FORMATION OF CONJUGATES AND HUMORAL ANTIBODIES IN CONTACT ALLERGY TO EPOXY COMPOUNDS

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Humoral antibodies against epidermal proteins of intact skin, hapten, and conjugate formed in the epidermis during the first few hours after contact between skin and allergen are found in guinea-pigs with contact allergy to epoxides.

An increasingly important role is being ascribed to the protein carrier in the induction of contact allergy (6, 7, 10]. It was previously considered that chemical haptens, when falling on the skin, conjugate only with the proteins of the epidermis (9, 12], but in recent years evidence has been obtained, including in the authors' laboratory, that conjugates are formed with globular proteins also [1, 3, 8, 11]. However, it must be remembered that these recent investigations were carried out with a limited number of chemical allergens: usually with 2,4-dinitrochlorobenzene (DNCB), and less commonly with soluble compounds of chromium and beryllium.

Accordingly, in the present investigation on guinea-pigs it was decided to study conjugation between various skin proteins and two epoxy compounds: monoepoxide, an epoxyfurfuryl ester (EF), and the diepoxy resin DEG-1, both of which are widely used in industry and possess well marked allergenic properties [5]. Considering the conflicting nature of published results regarding the production of humoral antibodies in contact allergy [5], the search was made, with the use of conjugates, for antibodies in the sera of guinea-pigs with contact allergy to EF.

TABLE 1. Results of Skin Tests with Haptens on Guinea-pigs Sensitized with Epoxide Conjugates

Material for skin test	Sensitizing conjugate	Total number of animals	No. of animals with reaction points			
			3	2	1	0
10% EF solution	EP plus epidermal protein (3 h)	3	—	—	3	—
	The same (16 h)	4	1	3	—	—
	EF plus globular proteins of dermis	9	1	3	1	4
	–	10	—	—	—	10
10% DEG-1 solution	DEG-1 plus epidermal proteins (3 h)	5	—	3	1	1
	The same (16 h)	3	—	1	1	1
	DEG-1 plus globular proteins of dermis	5	—	—	2	3
	–	5	—	—	—	5

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TABLE 2. Results of Determination of Humoral Antibodies in Sensitized Animals (log of titer, M ± m)

Day of determination after sensitization	Antigen used in PHT					
	EF hapten	EF conjugates			protein carriers	
		epidermal proteins		globular proteins	epidermal proteins	globular proteins
		3 [a]	16 [b]			
21st	0,37±0,03 (13/3)	0,78±0,06 (13/6)	0,21±0,01 (9/1)	— (12/0)	1,12±0,06 (12/8)	— (12/0)
30th	0,56±0,09 (8/3)	1,15±0,12 (8/5)	0,64+0,12 (8/3)	— (8/0)	0,3±0,09 (8/1)	— (8/0)
Control	— (18/0)	0,2+0,02 (13/2)	— (10/0)	— (13/0)	— (13/0)	— (13/0)

Note. Numerator shows total number of sera, denominator number of sera with antibodies.

EXPERIMENTAL METHOD

To obtain conjugates formed in vivowith the epoxide determinant, the method [9] of treating the skin of albino guinea-pigs with 10% solutions of EF and DEG-1 in acetone was used. Globular proteins were isolated from the dermis after 16 h by the method previously used in the writers' laboratory [3]. The epidermal proteins were isolated after 3 and 16 h by hydrolysis of the epidermis by Gel'fon's method [4]. The isolated fractions were used to sensitize 35 guinea-pigs weighing 220-250 g by the following scheme: each animal received 1.5-2 mg protein intraperitoneally, accompanied by 0.2 ml Freund's complete adjuvant intramuscularly, and 1 week later a second intraperitoneal injection of 750 μg/1 mg protein was given without adjuvant. Contact allergy was produced in 13 guinea-pigs by ten applications, each of three drops, of 10% acetone solution of EF to the skin (five times a week).

Skin tests with one drop of EF and DEG-1 solutions were carried out 14 days from the beginning of sensitization with the conjugates or hapten, and the results were assessed 24 h later in points [5]. Antibodies were determined in individual blood sera (on the 21st, and in some animals again on the 30th day of the experiment) by the passive hemagglutination test (PHT) with 200-600 μg epidermal and 5-72 μg globular proteins to 1 ml 2.5% suspension of tanninized sheep's erythrocytes. Antihapten antibodies were detected in the PHT by the method modified in the writers' laboratory [2] with 8% EF. A 0.2% solution of human serum albumin was used as the diluting fluid.

EXPERIMENTAL RESULTS

The results of the skin tests with haptens on giunea-pigs sensitized by the skin protein fractions are given in Table 1. The appearance of positive skin reactions of delayed type indicated the presence of conjugate in the proteins obtained from skin preliminarily treated in vivo with epoxy allergen. Conjugates with epidermal proteins were more active: allergic reactions to hapten developed in all animals to EF and in six of eight animals to DEG-1, while globular conjugates led to the appearance of reactions in only half of the animals. However, in contrast with the results of analogous experiments [3] with DNCB, no antibodies against conjugates, haptens, or the protein carrier could be found in any of the animals. If this result were due to an insufficient dose of conjugate for induction of antibodies, this would nevertheless mean that contact allegry arises more easily after administration of epoxide conjugates formed in homologous skin.

Nevertheless, it was important to use conjugates isolated from the skin in order to search for humoral antibodies in guinea-pigs with contact allergy to epoxides. The scheme of percutaneous sensitization with EF used led to the appearance of clearly defined allergic skin reactions to the hapten in all sensitized animals (mean number of points 2.8 ± 0.01). Humoral antibodies (Table 2), on the other hand, were found in only some of the guinea-pigs and not with all antigens: no antibodies were found against globular proteins

or their conjugates with EF. Antibodies appeared regularly against epidermal conjugates, especially those isolated 3 h after treatment of the skin of the donor EF conjugates with allergen, and theywere found about twice as often as antihapten antibodies.

Antibodies against epidermal proteins also were found, but the dynamics of their appearance was different: they reached a maximum on the 21st day (i.e., a week after the end of the cycle of sensitizing applications), and by the 30th day they had virtually disappeared. Meanwhile the content of antibodies against hapten and conjugate, on the contrary, had increased by the 30th day. The earlier appearance of antibodies against proteins agreed with results in the literature [10] indicating the primary role of the carrier in the induction of allergy to chemical compounds. In addition, the possibility that a true autoimmune process may develop as the result of absorption of breakdown products of the epidermis at the site of the sensitizing applications, under the influence of the primarily irritant properties of EF, likewise cannot be ruled out.

The absence of antibodies against "EF plus globular proteins" conjugates and the lower titers of antibodies against epidermal conjugates isolated after 16 h suggest that conjugation of epoxides in the epidermis takes place during the first few hours after contact between skin and allergen. Unlike DNCB [3], epoxides evidently do not penetrate at all into the dermis as haptens. Absorption of conjugates from the epidermis takes place rapidly, and after 16 h so little of the complete antigen remains in it that the fraction has low activity in the PHT. In the dermis, on the other hand, the conjugate is present in a smaller quantity than the working dose of antigen for the PHT.

Humoral antibodies are formed in very small quantities in contact allergy to epoxy compounds, and the serological diagnosis of this type of allergy, unlike that of allergy to DNCB [3], does not appear promising. However, in the writers' opinion, this conclusion in no way conflicts with the suggestion that antibodies against conjugates play a pathogenetic role in the development of allergic contact dermatitis to epoxy compounds.

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