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Coupling of proteins to liposomes and their role in understanding delayed type of hypersensitivity in human and mice

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Abstracts. Liposome-coupled lepromin was found to elicit a 3-week skin reaction in leprosy patients similar to that elicited by whole *Mycobacterium leprae.* The present study suggests that the presentation of antigens in a specific orientation is necessary for evoking delayed type hypersensitivity response in humans.

Keywords. Liposome-coupled lepromin; delayed type hypersensitivity.

Introduction

Lepromin test is a delayed type of hypersensitivity (DTH) test which is elicited as skin reaction after intradermal inoculation of lepromin. When whole *Mycobacterium leprae* remain as an integral component in lepromin, it elicits two types of skin reaction—early, Fernandez reaction (24–48 h reaction) and late, Mitsuda reaction observed after 3 to 4 weeks (Rees, 1964). However, when *M. leprae* are sonicated and the soluble components are injected, they evoke only an early reaction (Sinha *et al.,* 1979; Smelt *et al.,* 1981). Quite likely, if *M. leprae* soluble antigens are also made to present antigens in a manner mimicking the antigen presentation by *M. leprae* to the host then they may also evoke a late reaction. To investigate this possibility, we have coupled *M. leprae* soluble antigens to liposomes and used these as skin test antigens.

Materials and methods

Antigens

(i) *M. leprae* soluble antigens [MLSA, prepared by the described method (Smelt *et al.,* 1981)] was a kind gift from Dr. R. J. W. Rees, NIMR, London.

(ii) Standard Dharmendra lepromin (DL) was prepared as described (Sengupta *et al.,* 1979).

Antisera

Commercially available monoclonal antibodies against pan, helper and suppressor T cells, Langerhans cells, la antigens were obtained from Becton Dickinson, USA

Abbreviations used: DTH, Delayed-type hypersensitivity; MLSA, *M. leprae* soluble antigens; DL, Dharmendra lepromin; BSA, bovine serum albumin.

and Ortho Pharmaceuticals. FITC conjugated sheep antimouse Ig $F(ab')_2$ used as second antibody was obtained from New England Nuclear, Boston, USA.

Liposomes

Liposomes were prepared from egg lecithin (45 μM), cholesterol (45 μM) and gangliosides (9 μM) in 2 ml borate-buffered saline (pH 8·4) by sonication and fractionation (Gupta and Bali, 1981).

Liposome coupling with antigens

The soluble protein of MLSA was covalently coupled to the liposome surface according to a standard method (Heath *et al.,* 1981).

Skin biopsies

The patients with leprosy were selected from the outpatient clinic of Central JALMA Institute for Leprosy, Agra. They were intradermally injected with DL (1×10^6 bacilli in 100 μl); MLSA (1 μg in 100 μl); MLSA coupled to liposomes (1 μg in 100 μl); liposomes in saline (100 μl). The diameter of skin indurations were recorded in mm at 48 h and 21 days after injection. An induration of 5 mm or more was regarded as positive for the 21-day skin reaction. For immuno-fluorescence studies, the biopsies were collected from a separate group of patients.

DTH in mice

BALB/c mice were sensitized by intracutaneous injection of 2 mg of bovine serum albumin (BSA) with incomplete Freund's adjuvant at the base of the tail. After 28 days the DTH reactions were elicited by foot pad inoculations with 0·03 ml of BSA solution (600 μg) and liposomised BSA solution (15 μg) respectively. The administered dose of free BSA was selected according to published reports whereas the dose of liposomised BSA was selected arbitrarily.

Results and discussion

Skin reaction in 10 borderline tuberculoid patients at 48 h and at 21 days evoked by different antigens are depicted in table 1. While MLSA elicited only 48 h reaction, DL elicited mostly 48 h and 3-week reactions. In all these patients liposomised antigen elicited both early and late reactions similar to those in DL. Control liposomes induced insignificant reactions at 48 h in 4 patients. All 12 lepromatous leprosy patients showed negative skin reaction to these antigens.

Elicitation of a 3-week skin reaction with liposome-coupled lepromin supports the hypothesis that the presentation of eliciting antigens in a specific orientation is required for the development of late reaction. To find out whether the skin reactions induced by liposomised MLSA were similar to those induced by DL, indurations were biopsied, subjected to histological and immunohistological

Table 1. Skin reactions with different antigen preparations in borderline tuberculoid leprosy patients.

	Antigens							
	MLSA (10 μg/ml)		DL (10^7 bacilli/ml)		Liposomised MLSA		C	
Patient	48 h	3 weeks	48 h	3 weeks	48 h	3 weeks	48 h	3 weeks
1	29	0	28	10	28	10	0	0
5	20	0	25	7	27	7	5	0
3	28	0	22	0	32	0	5	0
4	0	0	0	6	15	0	5	0
5	23	0	18	0	30	0	5	0
6	12	0	12	0	13	0	0	0
7	15	0	12	5	12	5	0	0
8	26	0	24	12	27	6	0	0
9	30	0	22	5	19	10	0	0
10	18	0	16	5	14	6	0	0

Numbers in this table represent the diameter of skin indurations in mm.
C, Control liposomes.

analysis. It was noted that the 48 h skin reaction yielded a classical DTH picture, a predominant lymphocytic infiltration interspersed with a few polymorphonuclear cells. The 21-day reaction with liposomised MLSA also revealed a histological picture resembling the DTH reaction induced by DL with focal collection of epithelioid and giant cells (table 2).

Table 2.. Cellular characteristics of the DTh skin reactions

	DL lepromin		Liposomised MLSA	
Cell type	48 h	3 weeks	48 h	3 weeks
Ly[a]	+	+	+	+
PMN[b]	+	−	+	−
EC[c]	−	+	−	+
Ratio Leu 3a+/ T8+ (mean±SD)	1·18 ± 0·18	4·0 ± 1·5	1·21 ± 0·25	3·12 ± 1·3
Distribution Ly Leu 3a+	No pattern	Diffused in EC granuloma	No pattern	Diffused in EC granuloma
T8+	No pattern	Periphery of EC granuloma	No pattern	Periphery of EC granuloma
T6+ cells	+	+	+	+
Macrophages	−	Ia+, T6+	−	Ia+, T6+

[a] Ly, Lymphocyte; [b]PMN, polymorphonuclear leucocytes; [c]EC, epithelioid cell.

Immunohistologically, the 48 h skin reaction induced by liposomised MLSA was again akin to, the 48 h reaction induced by DL with infiltration of activated T lymphocytes expressing Leu 4, Leu 3a, OKT 8 and la like antigens. The ratio of Leu 3a + /OKT8+ cells was 1·21 ± 0·25. These results have confirmed the earlier findings in lepromin reaction (Narayanan *et al.,* 1985). In the 21-day skin reaction

evoked by liposomised MLSA the granuloma cell infiltration was similar to the 21-day skin reaction induced by DL. The predominant lymphocytes in these granulomas were activated T cells expressing Leu 4 and la like antigens. Epithelioid cell granulomas were surrounded by Leu 4+ positive cells. OKT8 + cells were lesser in number. The ratio of Leu 3a + /OKT8+ cells were 3·2 ± 1·3. Leu 3a + cells were found scattered amongst the epithelioid cells. OKT8 + cells were localised as a 'ring' in the periphery of the granuloma (table 2).

This study substantiates the fact that covalent liposomisation of protein molecules of MLSA presented the antigens in a manner similar to those in integral *M. leprae.* It was, however, not possible to define the molecules involved in the development of DTH reactions.

As the above *in vivo* experiments are very difficulty to perform on patients because of the difficult in obtaining their consent, an attempt was further made to establish an experimental model in mice using a simple protein like BSA. It was noted that liposomised BSA was capable of inducing larger 48 h reaction than BSA alone in BSA-sensitized mice. This was despite the fact that the administered dose of liposomised BSA was 40 times lesser than that of free BSA, indicating that liposomization enhanced the immunogenicity of BSA. However liposomised BSA did not elicit a 3-week reaction in mice. This could be due to the differences in nature of antigen or the host involved. Further studies on these lines are needed.

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