Communications to the Editor

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MITOGENIC ACTIVITY OF CHEMICALLY SYNTHESIZED TETRAACETYL-2-KETO-3-DEOXYOCTONIC ACID-(α 2→6)-D-GLUCOSAMINE ANALOGUES OF LIPID A[1)]

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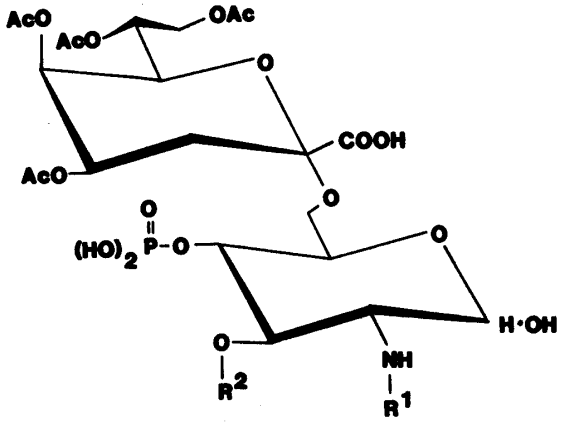
The mitogenic activity of chemically synthesized compounds, three derivatives of 4-O-monophosphorylglucosamine-linked tetraacetyl-2-keto-3-deoxyoctonic acid, was compared with that of a bacterial lipopolysaccharide or of lipid A. These compounds were capable of increasing the incorporation of ^{3}H-thymidine into splenocytes of C57BL/6 mice in vitro.

KEYWORDS ———— lipopolysaccharide; lipid A; monophosphoryl-glucosamine; 2-keto-3-deoxyoctonic acid (KDO); mitogenicity

Lipopolysaccharide (LPS) of Gram-negative bacteria is composed of three structural moieties; a O-polysaccharide region, a common core region, and lipid A. Lipid A exerts various biological effects such as an adjuvant effect, mitogenic activity, antitumor activity and so on.[2)] Recently, it has been shown that the biological activities of chemically synthesized lipid A analogues[3)] are almost the same as those of natural lipid A.[4)] Furthermore, it has been reported that the C-6 position of the nonreducing glucosamine moiety in lipid A of the Re mutants of Salmonella typhimurium[5)] and Escherichia coli[6)] is the attachment site to the C-2 position of 2-keto-3-deoxyoctonic acid (KDO), which is well known as the acidic sugar of the core region of LPS. However, the role(s) of KDO in the various biological activities of LPS is still unclear.

Previously, we reported the mitogenic activity[7)] of chemically synthesized monosaccharide analogues[8)] of lipid A containing two 3-acyloxytetradecanoyl and a phosphoryl group at the C-2, -3 and C-4 positions of the glucosamine skelton. In the present paper, we describe the mitogenic activity of chemically synthesized 4-O-monophosphorylglucosamine-linked tetraacetyl-KDO.

The compounds were synthesized as described previously[9] and their chemical structures are shown in Fig. 1. All compounds tested were suspended in pyrogen-free saline (Ohtsuka Seiyaku, Co., Ltd., Tokyo). Before the mitogenic assay, the suspensions were sonicated for 20-30 s.



Compounds	Substituents	
	R^1 (N-)	R^2 (3-O-)
A-203	C_{14}-O-C_{14}	C_{14}-O-C_{14}
A-204	C_{14}-O-C_{12}	C_{14}-O-C_{12}
A-205	C_{14}-O-C_{12}	C_{14}-O-C_{14}

Ac; acetyl, C_{14}-O-C_{12}; (R)-3-dodecanoyloxytetradecanoyl, C_{14}-O-C_{14}; (R)-3-tetradecanoyloxytetradecanoyl.

Fig. 1. Structure of Synthetic Monosaccharide Analogues, Tetraacetyl-KDO-(2→6)-glucosamine-4-phosphate, of Lipid A

The mitogenicity of the compounds was determined on the basis of in vitro ^{3}H-thymidine (^{3}H-TdR) uptake into splenocytes in C57BL/6 mice as described in a previous report.[10] In brief, the splenocytes were suspended in RPMI-1640 medium (Nissui Seiyaku, Co., Tokyo) supplemented with 10% fetal calf serum (GIBCO, N.Y.). One-tenth ml (5×10^5 cells) of the cell suspension and 0.1 ml of the suspension of the compound were placed in a 96-well microplate (Falcon, #3072). Each well was pulsed with 0.25 μCi of ^{3}H-TdR (New England Nuclear,

Boston, Mass) during the final 16 h of incubation. After the incubation for 64 h, the cells were harvested on filter paper and then the ^{3}H-TdR uptake of the cells was measured with a scintillation counter.

The LPS of S. typhimurium LT-2 and lipid A were used as the reference materials. Lipid A was obtanined from E. coli O55:B5 LPS (Difco Laboratories, Detroit, MI) by acetic acid hydrolysis according to the method of Galanos.[11] The lipid A was solubilized with triethylamine and then mixed with bovine serum albumin (BSA; Armour, Fraction V) to make a conjugate (lipid A-BSA) as described by Galanos et al.[12]

Table I. Mitogenic Effect of Synthetic Monosaccharide Analogues, Tetraacetyl-KDO-(2→6)-glucosamine-4-phosphate, of Lipid A on Cultured Spleen Cells of C57BL/6 Mice in Vitro

Preparations	Stimulation ratio[a]			
	10	25	50	100
		(μg/ml)		
A-203	1.8	2.8	3.3	5.2
A-204	2.0	3.8	4.4	1.9
A-205	5.3	9.7	7.9	3.8
E. coli O55:B5 lipid A-BSA	4.4	n.t.[b]	n.t.	n.t.
S. typhimurium LT-2 LPS	30.1	n.t.	n.t.	n.t.

a) Stimulation ratio = mean cpm of experimental group/mean cpm of control group.
b) n.t.: not tested.

The results of the mitogenic assays are summarized in Table I. Although the mitogenic activity of the compounds was weaker than that of S. typhimurium LPS, the three compounds as well as lipid A-BSA complex were capable of stimulating the incorporation of ^{3}H-TdR. These compounds, A-203, A-204 and A-205, showed the highest activity at doses of 100, 50, and 25 μg/ml, respectively. In the previous paper,[5] we showed that KDO-free 4-O-monophosphorylglucosamine derivatives had little or no mitogenicity. From the above

results, it indicates that the attachment of tetraacetyl-KDO enhances the mitogenic activity of 4-O-monophosphorylglucosamine.

On the other hand, the antitumor activity and lethal toxicity of the tetraacetyl-KDO-attached 4-O-monophosphorylglucosamine derivatives did not differ from those of KDO-free 4-O-monophosphorylglucosamine derivatives (T. Shimizu et al., unpublished data). To investigate the biological role(s) of KDO in LPS, synthesis of the derivatives of 4-O-monophosphorylglucosamine-linked acetyl-free KDO is in progress.

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