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Some Biochemical Properties of Pemphigoid Antigen Bound to the Surface of Dissociated Epidermal Basal Cells

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Bullous pemphigoid antigen (BP Ag) is a cell surface marker of epidermal basal cells. The functional role of this molecule is unknown. Epidermal cell suspensions obtained by trypsinization of skin show a population of epidermal basal cells with a polar rim of antigen as demonstrated by indirect immunofluorescence technique. This study shows that treatment of these cells suspensions with a variety of proteolytic and glycosidic enzymes failed to remove the antigen from these basal cells. BP Ag was also stable upon incubation with distilled water, Triton X-100, PBS, and 1 M NaCl. Treatment of epidermal basal cells with 2 N NaSCN, 1% periodic acid, and 4 M urea, as well as acidic pH or 56°C temperature, abolished the reactivity of these cells with BP antibodies.

Bullous pemphigoid antigen (BP Ag) is a cell surface constituent which is found at the dermal-epidermal junction (DEJ) of normal squamous epithelia of mammals and other vertebrates [1]. This antigen has been isolated from human skin and the urine of a patient with BP [2,3]. BP autoantibodies were obtained from patients with BP and used to detect BP Ag *in situ* or in solution in both studies.

In vitro epidermal cell cultures are commonly carried out using trypsin-dissociated epidermal cells [4,5]. The epidermal cell suspensions obtained by this method show a population of cells which bear BP Ag on one of the cell poles [6,7]. Furthermore, only those epidermal cells which attach to a substrate and grow and differentiate *in vitro* contain BP Ag on their surface [8]. Therefore, BP Ag may be considered a surface marker of epidermal basal cells.

We have previously shown that BP autoantibodies impair the attachment of epidermal cells growing *in vitro* [7]. This study suggested that BP Ag may be involved in epidermal basal cell-to-substrate adherence. One way to test this hypothesis *in vitro*, using cell adhesion technology would be studying a BP Ag-free population of basal cells as compared with BP Ag-positive basal cells. In this report, we have investigated the effects of different enzymes, and chemical reagents on BP Ag bound to the surface of trypsin-dissociated epidermal basal cells. It is demonstrated that when bound to basal cells, BP Ag is extremely resistant to a variety of enzymatic treatments. Chemical reagents such as periodic acid, 4 M urea, or 2 N NaSCN made basal cells unreactive with BP autoantibodies, whereas 1 M NaCl, 0.5% Triton X-100, or distilled water did not inhibit this reaction. The biological relevance of this unusual resistance of cell-bound BP Ag remains to be determined.

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Abbreviations:
IF: immunofluorescence
PBS: phosphate buffered saline

MATERIALS AND METHODS

Cell Preparation

Rat tail epidermal cells were prepared by trypsinization of 1 cm squares of rat tail skin in 0.5% trypsin in PBS pH 7.2 at 37° for 1 hr. The epidermis was separated from the dermis and epidermal cells gently scraped into 0.025% DNAase I (Sigma) in phosphate buffered saline (PBS) containing 0.1 M MgSO4, pH 7.2. The suspension of epidermal cells were filtered through glass wool and washed 3 times in PBS—.01 M EDTA pH 7.2. The cell pellet was resuspended in PBS and adjusted to 1–2 × 10^6 cells/ml. Approximately 90% of these cells excluded Trypan blue soon after they were obtained. These cells stored in PBS at 4°C for several days are unable to exclude this dye.

Origin and Source of Enzymes Used

Trypsin (Type III), Chymotrypsin (Type 1–5), and Deoxyribonuclease I from Bovine pancreas; Papain (Type IV) from papaya latex; Hyaluronidase (Type I) from Bovine testis; Galactose Oxidase (Type V) from Dactylium dendroides were obtained from Sigma Chemical Company (St. Louis, MO). Elastase (porcine pancreas) and β-galactosidase (*E. coli*) were purchased from Worthington Biochemical Corporation (Freehold, NJ). Pronase (Streptomyces grisens), from Calbiochem-Behring Corporation (La Jolla, CA). Callagenase (*Clostridium hystolyticum*) from Millipore Corporation (Bedford, MA). α-galactosidase (green coffee beans) from Boehringer Mannheim Biochemical (Indianapolis, IN). Condroitinase ABC (proteus Vulgaris) from Miles Laboratory, Incorporated (Elkhart, IN). Neuraminidase (Vibrio Cholera) was a gift from Dr. D. Aminoff (Department of Medicine, University of Michigan, Ann Arbor, MI).

Incubations

Aliquots of 1–2 × 10^6 cells were incubated with the enzymes previously listed and reagents described in Table I. Enzymes were prepared in buffers which allow optimal enzymatic activity according to the manufacturer. For each solution (chemical or enzyme) tested, a control of PBS pH 7.2 and incubation buffer without enzyme were run at the same time. Following incubation, the cells were washed 3 times in PBS—0.01 M EDTA pH 7.2.

Immunofluorescence (IF) Techniques

Rat epidermal cells were attached to glass slides by a Cytospin centrifuge (Shandon Southern Corporation). These cells were used as

TABLE I. *Effects of chemicals and buffers on in situ pemphigoid antigen*

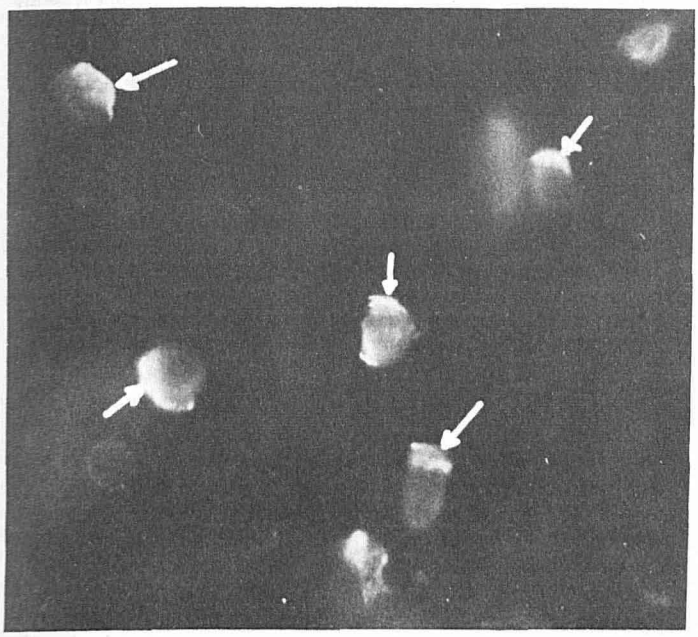
Treatment	Time (min[a])	Temperature (°C)	Indirect IF
Distilled water	60	20	+
PBS, pH 7.2	24 hr	4	+
PBS, pH 7.2	2 weeks	4	+
PBS, pH 7.2	24 hr	37	+
PBS, pH 7.2	24 hr	56	−
1 M NaCl	60	25	+
2 N NaSCN	60	25	−
4 M Urea, 0.1 M Tris/HCl, pH 8.1, 0/5 M NaCl	60	25	−
0.1 % Triton X-100 in PBS, pH 7.2	30	25	+
PBS, pH 6.0	60	37	+
0.01 M Acetate pH 5.0, 0.14 M NaCl	60	37	−
0.5% Triton X-100 in PBS, pH 7.2	60	37	+
1% Periodic acid in PBS, pH 7.2	60	25	−

[a] Unless otherwise stated.

substrates for BP serum (indirect IF titer, 1:10,000) at 1:80 dilution. A dilution or normal human serum was always included as a control. Binding of BP autoantibodies of rat tail epidermal cells was performed by routine indirect IF techniques described in detail elsewhere [3,9]. The slides were coded and read with a Zeiss fluorescent microscope equipped with epiillumination.

RESULTS

BP autoantibodies bind rat epidermal basal cells in a similar manner as reported for mouse and guinea pig epidermal cells [6–8]. A peculiar polar rim of fluorescence was noted in approximately 60% of the initial epidermal cell suspensions tested. This is shown in the Figure. The percentage of BP Ag-positive cells remained approximately constant in cell suspensions stored at 4°C in PBS for several days. The effect of various buffers, chaotropic agents, and other chemicals on the binding of BP autoantibodies on these cells is seen in Table I. Treatment of the epidermal cells with high salt concentrations (1 M NaCl) distilled water or solutions of Triton X-100 did not alter the polar fluorescence produced by BP autoantibodies. Incubation with 2 N NaSCN, 1% periodic acid, 4 M urea, buffers at low pH and 56°C temperature made these cells unreactive with BP autoantibodies. The effect of proteolytic, glycosidic, and other enzymes is shown in Tables II and III. None of the enzymes used nor the sequential combination of some of these enzymes prevented the staining produced by BP autoantibodies on rat epidermal cells.



Indirect IF staining of rat epidermal basal cells treated with bullous pemphigoid serum (1:80 dilution). *Arrows* show the "polar" distribution of BP antigen (reduced from × 310).

DISCUSSION

This report presents important information about the stability of BP Ag when bound to the cell surface of rat tail epidermal basal cells. A panel of proteolytic and glycosidic enzymes tested singly or sequentially did not abolish the reactivity of these cells with BP autoantibodies. These findings may have resulted from suboptimal experimental conditions in which these enzymes were tested. It is also possible, however, that BP Ag when embedded in the cell membrane of basal cells is unavailable to the active site of the enzymes.

Incubation of epidermal cells with a variety of buffers at different pH values and temperatures, showed that BP Ag reactivity was abolished by 56°C temperature and low pH, whereas it remained active at 4°C for several weeks in PBS, thus epidermal basal cells which no longer exclude trypan blue continue reacting with BP antibodies. It was also unchanged if incubated with distilled water, 1 M NaCl, and 0.5% Triton X-100. Since Triton X-100 did not solubilize BP Ag, it would appear that the common method of detergent-solubilization and immunoprecipitation of previously labeled cell surface molecules will not be successful for obtaining and characterizing labeled BP Ag.

It is known that 2 N NaSCN splits the DEJ at the level of the lamina lucida [10,11]. Its mechanism of action is unknown. In this study, we show that trypsin-dissociated cells became unreactive with BP autoantibodies when incubated with 2 N NaSCN, 4 M urea, or 1% periodic acid. Although BP Ag from these cells may have been denatured by these reagents, another possibility, however, which is more likely in the case of 2 N NaSCN and 4 M urea is that the antigen was released in solution. Both reagents have been used to purify BP Ag from human skin and urine [2,3]. This may indicate that BP Ag is

TABLE II. *Effects of proteolytic enzymes on in situ pemphigoid antigen*

Enzyme	Concentrations	Buffers	Incubation (min)	Temp. (°C)	Indirect IF
Trypsin (Type III)	up to 0.5%	PBS, pH 7.2	30	37	+
Chymotrypsin (Type 1–5)	0.05% to 0.5%	0.08 M Tris/HCl, pH 7.8, 01 M $CaCl_2$	30	37	+
Papain (Type IV)	0.5%	0.2 M Tris/HCl, pH 7.4, 0.05 M EDTA 0.15 M NaCl, 0.05 M cysteine	60	37	+
Elastase	0.1% to 0.3%	PBS, pH 7.4	30	37	+
Pronase	0.1%	PBS, pH 7.2	60	37	+
Collagenase	0.5%	0.05 M Tris/HCl, pH 7.5, 0.005 M $CaCl_2$	60	37	+

TABLE III. *Effects of other hydrolasas on in situ pemphigoid antigen*

Enzymes	Concentrations	Buffer	Incubation (min)	Temp. (°C)	Indirect IF
Hyaluronidase (Type I)	0.1%, 0.5%	PBS, pH 6.0 and PBS, pH 7.2	60	37	+
Condroitinase ABC	5 Units	Tris/HCl, pH 8, 0.5% BSA	60	37	+
Neuraminidase	1 Unit	0.79% NaCl, 0.1 M $CaCl_2$, pH 7.0	60	37	+
∝ Galactosidase	2 Units	PBS, pH 7.2, 0.01 M Mg SO_4, 0.02 M DTT	60	37	+
β-Galactosidase	50 Units	PBS, pH 7.2, 0.01 M Mg SO_4, 0.2 mM DTT	60	37	+
Galatose Oxidase	25 Units	PBS, pH 7.2	60	37	+
Deoxyribonuclease I	0.05%	PBS-0.01 M Mg SO_4	60	37	+
Neuraminidase/Trypsin	1 Unit/0.5%	0.79% NaCl, 0.01 M $CaCl_2$, pH 7.0 and PBS, pH 7.2	60	37	+
β-Galactosidase/Trypsin	50 Units/0.5%		60/60	37	+

relatively stable in these reagents when solubilized. Basal cells became unreactive with BP autoantibodies when incubated with 1% periodic acid. Oxidation and denaturation of the antigen may account for this finding.

It is known that certain proteolytic enzymes such as trypsin split epidermis from dermis by digesting the cutaneous basal lamina [12]. These enzymes and also glycosidic enzymes have been shown to increase the reactivity of skin sections with BP autoantibodies, when tested by indirect IF [13,14]. In dissociated cells, however, we have not detected this increase in reactivity. Therefore, if inflammatory immunological injury and release of these enzymes occurs in the upper dermis, the split of epidermis from the dermis may result from the digestion of structures located underneath the lamina lucida, which contains BP Ag.

REFERENCES

1. Diaz, LA, Weiss H, Calvanico, NJ: Phylogenetic studies with pemphigus and pemphigoid antibodies. Acta Dermatovener (Stockh) 58:537–540, 1979
2. Diaz LA, Calvanico NJ, Tomasi TB, Jr., Jordon RE: Bullous pemphigoid antigen: Isolation from normal human skin. J Immunol 119:455–460, 1977
3. Diaz LA, Patel H, Calvanico NJ: Bullous pemphigoid antigen. II. Isolation from the urine of a patient. J Immunol 122:605–608, 1979
4. Yuspa SH, Morgan DL, Walker RJ, Bates RR: The growth of fetal mouse skin in cell culture and transplantation to F_1 mice. J Invest Dermatol 55:379–389, 1970
5. Karasek MA, Charlton ME: Growth of post-embryonic skin epithelial cells on collagen gels. J Invest Dermatol 56:205–210, 1971
6. Brigden WD, Amos HE: The differential binding of antibody from the sera of patients with pemphigus and pemphigoid to isolated guinea pig epidermal cells. Br J Dermatol 93:425, 1975
7. Diaz LA, Marcelo CL: Pemphigus and pemphigoid antigens in cultured epidermal cells. Br J Dermatol 98:631, 1978
8. Stanley JR, Foidart JM, Murray JC, Martin GR, Katz, SI: The epidermal cell which selectively adheres to a collagen substrate is the basal cell. J Invest Dermatol 74:54–58, 1980
9. Buetner ER, Chorzelski TP, Jordon RE: Autosensitization in Pemphigus and Bullous Pemphigoid. Charles C Thomas, Springfield, Illinois, 1970
10. Felsher Z: Studies on the adherence of the epidermis to the corium. J Invest Dermatol 8:35–47, 1947
11. Diaz LA, Heaphy MR, Calvanico NJ, Tomasi TB, Jr., Jordon RE: Separation of epidermis from dermis with sodium thiocyanate. J Invest Dermatol 68:36–38, 1977
12. Fukuyama K, Black MM, Epstein WL: Ultrastructural studies of newborn rat epidermis after trypsinization. J Ultrastruct Res 46: 219–229, 1974
13. Remy W, Bockendahl H, Stüttgen G, Petersen G: The activation of antigens of the basement membrane zone by proteolytic enzymes in vitro. Acta Dermatovener (Stockh) 56:43–46, 1976
14. Remy W, Bockendahl H, Kunow H, Remy B, Stüttgen G: Investigations on the binding sites of the basement membrane zone for pemphigoid antibodies in vitro. I. Enzymatic actions on the active groups of human skin. Acta Dermatovener (Stockh) 56:423–428, 1976

Announcement

A Dermatopathology Foundation course in Gross and Microscopic Pathology of the Skin will be held June 15 to 19, 1981, (Monday-Friday) at the Resorts International Hotel Casino, Atlantic City, New Jersey. (40 hours credit—AMA category-1.) For information write Dermatopathology Foundation, PO Box 377, Canton, MA 02021.