

STUDIES ON CARRIER PROTEIN IN CONTACT DERMATITIS: IN VIVO SENSITIZATION WITH SOLUBLE EPIDERMAL PROTEINS AS CARRIER PROTEINS

Sachiko Miyagawa and Nobuyuki Miyagawa

Departments of Dermatology and Bacteriology, Nara Medical University, Kashihara, Nara, Japan

Abstract. Contact hypersensitivity to picrylchloride was induced in guinea pigs by the subcutaneous injection of heteroantigenic components which were isolated by antibody immunoadsorbent from soluble epidermal proteins of picrylchloride painted guinea pigs. The injection of non-heteroantigenic components obtained from the picrylchloride treated guinea pig epidermis did not elicit contact sensitization to picrylchloride in guinea pigs. These results corroborate previous findings obtained when utilizing the in vitro migration inhibition assay.

Key words: Contact dermatitis; Carrier protein; Soluble epidermal proteins

Many attempts have been made to elucidate the carrier substances active in allergic contact dermatitis, by utilizing in vivo sensitization methods and in vitro lymphocyte transformation or macrophage migration tests (1). While the characteristics of the carrier substances are still poorly defined, recent investigators are more inclined to locate carrier activity to cell structure (especially membranous) components (2, 3, 6, 7, 8).

We have recently reported on experimentation utilizing the macrophage migration test. The results suggested that carrier proteins in contact hypersensitivity resided on the surface of epidermal cells (4, 5).

The experiment reported in this paper is an attempt to investigate the in vivo response to the hapten-carrier conjugates active in the in vitro macrophage migration test.

MATERIALS AND METHODS

Hartley guinea pigs of both sexes weighing about 400 g were used throughout the experiments.

Sephadex fraction II of picrylchloride treated epidermal

extract of guinea pigs (pic.-Fr. II) was prepared as described elsewhere (4).

Antibody immunoadsorbent was prepared as described by Pass et al. (9). Rabbit antiserum against guinea pig epidermis was obtained as described previously and served as the source of antibody for the preparation of immunoadsorbent. The antibody-Sepharose immunoadsorbent was incubated with pic.-Fr. II at 4°C overnight, and then poured into a column. The unadsorbed material was washed with saline, and specific antigens were dissociated from the immunoadsorbent by eluting with 0.1 N acetic acid. Thus two fractions were separated from pic.-Fr. II: the fractions adsorbed (heteroantigenic pic.-Fr. II) and unadsorbed (non-heteroantigenic pic.-Fr. II) to anti-epidermis antibody. The fractions were collected and the protein content was monitored with an LKB Uvicord at 280 nm.

The material obtained by acid elution was adjusted to pH 7.0 with 1.0 N NaOH and dialysed against saline at 4°C for 3 days by changing saline twice daily.

Three antigens (viz. pic.-Fr. II, heteroantigenic pic.-Fr. II, and non-heteroantigenic pic.-Fr. II) were concentrated by ultrafiltration using seamless cellulose tubes and dialysed against saline at 4°C overnight.

The protein content of antigens was estimated by biuret analysis and ϵ -N-trinitrophenyl lysine/protein determined as described previously (4).

Guinea pigs were immunized with 0.4 mg protein of antigens in Freund's complete adjuvant. Preliminary studies revealed that 0.4 mg protein dose of pic.-Fr. II per animal in Freund's complete adjuvant was enough to sensitize all the guinea pigs. This antigen dose was therefore chosen for use in the present study. One and two weeks after the immunization the animals were skin tested. Animals were judged to be sensitized when erythema was induced after epicutaneous application of 0.1% picrylchloride in acetone solution.

RESULTS

Table I summarizes the amounts of ϵ -N-trinitrophenyl lysine/protein (mol/g) of antigens used for



Fig. 1. Positive skin reaction of a guinea pig immunized with 0.4 mg of heteroantigenic pic.-Fr. II.

immunization. They are as follows; pic.-Fr. II: 0.55×10^{-4} , heteroantigenic pic.-Fr. II: 0.83×10^{-4} and non-heteroantigenic pic.-Fr. II: 0.39×10^{-4} .

As shown in Table II, pic.-Fr. II and heteroantigenic pic.-Fr. II were capable of sensitizing all the guinea pigs tested, whereas non-heteroantigenic pic.-Fr. II sensitized none of guinea pigs. Fig. 1 indicates the positive skin reaction of a guinea pig immunized with 0.4 mg protein of heteroantigenic pic.-Fr. II.

Pic.-Fr. II contains serum components as revealed by immunoelectrophoresis (4) which may be active as carrier proteins. However, immunization with 0.4 mg of antigenic serum components obtained from pic.-Fr. II by eluting from anti-guinea pig serum-Sepharose immunoabsorbent did not induce contact hypersensitivity to picrylchloride in 5 guinea pigs tested.

DISCUSSION

The authors have attempted to identify carrier substances of picrylchloride contact allergy in guinea

Table I. Number of ϵ -N-TNP lysine/protein of antigens used for immunization

Antigens	ϵ -N-TNP lysine/protein (mol/g)
Pic.-Fr. II	0.55×10^{-4}
Heteroantigenic pic.-Fr. II	0.83×10^{-4}
Non-heteroantigenic pic.-Fr. II	0.39×10^{-4}

Table II. Contact sensitivity induced in guinea pigs by various antigens

Immunizing antigen	Immunizing dose (mg protein)	Positive skin test after immunization	
		1 w.	2 w.
Pic.-Fr. II	0.4	8/10	10/10
Heteroantigenic pic.-Fr. II	0.4	6/10	10/10
Non-heteroantigenic pic.-Fr. II	0.4	0/7	0/7

pigs, by utilizing the in vitro migration inhibition assay as an indicator of a specific immunological reaction for delayed hypersensitivity. As reported previously (4), 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 picrylchloride painted guinea pig epidermis. Further examination revealed that the hapten-protein conjugates were present in Sephadex fraction II, with molecular weights ranging between 10 000 and 200 000.

The rabbit antibody produced against the Sephadex fraction II of guinea pig epidermis specifically absorbed picryl proteins active as hapten-carrier conjugates in the macrophage migration test, and reacted with intercellular substances of guinea pig epidermis (5). These results appear to indicate that carrier substances in contact sensitivity are present in the intercellular space or associated with the cellular membrane.

In the experiment reported in this paper, the heteroantigenic components which were isolated by antibody immunoabsorbent from Sephadex fraction II of picrylchloride painted epidermis induced contact sensitivity to picrylchloride in guinea pigs. Injection of the heteroantigenic serum components or the non-heteroantigenic components of fraction II were incapable of eliciting contact sensitivity. These in vivo findings correlate well with the results of migration inhibition experiments in vitro and present additional prospects that carrier proteins in contact sensitivity are present in the cell surface membrane of epidermis.

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