

Mechanisms of Defective Delayed Cutaneous Hypersensitivity in Children
with Protein-Calorie Malnutrition

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INTRODUCTION: Cell-mediated immunity (CMI) plays an important role in determining the outcome of infections characterized by intracellular parasitism. Included among these infections are tuberculosis, monilliasis, herpes simplex, chicken pox, and measles, and these pathogens may produce unusually severe morbidity and mortality in malnourished individuals. Several investigators have recently reported defective CMI in African and Indian children with protein-calorie malnutrition (PCM). The possibility thus arises that a defective CMI response may be one immunological mechanism accounting for the severity of these selected infections.

One test commonly employed for assessing cell-mediated immunity is the delayed cutaneous hypersensitivity (DCH) reaction. According to current immunological concepts, DCH is a multistep reaction composed of at least 3 separate components (Figure 1). The sensitization (afferent) limb entails immunization of thymic-derived (T) lymphocytes against a macrophage-processed antigen. The recognition (efferent) limb is characterized by lymphokine production by sensitized T-lymphocytes after they recognize and interact with the antigen deposited in the skin. The inflammatory reaction, probably induced by lymphokines released at the skin site, is ultimately read as a positive DCH skin test. An intact inflammatory response is thus required for full expression of DCH, but it is not immunologically specific in that many irritants other than lymphokines can induce it. A defect in any one of the three components of DCH depicted in Figure 1 may account for the impaired skin test responses previously reported in PCM patients. The present study was designed to elucidate which of these three mechanisms was impaired in children with PCM, and to measure immunological recovery during hospitalization and treatment.

MATERIALS AND METHODS:

Malnourished Patients: The patients, 1 to 5 years of age, were admitted to the research ward of the Anemia and Malnutrition Research Center in Chiangmai, Thailand, where they remained throughout the 70 day study period. On admission the patients were diagnosed as having marasmus (M), marasmus-kwashiorkor (M-K), or kwashiorkor (K), using accepted criteria. Children admitted to the study had primary malnutrition and weighed between 3.0 and 12.0 Kg. All patients were treated for fluid and electrolyte imbalance during the first 7 hospital days and received supplemental vitamins and minerals. Virtually all patients on admission had bacterial infections and received appropriate antibiotic therapy until the infections cleared. Children were placed on gradually increasing calorie and protein milk-base formula diets, to a maximum intake of 175 calories-4 gm. protein/kg/day, which was started by hospital day 30 for all patients. Nutritional repair was judged complete when the clinical stigmata of PCM had disappeared, and the abnormal laboratory tests, such as low serum albumin, had returned to normal levels. Virtually all patients in this study had clinically recovered by 4 to 8 weeks after admission.

Dinitrofluorobenzene Skin Tests: 2,4-Dinitrofluorobenzene (DNFB), a potent skin irritant and contact allergen, was diluted in 90% acetone. A sensitizing and inflammatory dose of 2000 μ g was applied to the

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forearm, allowed to dry, and protected for 24 hours with an occlusive dressing. At 48 hours, the degree of non-specific inflammation at the DNFB contact site was graded as follows: Negative=no reaction or mild induration and/or erythema; Positive=induration, erythema, and vesicle or bulla formation.

At varying time intervals from 14 to 70 days after application of the 2000 μg DNFB sensitizing dose, DCH responses were elicited by applying 100 μg DNFB to the opposite forearm. The degree of inflammation at the 100 μg test dose site was graded 48 hours later. Presence of erythema, induration and vesiculation was considered skin test positive. No reaction or presence of only induration and/or erythema was considered skin test negative. These rigorous criteria for a positive skin test were selected in order to minimize possible confusion with the mild non-specific cutaneous inflammatory reactions occasionally produced by 100 μg DNFB.

Candida Albicans Skin Tests: 0.1 ml of a 1:100 dilution of *Candida albicans* skin test antigen (Hollister-Stier Laboratories, Spokane, Washington) was injected intradermally into the forearm. Reactions measuring greater than 5 mm induration at 48 hours were considered positive.

Peripheral blood lymphocyte counts, performed on the first 25 patients admitted to the study, were within normal limits.

RESULTS:

Cutaneous Inflammatory Response: Patients, presumed not to be immune to DNFB, were exposed to a 2000 μg dose on admission; only 13% had responded with a marked inflammatory reaction when measured 2 days after exposure (Table 1). More patients had an inflammatory reaction when first tested on day 15 (during clinical recovery), while the largest percentage responded on day 56 (after clinical recovery). The difference in the percentage of positive inflammatory responses between day 1 and day 56 is statistically significant ($\chi^2 = 9.4$, $P < .01$).

Evaluation of the Sensitization Component: We investigated the status of the sensitization component of the DCH reaction by attempting to sensitize 3 groups of patients against 2000 μg DNFB. These patients, exposed to DNFB for the first time either on day 1, 15, or 56, were all skin tested for possible sensitization on day 70. The results are shown in Table 2. The difference between the low proportion of patients sensitized on day 1 and the high proportion sensitized on day 56 was statistically significant ($\chi^2 = 4.7$, $P < .05$). The data also suggest that the impaired sensitizing component apparently existing on day 1 may not be repaired until after day 15. It was of interest to examine the relationship existing between DNFB inflammation and subsequent sensitization. Accordingly, these two reactions were examined in each of the 23 patients listed in Table 2, and the comparisons are shown in Table 3. Of the 10 patients who were inflammation positive, 7 were subsequently found to have been sensitized. A similar direct association exists for the negative reactors, so that of the 13 inflammatory negative patients, 10 failed to be sensitized. However the numbers of patients in each group are small and the differences do not reach statistical significance ($\chi^2 = 3.27$, $.05 < P < .10$).

Evaluation of Immunological Recall: Recall in DCH is a response characterized by recognition and interaction of sensitized lymphocytes with specific intracutaneous antigen which leads to inflammation at the skin site (Fig. 1). Recall was tested with *Candida albicans* skin test antigen. We assumed most patients had been naturally immunized against candida prior to their illness. This assumption is based on the observation that 70% of well-nourished Thai children over the age of 1 year are candida skin test positive.

Only 14% of PCM children were candida skin test positive on admission (Table 4). Skin test negative patients were retested on day 29 and 70 and an increasing percentage had converted to positive on these 2 days. Repeated skin tests with candida antigen did not induce DCH to candida in 7 well-nourished skin test negative children, thus indicating that the increasing number of positive skin tests during hospitalization was not simply due to immunization by skin test antigen. The differences in numbers of skin test positive

responders on days 29 and 70 compared to day 1 was statistically significant (McNemar test corrected for continuity; chi-square = 9.1, P < .01 for day 70).

SUMMARY & CONCLUSIONS: We attempted to evaluate the three principal components of the delayed cutaneous hypersensitivity (DCH) response in children hospitalized with protein-calorie malnutrition. The lymphocyte sensitization component (afferent limb) and the cutaneous inflammatory reaction were evaluated with the contact allergen and skin irritant, dinitrofluorobenzene (DNFB); the immune lymphocyte recognition component (efferent limb) was tested with *Candida albicans* skin test antigen. The results indicated that 60 to 80% of PCM patients on admission had malfunction of both their afferent limb and their cutaneous inflammatory response. Two patients with intact inflammatory responses to DNFB on admission, but with negative candida skin tests, later displayed positive candida skin tests, suggesting that the efferent limb was defective on admission. Except for these two patients, the impaired inflammatory reaction precluded independent evaluation of the efferent limb *in vivo*. The DCH components were intact in most patients after nutritional repair, 1 to 2 months later.

Table 1. Cutaneous Inflammatory Response to 2000 μg Dinitrofluorobenzene

Day Tested	No. of Patients	Skin Response		
		Positive	Negative	Percent Positive
1	30	4	26	13%
15	5	3	2	60%
56	8	6	2	75%

Table 2. Attempt to Sensitize PCM Patients against 2000 μg Dinitrofluorobenzene

Day Sensitizing Dose Applied	No. of Patients	Skin Test Response*		
		Positive	Negative	Percent Positive
1	10	2	8	20%
15	5	1	4	20%
56	8	7	1	88%

* Skin test dose of 100 μg DNFB applied on day 70 and read on day 72.

Table 3. Correlation of Cutaneous Inflammation and Sensitization by 2000 μ g Dinitrofluorobenzene

Sensitized*	Number of Patients Who Were Inflammation+	
	Positive	Negative
Yes	7	3
No	3	10

* DNFB skin test either positive or negative on day 70.

+ Inflammatory response graded 2 days after application of 2000 μ g DNFB on hospital day 1, 15, or 56.

Table 4. *Candida albicans* Skin Test Recall Response

Day Tested	Number of Patients	Skin Test Response*		Accumulated Percent Positive
		Positive	Negative	
1	14	2	12	14%
29	12	8	4	72%
70	4	3	1	92%

* Skin tests read 2 days after 0.1 ml 1:100 antigen injected intradermally (positive = > 5 mm induration).

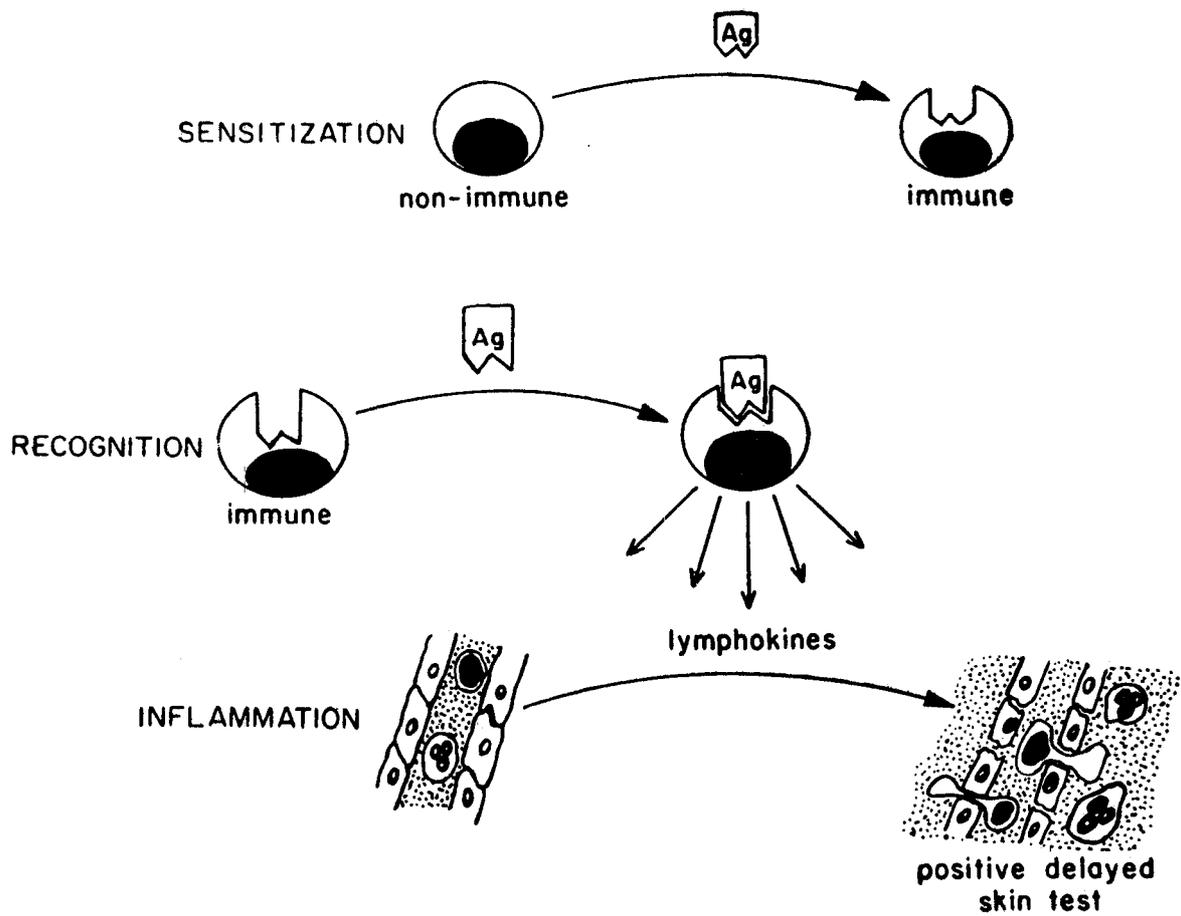


Figure 1. Schematic Drawing of Components of Delayed Cutaneous Hypersensitivity.