INTERFERON PRODUCTION IN MICE BY CELL WALL MUTANTS OF SALMONELLA TYPHIMURIUM. II. EFFECT OF PURIFIED GLYCOLIPIDS FROM S AND Re CHEMOTYPES

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A previous publication from this laboratory (Youngner and Feingold, 1967) showed that the interferon response elicited by Salmonella typhimurium mutants in mice is not dependent on the presence of a complete cell wall glycolipid. Whole killed cells of a mutant (G30/C21) (the glycolipid of which contains only 2-keto-3-deoxyoctonate and lipid) are indistinguishable in their interferon-stimulating ability from the cells of the wild type (LT-2) in which the glycolipid possesses a complete O antigen with polysaccharide side chains.

In this report we present data which show that the purified glycolipids from wild-type S. typhimurium and from the deficient mutant G30/C21 are identical in interferon-stimulating ability.

Materials and Methods

Salmonella typhimurium wild-type, strain LT-2, and mutant strain G30/C21 (which lacks side chains, basal core, and heptose in its cell-wall glycolipid) were obtained through the courtesy of Werner Braun. The organisms were grown for 18 hr at 37° with shaking in Todd-Hewitt broth. The cells were collected by centrifugation, washed twice in physiological saline, and dried with acetone. Glycolipid was extracted from the acetone-dried cells with phenol-water by the method of Westphal and Jann (1965) and purified by

repeated ultracentrifugation as described by Kim and Watson (1967) followed by cetavlon fractionation (Westphal and Jann, 1965) to remove nucleic acids.

The expected carbohydrate pattern was obtained from hydrolyzates of the glycolipid of strain LT-2 by paper (Volk, 1966) as well as by gas-liquid (Braude, personal communication) chromatography.

Analysis of the glycolipid obtained from G30/C21 showed that none of the carbohydrate constituents of the O chains or the basal core was present in the hydrolyzate. 2-keto-3-deoxyoctonate in both glycolipids was demonstrated by the method of Osborn (1963).

Interferon was produced in mice injected intravenously with 0.1 ml of the appropriate amount of glycolipid and bled 2 hr after inoculation. Pooled plasmas from 10 mice were tested for interferon content in L-cell cultures by a plaque reduction method with vesicular stomatitis virus as the challenge virus. The interferon assay has been described in detail (Youngner and Stinebring, 1966).

Results

Groups of mice were injected intravenously with 50, 5, or 0.5 μg of glycolipid from either the wild-type (LT-2) or the deficient mutant (G30/C21). Blood samples were obtained 2 hr later. Similar injections were given to uninfected mice and to mice infected 2 weeks earlier with M. tuberculosis strain BCG. Infected mice were employed since it has been demonstrated that infection with tubercle bacilli enhances the reactivity of mice to endotoxin when measured by the interferon response (Youngner and Stinebring, 1965). This increased production of interferon in BCG-infected mice is characteristic of the "endotoxin-type" of response and does not occur with viral stimuli.

The interferon responses obtained with the two glycolipid preparations injected into uninfected and infected mice are shown in Table 1. There was no significant difference in the dose responses obtained with the two glycolipid preparations. It can be seen that, characteristically, more interferon was produced in BCG-infected mice than in uninfected mice.

TABLE I

Interferon production in mice by cell wall glycolipids from *Salmonella typhimurium* mutants

S. typhimurium Strain and (Chemotype)	Dose of glycolipid (μg)	Interferon titer of plasma at 2 hr in Uninfected mice	Interferon titer of plasma at 2 hr in BCG-infected mice
LT-2 (S)	50	600	1,350
	5	350	1,750
	0.5	70	600
G30/C21 (Re)	50	450	1,600
	5	100	1,200
	0.5	60	110

Discussion and Summary

The results reported extend our previous finding (Youngner and Feingold, 1967) that the interferon response elicited in mice by killed cells of *S. typhimurium* mutants is not dependent on the presence of a complete cell wall glycolipid. As far as interferon production in mice is concerned, purified cell wall glycolipid from a heptoseless mutant (G30/C21) is indistinguishable from the glycolipid of the wild-type organism, which is a complete O antigen with polysaccharide side chains. On the basis of these results, further analysis is being made of the roles of 2-keto-3-deoxyoctonate and of lipid A in the production of interferon in mice.

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