Journal of Dermatological Science 65 (2012) 149–158

Contents lists available at SciVerse ScienceDirect

Journal of Dermatological Science

journal homepage: www.elsevier.com/jds

Letter to the Editor

Novel and recurrent *COL7A1* mutations in Chilean patients with dystrophic epidermolysis bullosa

Dystrophic epidermolysis bullosa (DEB) is a genodermatosis characterized by trauma-induced blister formation beneath the lamina densa and healing with scarring. In addition, patients often have nail dystrophy, pseudosyndactyly, corneal erosions, oesophageal strictures, anaemia and excessive caries [1].

Dystrophic EB is inherited either as an autosomal dominant (DDEB) or recessive (RDEB) trait. Both forms are caused by mutations in *COL7A1*, the gene encoding type VII collagen [2] which is the major component of anchoring fibrils. Patients with DEB may have absent or morphologically altered and functionally defective anchoring fibrils [3]. To date, more than 500 different disease-causing sequence variations, distributed over the entire *COL7A1* gene, are reported in the Human Gene Mutation Database (HGMD). Many of these have been found in large population studies enabling the development of *COL7A1* mutation detection strategies, to assist in confirming the diagnosis, predicting prognosis of new patients, as well as to permitting genetic counseling and prenatal testing for families at risk [4].

In Chile, the overall incidence of EB is 19.6 new cases per million live births (DebRA-Chile, unpublished data). In 2008 a total of sixty four patients with DEB had been recorded; however no studies regarding the nature of the *COL7A1* mutations in this population had been undertaken. Here, we present mutational analysis in the first South American series of patients with DEB, identifying two highly recurrent *COL7A1* mutations among a total of 12 novel mutations.

Approval was obtained from the Bioethical Committee of Facultad de Medicina Clínica Alemana-Universidad del Desarrollo as well as informed consent of 35 patients to undertake DNA analysis as previously described [5]. The study demonstrated 22 different changes in the normal sequence of the *COL7A1* gene (Table 1). Nine had been reported previously, whereas 13 were novel according to the HGMD.

To (i) confirm reported changes, (ii) rule out these alterations as normal polymorphic variants in the Chilean population, (iii) enlarge the number of studied patients and (iv) determine the pattern of inheritance, blood sample from 60 patients (35 who had initially enrolled plus 25 who enrolled later), 50 healthy control and 69 patient relatives, previous informed consent sign, were analyzed by restriction-endonuclease digestion of amplified genomic DNA or PCR amplification of specific allele assay (PASA). All reported sequence variations were confirmed with the screening assays indicated in Table 1 (data not shown). Substitution c.7313C>G was detected in alleles from healthy controls (9/100) suggesting it is a polymorphism. Screening of reported alterations in the remaining patients (25) showed 48 mutant alleles. Finally, analysis of patients' families showed 19 recessive and 2 dominant mutations. The two dominant mutations (c.5081G>A and c.6127G>T, italic in Table 1) were not detected in parents with non-paternity excluded, and could represent *de novo* mutations or parental germline mosaicism. Patient EBCh125 had both substitutions c.5081G>A and c.7313C>G. Since c.7313C>G is probably a polymorphism, dominance for c.5081G>A is suggested, a recessive effect of this mutation cannot be completely ruled out though.

Population frequency analysis showed that 87% (52/60) of enrolled patients with DEB carried c.6527dupC or c.7708delG mutation. While the former was previously described [6], the latter is a novel *COL7A1* mutation. Allele frequencies of recurrent mutations were 42% (50/120) for c.6527dupC and 28% (34/120) for c.7708delG. It was noteworthy that 51% of c.6527dupC mutation carriers were concentrated in the southern region of Chile, which represents 38% of the country (2002 census, INE[1]).

The presence of c.6527dupC in both alleles resulted in RDEB-severe generalized (RDEB-sev gen) phenotype in 13 out of 15 patients. Histological and clinical features observed in these patients concurred with those described by Escámez et al. [6] in Spanish patients homozygous for c.6527dupC.

Homozygosity for c.7708delG was also associated with a RDEB-sev gen phenotype in 10 of 11 patients. Absence of type VII collagen immunoreactivity or marked reduction of anchoring fibrils (Fig. 1a and b) was also observed. In all homozygous patients, disease onset was from birth, with lesions progressing to a generalized distribution, and other features associated with this subtype of EB (Fig. 1c).

Recurrence of mutations may be due to propagation of an ancestral allele or to different mutational events. In order to establish the cause of the recurrence of c.6527dupC and c.7708delG we performed haplotype analysis for 16 and 11 carrier families, respectively. One intragenic single nucleotide polymorphism (c.2820G/A) and three informative microsatellite markers (D3S1581, D3S1289 and D3S1029) in linkage with the *COL7A1* locus were studied [7,8]. Results showed that c.6527dupC segregated with a single haplotype, demonstrating a common ancestral origin. A founder effect was also demonstrated for c.6527dupC in Spain [9], although since haplotype markers used in both studies were different, we cannot conclude that the ancestral progenitor of the Chilean mutation came from Spain. Nevertheless, we might suggest this because most of the colonizers that arrived in Chile (1601–1810) were native of Andalusia and Extremadura, Spanish regions where c.657dupC is very common [6]. In addition, nearly 11,000 Spanish immigrants arrived during the period 1883 to 1901,[2] settling in the southern part of Chile where a high incidence of c.6527dupC mutation was detected.

Analysis of the c.7708delG showed association with two haplotypes that differ only for the D3S1029 marker (Fig. 1d). The latter is 10 cM telomeric, far from the *COL7A1* locus, meanwhile markers D3S1581 and D3S1289 are within 2 cM of

[1] National Institute of Statistics (Chile).
[2] Emilio Held Winkler Archive; German – Chilean Association (Chile).

Table 1
COL7A1 mutations elucidated in Chilean patients with DEB.

Changes in normal sequence	Localization		Predicted consequence	Screening assay	Freq.	Reference
	Ex/Int	PD				
Duplication						
c.6527dupC	Ex 80	THC	PTC (p.2289)	BglI	42% (35)	[11]
Insertion						
c.325_326insCG	Ex 3	CMP	PTC (p.147)	Cac8I	3% (1)	[11]
Deletion						
c.3970delC	Ex 32	THC	p.L1324X	PASA	2% (1)	–
c.4317delC	Ex 39	THC	PTC (p.1709)	PASA	2% (1)	[12]
c.7708delG	Ex 103	THC	PTC (p.2630)	RsaI	28% (23)	–
Substitution			Nonsense			
c.2005C>T	Ex 15	Fn3	p.R669X	HpyCH4III	3% (2)	[13]
c.5932C>T	Ex 72	THC	p.R1978X	TaqI	3% (1)	[12]
c.7234C>T	Ex 94	THC	p.R2412X	XhoI	2% (1)	–
c.8329C>T	Ex 112	THC	p.R2777X	XhoI	2% (1)	[14]
			Missense			
c.5081G>A	Ex 55	THC	p.G1694V	BstEII	2% (1)	–
c.5344G>A	Ex 61	THC	p.G1782R	BsrBI	2% (1)	[15]
c.6127G>T	Ex 73	THC	p.G2043W	SmaI	2% (1)	[16]
c.7313C>G*	Ex 95	THC	p.P2438R	BsaHI	2% (1)	–
c.8245G>A	Ex 111	THC	p.G2749R	MspI	3% (2)	[15]
c.8393 T>G	Ex 113	Acidic	p.M2798R	BseRI	2% (1)	–
			Splice site			
c.2992+2T>G	Int 22	Fn3	(a) Novel CDS 77 bp US → OF PTC in p.989 (b) Skip Ex 22 → IF	HphI	2% (1)	–
c.3760+2T>G	Int 28	C/P	(a) Novel CDS 19 bp US → IF[a] (b) Skip Ex 28 → IF	MseI	7% (2)	–
c.4342-2A>G	Int 39	THC	(a) Novel CAS 34 bp DS → OF PTC in p.1698 (b) Skip Ex 40 → IF	AcuI	3% (2)	–
c.5532+1G>T	Int 64	THC	(a) Novel CDS 714 bp DS → OF PTC in p.1872 (b) Novel CDS 1274 bp DS → OF PTC in p.1872 (c) Skip Ex 64 → IF	HpyCH4IV	5% (3)	–
c.5857+1G>T	Int 71	THC	(a) Novel CDS 20 bp US → OF PTC in p.1972 (b) Skip Ex 71 → IF	MslI	2% (1)	–
c.7876-1G>A	Int 105	THC	(a) Novel CAS 1 bp DS → OF PTC in p.2630 (b) Novel CAS 160 bp US → OF PTC in p.2640 (c) Skip Ex 106 → IF	SmlI	2% (1)	–
c.8528-1G>A	Int 115	Acidic	(a) Skip Ex 116 → IF	BsrBI	3% (2)	–

Changes in normal sequence: Nucleotides are numbered according to *COL7A1* cDNA sequence at accession NM_000094.2 (GenBank). In bold sequence variations previously unreported. *Possible polymorphism. In italic autosomal dominant mutations. *Localization*: Ex: Exon. Int: Intron. PD: protein domain. THC: triple helical collagenous domain. CMP: cartilage matrix protein. Fn3: fibronectin III-like domains. C/P: cysteine and proline rich region. *Predicted consequence*: Amino acids are numbered according to protein sequence at accession NP_000085 (GenBank). PTC: premature termination codon (position in parenthesis). Splice site changes have been analyzed with the Splice-Site Prediction by Neural Network software available at http://www.fruitfly.org/seq_tools/splice.html. CDS: cryptic donor splice site. CAS: cryptic acceptor splice site. bp: base pair. DS: downstream from canonical splice site. US: upstream from canonical splice site. IF: in-frame translation. OF: out-of-frame translation.

[a] Insertion of heptapeptide (GRVPHRE). *Screening assay*: PASA: PCR amplification of specific alleles. *Frequency*: 100% correspond to 120 alleles from 60 DEB patients; number of carrier patients in parenthesis.

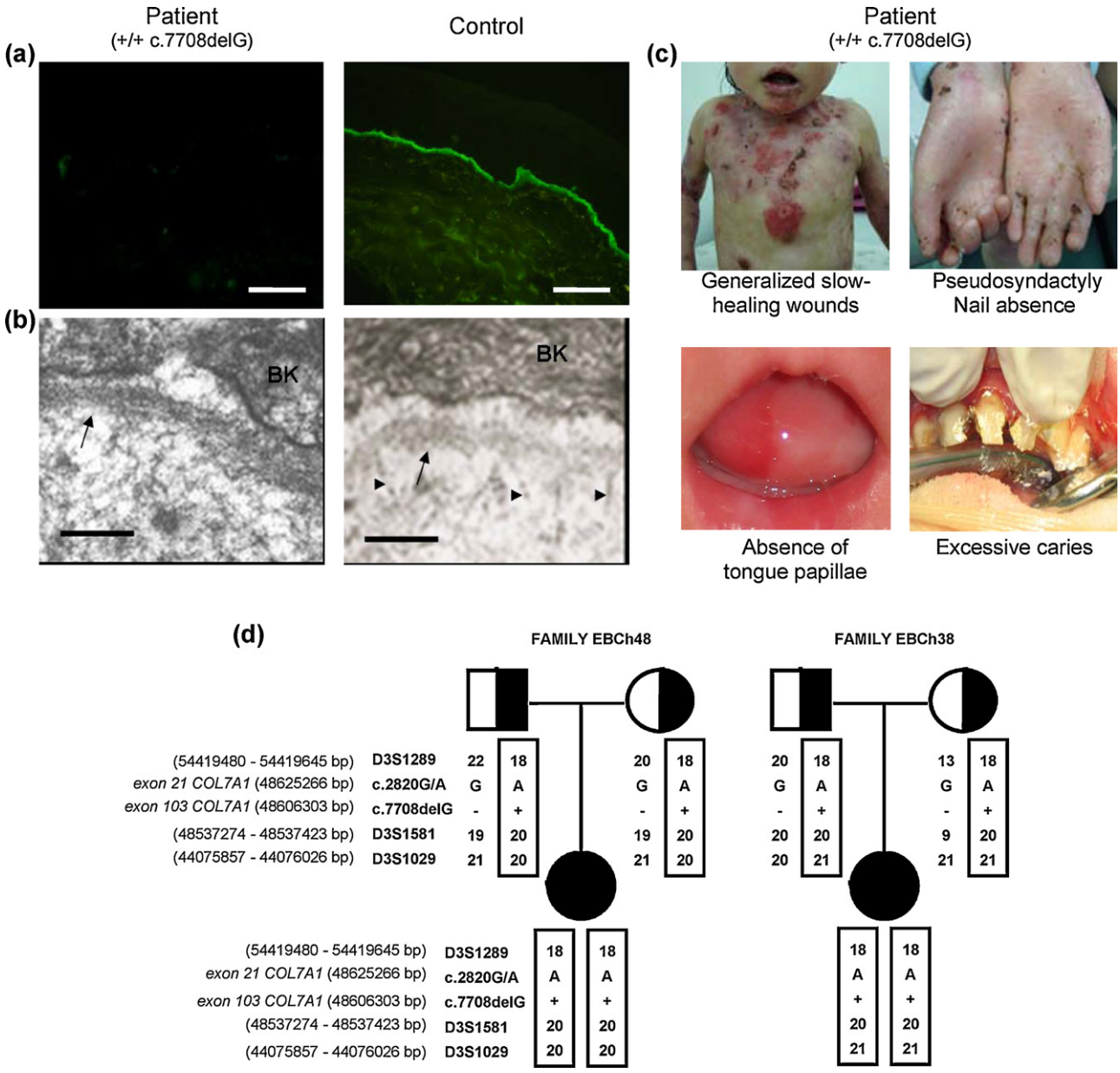

Fig. 1. Characterization of RDEB-sev gen patients homozygous for mutation c.7708delG. (a) Immunofluorescence staining with monoclonal anti-collagen VII antibody (LH7.2, Novocastra) showed complete absence of type VII collagen in patient skin but bright linear fluorescence along the dermal–epidermal junction in control skin. Scale bar = 200 μm. (b) Transmission electron microphotograph of patient skin showing basal keratinocytes (BK), lamina densa (arrow) but absent anchoring fibrils. In control skin, anchoring fibrils are indicated by arrowheads. Scale bar = 400 nm. (c) Clinical features. (d) Haplotypes were built with the Family-Based Association Tests software. Haplotypes that segregate (boxes) with c.7708delG (+) in Chilean patients with DEB are shown in two representative pedigrees (patients EBCh48 and 38). The alleles at the microsatellite markers are numbered according to the amount of repetitive sequence. Markers are organized from centromere (up) to telomere (down), with its chromosomal position according to *Homo sapiens* GRCh37.p5 Primary Assembly (July 2011) indicated at left. Symbols are encoded as follows: black, DEB patients; white and black, carrying progenitors.

the gene, telomeric and centromeric, respectively. It suggests a possible recombination episode between the D3S1581 and D3S1029 markers in a common ancestral allele and segregation of two haplotypes [10].

In summary, characterization of *COL7A1* mutations in Chilean patients with DEB allowed (i) the detection of 12 novel mutations, half of which were substitutions affecting splice sites; (ii) the identification of two recurrent mutations: c.6527dupC and c.7708delG, that together comprise 70% of mutated alleles in Chilean patients with DEB, resulting in RDEB-sev gen phenotype in the homozygous state. In addition, for the first time in our country a rapid and inexpensive molecular-based screening strategy is available; useful for confirming DEB diagnosis, to perform prenatal testing and to support genetic counseling for Chilean families at risk.

Acknowledgments

We are grateful to the patients and their families for helping us to carry out this study and compile this report. This work was supported by a grant from *Fondo Nacional de Desarrollo Científico y Tecnológico-Chile* for the Initiation Research Project No. 11085036 (to F.R.). JAM and JEM acknowledge financial support from the Department of Health *via* the UK National Institute for Health Research (NIHR) comprehensive Biomedical Research Centre award to Guy's and St Thomas' NHS Foundation Trust in

partnership with King's College London and King's College Hospital NHS Foundation Trust.

References

[1] Fine JD, Bauer EA, Gedde-Dahl Jr T. In: Fine JD, Bauer EA, McGuire J, Moshell A, editors. Inherited epidermolysis bullosa: definition and historical overview. In the epidermolysis bullosa: clinical, epidemiologic, and laboratory advances and the findings of the National Epidermolysis Bullosa Registry. Baltimore: The Johns Hopkins University Press; 1999. p. 1–19.
[2] Hovnanian A, Duquesnoy P, Blanchet-Bardon C, Knowlton RG, Amselem S, Lathrop M, et al. Genetic linkage of recessive dystrophic epidermolysis bullosa to the type VII collagen gene. J Clin Invest 1992;90:1032–6.
[3] Bruckner-Tuderman L, Hopfner B, Hammami-Hauasli N. Biology of anchoring fibrils: lessons from dystrophic epidermolysis bullosa. Matrix Biol 1999;18:43–54.
[4] Dang N, Murrell DF. Mutation analysis and characterization of *COL7A1* mutations in dystrophic epidermolysis bullosa. Exp Dermatol 2008;17:553–68.
[5] Christiano AM, Hoffman GG, Zhang X, Xu Y, Tamai Y, Greenspan DS, et al. Strategy for identification of sequence variants in *COL7A1* and a novel 2-bp deletion mutation in recessive dystrophic epidermolysis bullosa. Hum Mutat 1997;10:408–14.
[6] Escámez MJ, García M, Cuadrado-Corrales N, Llames SG, Charlesworth A, De Luca N, et al. The first *COL7A1* mutation survey in a large Spanish dystrophic epidermolysis bullosa cohort: c.6527insC disclosed as an unusually recurrent mutation. Br J Dermatol 2010;163:155–61.
[7] Christiano AM, LaForgia S, Paller AS, McGuire J, Shimizu H, Uitto J. Prenatal diagnosis for recessive dystrophic epidermolysis bullosa in 10 families by mutation and haplotype analysis in the type VII collagen gene (COL7A1). Mol Med 1996;2:59–76.
[8] Fassihi H, Renwick PJ, Black C, McGrath JA. Single cell PCR amplification of microsatellites flanking the COL7A1 gene and suitability for preimplantation genetic diagnosis of Hallopeau—Siemens recessive dystrophic epidermolysis bullosa. J Dermatol Sci 2006;42:241–8.
[9] Cuadrado-Corrales N, Sánchez-Jimeno C, García M, Escámez MJ, Illera N, Hernández-Martín A, et al. A prevalent mutation with founder effect in Spanish recessive dystrophic epidermolysis bullosa families. BMC Med Genet 2010;11:139.
[10] Mellerio JE, Dunnill MG, Allison W, Ashton GH, Christiano AM, Uitto J, et al. Recurrent mutations in the type VII collagen gene (COL7A1) in patients with recessive dystrophic epidermolysis bullosa. J Invest Dermatol 1997;109:246–9.
[11] Hovnanian A, Rochat A, Bodemer C, Petit E, Rivers CA, Prost C, et al. Characterization of 18 new mutations in *COL7A1* in Recessive Dystrophic Epidermolysis Bullosa provides evidence for distinct molecular mechanisms underlying defective anchoring fibril formation. Am J Hum Genet 1997;61:599–610.
[12] Whittock NV, Ashton GH, Mohammedi R, Mellerio JE, Mathew CG, Abbs SJ, et al. Comparative mutation detection screening of the type VII collagen gene *(COL7A1)* using the protein truncation test, fluorescent chemical cleavage of mismatch, and conformation sensitive gel electrophoresis. J Invest Dermatol 1999;113:673–86.
[13] Cserhalmi-Friedman PB, McGrath JA, Mellerio JE, Romero R, Salas-Alanis JC, Paller AS, et al. Restoration of open reading frame resulting from skipping of an exon with an internal deletion in the *COL7A1* gene. Lab Invest 1998;78:1483–92.
[14] Kern JS, Grüninger G, Imsak R, Müller ML, Schumann H, Kiritsi D, et al. Forty-two novel *COL7A1* mutations and the role of a frequent single nucleotide polymorphism in the MMP1 promoter in modulation of disease severity in a large European dystrophic epidermolysis bullosa cohort. Br J Dermatol 2009;161:1089–97.
[15] Christiano AM, McGrath JA, Tan KC, Uitto J. Glycine substitutions in the triple-helical region of type VII collagen result in a spectrum of dystrophic epidermolysis bullosa phenotypes and patterns of inheritance. Am J Hum Genet 1996;58:671–81.
[16] Mecklenbeck S, Hammami-Hauasli N, Höpfner B, Schumann H, Kramer A, Küster W, et al. Clustering of *COL7A1* mutations in exon 73: implications for mutation analysis in Dystrophic Epidermolysis Bullosa. J Invest Dermatol 1999;112:398–400.

Fernando A. Rodríguez[a,b,*], María José Gana[a], María Joao Yubero[a], Gisela Zillmann[a,c], Susanne M. Krämer[a,c], Javiera Catalán[a], Julia Rubio-Astudillo[b], Sergio González[d], Lu Liu[e], Linda Ozoemena[e], Jemima M. Mellerio[e], John A. McGrath[e], Francis Palisson[a,b], Paulette Conget[a,b]

[a]*Dystrophic Epidermolysis Bullosa Research Association-Chile (DebRA-Chile), Santiago, Chile*;
[b]*Instituto de Ciencias, Facultad de Medicina, Clínica Alemana – Universidad del Desarrollo, Santiago, Chile*;
[c]*Facultad de Odontología, Universidad de Chile, Santiago, Chile*;
[d]*Departamento de Patología, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile*;
[e]*St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, St Thomas' Hospital, London, UK*

*Corresponding author at: Instituto de Investigación Materno Infantil (IDIMI), Hospital Clínico San Borja-Arriaran, Avenida Santa Rosa 1234 Santiago, Chile. Tel.: +56 2 9770855
E-mail addresses: frodriguezr@med.uchile.cl (F.A. Rodríguez)
pconget@udd.cl (P. Conget)
faro_78@hotmail.com (F.A. Rodríguez)

1 November 2010

doi:10.1016/j.jdermsci.2011.11.010

Letter to the Editor

Histone deacetylase activity is required for skin Langerhans cell maturation and phagocytosis

Keywords:
Langerhans cells; Phagocytosis;
Maturation; Histone deacetylases

To the Editor

Histone acetylation plays key roles in modulating chromatin structure and controlling the gene expression. It is widely accepted that densely packed DNA structure is related to histone acetylation status. Histone acetylation is a dynamic process controlled by the antagonistic actions of two classes of enzymes – the histone acetyltransferases (HATs) and the histone deacetylases (HDACs), which function to add acetyl groups or removed them from target histones, respectively [1,2]. The balance between the actions of HATs and HDACs represents a key epigenetic regulatory mechanism in the gene expression regulations, which controls numerous developmental processes, biological pathways and disease states [1,2]. Inhibition of HDACs has been shown to modulate gene transcription, induce growth arrest and apoptosis, or differentiation in the cancer cells. Several recent studies have indicated that HDAC inhibitors also have anti-inflammatory effects in the mouse models of different immune disorders, including atopic dermatitis, suggesting that HDAC inhibitors have immunosuppressive properties [3,4]. However, the underlying mechanisms for HDAC inhibitors-mediated immune regulation remain poorly understood.

Langerhans cells (LCs) are skin-resident dendritic cells (DCs) with a life cycle distinct from other types of DCs, and have long been considered to be prototypic sentinel DCs due to their prominent position in environmental barrier function [5,6]. LCs have the ability to transport peripherally acquired antigens to