Development of anti-type VII collagen ELISA for the diagnosis and monitoring the disease activity of epidermolysis bullosa acquisita patients

Shunsuke Kurei[1], Ritsuko Kurei[1], Akihiro Murakami[1], Marwah Adly Saleh[2,3], Ken Ishii[2], and Masayuki Amagai[2]

[1]Medical and Biological Laboratories Co. Ltd., Nagoya, Japan
[2]Department of Dermatology, Keio University School of Medicine, Tokyo, Japan
[3]Department of Dermatology, Cairo University School of Medicine, Cairo, Egypt

Introduction

Epidermolysis bullosa acquisita (EBA) is an autoimmune blistering skin disease characterized by the presence of IgG against type VII collagen. Type VII collagen is a major component of anchoring fibrils that are attachment structures within the basement membrane between epidermis and dermis. Type VII collagen consists of a central **collagenus domain (COL)** flanked by a 145-kDa amino-terminal **non-collagenous domain 1 (NC1)** and a 34-kDa carboxyl-terminal **non-collagenous domain 2 (NC2) (Fig. 1)**. Major epitopes for anti-type VII collagen autoantibodies reside within NC1. Mei Chen *et al.* reported that minor epitopes reside within NC2 (1). The aim of this study is to develop an ELISA using recombinant NC1 and NC2 and to evaluate its performance in the diagnosis of EBA and monitoring disease activity.

Methods

Recombinant NC1 and NC2 were expressed in CHO and *E. coli* cells, respectively, and purified by immobilized metal ion affinity chromatography. We also tried to express NC2 in mammalian cells but the expression level was very poor.

We constructed three ELISA systems (NC1 ELISA, NC2 ELISA, NC1+NC2 ELISA) by using purified NC1 and NC2. They were tested with 49 EBA sera, 30 **pemphigus vulgaris (PV)** sera, 30 **pemphigus foliaceus (PF)**, 60 **bullous pemphigoid (BP)** sera, and 265 normal control sera. A cut off value was determined as a value of 10SD above the mean of the normal sera. The Index value of NC1+NC2 ELISA was calculated with the following equation: (sample - negative control)/(positive control - negative control) x 100.

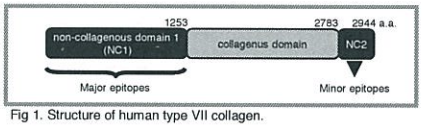

Fig 1. Structure of human type VII collagen.

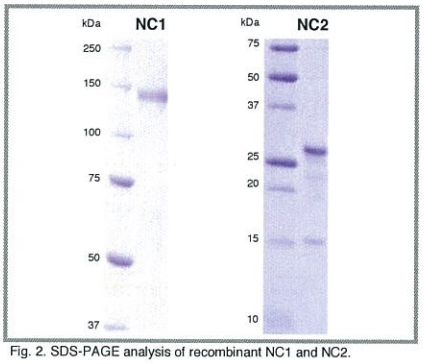

Fig. 2. SDS-PAGE analysis of recombinant NC1 and NC2.

ELISA	Patient (n=49)	Sensitivity
NC1	45	91.8%
NC2	10	20.4%
NC1 + NC2	46	93.9%

Table 1. Comparison of the sensitivity of three ELISA systems.

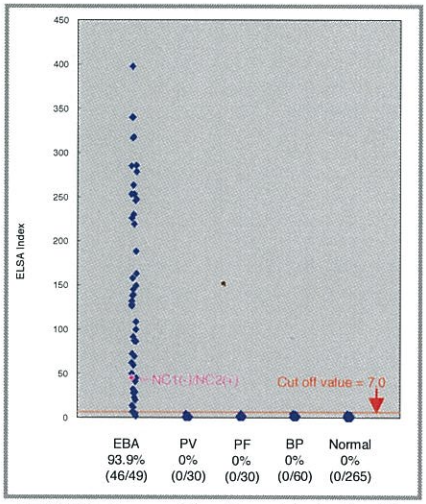

Fig. 3. Scatter plot representation of the reactivity of anti-type VII collagen ELISA (NC1+NC2) with sera from EBA, PV, PF, BP patients and normal controls.

Results

1) Yields of purified recombinant NC1 and NC2 were ~5.3 mg and ~0.35 mg, respectively, from 1.0 L culture. Their purity was estimated to be over 90% as determined by SDS-PAGE followed by Coomassie blue staining (Fig. 2).

2) The performance of three ELISA systems were clinically evaluated with EBA sera. The sensitivity of NC1 ELISA, NC2 ELISA, and NC1+NC2 ELISA was found to be 91.8%, 20.4% and 93.9%, respectively (Table 1). NC1+NC2 ELISA appeared to exhibit the best performance since it reacted only with EBA sera, not with sera from PV, PF, BP and normal controls (Fig. 3). Notably, it detected one NC1(-)/NC2(+) EBA serum.

3) We examined the correlation between the time course of the disease activity and the amount of anti-type VII collagen antibodies with NC1+NC2 ELSA in two EBA patients (Fig. 4). During therapy, the disease activity and autoantibody titer in patient A (a 25-years old female with extensive blisters and erosions all over her body) tended to decrease while those in patient B (a 49-years old female with oral elosions localized on her extremities) were fluctuated in parallel.

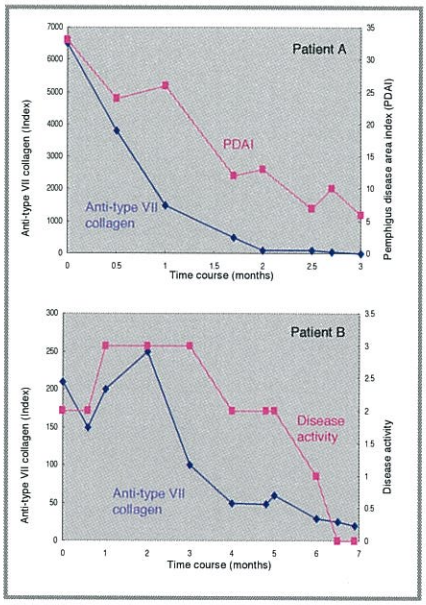

Fig. 4. Correlation between time course of disease activity and amounts of anti-type VII collagen antibodies in two EBA patients.

Conclusion

Here we developed the NC1+NC2 ELISA system which can specifically diagnose EBA patients with anti-type VII collagen autoantibodies. Although EBA and BP are similar in terms of clinical symptoms, those diseases are sometimes different in therapy. EBA cases often require high dose of systemic corticosteroids and a variety of immunosuppressants. Therefore, it is important to distinguish EBA from BP and our NC1+NC2 ELISA is more useful than NC1 ELISA in clinical practice.

"Anti-Type VII collagen ELISA Kit" is now commercially available from Medical & Biological Laboratories (MBL) and will be useful in the diagnosis of EBA patients (especially distinguishing EBA from BP) and in monitoring EBA disease activity.

Reference

1)Mei Chen *et al.* : The carboxyl terminus of type VII collagen mediates antiparallel dimer formation and constitutes a new antigenic epitope for epidermolysis bullosa acquisita autoantibodies.
J. Biol. Chem. 276: 21649-21655, 2001

MBL MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
http://www.mbl.co.jp/

5-3 Sakae 4 chome, Naka-ku, Nagoya, 460-0008, JAPAN
TEL : +81-52-238-1901 FAX : +81-52-238-1440
E-mail : IVD@mbl.co.jp