ADOPTIVE TRANSFER OF CELL-MEDIATED IMMUNITY TO *TRICHOPHYTON MENTAGROPHYTES* IN GUINEA PIGS

VIJAYA MANOHAR, G. RAMANANDA RAO AND M. SIRSI

Mycology and Cell Biology Laboratory, Indian Institute of Science, Bangalore 560 012

ABSTRACT

Sensitized lymphocytes and their cell-free extract were shown to transfer cell-mediated immunity (CMI) to *Trichophyton mentagrophytes* in guinea pigs. Significant levels of delayed-type hypersensitive reactions (DTH) were also observed against antigens from heterologous dermatophytes, *viz. Trichophyton violaceum, Trichophyton rubrum* and *Microsporum canis* suggesting the cross reactivity.

INTRODUCTION

THE defense mechanism in response to infectious agents involves the differentiation and proliferation of B-cells (antibody-mediated immunity, AMI) or T-cells (cell-mediated immunity, CMI). In recent years, operation of these immune mechanisms in a number of infectious diseases, autoimmune processes, rejection of homografts and neoplastic tissue is well documented[1-3]. Resistance to some of the systemic mycotic infections like histoplasmosis and coccidioidomycosis has been attributed to the operation of both the mechanisms[4,5]. Although humoral antibodies and skin hypersensitive reactions have been reported in patients with ringworm infections, the role and nature of immune mechanism are not clearly understood[6]. Our studies on the immunity in experimental dermatomycoses in guinea pigs have demonstrated a low level of AMI and a high level of dermal sensitivity of delayed-type, implying the role of CMI during the recovery from infection (unpublished data).

With the exploration of cellular and biochemical aspects of immune mechanisms, the role of "transfer factor" in transferring the immunity has been extensively studied[7], and its clinical value in a number of diseases recognized[8,9]. In the present communication, the transfer of delayed hypersensitivity to *Trichophyton metagrophytes* in guinea pigs, by sensitized lymphocytes and a cell-free extract from them are reported.

MATERIALS AND METHODS

Immunization of guinea pigs.—The mycelium from a 7-day old culture of *Trichophyton mentagrophytes* was washed with acetone at — 15° C and dried under vacuum. The acetone-dry powder was extracted with 0·1 M phosphate bufferred saline (PBS) of pH 7·2. The extract was dialyzed, and its protein content was estimated by the method of Lowry *et al.*[10]. From the soluble extract, the proteins were separated by the method of Schneider[11], and lipopolysaccharide by the method of Westphal *et al.*[12]. The guinea pigs (350–400 g body wt.) were immunized by injecting the soluble extract (12 mg protein) from *T. mentagrophytes* with Freund's Complete Adjuvant in three doses at weekly intervals. Two weeks after the last dose of antigen, the animals were tested for delayed-type of hypersensitive reactions (DTH).

Transfer of CMI to non-sensitized guinea pigs.—The spleens from the animals showing a positive skin reaction (14–15 mm of induration and edema) were collected aseptically, minced and squashed in PBS. The suspension was filtered through a sterile stainless steel mesh and the cells were washed thrice with PBS and suspended in the same at a cell density of 90 × 10^6 lymphocytes per ml. A cell-free extract was prepared from lymphocytes (180 × 10^6 cells in 2 ml) by repeated freezing and thawing for 10 times. Either whole cells or cell-free extract (0·2 ml) were administered subcutaneously into the clipped and shaved flanks of non-sensitized guinea pigs. After an incubation period of 20 hr, the animals were challenged intradermally with 0·1 ml of soluble extract or protein fraction of lipopolysaccharide fraction from *T. mentagrophytes*. The cross reactivity with the related dermatophytes was examined by injecting the soluble extracts from *Trichophyton violaceum, Trichophyton rubrum* and *Microsporum canis*. The hypersensitive reactions developing 24 hr later were recorded.

RESULTS AND DISCUSSION

Guinea pigs injected with sensitized lymphocytes showed a positive DTH reaction on challenge with a soluble extract prepared from *T. mentagrophytes* (Table I). A similar reaction was also seen in animals which received the lymphocyte-extract.

The soluble extract contained both proteins as well as lipopolysaccharides. While the protein fraction elicited reactions comparable with soluble extract, no reactions were observed with lipopolysaccharide fraction.

TABLE I

Adoptive transfer of CMI to T. mentagrophytes *in guinea pigs*

Antigenic material	Skin reaction (in mm) in animals injected with	
	Lympho-cytes	Lympho-cyte-extract
T. mentagrophytes		
Soluble extract (20 μg)+	12	11
Protein fraction (20 μg)	11	10
Lipopolysaccharide fraction (20 μg)++	0	0
T. violaceum		
Soluble extract (20 μg)+	10	not tested
T. rubrum		
Soluble extract (20 μg)+	8	not tested
M. canis		
Soluble extract (20 μg)+	9	not tested

+ In terms of protein ++ In terms of hexoses

Soluble extracts prepared from other dermatophytes like *T. violaceum, T. rubrum* and *M. canis* also showed significant levels of DTH reactions suggesting cross reactivity among these fungi. But the degree of reactions do differ markedly as compared with the homologous system.

The present findings reveal the occurrence of a "transfer factor" in lymphocytes sensitized to *T. mentagrophytes* as observed in histoplasmin and coccidioidin sensitivity[13]. The chemical nature of this "transfer factor" and its *in vivo* efficacy in curing experimental dermatomycoses are being investigated.

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