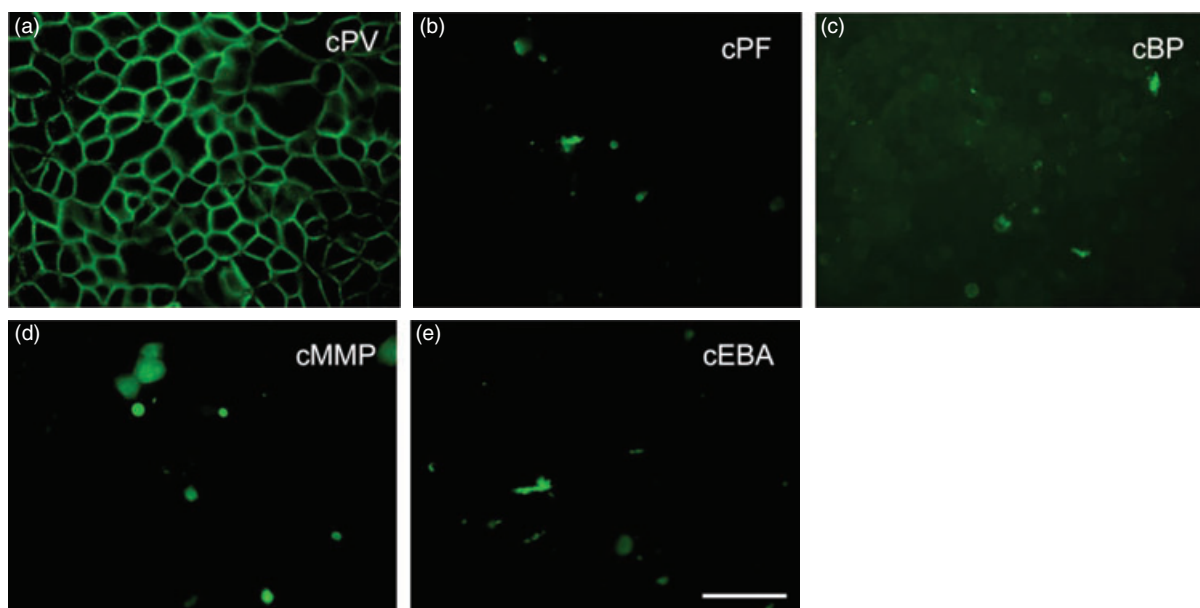


Figure 6. Canine PF and AISBD sera showing high index values of IgG against cDsg3 do not stain the surface of MCA-B1 cells. MCA-B1 cells were incubated with sera from a dog with pemphigus vulgaris (a), pemphigus foliaceus (b), bullous pemphigoid (c), mucous membrane pemphigoid (d) and epidermolysis bullosa acquisita (e). The figure is shown at 1 : 20 dilution of the patient's serum. Bar = 50 µm.

have circulating IgG that recognizes molecules other than Dsg3 or Dsg1. Further studies are needed to define the exact target molecules for circulating autoantibodies in canine PV sera negative in the anti-cDsg3 ELISA.

The results of this study also indicate that certain sera obtained from dogs with PF or AISBD showed positive IgG immunoreactivity against cDsg3 by ELISA, although none of these sera with index values above 50 stained the surface of MCA-B1 cells. The IgG immunoreactivity in the anti-cDsg3 ELISA could be blocked by preincubation with cDsg3, but not by a recombinant polypeptide tags including the hinge region of human IgG₁, E tag and 6 × His tag. Thus, immunoreactivity against cDsg3 in canine PF and AISBD sera is antigen-specific, but may not correspond to reactivity against native canine Dsg3. Possible explanations why some canine PF and AISBD sera were positive in the anti-cDsg3 ELISA include that circulating IgG in those sera recognized neo-epitopes including signal peptide or prosequence on cDsg3 expressed during baculovirus expression or some minor contaminants in the preparation of cDsg3.¹⁰ The exact reason why our anti-cDsg3 ELISA was positive in canine PF or AISBD sera remains to be further elucidated. We also found that anti-cDsg3 ELISA reactivity was not present in sera obtained from dogs with non-autoimmune bullous dermatoses such as superficial pyoderma and atopic dermatitis.

In conclusion, although of low specificity for sera from dogs with PF and AISBD, our anti-cDsg3 ELISA may be useful as a screening test to detect circulating IgG against canine Dsg3 in dogs with PV and PNP. Careful interpretation of the results of the anti-cDsg3 ELISA, along with clinical and histopathological findings, would provide an advantage in achieving a proper diagnosis of canine PV or PNP. The anti-cDsg3 ELISA will help provide us with a better understanding of the relationship between clinical phenotype and circulating autoantibody profile in dogs with pemphigus.

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Résumé Les pemphigus, groupe de dermatoses autoimmunes décrit chez l'homme et le chien, est subdivisé en pemphigus vulgaire (PV), pemphigus foliacé (PF) et pemphigus paraneoplasique (PNP). Précédemment, il a été démontré que les IgG anti-desmogleïne 3 (Dsg3) circulantes chez les chiens atteints de PV ou de PNP interfèrent avec l'adhésion inter-kératinocytaire liée à la Dsg3. Le but de cette étude était de développer un test ELISA de détection des IgG circulantes dirigées contre la Dsg3 canine des chiens atteints de pemphigus. Une forme sécrétée de protéine recombinante représentant le domaine extracellulaire entier de la Dsg3 canine (cDsg3) a été produit par expression de baculovirus et utilisé comme l'antigène de couverture dans le test ELISA. Le titre en cDsg3 était significativement plus élevé chez les chiens atteints de PV ($n = 14$) que chez les chiens sains ($n = 44$) ($P < 0.05$). Les sérums de 11 chiens sur 14 (79%) atteints de PV et le sérum du chien atteint de PNP, étaient positifs pour le test ELISA anti-cDsg3. D'autre part, 14/37 (38%) des sérums de chiens atteints de PF et 9/18 (50%) des sérums de chiens atteints de dermatoses bulleuses sous-épidermiques présentaient un test ELISA positif. Les IgG circulantes anti-cDsg3 n'ont pas été reconnus chez les chiens atteints de pyodermite superficielle ($n = 5$), de dermatite atopique ($n = 8$), ou chez les chiens sains ($n = 44$). De plus, l'immunoréactivité des IgG anti-cDsg3 a été complètement absorbée dans les 21 cas après incubation avec cDsg3. Ces résultats suggèrent que le test ELISA anti-cDsg3 peut être utilisé comme test rapide de dépistage des IgG anti-Dsg3 circulante dans le pemphigus canin.

Resumen Pénfigo, un grupo de dermatosis autoinmunes vesiculares reconocido en humanos y perros, se divide en tres grupos: pénfigo vulgar (PV), pénfigo foliaceo (PF) y pénfigo paraneoplásico (PNP). Previamente hemos demostrado que IgG frente a desmogleína 3 circulante en perros con PV y PNP altera la adhesión intercelular en queratinocitos mediada por esa proteína. El propósito de este estudio fue desarrollar una prueba de ELISA para la detección de IgG circulante frente a la desmogleína 3 en perros con pénfigo. Se produjo mediante expresión en baculovirus la forma secretada de la proteína recombinante que representa el dominio extracelular de desmogleína 3 canina (cDsg3), y se utilizó como antígeno en la prueba de ELISA. El título de anti-cDsg3 en ELISA fue significativamente mayor en perros con PV ($n = 14$) que en perros sanos ($n = 44$) ($P < 0.05$). De los sueros caninos de PV y PNP 11/14 (79%) sueros de PV y los sueros de PNP fueron positivos en la prueba de ELISA frente a cDsg3. Por otra parte, 14/37 (38%) sueros de perros con PF and 9/18 (50%) sueros de perros con dermatosis bullosas subepidermales autoinmunes fueron positivos en la prueba de ELISA. IgG circulante frente a cDsg3 no fue reconocida en perros con pioderma superficial ($n = 5$), dermatitis atópica ($n = 8$) o en perros sanos ($n = 44$). Además, la inmuoreactividad de IgG frente a cDsg3 en los 21 sueros fue completamente neutralizada mediante preincubación con cDsg3. Estos hallazgos en su conjunto sugieren que el ELISA frente a cDsg3 puede utilizarse como prueba rápida de muestreo para la detección de autoanticuerpos IgG circulantes frente a Dsg3 en perros con pénfigo.

Zusammenfassung Pemphigus, eine Gruppe von autoimmunen blasenbildenden Dermatosen, die bei Menschen und Hunden vorkommt, wird in drei Untergruppen eingeteilt: Pemphigus vulgaris (PV), Pemphigus foliaceus (PF) und paraneoplastischer Pemphigus (PNP). In früheren Studien haben wir gezeigt, dass zirkulierende Anti-Desmoglein 3 (Dsg3) IgG bei Hunden mit PV und PNP die Dsg3-vermittelte Keratinozyten Zell-Zell Adhäsion stören. Das Ziel dieser Studie war es, einen Enzyme-linked Immunosorbent Assay (ELISA) zu entwickeln, um zirkulierende IgG gegen canines Dsg3 bei Hunden mit Pemphigus zu ermitteln. Mittels Baculovirus-Expression wurde eine sezernierte Form eines rekombinanten Proteins produziert, welches die gesamte extrazelluläre Domäne des caninen Dsg3 (cDsg3) repräsentierte und als Coating-Antigen im ELISA verwendet wurde. Der Titer des Anti-cDsg3 ELISA war beim caninen PV ($n = 14$) signifikant höher als bei gesunden Hunden ($n = 44$) ($P < 0.05$). Von den caninen PV und PNP Seren waren 11/14 (79%) der caninen PV ($n = 14$) und das PNP Serum positiv im Anti-cDsg3 ELISA. Andererseits waren 14/37 (38%) der Seren von caninem PF und 9/18 (50%) der Seren von caninen autoimmunen subepidermalen bullösen Dermatosen im ELISA positiv. Zirkulierende IgG gegen cDsg3 wurden bei Hunden mit oberflächlicher Pyodermie ($n = 5$), mit atopischer Dermatitis ($n = 8$) oder bei gesunden Hunden ($n = 44$) nicht gefunden. Überdies wurde die IgG Immunreaktivität gegen cDsg3 bei allen 21 Seren durch eine Präinkubation mit cDsg3 völlig absorbiert. Zusammengefasst weisen diese Ergebnisse darauf hin, dass der Anti-cDsg3 ELISA als rascher Screening Test für die Bestimmung von zirkulierenden Anti-Dsg3 IgG Autoantikörpern beim caninen Pemphigus verwendet werden kann.