# RECOVERY OF IMMUNOLOGICAL COMPETENCE IN HOSPITALIZED CHILDREN WITH PROTEIN-CALORIE MALNUTRITION

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## Introduction

Numerous studies have established that in the malnourished host, independent of intercurrent infection, there is depressed cell-mediated immunity (CMI) (Scrimshaw, et. al. 1968; Smythe, et. al. 1971). This is manifested by both absent delayed hypersensitivity skin responses and depressed general inflammation. As malnutrition becomes severe, the host's circulating lymphocytes decrease and lymphoid tissue especially the thymus becomes atrophied. The discovery of a specific defect in the lymphocyte population has continued to be elusive.

Few studies have continued to follow a child's immunological status more than 4 months after recovery from severe malnutrition. The Children's Nutrition Research Unit (CNRU) permits prolonged follow-up, during which we hope to assess the duration and degree of immunologic depression. Therefore, this protocol will outline a survey of parameters of both the afferent and efferent limbs of the immune response and the general inflammatory response.

Studies in Calcutta and Hyderabad (Bang 1974; Hyderabad 1974) have shown that the percentage of circulating T cells is depressed in kwashiorkor and marasmus. The latter study suggested this depression persisted for years after recovery from protein-calorie malnutrition (PCM). Whether reduced numbers of circulating T cells reflects diminished function can be answered by two independent techniques (Catalona, et. al. 1972; Miller and Levis, 1973). The test for lymphocyte function is the ability to become sensitized to the chemical dinitrochlorobenzene (DNCB). Previous studies in Calcutta and Chiang Mai (Bang 1974; Edelman et. al., 1973) suggested that "tolerance" to DNCB was established if the

DNCB was given to a child in a severely malnourished state. If this is true, repeated skin doses of DNCB would not sensitize the patient. We plan to repeatedly challenge the patient with DNCB, if the initial sensitization attempt fails. Such skin test reactivity can be correlated with the ability of the patient's lymphocytes to recognize DNCB antigen in vitro (Miller & Levis, 1973). In this test, DNCB is coupled to autologus or allogeneic peripheral blood leukocytes, forming a complex DNCB-antigen, that induces transformation in lymphocyte cultures from subjects sensitized to DNCB. If recognition is intact, then the defect is not in lymphocyte sensitization (i.e. is not immunological tolerance) but in the peripheral skin stage of expression of hypersensitivity.

A cytotoxicity technique will be used to measure the ability of lymphocytes sensitized to vaccinia virus to kill chick fibro blast cells infected with the same virus (Gardner, et. al. 1974). Thus, after smallpox (vaccinia) vaccination, the presence of specifically sensitized lymphocytes are expected in the bloodstream, and their absence would indicate failure of sensitization.

Methods and Procedures

Subjects: All subjects will be among those admitted to the CNRU. The age of the subjects will range from ten months to five years. Recent serious or prolonged infections or renal disease is cause for exclusion from this particular study. On admission as feeding is commenced, physical exam., appropriate lab tests, X-ray evaluation when necessary, nutrition profile and parental permission for the study will be obtained. The nutrition profile includes anthropomorphic measurements (height and daily weight), serum proteins, hematocrit and white blood cell count. The anthropomorphic measurements are to be repeated at weekly intervals and the lab tests at bi-weekly to monthly intervals. Children will be classed as marasmic, marasmic-kwashiorkor and kwashiorkor. This classification is designed on a simple scoring system which correlates serum-albumin and physical signs such as oedema, dermatosis, hair change and hepatomegaly (MacLaren and Pellett 1970).

The study begins prior to feeding, with the routine admission medical assessment, but feeding will not be delayed.

On day one, three "ubiquitous" antigens will be applied as skin tests: streptokinase - dornase, Candida and PPD, and in addition, the first sensitizing dose of a unique antigen. The "unique" test antigens are DNCB and keyhole limpet hemocyanin (KLH), in common use for at least five years for experimental purposes. The dosages and expected responses to these antigens are well-known (Catalona & Taylor 1972). The sensitizing dose of DNCB is 2 mg and the challenge dose is 50 mcg. Sensitizing dose of KLH is 500 mcg and the challenge dose is 50 mcg. All time intervals between challenge and sensitizing doses will be 20 days.

The plan will randomly allocate children into one of five groups, illustrated in the table below.

S = Sensitizing Dose  C = Challenging Dose

| | S-1 | C-1 | S-2 | C-2 | S-3 | C-3 |
|---|---|---|---|---|---|---|
| Group 1 | DNCB | DNCB | DNCB | DNCB | DNCB | DNCB |
| Group 2 | DNCB | DNCB | KLH | KLH | DNCB | DNCB |
| Group 3 | KLH | KLH | DNCB | DNCB | KLH | KLH |
| Group 4 | KLH | KLH | KLH | KLH | KLH | KLH |
| Group 5 | — | — | DNCB | DNCB | DNCB | DNCB |

Each group will have an S-4/C-4 phase identical to S-3. Groups 1 and 4 will establish whether repeated sensitization either eventually elicits a response by recruiting from a growing T cell population or whether T cells have been rendered unresponsive. Groups 2 and 3 check whether unresponsiveness is specific for the first antigen. Group 5 checks for consistency of the return to normalcy (capable of being sensitized) by 20 days. This group can be expanded to include different waiting periods before the first sensitization. Fifty subjects followed for five to six months will complete the study.

An <u>in vitro</u> test will be used to determine the on-set of recognition by lymphocytes sensitized to the antigen DNCB. It will also be possible to measure memory to DNCB by taking samples from sensitized subjects, three or more months after exposure. The individual may or may not be subjected to a challenge dose of DNCB at that time.

Lymphocytes from blood group O Rh- volunteers will be coupled to DNCB. The lymphocyte - DNCB complex will then be introduced to a lymphocyte culture obtained from test subjects exposed to a sensitizing dose of DNCB. If the lymphocytes are sensitive to DNCB blastogenic transformation will occur. The degree of transformation can be measured by the incorporation of radioactive thymadine in 38 to 72 hour lymphocyte cultures. One ml samples will be taken on admission; and 3, 6, 9, 12 and 20 days after each sensitizing dose.

The cytotoxicity test measures the ability of sensitized lymphocytes to translate recognition into an appropriate "killer cell" response (Gardner, et. al., 1974). This is one of the few tests which measures only T cell function and does not depend on B cell interaction.

Confluent monolayers of chick fibroblast tissue cultures which have incorporated Chromium 51 ($^{51}Cr$) will be infected with vaccinia virus. Lymphocytes, separated from hepranized whole blood on a ficalhypaque gradient, from individual vaccinated with vaccinia virus will be layered on to the infected cell sheet. Lymphocytes which have been sensitized to vaccinia will destroy the virus infected cells releasing chromium 51 into the media. The degree of lysis is measured by assaying the amount of $^{51}Cr$ released from the cell culture. The percentage of $^{51}Cr$ released from the target cells in each culture will be calculated using the formula: counts in supernatant/counts in supernatant and counts in cells X 100. These results will then be compared to proper controls.

Subjects, who have depressed skin reaction to DNCB and KLH, will be selected for this study and compared with individuals who have normal skin reactivity to the two antigens.

In addition to the delayed hypersensitivity, general assessment of the CMI response will be measured using the following techniques:

1. Quantitative counts of the small lymphocytes as an indicator of gross cellular deficits.

2. Blastogenic transformation of peripheral leucocytes by PHA as measured by radioactive thymadine

incorporation in 38 hour cultures (Patke, Good 1970).

3. Rosette formation by peripheral lymphocytes with sheep red blood cells (Brain, et. al. 1970, Pincus, et. al. 1970).

Benefits

Justification for this study is to give some additional insight into the causes of the depression of CMI response in severely malnourished children. New information will be obtained concerning the ability of lymphocytes, from PCM subjects, to recognise new antigens and to translate recognition into an appropriate response such as the "killer cell" response. It may also be possible to determine if "tolerance" does occur in children when new antigens are introduced to the child during the acute periods of depressed CMI response. We are looking for the most vulnerable link in the chain of events leading to immunologic competency. The identification of this link may help in designing more rational, efficient refeeding programs, since many malnourished children in feeding centers die of overwhelming infections.

Guarantees

The risks to the patient are restricted to problems associated with the skin tests. DNCB is capable of eliciting a strong vesiculating inflammatory response in the skin, with itching and discomfort which can be ameliorated by topical steroids.

Parental permission will be obtained prior to inclusion into the study. The parent may withdraw the child from the study at any time without compromising the child's further care. Direct benefit may occur through closer medical surveillance.

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