Advance Publication

Journal of Toxicologic Pathology

Received : August 11, 2021

Accepted : September 27, 2021

J-STAGE Advance Published Date : October 16, 2021

Japanese Society of Toxicologic Pathology

Title: A case of spontaneous autoimmune skin disease in a cynomolgus monkey (*Macaca fascicularis*)

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Running head:

Spontaneous autoimmune skin disease in a cynomolgus monkey

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Abstract

Pemphigus is an autoimmune blistering disease characterized by lesions on the skin and mucous membranes. To date, no spontaneous cases of this disease have been reported in cynomolgus monkeys. This report describes the histopathological characteristics of spontaneous pemphigus in a cynomolgus monkey. Macroscopically, redness and scaling with pruritus were observed on the skin of the entire body. Histopathologically, the epidermis showed intercellular edema, and eosinophils and mononuclear cells infiltrated the epidermis. There was no obvious acantholysis in the epidermis. The perivascular area showed edema, and eosinophils and mononuclear cells infiltrated the vessels in the dermis. Immunohistochemically, the intercellular area in the epidermis was positive for Immunoglobulin G and Complement component 3. Serologically, anti-desmoglein 1 and desmoglein 3 antibodies in the serum were negative. From these findings, this case was diagnosed as an autoimmune skin disease, suspected to be pemphigus, and concluded as lesions being similar to those in human “pemphigus herpetiformis.”

Key word

Autoimmune skin disease, Pemphigus herpetiformis, Cynomolgus monkey, Spongiosis

Pemphigus is a group of autoimmune blistering diseases characterized by lesions on the skin and mucous membranes. Blisters are formed by acantholysis due to autoantibodies that target cell-cell adhesion factors of the epidermis and mucosal epithelium.^{1,2}

Pemphigus is classified into two major types: pemphigus vulgaris and pemphigus foliaceus.¹
² Naturally occurring cases of pemphigus vulgaris have been reported in dogs, cats, horses, goats, monkeys, and llamas; cases of pemphigus foliaceus have been reported in dogs, cats, horses, goats, and sheep.² However, no spontaneous cases have been reported in experimental animals, including cynomolgus monkeys. In this report, we describe a case of spontaneous pemphigus in a cynomolgus monkey.

A male cynomolgus monkey (*Macaca fascicularis*), originally from Cambodia, was purchased from Shin Nippon Biomedical Laboratories, Ltd. This animal was not treated with any of the test articles. At the age of five years, this animal showed redness and scaling with pruritus on the skin of the entire body, including the extremities, trunk, and face (Fig.1a-c). This symptom was temporarily relieved by treatment with shampoo, but it kept recurring. No pathogens, such as parasites or dermatophytes, were detected by scratch tests or culture. Blood tests showed an increased eosinophil count in the peripheral blood (Fig.2). Because the skin symptoms continued for approximately five months and locomotor activity decreased, necropsy was performed from the viewpoint of animal welfare. No abnormality was observed in the oral mucosa, during necropsy. All animal procedures were conducted in accordance with the Chugai Pharmaceutical Guide for the Care and Use of Laboratory Animals, and all the experimental protocols were approved by the Institutional Animal Care and Use Committee.

For histopathological examination, all organs, including the skin and the oral mucosa, were fixed in 10 % neutral-buffered formalin and embedded in paraffin. The sections were stained with hematoxylin and eosin. For immunohistochemical analysis,³ primary antibodies against Human

Immunoglobulin G (IgG) (γ -Chain) (0.05 $\mu\text{g/mL}$; OriGene Technologies, Maryland, USA) and Complement component 3 (C3) (0.1 $\mu\text{g/mL}$; Abcam, Cambridge, UK) were used, and immunohistochemical staining was performed using the polymer-immuno complex method with an ENVISION+ kit (Agilent Technologies, California, USA). Immunoreactivity was visualized using a peroxidase-diaminobenzidine reaction. Finally, the sections were counterstained with hematoxylin.

For serological examination, anti-desmoglein (Dsg) 1 and Dsg3 in the serum were tested using an enzyme linked immunosorbent assay (ELISA) kit (MBL, Nagoya, Japan, RG-M7593-D), following the manufacturer's protocol.

Histopathologically, the lesional epidermis was thickened and showed intercellular edema (spongiosis) (Fig. 3a, b). Eosinophils and mononuclear cells, such as lymphocytes and macrophages, infiltrated the dermis and epidermis (Fig. 3c, d). Occasionally, epidermal cells showed degeneration and necrosis accompanied by inflammation (Fig. 3e). There was no obvious acantholysis in the epidermis. In the dermis, perivascular edema was observed, and eosinophils and mononuclear cells, such as lymphocytes and macrophages, infiltrated around the vessels (Fig. 3f-g). No related lesions were observed in other organs, including the oral mucosa. Pemphigus was suspected based on the histopathological findings, and immunohistochemistry for IgG and C3 in the skin was performed. Immunohistochemically, the intercellular area in the epidermis was positive for IgG (Fig. 4a) and C3 (Fig. 4b).

To analyze the target of IgG deposited between epidermal cells, anti-Dsg1 and Dsg3 antibodies in the serum of this animal were analyzed by ELISA. The serum was negative for both anti-Dsg1 and Dsg3 antibodies. (data not shown).

In pemphigus, IgG and occasionally C3 deposition in the intercellular area of epidermal cells and/or mucosal cells can be detected by immunohistochemistry.^{4,5} Based on the histopathological

findings and immunohistochemistry, the animal was diagnosed with an autoimmune skin disease suspected to be pemphigus.

Classical pemphigus can be classified as either pemphigus vulgaris or pemphigus foliaceus according to the site of the lesion.^{1,2} In general, Dsg1 is expressed predominantly in the epidermis, and Dsg3 is expressed predominantly in the mucosa. Pemphigus vulgaris produces autoantibodies to Dsg3 and sometimes Dsg1, so that lesions occur in the mucosa and sometimes the skin. In contrast, pemphigus foliaceus produces autoantibodies to Dsg1 alone, and lesions occur only in the skin.^{4,5} These autoantibodies can be detected in the serum by ELISA.^{1,6} In human medicine, pemphigus herpetiformis has been reported to be different from classical pemphigus.^{7,8} In general, acantholysis is characteristic of both pemphigus vulgaris and pemphigus foliaceus,^{1,2} whereas acantholysis is absent or minimal in pemphigus herpetiformis.^{7,8} Many cases of pemphigus herpetiformis are characterized by eosinophilic spongiosis, which is not common in pemphigus vulgaris and pemphigus foliaceus.^{7,8}

Autoantibodies in sera were positive for Dsg3 and sometimes Dsg1 in pemphigus vulgaris, and positive for only Dsg1 in pemphigus foliaceus. However, pemphigus herpetiformis has been reported to be positive not only for Dsg1 and Dsg3, but also for desmocollin (Dsc) 1 and Dsc3, which are other epidermal adhesion molecules.^{5,9} Although rare, some cases of pemphigus herpetiformis with autoantibodies negative for Dsg and positive for Dsc1 or Dsc3 have been reported in humans.^{10,11} In the current report, anti-Dsg antibodies in the serum could not be detected. There are two possible explanations for this finding. First, anti-monkey Dsg antibodies could not be detected because we used an ELISA kit for humans. However, the amino acid sequence homology between Dsg1 and Dsg3 in humans and monkeys is 97 % and 99 %, respectively, and we confirmed that anti-human IgG in this kit recognized monkey IgG by ELISA (data not shown). Another possibility is that this animal had only anti-Dsc antibodies, similar to some rare human cases.

In this case, the lesion was located in the skin, acantholysis was not evident, and eosinophilic spongiosis was prominent, consistent with pemphigus herpetiformis in humans. Therefore, we concluded that this case was similar to pemphigus herpetiformis in humans; however, because such spontaneous cases are rare, more cases need to be evaluated to fully understand the phenomenon of pemphigus in experimental animals.

Acknowledgments

We would like to thank Ms. Kiyoka Katsuyama and Mr. Toshihiko Shiga for their scientific and technical assistance.

Disclosure of Potential Conflicts of Interest

The authors declare that they have no potential conflicts of interest.

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Figure legends

Fig. 1. Redness and scaling (arrows) on the skin of the (a) chest, (b) forearm, and (c) face.

Fig. 2. The number of eosinophils in peripheral blood was increased compared to before onset.

Fig. 3. (a) Epidermis was diffusely thickened in lesioned skin. Hematoxylin Eosin (HE), Bar = 100 μ m. (b) Intercellular area of the epidermis showed edema (spongiosis). HE, Bar = 50 μ m. (c) Eosinophils infiltrated in the dermis and epidermis. HE, Bar = 50 μ m. (d) Mononuclear cells such as lymphocytes and macrophages infiltrated the dermis and epidermis. HE, Bar = 50 μ m. (e) Occasionally, epidermal cells showed degeneration and necrosis accompanied by inflammation. HE, Bar = 50 μ m. (f) Perivascular edema (arrows) was observed in the dermis. HE, Bar = 100 μ m. (g) Higher magnification of (f). Eosinophils and mononuclear cells infiltrated around the vessels. HE, Bar = 50 μ m.

Fig. 4. The intercellular area in epidermis was positive for (a) IgG and (b) C3. The images shown are the magnified views of each figure. Bar = 100 μ m.

Fig. 1

Fig. 2

Fig. 3

Fig. 4