BACTERIAL CHARACTERISATION

Demonstration of the Rb₁ lipopolysaccharide core structure in *Salmonella* strains with the monoclonal antibody M105

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The lipopolysaccharide (LPS) from 42 strains representing 19 *Salmonella* serogroups was differentiated into characteristic ladder-like profiles by SDS-PAGE analysis. The core-specific antibody M105 (Ra, Rb₁ and Rb₂) was used in an immunoblot assay of SDS-PAGE-separated LPS molecules. The M105 antibody bound to the R-type LPS of 18 of the 20 *Salmonella* strains tested. The results demonstrate that *S. enterica* serotype Godesberg, *S. Adelaide* (one of two strains), *S. Milwaukee*, *S. Niarembe*, *S. Bere* and *S. Arizonae* (serogroup 63) have an atypical LPS core structure which is Rb₁ type.

Introduction

The genus *Salmonella* has been differentiated into >2400 serotypes according to the Kauffmann-White Scheme [1]. This is based on the biochemical diversity of the flagellar and somatic antigens (H and O respectively). The O antigens are located on the lipopolysaccharide (LPS) molecules which project from the outer leaflet of the outer membrane of the gram-negative bacterial cell. LPS is divided into three regions – a hydrophobic lipid A residue linked to an oligosaccharide core region and a hydrophilic O-polysaccharide or O-chain (Fig. 1). The O-chain region is composed of repeating subunits of differing chain lengths. Polysaccharide chains with the same chain lengths co-migrate in SDS-PAGE gels to form a 'ladder' pattern [2]. The 3-D architecture and conformational flexibility of complete LPS molecule (S-type) has been predicted [3]. It is generally accepted that all *Salmonella* spp. have the same core structure, termed Ra type LPS [4] (Fig. 1). However, there have been a few reports proposing that non-Ra type core structures also occur [5–7].

Initial LPS core studies have used a monoclonal antibody (MAb) T6 [8] which binds to the N-acetylglucosamine → glucose portion of the salmonella Ra type LPS (Fig. 1). This antibody has been used for salmonella identification purposes [9, 10]. MAb T6 did not react with 29% of the 96 serotypes belonging to

serogroups F to 67 [5]. This indicated that a significant number of salmonellae may lack the Ra type core. However, this does not identify the structure of the non-Ra type core. The work reported here used the MAb M105 which binds to the three sugar residues of N-acetylglucosamine → glucose → galactose in the outer core region of salmonella LPS (Fig. 1). It binds to Ra and less effectively to the Rb₁ and Rb₂ regions of LPS [11].

Previous studies have used dot-blots to measure reactivities of antibodies with LPS [5]. However, when this method is used the LPS core region may be inaccessible because of the presence of the heterogeneous LPS chain lengths and other cellular material. In the present study, extracted LPS was separated according to chain length by SDS-PAGE before determining its reactivity with MAb M105.

Materials and methods

Bacterial strains

Rough mutant strains of *S. enterica* serotype Minnesota were provided by Professor I. Poxton (University of Edinburgh). One strain of *S. Ealing* was donated by Professor K. Nielsen (Animal Diseases Research Institute of Agriculture, Canada). *S. Gallinarum* was from Campden & Chorleywood Food Research Association, UK. All other strains were from the culture collection at the Nottingham Trent University and Dynal Research and Development Laboratories (Dynal A/S, Norway). All cultures were maintained on tryptone soya agar (TSA) slopes and were grown

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Fig. 1. (a) The chemical structure of salmonella LPS. Eth, ethanolamine; Gal, D-galactose; Glc, D-glucose; GlcN, D-glucosamine; GlcNAc, N-acetyl-D-glucosamine; Hep, heptose; KDO, 3-deoxy-D-manno-octo-2-ulopyranosonic acid; P, phosphate. (b) Structure of MAb M105 binding site in the outer core region. Antibody T6 binding site is also indicated.

overnight in 10 ml of buffered peptone water (Oxoid) at 37°C.

LPS preparation and analysis by SDS-PAGE and immunoblotting

Pure LPS was obtained from Sigma. Dr O. Holst (The Forschungs Zentrum, Germany) kindly donated the same purified LPS material as previously used with T6 [5]. Pure LPS was dissolved in distilled water (1 mg/ml) before analysis. LPS extracts from bacteria were prepared by proteinase K digestion of whole-cell lysates [12]. Procedures for SDS-PAGE and immunoblot analysis of LPS have been described previously [12].

Immunology

Protein A affinity-purified murine MAb M105 (from Professor K. Nielsen) was diluted in bovine serum albumin (BSA) 1% in Tris-buffered saline (TBS). MAb M105 was detected with an alkaline phosphatase-labelled rabbit anti-mouse IgG 'secondary' antibody (Dako A/S, Denmark).

Results

LPS banding profiles

LPS was extracted from 42 strains by proteinase K digestion and by the phenol-water process, and analysed by SDS-PAGE. It was evident that the LPS profiles could be grouped according to the distribution of LPS chains (Fig. 2). In profile A, the LPS bands had

equal intensity from short to long chains. Profile B had predominantly short and medium size chains, which resulted in more intense bands in the lower half of the gel. In profile C, there was a concentration of very short chains; medium and long chains were also present. Profile D had predominantly short and long chains. Six strains had profile A, 10 strains profile B, 12 strains profile C and 14 strains had profile D (Table 1).

Immunoblot assays

Purified LPS from rough mutant strains of *S. Typhimurium* (Ra, Rb₁, Rc and Re) and *S. Minnesota* (Ra, Rb₂, Rc, Rd₁, Rd₂ and Re) were analysed by immunoblot assays with MAb M105. MAb M105 reacted with Ra and Rb₁ type LPS and failed to react with LPS from Rb₂, Rc, Rd₁, Rd₂ and Re mutant strains of *Salmonella*.

Immunoblot binding profiles

As described previously [12], the MAb M105 immunoblot reaction with salmonella LPS resulted in one of three reactions (Fig. 3). In type 1, the MAb reacted with all LPS molecules regardless of O-chain length. In type 2, the MAb reacted strongly with R-type LPS and weakly with SR-type LPS and short-chain LPS. This weak reaction was often seen as a smear rather than a band. In type 3, the MAb reacted weakly with R-type LPS. Table 2 summarises the immunoblot reactions of all *Salmonella* strains tested. All serotypes belonging to serogroups B, C₂, C₃, D and E gave reaction type 1. *Salmonellae* of serogroups C₁, F and L reacted with short-chain and R-type LPS only (reaction type 2), whereas strains belonging to serogroups G, K, O, U, V, X and 63 expressed the third reaction type. Strains of the same serogroup in some cases produced different reactions. For example, MAb M105 bound with the R-type LPS only of *S. Godesberg* strain 1140, whereas it bound with SR- and R-type LPS from strain 1431. The antibody reacted with R-type LPS of *S. Arizonae* serogroup 63 (strain 10463) and not with strain 1803 (serogroup 51). Moreover the M105 antibody did not react with *S. Djakarta* (serogroup Y) LPS. The M105 antibody did not react with the LPS profiles of any of the non-salmonella Enterobacteriaceae analysed.

Discussion

SDS-PAGE and immunoblot assay techniques were used to study the binding of MAb M105 with LPS from various strains of *Salmonella* belonging to different serogroups and also rough mutants. MAb M105 bound strongly to LPS prepared from rough mutant Ra strains of *S. Typhimurium* and *S. Minnesota*. The antibody bound to a lesser degree to a Rb₁ rough *S. Typhimurium* mutant strain. MAb M105 failed to bind to LPS from Rb₂, Rc, Rd₁, Rd₂, Re mutant *S.*

Fig. 2. SDS-PAGE profile types A–D of *Salmonella* LPS. Lane 1, *S. Typhimurium* (type A); 2, *S. Agona* (type B); 3, *S. Newport* (type C); 4, *S. Arizonae* (type D). This figure is a composite photograph from different gels.

Table 1. LPS profiles of *Salmonella* strains

Serogroup	Serotype (strain)	Profile	Serogroup	Serotype (strain)	Profile
B	<i>S. Abortusequi</i> (L-5886)	D	E ₄	<i>S. Senftenberg</i>	B
	<i>S. Agona</i>	B		<i>S. Arizonae</i> (1823) IIIa	D
	<i>S. Bredeney</i>	B	F	<i>S. Arizonae</i> (10455) IIIb	D
	<i>S. Gallinarum</i>	C		<i>S. Arizonae</i> (10466) IIIa	D
	<i>S. Saintpaul</i>	B	G	<i>S. Cerro</i> (10390)	A
	<i>S. Typhimurium</i>	A	K	<i>S. Minnesota</i>	D
	<i>S. Typhimurium</i> (L-6511)	A		<i>S. Minnesota</i> (L-6261)	D
	<i>S. Typhimurium</i> (1135)	A	L	<i>S. Godesberg</i> (1140)	C
C ₁	<i>S. Typhimurium</i> (1835)	A		<i>S. Godesberg</i> (1431)	C
	<i>S. Infantis</i>	D	O	<i>S. Adelaide</i>	B
	<i>S. Livingstone</i>	D		<i>S. Adelaide</i> (1464)	B
	<i>S. Virchow</i>	D		<i>S. Ealing</i>	B
C ₂	<i>S. Hadar</i>	A		<i>S. Ealing</i> (Canada)	B
	<i>S. Manchester</i>	C	U	<i>S. Milwaukee</i> (1139)	B
	<i>S. Newport</i>	C	V	<i>S. Niarembé</i> (1660)	C
C ₃	<i>S. Kentucky</i>	C	X	<i>S. Bere</i> (10392)	D
D ₁	<i>S. Dublin</i>	C	Y	<i>S. Bere</i> (10403)	D
	<i>S. Enteritidis</i>	C		<i>S. Djakarta</i>	C
	<i>S. Enteritidis</i> (L-6011)	B		<i>S. Arizonae</i>	D
	<i>S. Panama</i>	D		<i>S. Arizonae</i> (1803) IIIa	C
E ₄	<i>S. Give</i>	C	51	<i>S. Arizonae</i> (10463) IIIb	D
			63

Fig. 3. SDS-PAGE (lanes 1, 3, 5) and immunoblots (lanes 2, 4, 6) of *Salmonella* LPS with the MAb M105. Lanes 1 and 2, *S. Manchester* (reaction type 1); 3 and 4, *S. Infantis* (reaction type 2); 5 and 6, *S. Adelaide* (reaction type 3). This figure is a composite photograph from different gels.

Typhimurium and *S. Minnesota* strains. This confirmed the epitope site for M105 as shown in Fig. 1.

In reaction type 1, MAb M105 bound to all bands in the LPS profile and demonstrated that the M105 epitope was present and accessible. Reaction type 1 was obtained with *Salmonella* strains from serogroups B, C₂, C₃, D and E (Table 2). In reaction type 2, MAb M105 bound to LPS molecules with short O-chain length and R-type LPS only (Table 2). The restricted binding of serogroup C₁, F and L salmonellae could be

explained by steric hindrance to the epitope recognised by MAb M105, caused by the addition of a few or more O-chain units. Although they share common O-antigen O:6, the O-chains of subgroups C₂ and C₃ (reaction type 1) and C₁ (reaction type 2) are composed of different sugars. For example, the O-chains of *Salmonella* subgroups C₂ and C₃ have main chains composed of tetrasaccharide repeating units and two lateral monosaccharide substituents (abequose and glucose), whereas the O-chains of *Salmonella* subgroup C₁ have a main chain with pentasaccharide repeating

Table 2. Reactivities of a range of *Salmonella* strains with M105 and T6 antibodies in immunoblot assays

Serogroup	Serotype (strain)	MAb M105 immunoblot reaction type*	Serogroup	Serotype (strain)	MAb M105 immunoblot reaction type
B	<i>S. Abortusequi</i> (L-5886)	1	E ₄	<i>S. Senftenberg</i>	1
	<i>S. Agona</i>	1		<i>S. Arizonae</i> (1823) IIIa	2 (+) [†]
	<i>S. Bredeney</i>	1	F	<i>S. Arizonae</i> (10455) IIIb	2
	<i>S. Gallinarum</i>	1		<i>S. Arizonae</i> (10466) IIIa	3
	<i>S. Saintpaul</i>	1	K	<i>S. Cerro</i> (10390)	3 (+)
	<i>S. Typhimurium</i>	1		<i>S. Minnesota</i>	2
	<i>S. Typhimurium</i> (L-6511)	1	L	<i>S. Minnesota</i> (L-6261)	2
	<i>S. Typhimurium</i> (1135)	1		<i>S. Godesberg</i> (1140)	3 (+)
C ₁	<i>S. Typhimurium</i> (1835)	1	N	<i>S. Godesberg</i> (1431)	2 (+)
	<i>S. Infantis</i>	2		<i>S. Adelaide</i>	3
	<i>S. Livingstone</i>	2		<i>S. Adelaide</i> (1464)	3 (-)
C ₂	<i>S. Virchow</i>	2	O	<i>S. Ealing</i>	3
	<i>S. Hadar</i>	1		<i>S. Ealing</i> (Canada)	3
	<i>S. Manchesters</i>	1		<i>S. Milwaukee</i> (1139)	3 (-)
C ₃	<i>S. Newport</i>	1	U	<i>S. Niarembe</i> (1660)	3 (-)
	<i>S. Kentucky</i>	1		<i>S. Bere</i> (10392)	3 (-)
D ₁	<i>S. Dublin</i>	1	X	<i>S. Bere</i> (10403)	3 (-)
	<i>S. Enteritidis</i>	1		<i>S. Djakarta</i>	No reaction (-)
	<i>S. Enteritidis</i> (L-6011)	1		<i>S. Arizonae</i>	3
E ₄	<i>S. Panama</i>	1	51	<i>S. Arizonae</i> (1803) IIIa	No reaction (-)
	<i>S. Give</i>	1		<i>S. Arizonae</i> (10463) IIIb	3 (-)

*Reaction types are explained in the text.

[†]Results with T6 antibody are given in parentheses.

units and side chains [13]. Hence, differences in the reactivities of the subgroups C₂ and C₃ could also be explained by differences in the 3-D architecture and rigidity of their O-chains [3].

The immunoblots of *Salmonella* strains from serogroups G, K, O, U, X and 63 revealed that MAb M105 bound to R-type LPS with reduced reactivity (reaction type 3). Failure of MAb M105 to bind to SR-type and S-type LPS suggested that the epitope became unavailable in the presence of one O-chain repeat unit [14].

The antibody T6 is specific for the outer core region of Ra-type LPS [8] and, unlike MAb M105, it does not bind to Rb₁-type LPS (Fig. 1b). The T6 antibody specificity is not only defined by the presence of the N-acetyl glucosamine (GlcNAc) residue, but also the bonding to the core oligosaccharide [8]. As all strains in this study bound MAb M105, their LPS core structures were taken to be Ra or Rb₁ type. The T6 antibody has confirmed the presence of typical Ra-type LPS core structures in many *Salmonella* serotypes from serogroups A, B, C, D and E [5, 8, 15, 16]. Subsequently, it was predictable that MAb M105 would bind to these serogroups (Table 2). In contrast, a large proportion of the *Salmonella* strains from other serogroups analysed lacked the T6 epitope [8, 16, 17]. Many of these samples were the same LPS material as previously used [5]. The R-type LPS from *Salmonella* strains which reacted with MAb M105 were previously reported not to bind T6 antibody [5] (Table 2). Hence they probably contain atypical Rb₁-type core LPS. These included *S. Adelaide* (1464, serogroup O), *S. Milwaukee* (serogroup U), *S. Niarembe* (1660, sero-

group V), *S. Arizonae* (10463, serogroup 63) and both strains of *S. Godesberg* (serogroup N) and *S. Bere* (serogroup X). The S-, SR- and R-type LPS of *S. Djakarta* (serogroup Y) and *S. Arizonae* (1803; serogroup 51) failed to react with T6 and M105 antibodies. Based on the known specificities of these antibodies (Fig 1b), it can be concluded that the core LPS of *S. Djakarta* (10403) and *S. Arizonae* (1803) did not contain Ra- or Rb₁-type core structures.

Chemical analysis of LPS composition and structure requires considerable expertise and facilities [7]. However, immunological studies of LPS are less demanding and, therefore, could be used as an initial screening method before chemical analysis. The present study has confirmed that MAb M105 bound to a wider range of salmonellae than the T6 antibody. Thus, these results provided immunological evidence that Rb₁ and other atypical core structures exist in the R-type LPS of certain wild-type *Salmonella* strains.

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