

STUDIES ON CARRIER PROTEIN IN CONTACT DERMATITIS:  
LOCALIZATION OF SOLUBLE EPIDERMAL CARRIER  
PROTEINS BY MACROPHAGE MIGRATION TEST

Nobuyuki Miyagawa, Sachiko Miyagawa, Hiromasa Ishii, Koji Hayashi,  
Kuniki Sakamoto and Toshiyuki Aoki

*Departments of Bacteriology and Dermatology, Nara Medical University, Kashihara, Nara,  
and Department of Allergy and Dermatology, Habikino Hospital, Habikino, Osaka, Japan*

**Abstract.** The migration of peritoneal exudate cells from picrylchloride-sensitive guinea pigs was inhibited by heteroantigenic components which were isolated by anti-normal guinea pig epidermis antibody immunoadsorbent from soluble epidermal proteins of picrylchloride painted guinea pigs. The components were demonstrated by indirect immunofluorescence study to localize in the intercellular spaces of guinea pig epidermis. It is consequently suggested that carrier protein(s) of contact hypersensitivity reside on the cell surface of epidermis.

**Key words:** Contact dermatitis; Carrier protein; Cell surface membrane; Migration inhibition assay; Soluble epidermal proteins

Most substances which cause allergic contact dermatitis appear to act as haptens, that is to say they are simple chemicals of relatively low molecular weight which require conjugation with proteins in order to become complete antigens. One of the important problems hindering an understanding of the sensitization mechanism of allergic contact hypersensitivity is the nature of carrier proteins.

The authors have attempted to isolate and identify carrier substances of picrylchloride contact allergy in guinea pigs by utilizing the *in vitro* migration inhibition assay as an indicator of a specific immunological reaction. As reported previously (8), hapten-protein conjugates which can inhibit the migration of peritoneal exudate cells from picrylchloride-sensitive guinea pigs were obtained in solubilized form by repeated freezing and thawing of the picrylchloride-painted guinea pig epidermis. Further examination revealed that the hapten-protein conjugates were in the Sephadex fraction II with molecular weights ranging between 10 000 and

200 000. The Sephadex fraction II from guinea pig epidermis was still a complex mixture containing several proteins demonstrable by immunoelectrophoresis. The experiment reported in this paper is an attempt to extend the analysis of the hapten-protein conjugates and to study their localization in epidermal cells.

#### MATERIALS AND METHODS

##### *Sensitization of guinea pigs*

Hartley guinea pigs weighing about 400 g were immunized with 40  $\mu$ g of sodium picrylsulfonate in Freund's complete adjuvant. Animals were judged as sensitized to picrylchloride when erythema was induced 48 hours after epicutaneous application of 0.1% picrylchloride in acetone solution.

##### *Preparation of rabbit antiserum to guinea pig epidermis or serum*

Rabbit antisera against guinea pig epidermis, guinea pig serum and Sephadex fraction II of guinea pig epidermis were used in these experiments. Epidermal antigen and the fraction II were produced as described previously (8).

Immunization was carried out as follows: rabbits weighing about 2 kg were injected in four foot pads with 4 mg of antigen emulsified in Freund's complete adjuvant twice with a 5 week interval and the antisera were obtained by heart puncture between 10 and 14 days after the last injection.

##### *Preparation of antigens for migration inhibition assay*

Sephadex fraction II of picrylchloride-treated epidermal extract of guinea pigs (pic-Fr. II) was prepared as described in the previous paper (8). Antibody immunoadsorbents were prepared as described by Pass et al. (11). Rabbit antisera against guinea pig epidermis and against guinea pig serum served as the source of antibodies for the preparation of immunoadsorbents.

Table I. Mean macrophage migration indices of peritoneal exudate cells from normal and from picrylchloride-sensitive guinea pigs

Results are the mean migration indices and standard deviation of 40 observations on 10 animals in each group. The degree of significance was determined by Student's *t*-test

Source of PEC	Antigen added to PEC			
	pic-Fr. II (protein = 1.89 mg/ml)			
	×32 <sup>a</sup>	×64	×128	×256
Normal g.p.	81.6±9.1 <sup>b</sup>	92.7± 8.7	100.9± 7.8	96.1±7.7
Picrylchloride-sensitive g.p.	64.0±8.5	78.2±10.2	88.2±12.7	95.9±7.7
Degree of significance	0.001< <i>P</i> <0.01	0.02< <i>P</i> <0.05	0.05< <i>P</i>	0.05< <i>P</i>

<sup>a</sup> Dilution with medium.

<sup>b</sup> Migration index (means ±S.D.).

The antibody-Sepharose immunoabsorbents were incubated with pic-Fr. II overnight at 4°C, and then poured into columns. The unabsorbed material was washed with phosphate-buffered saline (PBS, pH 7.0, 0.02 M) and specific antigens were dissociated from the immunoabsorbent by eluting with 0.1 N acetic acid. Thus three fractions were separated from pic-Fr. II: the fractions adsorbed (antigenic epidermal component) and unadsorbed (non-antigenic epidermal component) to anti-epidermis antibody, and the fraction adsorbed to anti-whole serum antibody (antigenic serum component). The fractions were collected and their protein contents monitored with an LKB Uvicord at 280 nm.

The material obtained by acid elution was brought to pH 7.0 with 1.0 N NaOH, dialysed against PBS, and concentrated by ultrafiltration using seamless cellulose tubes.

#### Macrophage migration inhibition assay

The method used was the same as that reported previously (8).

The migration index (%): (Mean migration area in the presence of antigen/Mean migration area in the absence of antigen×100) was used to express the effect of antigen on macrophage migration.

#### Immunofluorescence study

The indirect immunofluorescence technique was used to study the localization of Sephadex Fr. II of guinea pig epidermal extract. Anti-Sephadex Fr. II rabbit serum was absorbed with aggregated normal guinea pig serum in order to remove antibodies to serum components. Excision biopsy material from lips of normal guinea pigs was quickly frozen at -70°C and sectioned in a cryostat at 4 μm thickness at -20°C. These sections were incubated with the absorbed anti-Sephadex Fr. II rabbit serum in a moist chamber for 30 min at 37°C and washed thoroughly with PBS. The sections were incubated with FITC-labelled goat anti rabbit 7S gammaglobulin (Hyland, Lot 2223×001A1) for 30 min at 37°C, washed with PBS, mounted with one drop of a medium consisting of 9 parts glycerol and 1 part of PBS (pH 9.0), and examined with an

Table II. Mean macrophage migration indices of peritoneal exudate cells from normal and from picrylchloride-sensitive guinea pigs

Results are the mean migration indices and standard deviation of 40 observations on 10 animals in each group. Antigens: pic-Fr. IIa: antigenic epidermal components, pic-Fr. IIb: non-antigenic components, and pic-Fr. IIc: antigenic serum components, of pic-Fr. II

Source of PEC	Antigens added to PEC							
	pic-Fr. IIa (protein = 1.68 mg/ml)				pic-Fr. IIb (protein = 2.40 mg/ml)			
	×32 <sup>a</sup>	×63	×128	×256	×32	×64	×128	×256
Normal g.p.	67.4±6.7 <sup>b</sup>	83.0±13.7	84.5±6.6	80.1±4.0	75.9± 7.4	89.4± 8.5	100.8±14.3	93.9±11.7
Picrylchloride sensitive g.p.	44.0±9.4	67.3± 8.0	78.2±9.0	81.7±6.9	68.6±12.0	79.7±15.7	87.5± 9.7	87.8± 7.8
Degree of significance	0.001< <i>P</i> <0.01	0.02< <i>P</i> <0.05	0.05< <i>P</i>	0.05< <i>P</i>	0.05< <i>P</i>	0.05< <i>P</i>	0.05< <i>P</i>	0.05< <i>P</i>

<sup>a</sup> Dilution with medium.

<sup>b</sup> Migration index (means ±S.D.).

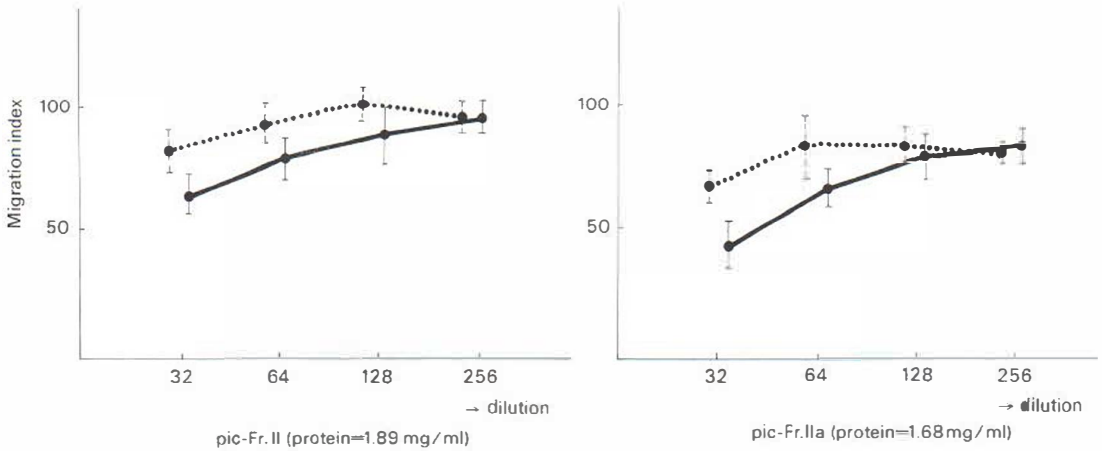


Fig. 1. Macrophage migration indices (means  $\pm$  S.D.) of PEC from normal (---) and from picrylchloride-sensitive (—) guinea pigs. Antigens are Sephadex G-200 fraction II

of picrylchloride-painted guinea pig epidermis (pic-Fr. II) and acid eluate of pic-Fr. II from antibody-Sephadex immunoadsorbent (pic-Fr. IIa).

UV fluorescence microscope (Olympus, type FLM). The experiment was controlled by sections incubated with normal rabbit serum followed by FITC-labelled goat antiserum.

RESULTS

Migration inhibition of peritoneal exudate cells (PEC) from hypersensitive guinea pigs

As shown in Table I and Fig. 1, inhibition of the migration of PEC from sensitive guinea pigs took place with pic-Fr. II. At  $\times 32$  and  $\times 64$  dilutions of this fraction, migration of sensitive PEC was inhibited to a significantly lesser extent than that of normal cells.

Migration inhibition experiments were further advanced using the three fractions of pic-Fr. II. As shown by Table II and Fig. 1, acid eluate of anti-epidermis antibody-Sephadex immunoadsorbent inhibited the migration of sensitive PEC, whereas it had no effect on cells from normal controls. The difference was statistically highly significant ( $0.001 < P < 0.01$ ) at the dilution  $\times 32$  and significant ( $0.02 < P < 0.05$ ) at  $\times 64$ .

The minimum dose of  $29.5 \mu\text{g/ml}$  of pic-Fr. II represented the inhibitory activity of PEC migration, whereas the  $26.3 \mu\text{g/ml}$  dose of antigenic epidermal component of pic-Fr. II represented the same activity. The non-antigenic epidermal component of pic-Fr. II (viz. unabsorbed materials of antibody-Sephadex immunoadsorbent) did not inhibit the migration of sensitive PEC (Table II). Antigenic serum components obtained from pic-Fr. II by eluting from anti-guinea pig serum-Sephadex immunoadsorbent also failed to inhibit the migration of sensitive PEC (Table II).

It was therefore concluded that antigenic epidermal proteins of pic-Fr. II acted as carrier proteins in the migration inhibition assay.

Immunofluorescence study

The rabbits immunized with Sephadex Fr. II showed circulating antibodies directed against intercellular components of guinea pig epidermis (Fig. 2), indicating that the antigenic components of Sephadex Fr. II localize in the intercellular spaces of the epidermis.

pic-Fr. IIc protein = 1.75 mg/ml				
<32	$\times 64$	$\times 128$	$\times 256$	
$7.3 \pm 6.0$	$99.2 \pm 10.7$	$97.2 \pm 9.3$	$103.3 \pm 7.2$	
$4.7 \pm 11.4$	$93.8 \pm 12.0$	$97.0 \pm 12.2$	$92.6 \pm 11.7$	
$0.05 < P$	$0.05 < P$	$0.05 < P$	$0.05 < P$	



*Fig. 2.* Normal guinea pig oral mucosa reacted with absorbed anti-Sephadex fraction II rabbit sera and stained with FITC-labelled anti-rabbit 7S gammaglobulin. The photograph shows intercellular staining.  $\times 400$ .

## DISCUSSION

In this experiment, rabbit antibody produced against fraction II of guinea pig epidermis specifically adsorbed picryl protein active as hapten-carrier conjugate on one hand and reacted with intercellular substances of guinea pig epidermis on the other. These results seem to indicate that carrier substances in contact sensitivity are present in the intercellular space or associated with cell surface membrane.

Recently, several attempts have been made to determine the carrier substances in allergic contact dermatitis by utilizing an *in vitro* system, *viz.* lymphocyte transformation or macrophage migration tests.

In reports on the utilization of lymphocyte transformation, serum proteins (2, 3), cells such as lymphocytes (2, 4, 5) or erythrocytes (2, 5), lymphocyte stroma (3) and soluble epithelial extract (6, 7) were tested for carrier activity. When serum proteins were used as the carrier substance, the authors failed to stimulate sensitized lymphocytes. The use

of homologous epithelial tissue extract conjugated with dinitrofluorobenzene promoted specific lymphocyte transformation (6). Lymphocyte transformation has also been observed in sensitized guinea pigs on the addition of free chemical to the *in vitro* culture (2). In the latter experiments direct lymphocyte conjugation may occur *in vitro* and the formation of required antigen may take place in culture. Support for this speculation may be found in the work of Miller & Levis (5), who employed dinitrophenylated lymphocytes as the antigen in lymphocyte transformation. Hinrichs & Gibbins (3) also showed that hapten conjugated homologous lymphocyte stroma could stimulate sensitized lymphocytes.

On the other hand, there are some reports of the successful detection of contact sensitivity by utilizing a macrophage migration inhibition technique. In the guinea pig system, contact sensitivity was detected *in vitro* by using serum proteins (1), or subcellular fractions of guinea pig epidermis (8, 9) as carrier substances. DNCB contact sensitivity was detected *in vitro* by using dinitrophenylated microsomes derived from guinea pig epidermis which were seen electron-microscopically to contain membranous particles (9).

To summarize, recent investigators have tended to detect carrier activity in cellular or subcellular structural components. In our previous investigations, carrier activity was found in soluble forms in the extract of picrylchloride painted epidermis. In the present report, however, the soluble carrier substances were shown to be present in the intercellular spaces of the epidermis, thus suggesting the possibility of its association with cell surface membrane. It is interesting, in this respect, that Nishioka repeatedly claims that subcellular components are active carrier substances (9, 10). It has been much debated whether carrier substance is soluble or insoluble. However, present investigation indicates that it is a matter of degree. This situation is quite similar to that of transplantation antigen, which has been found in subcellular structures and in the soluble forms at the same time.

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S. Miyagawa, M.D.  
 Department of Dermatology  
 Nara Medical University  
 Nara  
 Japan