Journal of Translational Autoimmunity 7 (2023) 100208

Contents lists available at ScienceDirect

Journal of Translational Autoimmunity

journal homepage: www.sciencedirect.com/journal/journal-of-translational-autoimmunity

Ultrastructural skin alterations of healthy subjects with *anti*-desmoglein 1 antibodies in endemic areas to pemphigus foliaceus: A case series

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ARTICLE INFO

Handling Editor: M.E. Gershwin

Keywords:
Endemic pemphigus foliaceus
Anti-desmoglein 1 antibodies
Ultrastructural skin alterations

ABSTRACT

Background: Endemic pemphigus foliaceus and endemic pemphigus vulgaris are autoimmune dermatologic disorders endemic to the Peruvian Amazon.
Objective: To determine the ultrastructural skin alterations of three healthy subjects with anti DSG-1 antibodies in areas endemic to pemphigus foliaceus and pemphigus vulgaris in the Peruvian Amazon.
Patients and methods: Case series carried out from data of three clinically healthy subjects positive to anti DSG-1 antibodies, from Peru. This study consists of a sub-analysis of data gathered in a previous study.
Results: Ultrastructural results are presented from the skin biopsies of three clinically healthy patients positive to *anti*-desmoglein 1 (DSG-1) antibodies. High Resolution Optical Microscopy (HROM) showed the absence of acantholysis. Transmission Electron Microscopy (TEM) showed the widening of intercellular space between keratinocytes, the presence of vacuoles in intercellular space with granular material and cytoplasmic vacuolization, loss of desmosome structure, loss of normal distribution among tonofilaments and lateral separation among cells in the stratum basale.
Conclusion: According to our results, healthy subjects that present *anti*-desmoglein 1 antibodies can develop ultrastructural alterations that are visible through transmission electron microscopy but not through conventional optical microscopy.

1. Introduction

Endemic pemphigus foliaceus and endemic pemphigus vulgaris are autoimmune dermatologic disorders, characterized by acantholysis and the presence of *anti*-desmoglein 1 (DSG-1) and *anti*-desmoglein 3 antibodies (DSG-3) [1,2]. In both cases, the disorder affects children and young adults upon being exposed to possible environmental factors present in endemic areas such as the repeated exposure to hematophagous insect bites [3–6].

The studies previously done by our research group have demonstrated that healthy individuals in areas endemic to pemphigus foliaceus in the Peruvian Amazon can develop anti DSG-1 (31–46%) and anti DSG- 3 antibodies (31.7%) [7–9]. Additionally, it has been shown that despite these subjects being disease-free, they present biochemical anomalies, such as elevated lipid peroxidation levels, which could be attributed to the presence of anti DSG-1 and anti DSG- 3 antibodies [10].

In the case of the anti DSG-1 antibodies, previous studies showed that healthy subjects in endemic areas of Ucayali and Amazonas, areas of the Peruvian Amazon, presented mainly IgG1 and IgG2 subclasses, rarely finding IgG4, which are usually considered pathogenic in the

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https://doi.org/10.1016/j.jtauto.2023.100208
Received 31 May 2023; Accepted 11 July 2023
Available online 13 July 2023

development of endemic pemphigus foliaceus [8,9].

Since immunological and biochemical disorders exist [8–10], it is feasible that, as a consequence of an incipient autoimmune phenomenon, healthy patients positive to anti DSG-1 antibodies develop ultrastructural alterations in the epidermis only visible through transmission electron microscopy but not optical microscopy. The manifestation of ultrastructural skin alterations may justify the recommendation to abandon their residence in the endemic areas and settle in non-endemic zones, where the environment does not play a negative role on the induction of cutaneous autoimmunity from endemic pemphigus foliaceus [8].

The objective of this study is to determine the ultrastructural skin alterations of three healthy subjects with anti DSG-1 antibodies in areas endemic to pemphigus foliaceus and pemphigus vulgaris in the Peruvian Amazon.

2. Materials and methods

Case series carried out from data of three clinically healthy subjects, positive to anti DSG-1 antibodies from the Ucayali department (Peru). This study consists of a sub analysis of data gathered in the study "Ultrastructural Skin Alterations in Patients with Pemphigus Foliaceus and Pemphigus Vulgaris: a comparative study between endemic and non-endemic disease" (code approval San Marcos National University: 080103211) [11].

We reviewed the database of the participants in this study, obtaining epidemiological, immunopathological, high resolution optical microscopy (HROM) and transmission electron microscopy (TEM) variables, to determine the ultrastructural characteristics with an emphasis on desmosomes.

- Epidemiological variables: Age, sex, origin, occupation, drinking water source, housing material, and housing location.
- Immunopathological variables: Results of IIF normal human skin (NHS), IIF monkey esophagus (ME), IP anti DSG-1, IP anti DSG-3, IgG total anti DSG-1 and subclasses, IgM total anti DSG-1 and IgG total anti DSG-3.
- HROM: Angiogenesis in papillary dermis, capillary thickening, inflammatory infiltrate, subcorneal and suprabasal acantholysis.
- MET: Fibrillar disarray, intercellular edema and space widening, intracellular vacuoles, loss of desmosome structure, loss of normal distribution among tonofilaments, cytoplasmic inclusions, perinuclear halo and vacuolization, cytoplasmic vacuolization, and stratum corneum, stratum spinosum and stratum basale separation.

We also reviewed the microphotographs archives obtained at different magnifications with a Philips EM300 electron microscope operated by the Universidad Nacional de Ingeniería (Lima- Peru) [11].

The research was approved by the Research Ethics Committee of the Ricardo Palma University, School of Medicine (Expedited Review: PG-033-2023). The study did not require informed consent because it was based on secondary sources, the confidentiality of the information obtained was guaranteed and used only for this study's purposes.

3. Results

The age of the subjects was between 13 and 45 years old, two were females and one was male. The rest of the epidemiologic variables are shown in Table 1. With relation to the immunopathologic characteristics of the subjects, they all presented IgG anti DSG-1 antibodies, shown with either ELISA or IP, one of them (PL-2) was also positive for IgM anti DSG-1. In two subjects, (PL-1 and NR-1), IgG total anti DSG-3 antibodies were also detected by ELISA, and one of the subjects presents a value superior to 100 (Table 1).

The HROM was done with toluidine blue stain (magnification 1800×), did not show the presence of subcorneal or suprabasal acantholysis. Angiogenesis in papillary dermis, the thickening of capillaries, and the presence of inflammatory infiltrate were also observed (Table 2).

The most common ultrastructural anomalies observed under transmission electron microscopy (TEM) were the widening of intercellular space, additionally, the presence of intercellular edema, vacuoles with granular tissue and cytoplasmic vacuolization. Furthermore, the loss of desmosome structure, loss of normal distribution among tonofilaments and the lateral separation among cells in the stratum basale were also observed. Less frequently found were fibrillar disarray, the separation of the stratum corneum and the presence of apoptotic cells (Table 2, Fig. 1a and b).

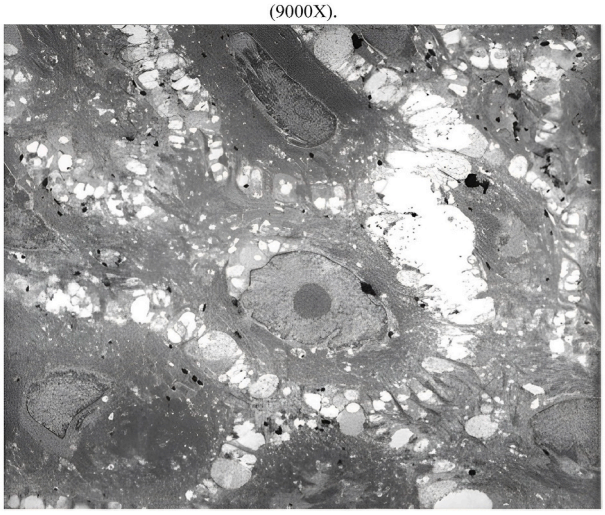
Table 1
Immunologic and epidemiologic characteristics among healthy subjects with DSG-1 antibodies from the pemphigus foliaceus and pemphigus vulgaris endemic areas of Peru.

CHARACTERISTICS	SUBJECTS		
	PL-1	NR-1	PL-2
Epidemiologic Characteristics			
Age (years)	45	13	24
Sex	Male	Female	Female
Origin	Pueblo libre	Nueva Requena	Pueblo libre
Occupation	Farmer	Student	Housewife
Water Source	Well	River/Well	Well
Housing Material	Wood, Straw	Wood, Leaves	Wood, Calamine
Housing Location	Rural Community	River bank	Rural Community
Inmunologic characteristics			
IIF NHS	Negative	Negative	Negative
IIF ME	Negative	Negative	Negative
IP anti DSG-1	1/2w+	Negative	1/2w+
IP anti DSG-3	Negative	Negative	Negative
IgG total anti DSG-1	39.02	50.26	30.06
IgG1 anti DSG-1	39.67	25.72	1.63
IgG2 anti DSG-1	24.44	40.97	18.47
IgG3 anti DSG-1	4.15	4.58	2.17
IgG4 anti DSG-1	−19.68	−1.26	−22.88
IgM total anti DSG-1	12.79	32.77	64.61
IgG total anti DSG-3	75.09	128.26	Negative

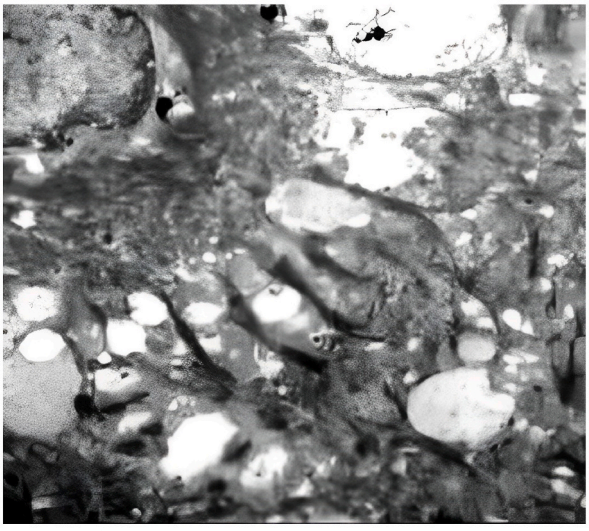
Table 2
Ultrastructural characteristics under microscopy among healthy subjects with DSG-1 antibodies from the pemphigus foliaceus and pemphigus vulgaris endemic areas of Peru.

ULTRASTRUCTURAL CHARACTERISTICS	SUBJECTS		
	PL-1	NR-1	PL-2
HROM			
Angiogenesis in papillary dermis	Present	Absent	Present
Capillary thickening	Present	Absent	Present
Inflammatory Infiltrate	Present	Absent	Present
Subcorneal acantholysis	Absent	Absent	Absent
Suprabasal acantholysis	Absent	Absent	Absent
Tem			
Fibrillar disarray	Absent	Present	Absent
Intercellular space widening	Present	Present	Present
Intercellular Edema	Present	Present	Present
Intracellular vacuoles with granular tissue	Present	Present	Present
Loss of desmosome structure	Present	Present	Absent
Cytoplasmic inclusions	Absent	Absent	Absent
Perinuclear halo	Absent	Absent	Absent
Perinuclear vacuolization	Absent	Present	Absent
Cytoplasmic vacuolization	Present	Present	Present
Loss of normal distribution among tonofilaments	Present	Present	Absent
Irregular nuclear contours	Absent	Absent	Absent
Increased heterochromatin	Absent	Absent	Absent
Presence of apoptotic cells	Present	Absent	Absent
Stratum corneum separation	Present	Absent	Absent
Stratum spinosum separation	Absent	Absent	Absent
Lateral separation among cells in the stratum basale	Present	Present	Absent
Separation beneath the stratum basale	Absent	Absent	Absent

A) Loss of normal distribution among tonofilaments and significant intercellular edema (9000X).



B) Loss of desmosome structure and bundles of tonofilaments (18 900X).



C) Fibrillar disarray of stratum spinosum cells (18 900X).

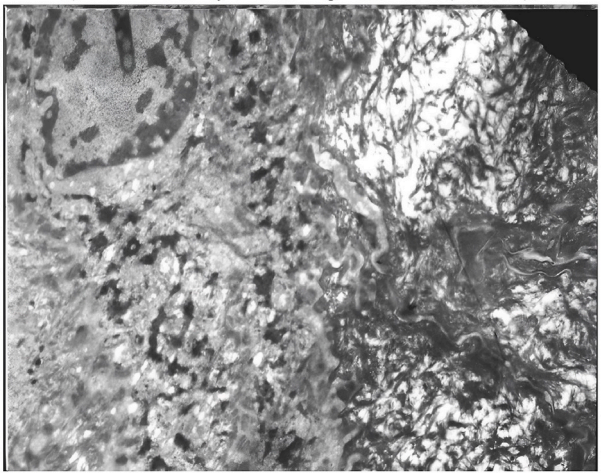


Fig. 1. Ultrastructural characteristics among healthy subjects with DSG-1
A) Loss of normal distribution among tonofilaments and significant intercellular edema (9000X).

4. Discussion

This case series finds that the three healthy subjects, residents of endemic zones for pemphigus foliaceus in Peru, presented ultrastructural skin alterations, possibly as a consequence of the presence of anti DSG-1 and anti DSG-3 antibodies (present in two of the three subjects evaluated). Frequent and relevant findings in the pathogenesis of endemic pemphigus were the intercellular space widening between keratinocytes, intercellular edema, loss of normal desmosome and tonofilament structure, as well as the lateral separation of the cells in the stratum basale.

A controversy exists in the role that the other subclasses of anti DSG-1, not IgG4, antibodies could play in the pathogenesis of endemic pemphigus foliaceus. While it is true that IgG4 anti DSG-1 has shown to reproduce the disease in murine models and the appearance of IgG4 in healthy subjects constitutes a strong predictor of clinical disease, this does not necessarily indicate that other subclasses do not have some role in blistering and induction of acantholysis, especially during the steps from being a healthy subject positive for anti DSG-1 up to the outbreak of endemic pemphigus foliaceus [12,13].

Our results showed the possibility that the IgG1 and IgG2 subclasses have certain pathogenic capacity to induce ultrastructural changes, despite not being enough for the development of lesions with clinical relevance [14]. According to our results, healthy subjects that present anti DSG-1 antibodies can develop ultrastructural alterations that are visible through TEM but not through HROM. Our results confirm the outcomes of *Yoshida* et al. who found that the injection of non-pathogenic IgG anti DSG-1 antibodies would attach in a linear manner to the keratinocytes cultivated from normal human skin but would not produce acantholysis [15].

The fact that we observe alterations such as the lateral separation of cells in the stratum basale, as well as the amplification of intercellular space widening and disarray of desmosomes and tonofilaments in deeper layers of the epidermis could be an indication that the effects of anti DSG-3 antibodies are in a similar way to that observed with anti DSG-1 antibodies which exerts its effects on the most superficial layers of the epidermis.

According to our results, it is recommended that the residents of areas endemic to pemphigus foliaceus and vulgaris who present non-pathogenic DSG-1 antibodies move to areas not endemic to these diseases since the anti DSG-1 antibodies are affecting the patients' skin, as seen at an ultrastructural level, and could contribute to a possible later onset of endemic pemphigus foliaceus [8].

A limitation of this study is the number of participating patients, however, we are not intending to make a statistical inference but rather show certain characteristics of patients' skin, without making population generalizations, for which the study meets its objective.

5. Conclusion

According to our results, healthy subjects that present anti DSG-1 antibodies can develop ultrastructural alterations that are visible through transmission electron microscopy but not through conventional optical microscopy.

Funding statement

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

IRB approval status

This study involves human participants and was approved by Institutional Committee on Research Ethics of the Ricardo Palma University, School of Medicine (Expedited Review: PG-033-2023).

Data sharing

All the data used in this review have been presented in the main text as either tables or figures. Additional requests for the data can be made electronically.

Contributors

WR, NR, and ELG were responsible for conceptualization of the study. WR, AOL, MT, JDCV, and JR were responsible for formal data analysis. NCC, VVP, NG, and GJ were responsible for methodology and project administration. WR was responsible for obtaining ethics approval. WR, MT, and NCC were responsible for writing the first draft of the report. WR, EG, AOL, and JDCV were responsible for verification of all the underlying data. The corresponding authors had the final responsibility of submission for publication.

Declaration of competing interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data availability

Data will be made available on request.

References

[1] V. Aoki, E.A. Rivitti, L.A. Diaz, Update on fogo selvagem, an endemic form of pemphigus foliaceus, J. Dermatol. 42 (1) (2015) 18–26.
[2] D.P. Pavoni, V.M. Roxo, A. Marquart Filho, M.L. Petzl-Erler, Dissecting the associations of endemic pemphigus foliaceus (Fogo Selvagem) with HLA-DRB1 alleles and genotypes, Gene Immun. 4 (2) (2003) 110–116.
[3] G. Hans-Filho, V. Aoki, N.R.H. Bittner, G.C. Bittner, Fogo selvagem: endemic pemphigus foliaceus, An. Bras. Dermatol. 93 (5) (2018 Sep-Oct) 638–650.
[4] A.G. Ortega-Loayza, W. Ramos, E.L. Gutierrez, G. Jimenez, I. Rojas, C. Galarza, Endemic pemphigus foliaceus in the peruvian Amazonia, Clin. Exp. Dermatol. 38 (6) (2013) 594–600.
[5] W. Ramos, G.R. Chacon, C. Galarza, E.L. Gutierrez, M.E. Smith, A.G. Ortega-Loayza, Endemic pemphigus in the Peruvian Amazon: epidemiology and risk factors for the development of complications during treatment, An. Bras. Dermatol. 87 (6) (2012) 838–845.
[6] N. Li, V. Aoki, Z. Liu, P. Prisayanh, J.G. Valenzuela, L.A. Diaz, From insect bites to a skin autoimmune disease: a conceivable pathway to endemic pemphigus foliaceus, Front. Immunol. 13 (2022), 907424.
[7] A. Ortega-Loayza, W. Ramos, G. Elgart, P. Bouman, G. Jiménez, J. Ávila, et al., Antibodies against desmoglein 1 in healtly subjects in endemic and nonendemic areas of pemphigus foliaceus (fogo selvagem) in Peru, Int. J. Dermatol. 45 (2006) 538–542.
[8] W. Ramos, C. Galarza, E.L. Gutierrez, G. Jiménez, I. Rojas, J. Hancco, et al., Epidemiological and immunopathologic characteristics in a cohort of healthy subjects for desmoglein 1 autoantibodies from endemic areas for endemic pemphigus foliaceus and vulgaris of Peru, Dermatol. Peru. 19 (1) (2009) 12–21.
[9] W. Ramos, J. Díaz, E.L. Gutierrez, J.S. Lazarte, M.C. Bohnett, G. Ronceros, et al., Antidesmoglein 1 and 3 antibodies in healthy subjects of a population in the Peruvian high amazon, Int. J. Dermatol. 57 (2018) 344–348.
[10] E.L. Gutierrez, W. Ramos, L. Seminario-Vidal, M. Tello, G. Ronceros, A.G. Ortega-Loayza, Oxidative stress in patients with endemic pemphigus foliaceus and healthy subjects with anti-desmoglein 1 antibodies, An. Bras. Dermatol. 93 (2018) 212–215.
[11] C. Galarza, W. Ramos, N. Rojas, E.L. Gutierrez, E.L. Neira, G. Ronceros, et al., Ultrastructural aspects of patients's skin with endemic pemphigus foliaceus and endemic pemphigus vulgaris from the peruvian amazonia. Preliminary report, Dermatol. Peru. 20 (2010) 228–235.
[12] C. Sitaru, S. Mihai, D. Zillikens, The relevance of the IgG subclass of autoantibodies for blister induction in autoimmune bullous skin diseases, Arch. Dermatol. Res. 299 (1) (2007) 1–8.
[13] M.K. Hacker-Foegen, M. Janson, M. Amagai, J.A. Fairley, M.S. Lin, Pathogenicity and epitope characteristics of antidesmoglein-1 from pemphigus foliaceus patients expressing only IgG1 autoantibodies, J. Invest. Dermatol. 121 (2003) 1373–1378, 2003.
[14] G. Van der Wier, M.F. Jonkman, H.H. Pas, G.F. Diercks, Ultrastructure of acantholysis in pemphigus foliaceus re-examined from the current perspective, Br. J. Dermatol. 167 (2012) 1265–1271.
[15] K. Yoshida, K. Ishii, A. Shimizu, M. Yokouchi, M. Amagai, K. Shiraishi, et al., Non-pathogenic pemphigus foliaceus (PF) IgG acts synergistically with a directly pathogenic PF IgG to increase blistering by p38MAPK-dependent desmoglein 1 clustering, J. Dermatol. Sci. 85 (2017) 197–207.