

## BINDING SPECIFICITY OF RABBIT ANTI-GUINEA PIG EPIDERMAL CELL SERA: COMPARISON OF THEIR RECEPTORS WITH THOSE OF CONCAVALIN A AND PEMPHIGUS SERA

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**Abstract.** Rabbit anti-guinea pig epidermal cell sera (AES) which were prepared by immunizing rabbits with enzymatically dispersed viable guinea pig epidermal cells were shown to react to the intercellular substances of stratified squamous epithelia of guinea pigs and monkeys in a pattern similar to that seen with concanavalin A (ConA) or pemphigus sera (PS) by immunofluorescence. Reciprocal blocking tests were performed on guinea pig lip mucosa after removal of the non-specific binding substances between preincubated substances and subsequently incubated ones. No definite inhibition was noted in the subsequent reactions upon preincubation with AES or PS. However, preincubation with ConA weakened the subsequent reaction with PS, but did not block the reaction with AES. Effects of solvents on the receptors for AES, ConA and PS were also examined. ConA receptor was resistant to both saline solution and ethanol (95 % and 99 %). PS receptor was labile to both saline solution and ethanol, while AES receptor was labile to ethanol, but resistant to saline solution. These observations suggest that the receptor for AES differs from that for ConA or PS.

**Key words:** Anti-guinea pig epidermal cell sera; Concanavalin A; Pemphigus sera; Receptor; Reciprocal blocking test; Effect of solvent

It has been demonstrated that a variety of antigens, including alloantigens (2, 11, 17) and heteroantigens (5, 16, 18), and receptors for plant lectin such as concanavalin A (ConA) and phytohemagglutinin P (8) are present on the surface of epidermal cells of many species. Rabbit anti-guinea pig epidermal cell serum (AES) which was prepared by immunizing rabbits with enzymatically dispersed viable guinea pig epidermal cells was demonstrated by means of complement-mediated cytotoxicity test and immunofluorescence as being directed toward cell surface antigens specific for stratified squamous epithelia of guinea pigs, monkeys and humans (13).

On the other hand, it is generally accepted that pemphigus serum (PS) has receptors within the intercellular spaces of stratified squamous epithelia from a wide range of species other than humans (1).

Since pemphigus antibodies are considered to play an important part in the pathogenesis of pemphigus lesions, a comparison of the properties of receptors for AES, ConA and PS seemed to be warranted. We report here the results of reciprocal blocking tests between AES, ConA and PS, and of the effect of solvents on their receptors.

### MATERIALS AND METHODS

#### *Specimens*

Tissue specimens were snap frozen in an acetone-dry ice mixture and stored at  $-80^{\circ}\text{C}$  until use. Sections were cut at a thickness of  $6\text{ }\mu\text{m}$  in a cryostat at  $-20^{\circ}\text{C}$ .

#### *Rabbit anti-guinea pig epidermal cell serum (AES)*

Preparation of AES has been described previously (13). Briefly, guinea pig epidermal cells were dispersed by a 0.3 % solution of trypsin. The dye exclusion test showed that more than 95 % of the cells were viable. Rabbits were given three intravenous injections of  $1.5 \times 10^7$  cells in 5 ml of 0.9 % saline solution at intervals of 2 weeks. One week after the last injection, blood was drawn and sera were collected and heat inactivated. The antiserum was absorbed with guinea pig red, spleen, and thymus cells, and liver powders. Complement-mediated cytotoxicity tests showed that the absorbed antiserum (AES) killed more than 90 % of dispersed guinea pig epidermal cells at a dilution of 1:10 (13).

#### *Fluorescein-labelled concanavalin A (ConA-FITC)*

ConA-FITC was prepared according to the methods of Tkacz, Cybulska & Lampen (14) with some modifications. Two mg of fluorescein isothiocyanate (FITC) was dissolved in 1 ml of 0.1 M  $\text{Na}_2\text{HPO}_4$ . Twenty-four mg of ConA (Sigma, Grade III) was dissolved in 1 ml of 1 M NaCl. 0.5 ml each of FITC and ConA solution were gently mixed together at pH 8.3, using a magnetic stirrer at room temp-

Table I. Intercellular reactivity of AES, ConA and PS on tissue sections

|                       | AES                  | ConA | PS      |
|-----------------------|----------------------|------|---------|
| Guinea pig lip mucosa | + (640) <sup>a</sup> | +    | + (320) |
| Guinea pig esophagus  | + (640)              | +    | + (320) |
| Guinea pig skin       | + (80)               | +    | + (160) |
| Monkey esophagus      | + (20)               | +    | + (640) |
| Rabbit oral mucosa    | ±                    | +    | + (320) |
| Rat oral mucosa       | ±                    | +    | + (160) |
| Mouse oral mucosa     | ±                    | +    | + (80)  |
| Human skin            | ±                    | +    | + (160) |

<sup>a</sup> Titre of antiserum giving positive reaction.

erature for 2 hours, and the reaction mixture was dialysed against 1 M NaCl at 4°C overnight. After a small amount of precipitate which had formed on dialysis was removed by centrifugation, the mixture was placed on a column of Sephadex G-75 (Pharmacia) previously equilibrated with 1.0 M NaCl. After removing the eluent with 1 M NaCl, 0.1 M glucose in 1.0 M NaCl was applied. The resultant eluent was pooled and dialysed against 1 M NaCl at 4°C overnight. The optical absorbancy of the dialysed pool was 0.069 at 490 nm and 0.87 at 280 nm. The estimated ConA concentration was 600 µg/ml. ConA-FITC was used without further dilution.

#### *Pemphigus serum (PS)*

The serum from a 66-year-old male patient with pemphigus foliaceus was used. The pemphigus antibody titre of the serum was 320 when guinea pig lip mucosa was employed as the substrate.

#### *Immunofluorescence staining*

Unfixed frozen tissue sections were stained with ConA-FITC by the direct method, or AES (1:10 dilution) followed by goat anti-rabbit Ig-FITC (BBL) or PS (1:10 dilution) followed by rabbit anti-human IgG-FITC (Dakopatts) by the indirect method. F/P molar ratio, protein concentration and dilution of goat anti-rabbit Ig-FITC and rabbit anti-human IgG-FITC were 3.7, 7.8 mg/ml, 1:20 and 1.7, 4.4 mg/ml, 1:10 respectively. Examinations were made with a Zeiss fluorescence microscope equipped with an Osram HBO 200 supermercury lamp and UV-5 exciter and 41 and 65 barrier filters.

#### *Reciprocal blocking tests*

Specific inhibition of the intercellular reaction by AES, ConA or PS was tested on the sections of guinea pig lip mucosa, since AES revealed highest reactivity to this tissue (see Results). Unfixed frozen sections were layered with preincubated substance for 30 min at 37°C, and after being washed with PBS (pH 7.2) for 10 min, were stained with AES (1:10 dilution) followed by goat anti-rabbit Ig-FITC, ConA-FITC or PS (1:10 dilution) and then by rabbit anti-human IgG-FITC. As preincubated substance, either AES, ConA solution or PS was used, and for con-

trol purposes, normal rabbit serum (NRS), normal human serum (NHS), or distilled water. Preincubated sera were either non-diluted or diluted to 1:5 or 1:10. The concentration of ConA solution was 1 mg/ml or 5 mg/ml. When PS was used as the subsequently incubated substance, distilled water was used as the washing reagent for PBS, since PS receptor was labile to saline solution (see Results). In these tests, the non-specific binding substances between preincubated substances and subsequently incubated ones were removed by immunoadsorbent columns (3). ConA-Sepharose was obtained from Pharmacia Fine Chemicals AB, Uppsala, Sweden. The human or rabbit sera were coupled to CNBr-activated Sepharose 4B (Pharmacia). When AES or NRS was preincubated, ConA-FITC, PS and rabbit anti-human Ig-FITC were incubated with the rabbit sera coupled immunoadsorbent. When ConA was preincubated, AES, goat anti-rabbit Ig-FITC, PS and rabbit anti-human IgG-FITC were incubated with ConA-Sepharose. When PS or NHS was preincubated, AES, goat anti-rabbit Ig-FITC and ConA-FITC were incubated with human sera coupled immunoadsorbent.

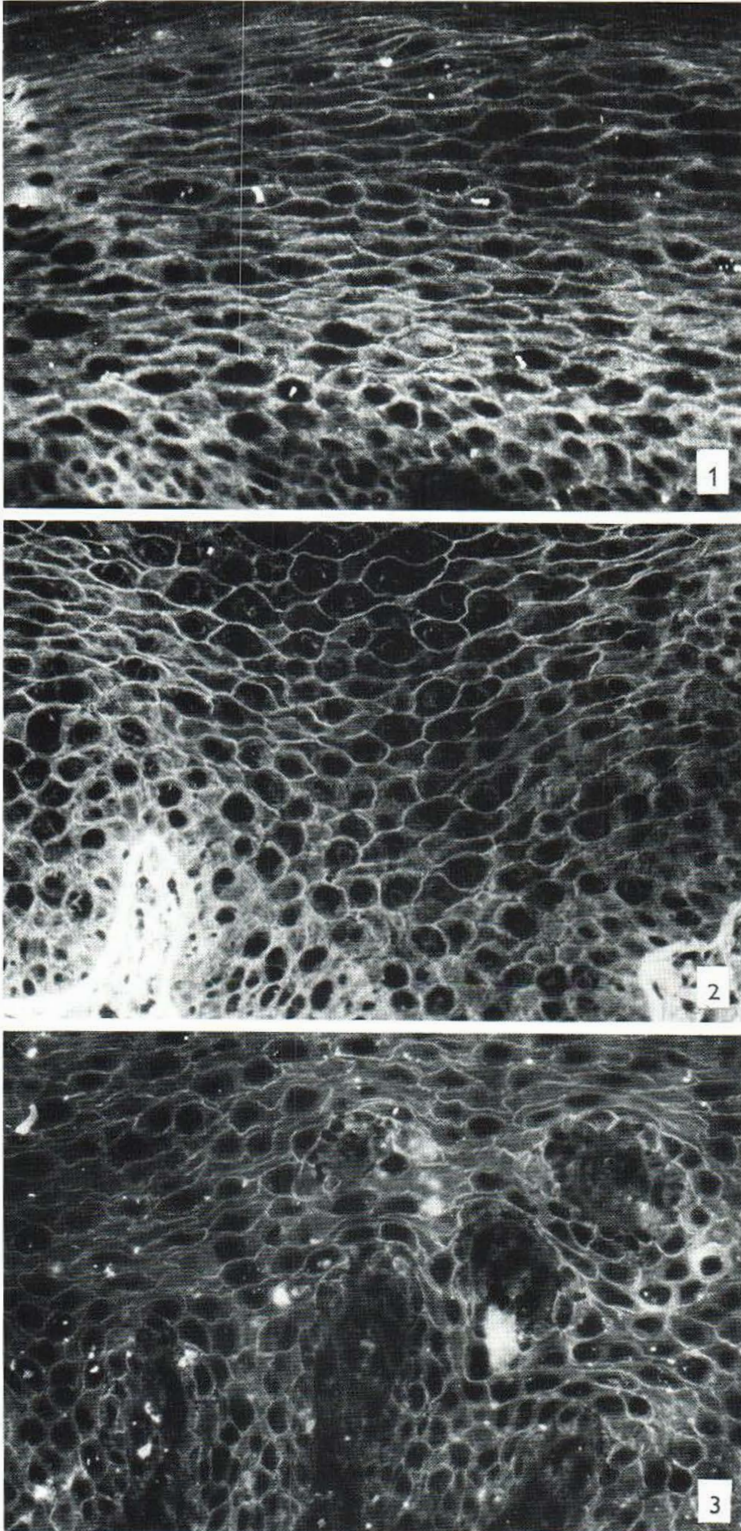
## RESULTS

#### *Reactivity of AES, ConA and PS*

Unfixed frozen sections from a variety of tissues were stained with AES followed by goat anti-rabbit Ig-FITC, ConA-FITC or PS followed by rabbit anti-human IgG-FITC. The results are shown in Table I. AES reacted intensely with intercellular spaces of guinea pig skin (antibody titre: 80), lip mucosa (640) (Fig. 1) and esophagus (640). In addition to guinea pig tissues, AES cross-reacted to monkey esophagus at the low titre (20), but did not reveal any clear reactions to rabbit, rat, or mouse oral mucosa or human skin. On the other hand, ConA and PS revealed intense intercellular fluorescence in all specimens examined, and these fluorescence patterns appeared similar to or even identical with that seen with AES (Figs. 2, 3). However, in the case of ConA, a faint fluorescence of the basement membrane, collagen fibres, and blood vessels was also noted (Fig. 2).

#### *Reciprocal blocking tests*

Reciprocal blocking tests on the epithelial intercellular reaction were performed on tissue sections of guinea pig lip mucosa. The results are shown in Table II and Figs. 4-6. Control experiments showed that preincubation with NRS, NHS or distilled water did not influence the subsequent reaction with AES, ConA or PS. Preincubation with AES did not block the subsequent reaction with ConA or with



*Figs. 1-3.* Reactivity of: AES (1 : 10 dilution) ( $\times 184$ ), Fig. 1; ConA (600  $\mu\text{g/ml}$ ) ( $\times 184$ ), Fig. 2; and PS (1 : 10 dilution) ( $\times 184$ ), Fig. 3, to the guinea pig lip mucosa.

Table II. Reciprocal blocking test on intercellular reactions to guinea pig lip mucosa

| Preincubated substances | Subsequently incubated substances |                  |                    |
|-------------------------|-----------------------------------|------------------|--------------------|
|                         |                                   | ConA (600 µg/ml) | PS (1:10 dilution) |
| AES                     |                                   |                  |                    |
| Non-dilution            |                                   | ++               | ++                 |
| 1:5 dilution            |                                   | ++               | ++                 |
| 1:10 dilution           |                                   | ++               | ++                 |
|                         | AES (1:10 dilution)               | ConA (600 µg/ml) | PS (1:10 dilution) |
| ConA                    |                                   |                  |                    |
| 1 mg/ml                 | ++                                | ±                | +                  |
| 5 mg/ml                 | ++                                | —                | +                  |
|                         | AES (1:10 dilution)               | ConA (600 µg/ml) |                    |
| PS                      |                                   |                  |                    |
| Non-dilution            | +~++                              | ++               |                    |
| 1:5 dilution            | ++                                | ++               |                    |
| 1:10 dilution           | ++                                | ++               |                    |

PS. Nor did preincubation with PS block the subsequent reaction with ConA, whereas non-diluted PS appeared to weaken slightly the subsequent reaction with AES—although 1:5 or 1:10 diluted PS did not block the reaction at all. Reaction with ConA was almost completely blocked by preincubation with ConA solution at the concentration of 1 mg/ml and was completely blocked at the concentration 5 mg/ml. Preincubation with ConA did not block the subsequent reaction with AES at either concentration, while the reaction with PS was weakened by this substance at the concentration 1 mg/ml, but was never completely blocked even at a concentration of 5 mg/ml.

Effect of pretreatment with solvents on tissue sections

Frozen tissue sections of guinea pig lip mucosa were immersed in distilled water, 0.3%, 0.9% or 2.7% saline solution, or 70%, 95% or 99% ethanol for 2 hours at room temperature, and stained with AES, followed by goat anti-rabbit Ig-FITC, ConA-FITC or PS followed by rabbit anti-human IgG-FITC. As shown in Table III, pretreatment with distilled water hardly influenced the reactivity with AES, ConA or PS, while pretreatment with

70% ethanol completely abolished the reactivity with all of these reagents. Although pretreatment with various concentrations of saline solution did not alter the reactivity with AES or ConA, this pretreatment resulted in a complete loss of the reactivity with PS. Pretreatment with 95% or 99% ethanol did not influence the reactivity with ConA, whereas such pretreatment completely abolished the reactivity with PS and markedly diminished the reactivity with AES.

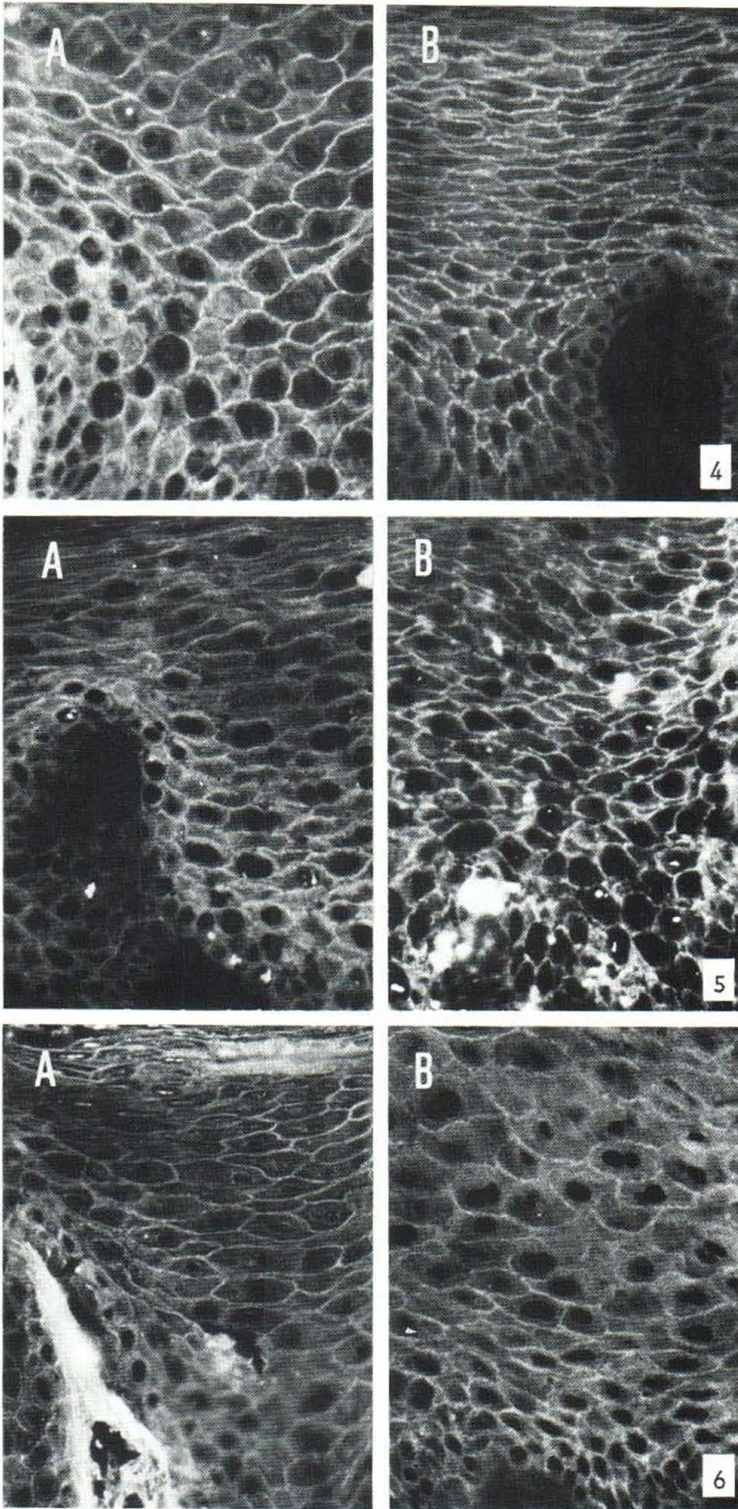
DISCUSSION

AES revealed intense reactions with the intercellular spaces of stratified squamous epithelia of guinea pigs and weak cross-reactions to monkey esophagus, but did not reveal any clear reactions to human skin, although it showed weak cytotoxicity against isolated human epidermal cells (13). The intercellular reactions with AES appeared morphologically similar to or even identical with those seen with ConA or PS. However, reciprocal blocking tests and the effect of solvents on the tissue sections disclosed distinct differences among them.

In the reciprocal blocking tests, special attention was given as follows: (a) Exclusion of non-specific binding substances between preincubated substances and subsequently incubated ones. This procedure was necessary as there are slight cross-reactions among human, rabbit and goat serum and non-specific binding between these sera and ConA. (b) Exclusion of PBS as the washing reagent when PS was used as the subsequently incubated substance, since PS receptor was found to be labile to saline solution.

No definite inhibition was noted in the subsequent reactions on preincubation of guinea pig lip mucosa with AES or PS, and no visible inhibition was found in the intercellular reaction with ConA and AES. However, preincubation with ConA at the concentrations 1 mg/ml and 5 mg/ml resulted in a considerable, though incomplete, blocking effect against the subsequent reaction with PS. These findings imply that AES receptor is not dependent on ConA or PS receptor, while the latter two receptors share a common part.

Recently, Lloyd & Darnule (6) reported that anti-rat epidermal cell sera prepared in a manner similar to ours reacted with different components



*Figs. 4-6. Reciprocal blocking test on the guinea pig lip mucosa. Fig. 4 A: Preincubated with non-diluted AES and subsequently incubated with ConA (600  $\mu$ g/ml) ( $\times 184$ ). Fig. 4B: Preincubated with ConA (5 mg/ml) and subsequently incubated with AES (1:10 dilution) ( $\times 184$ ).*

*Fig. 5 A: Preincubated with non-diluted AES and subsequently incubated with PS (1:10 dilution) ( $\times 184$ ). Fig. 5 B: Preincubated with non-diluted PS and subsequently incubated with AES (1:10 dilution) ( $\times 184$ ).*

*Fig. 6 A: Preincubated with non-diluted PS and subsequently incubated with ConA (600  $\mu$ g/ml) ( $\times 184$ ). Fig. 6B: Preincubated with ConA (5 mg/ml) and subsequently incubated with PS (1:10 dilution) ( $\times 184$ ).*

Table III. Intercellular reactions to guinea pig lip mucosa after 2 hours' incubation with solvents

|      | Distilled water | Saline solution |      |      | Ethanol |     |     |
|------|-----------------|-----------------|------|------|---------|-----|-----|
|      |                 | 0.3%            | 0.9% | 2.7% | 70%     | 95% | 99% |
| AES  | ++              | ++              | ++   | ++   | —       | ±~+ | ±~+ |
| ConA | ++              | ++              | ++   | ++   | —       | ++  | ++  |
| PS   | +~++            | —               | —    | —    | —       | —   | —   |

from ConA receptor on epidermal cells, using radio-immunoprecipitation method. Williams et al. (16) reported that the binding of antisera to human oral epithelial cells was not affected by preincubation with ConA. Our present data would seem to confirm their results.

Concerning the identity of ConA receptor and PS receptor, previous reports were not consistent: Hashimoto et al. (4) and Van Lis & Kalsbeek (15) described these two receptors as being identical, while Nishikawa et al. (9, 10) reported them to be non-identical. However, it should be pointed out that in their blocking experiments, non-specific binding substances between preincubated substances and subsequently incubated ones had not been removed. Recently, Miyagawa et al. (7) reported that ConA reactive soluble epidermal glycoproteins were not identical with pemphigus antigen.

The effect of solvents on the receptors seems to support the results of the reciprocal blocking tests. As Shu & Beutner (12) have already reported, PS receptor was labile to both saline solution and ethanol. On the other hand, ConA receptor was resistant to both saline solution and ethanol (95% and 99%), while AES receptor was labile to ethanol but resistant to saline solution. Ethanol at the concentration of 70% completely abolished intercellular reactions with AES, ConA and PS, suggesting that this concentration of ethanol will degenerate all of these receptors.

We have already reported two differences between AES and PS receptors in a previous paper (13): firstly, the reactivity of AES to isolated guinea pig epidermal cells is less influenced by trypsinization than that of PS; and secondly, AES shows complement-mediated cytotoxicity to the isolated guinea pig epidermal cells, whereas PS does not. The present data seem to further suggest that the receptor for AES differs from that for ConA or PS.

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