MOLECULAR STRUCTURE OF BULLOUS PEMPHIGOID ANTIGEN

Sakuhei Fujiwara, Hiroshi Shinkai.

Department of Dermatology, Medical College of Oita, Oita, Japan

Bullous pemphigoid (BP) is an autoimmune blistering disease characterized by the presence of circulating antibasement membrane zone (BMZ) antibodies (1). Biochemical studies of BP antigens are still controversial. Stanley et al. (2) immuno-precipitated a component from buffered saline extracts from cultured human epidermal cells that consisted of chain(s) with an approximate molecular weight of 220-240 kD. In contrast, Labib et al. (3) and Bernard et al. (4) showed that BP autoantibodies react with several protein bands, especially at 220-240 kD and 165-180 kD, suggesting a molecular heterogeneity of BP antigen. In the present study, two polypeptide chains with molecular weights of approximately 230 kD and 160 kD were electroeluted from human epidermal extracts and amino acid composition, peptide cleavage pattern and antigenic determinants were compared.

MATERIALS AND METHODS

We selected 26 BP patients by clinical criteria. Epidermal sheets were taken from normal human back or leg skin after heating at 56°C for one minute (3). They were homogenated and then extracted with Tris-HCL buffer pH 6.8 containing protease inhibitors, 4% SDS, and 0.2 % CHAPS for four hours at 4°C. The solubilized proteins were subjected to electrophoresis and immunoblotting and were further purified by electroelution. After electrophoresis, the proteins were transferred to the PVDF membrane. Both sides of the transferred membrane were cut and stained with amido black, and the protein bands corresponding to 230 kD or 160 kD antigen were cut, followed by hydrolysis for amino acid analysis. Both protein bands were electroeluted
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and cut after reelectrophoresis. They were iodinated and digested with
trypsin or endoproteinase Glu-C, and then subjected either to two
dimensional separation of the peptides or immunoblotting after SDS-PAGE
using Tris-Tricin buffer system (5).

RESULTS AND DISCUSSION

Six out of 26 sera recognized the 230kD band, and six bound to 160kD
alone. Seven of 26 sera reacted with both bands. Amino acid analysis of two
antigens demonstrated a similar composition, except for the amounts of
Thr, His and Arg. The peptide mapping patterns of 230kD and 160kD antigen
after digestion with either enzyme were similar, although minor spots were
different. After immunoblotting, both of the peptides which derived from
230kD and 160kD were cleaved with endo-proteinase Glu-C reacted with the
sera which recognize either 160kD alone or both antigens. These data show
that both antigens are closely related. The antigenic determinants, ranging
between 34kD and 21kD in molecular weight, are shared by both antigens,
although it is not yet clear that they have the same amino acid sequences
as each other. They are usually masked in 230kD antigen in genuine size.
The present study suggests the strong possibility that 230kD and 160kD
antigen are common gene products.

REFERENCES