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Endotoxin-like Activities in *Myxococcus xanthus*

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Abstract. Vegetative cells as well myxospores of *Myxococcus xanthus* have shown anticomplementary activity and the capacity to be used as active agents in the skin preparation of the Shwartzman reaction and in its intravenous induction. These endotoxin-like properties were not extractable by the hot phenol–water methods. Our results suggest the presence of a lipid A analog in both vegetative cells and myxospores, and emphasize the difficulty of lipopolysaccharide detection; this is perhaps a consequence of a developing associated change in polysaccharide moiety of the myxobacterial lipopolysaccharides; this may be the basis of the special immunomodulation pattern shown by *M. xanthus* myxospores.

The vegetative cells of myxobacteria resemble other Gram-negative species in containing lipopolysaccharide (LPS) in their walls; however, it could not be detected in myxococcal spores by standard methods [11, 17]. Although changes in the polysaccharide moiety during myxobacteria sporulation have been reported [18], the situation is unclear at the present in relation to possible changes in the lipid A content of *Myxococcus* versus vegetative cells [11, 17].

For many years, attempts have been made to determine which component of the LPS molecule is responsible for biological activity. Most of the biological effects of LPS have been associated with the lipid A moiety [15].

In previous work we demonstrated that the inoculation of mice with myxospores of *Myxococcus xanthus* caused modulation of the humoral and cellular immune responses against unrelated antigens, and we found that the modulation pattern of the antibody-forming cell response of mice to *M. xanthus* myxospores was quite different from that attributed to the LPS from Gram-negative bacteria [16].

The object of the present paper was to study the presence of two endotoxin-like activities, the anticomplementary activity and the Shwartzman reaction in both vegetative cells and myxospores of *M. xanthus*.

Materials and Methods

Animals. Rabbits weighing 2.5–3.0 kg were purchased locally.

Microorganisms. *Myxococcus xanthus* CCM 3494 was grown with aeration at 30°C in liquid medium CT [3]. Myxospore formation was induced by the addition of glycerol to a final concentration of 4.6% (wt/vol) as described by Dworkin and Gibson [4]. The myxospores were separated from the vegetative cells by exposure of the suspension to sonic vibration [5].

Strain K-12 of *Escherichia coli* was grown in trypticase soy broth at 35°C.

Extraction methods of LPS. By extraction with hot phenol–water, fractions from the aqueous and the phenol phases were prepared from whole myxospores of *M. xanthus*, and the aqueous phase was purified according to Lüderitz et al. [13].

Anticomplementary test. The test for the interaction of LPS with complement was a modification of the method described by Moreno et al. [14].

Three bacterial suspensions were prepared with *M. xanthus* vegetative cells and myxospores, and *E. coli* cultures, respectively. The suspension was adjusted to 10^8 cells/ml, and 0.75 ml was used in each assay. Also, 0.1 mg of both the phenol and aqueous phases was dissolved in 0.75 ml of the barbital buffer (pH 7.4) to be tested. This buffer was also used to prepare the bacterial suspension. Bacterial suspensions were boiled previously to avoid possible enzymatic interference [8].

The samples were incubated at 37°C for 30 min. With 0.25 ml of guinea pig complement reconstituted in barbital buffer, supernatants were removed by centrifugation at 3000 rpm for 10 min; 40 μl of the supernatants was added to 2 ml of a suspension containing 2.5×10^8 sensitized sheep erythrocytes in barbital buffer, and these mixture were incubated at 37°C for 1 h. After centrifugation at 3000 rpm and 4°C for 10 min, supernatants were harvest and hemoglobin release was recorded as absorbance by the supernatant fluids at 546 nm. Under these conditions the hemolytic activity of the complement without LPS corresponded to lysis of 40%–50% of the standard erythrocyte suspension. Anticomplementary activity was recorded as the percentage decrease in lysis from the control level of 40%–50%.

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Table 1. Anticomplementary activity of bacterial suspension

Bacteria	% Loss of hemolytic activity ± SE[a]
Escherichia coli	72.77 ± 4.05
Myxococcus xanthus Vegetative cells	81.11 ± 6.63
Myxococcus xanthus Myxospores	84.44 ± 3.02

[a] SE, standard error, calculated from five different assays.

Shwartzman reaction. The dermal Shwartzman reaction was performed as described by Ito [10]. The skin preparatory dose used was 0.2 ml of various samples that contained 2×10^9 vegetative cells or myxospores of *M. xanthus*, or 200 μg *E. coli* LPS (Difco). The reactions were induced 24 h later by the intravenous injection of 2 ml of saline solution containing 100 μg *E. coli* LPS, or 2 ml of a suspension in saline of 10^9 vegetative cells or myxospores of *M. xanthus*.

Results and Discussion

The anticomplementary activities in *Myxococcus xanthus* myxospores and vegetative cells, with the inclusion of *Escherichia coli* as a positive control, are shown in Table 1. *Myxococcus xanthus* myxospores showed anticomplementary activity quantitatively comparable to the vegetative cells or to *E. coli*. It has been reported that the degree of aggregation and the solubility of LPS are important factors in its anticomplementary activity [6, 7]. These factors may be different in *E. coli* and *M. xanthus*; this should be considered in the interpretation of the few differences between them.

Generally, when smooth strains of enterobacteria are extracted by the phenol–water procedure, the biologically active LPS are isolated from the aqueous phase [19]. However, phenol-soluble endotoxins have been isolated from other Gram-negative bacteria [9, 12]. Therefore, we studied the anticomplementary activity in the fractions isolated from the aqueous and phenol phase from *M. xanthus* myxospores. Practically, no anticomplentary activity was observed in both these phases.

The Shwartzman reaction occurred with all of the samples assayed (Table 2). Myxospores were less efficient than soluble *E. coli* LPS, but were similar to *M. xanthus* vegetative forms in both induction and elicitation of the Shwartzman reaction.

Our results showed that *M. xanthus* CCM 3494 myxospores possess two endotoxin-like activities and suggest that lipid A may be present in glycerol-induced myxospores. This is not in opposition to the literature, since changes in LPS composition during myxospore formation, which affect mainly the polysaccharide moiety [18], may be responsible for difficulty in LPS detection [11] and for changes in immunomodulation patterns of myxospores in comparison with typical LPS-containing bacteria [2, 16]. Alternatively, it has been repeatedly reported that the polysaccharide moiety of LPS possesses immunomodulatory properties [1]; if these properties contribute significantly to the immunomodulation pattern of the whole LPS molecule, it is reasonable to suppose that the partial modification of the polysaccharide moiety in myxospore LPS causes an immunomodulation pattern different from those of the typical Gram-negative bacteria LPS.

Table 2. Local Shwartzman reaction with *Escherichia coli* LPS and bacterial suspensions

Inducing agent (i.v.)	Reaction to given skin-preparatory agent (i.d.)[a] ± SE		
	LPS	Vegetative cells	Myxospores
LPS	22.3 ± 1.0	12.5 ± 0.7	13.6 ± 2.0
Vegetative cells	13.5 ± 0.6	7.4 ± 2.0	7.6 ± 1.9
Myxospores	13.0 ± 0.9	6.9 ± 0.5	7.0 ± 0.7

[a] Diameter of hemorrhagic necrosis (9 mm). SE, standard error of results from four rabbits.

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