Skin Test Potency in Guinea Pigs of *Entamoeba histolytica* Antigen (histolyticin) Prepared from Axenically Cultivated Amebas (33578)

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(Introduced by T. Von Brand)

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A skin test as an aid in the diagnosis of amebiasis would be of great value in areas where the disease is endemic. To date antigens employed in skin tests for amebiasis have been prepared from *Entamoeba histolytica* infected tissue or exudate, or from *E. histolytica* grown in the presence of one or more microbial associates (1–3). To obtain the highest degree of specificity and to avoid any possibile influence of tissue or microbial antigens, we have developed a skin test antigen using axenically cultivated *E. histolytica* (4, 5).

Before testing the antigen in humans we attempted to determine its potency in guinea pigs. The use of sensitized guinea pigs for potency testing of skin test antigens is a well documented procedure (6), however, it apparently has not been employed previously for amebiasis skin test antigens used to date. The results of studies carried out preliminary to testing the antigen in humans is presented in this report.

Materials and Methods. The antigen (histolyticin) employed was a water soluble extract of axenically cultivated *E. histolytica* strains 200:NIH and HK-9 prepared as described previously (5). The medium used in the cultivation of strain 200:NIH ameba contained 10% horse serum while that used for strain HK-9 contained 10% rabbit serum.

Hartley strain guinea pigs weighing 350–400 g were divided into seven groups of four each and sensitized with 200:NIH antigen. Groups 1, 3, and 5 were sensitized with 2.8, 1.4, and 0.35 mg of antigen protein (7), respectively. A total of 0.5 ml was administered: 0.1 ml subcutaneously (s.c.) in each footpad and 0.1 ml s.c. in the nuchal area. Groups 2, 4, and 6 received the same amounts of antigens s.c. in the nuchal area in a single 0.5-ml dose. In all cases the antigen was incorporated in complete Freund's adjuvant containing 10 mg of heat-killed BCG/ml and made with equal volumes of aqueous phase and oil phase. The oil phase was made with 35 parts Arlacel A and 65 parts Drakeol 6VR. One additional group received only the Freund's adjuvant without antigen via the footpad route.

Seven weeks after sensitization the animals were challenged intradermally (i.d.) with 1.25, 5, and 20 μg of antigen protein in a 0.1-ml volume. Areas of erythema were recorded after 24 hr. Forty-eight hr after skin testing the guinea pigs were exsanguinated. Antibody determinations were made by means of hemagglutination (HA) (8) and double diffusion in gel (9). In the studies using antigen prepared from HK-9 strain ameba, the guinea pigs were sensitized with doses of 0.2, 0.4, and 0.8 mg of antigen protein and challenged 7 weeks later with doses of 0.5, 2, and 8 μg of antigen protein.

Results. Guinea pigs injected with histolyticin prepared from strain 200:NIH *E. histolytica* reacted to skin test challenge doses of histolyticin in a typically delayed fashion (Fig. 1). Reactions were maximal at 24 hr after the skin test and were recorded at this time (Table I). The animals that were sensitized by a single nuchal injection (groups 2, 4, and 6) showed larger reactions than animals sensitized with the same dose via the combination footpad–nuchal route (groups 1, 3, and 5). Sensitizing doses of histolyticin ranging from 0.35 to 2.8 mg introduced in-

TABLE I. Intradermal Reaction in Guinea Pigs to *E. histolytica* Antigen[a] (strain 200:NIH).

Group[b] (4 animals each)	Sensitizing dose of histolyticin (mg)	Av reaction size (mm)[2] challenge			
		Control 1:10 dil. medium	Histolyticin (μg)		
			1.25	5	20
1	2.8	6	66	188	318
3	1.4	49	101	284	324
5	0.35	4	45	98	197
2	2.8	92	188	364	551
4	1.4	70	219	292	531
6	0.35	29	208	475	515

[a] Culture medium contained 10% horse serum.

[b] Groups 1, 3, 5 sensitized s.c. via combination footpad–nuchal route; groups 2, 4, 6 sensitized s.c. in the nuchal area.

tranuchally only induced similar reactivity in all animals at a given challenge dose. However, with the combination route of sensitization the 1.4-mg sensitizing dose appeared to be optimal. Challenge injections of culture medium (4) (containing 10% horse serum) diluted 1:10 induced small pale early reactions that had virtually disappeared by 24 hr. A challenge injection of 5 μg of toxoplasmin skin test antigen (6) produced no reaction.

The HA titers (Table II) of the sera from the animals sensitized by intranuchal injection (groups 2, 4, and 6) were higher than from those sensitized by intranuchal and footpad injections (groups 1, 3, and 5). It is interesting also to note that the HA titers were higher in those animals sensitized with 1.4 mg of antigen than in those sensitized with 2.8 mg. Fewer precipitin lines in agar gel were noted in sera from those guinea pigs that were sensitized via the combination route.

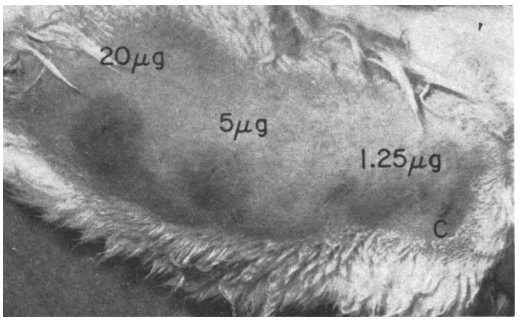

FIG. 1. Appearance of skin reactions in guinea pig sensitized with 2.8 mg of histolyticin and challenged with 20, 5, and 1.25 μg of histolyticin.

Although all guinea pigs initially were approximately the same weight, the guinea pigs sensitized by the combination route weighed an average of 120 g less than those sensitized by the intranuchal route alone when sacrificed 48 hr after skin testing. Severe localized lesions developed, especially at sensitization sites on the footpads, accompanied by ventral alopecia in some of the guinea pigs.

TABLE II. Serum HA Titers from Guinea Pigs Sensitized with *E. histolytica* Antigen (strain 200:NIH).

Group	Sensitizing dose (mg)	Reciprocal titers of individual guinea pigs[a]			
1	2.8	Neg	Neg	Neg	
3	1.4	Neg	162	486	Neg
5	0.35	Neg	Neg	Neg	162
2	2.8	486	486	54	162
4	1.4	486	1458	162	486
6	0.35	162	Neg	Neg	54

[a] 1:18 was the lowest dilution tested.

The results of skin tests using antigen prepared from *E. histolytica* strain HK-9 in which rabbit serum rather than horse serum was used in the culture media are shown in Table III.

Discussion. Entamoeba histolytica antigen

TABLE III. Intradermal Reaction in Guinea Pigs to *E. histolytica* Antigen[a] (strain HK-9).

Sensitizing dose (mg) (4 animals each)	Av reaction size (mm)[2] challenge			
	Control 5 μg of normal rabbit serum protein	Histolyticin (μg)		
		0.5	2	8
0.8	9	133	225	316
0.4	9	83	168	241
0.2	9	119	178	240

[a] Culture medium contained 10% rabbit serum.

(histolyticin) prepared from axenically grown amebas reacted well in guinea pigs sensitized with the antigen emulsified with BCG organisms in water-in-oil. No cross reactions of a nonspecific nature were observed when the guinea pigs were challenged with antigen from another protozoan parasite (toxoplasmin). Although the footpad has been used successfully to sensitize animals to other antigens (10), we found that animals sensitized via the combination footpad–nuchal route developed adjuvant disease similar to that described by Waksman, *et al.* (11) which was accompanied by decreased skin reactivity and lower antibody titers.

Suppression of skin reactivity by sensitizing doses of antigen greater than optimum was observed in the guinea pigs in group 1. This same effect has been observed also in guinea pigs sensitized with BCG (12). Although circulating antibodies were demonstrated by both HA and gel diffusion they did not appear to be related to skin sensitivity.

Skin testing with histolyticin containing small traces of horse serum may result in sensitization to the horse serum. Since antitoxins for human use prepared in horses are still commercially available, a histolyticin prepared from amebas cultivated in the presence of serum other than horse would be preferable. To this end we tested histolyticin made from amebas (HK-9 strain) grown in the presence of rabbit serum, a serum used only infrequently in the production of biologicals for humans. The results were comparable to those using histolyticin prepared from amebas cultivated in horse serum (Tables I and III). The HK-9 antigen was used because of its availability at the time of testing and furthermore because it had been found that the HK-9 strain yielded heavier growth in culture than 200:NIH. The use of smaller sensitizing and challenging doses in this instance was suggested by the results of our work with strain 200:NIH antigen indicating the feasibility of using such doses.

Summary. Delayed skin reactivity has been induced in guinea pigs sensitized with a water soluble extract of *Entamoeba histolytica* (histolyticin) grown axenically, and incorporated in complete Freund's type of adjuvant. These guinea pigs were suitable for dose response determinations of the antigen. Little, if any difference in induced skin reactivity was noted between antigen from organisms cultivated in the presence of horse serum and rabbit serum.

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