Antigenic and immunogenic differences in lipopolysaccharides of *Escherichia coli* J5 vaccine strains of different origins

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Escherichia coli strain J5 mutants of various origins have often been used as vaccines for induction of cross-reactive, cross-protective antibodies directed against the lipopolysaccharide (LPS) core region. The antigenic composition of LPS from J5 strains of different origin, i.e. strains J5(U), J5(UK), J5(2877) and J5(a), was investigated using monoclonal antibodies (mAbs) reactive only with LPS of a given chemotype, i.e. one specific for the incomplete *E. coli* core of the Rc chemotype, a second mAb reactive only with the *E. coli* R3 complete core, and a third specific for the O-antigen of *E. coli* serovar O111. The LPS of strains J5(U) and J5(a) is almost exclusively composed of LPS of the Rc chemotype, LPS of the J5(UK) strain is composed of Rc LPS and R3 complete core, while LPS of the J5(2877) strain contains Rc, R3 complete core and O-antigen. Growth of the bacteria in medium supplemented with galactose led to increased expression of complete core. The immune responses to the various strains were investigated. Antiserum to the J5 strain expressing the largest amount of R3 core [J5(UK)] had much higher anti-R3 LPS antibody titres compared to antiserum to the other strains. mAb 53, representative of the anti-R3 response to J5 strains containing complete core, bound to those *E. coli* LPS expressing the R3 core. Thus, the R3 LPS, present in some J5 vaccine strains is at least partially responsible for some of the cross-reactivities exhibited by some anti-J5 antisera. The present findings may explain some of the discrepant outcomes reported for cross-protection studies with J5 antisera, where J5 strains of various origins grown under different culture conditions have been used as vaccines and where LPS or bacteria expressing various core types have been used for challenge.

Introduction

The still very high mortality of Gram-negative sepsis (GNS) has led to intensive research for novel adjunctive forms of therapy. Given the fact that bacterial endotoxin (lipopolysaccharide, LPS) plays a central role in the pathogenesis of GNS, attempts have been made to prepare endotoxin-neutralizing antibodies. Attention has been paid particularly to preparing antibodies to common elements in the core/lipid A part of LPS. For preparing antisera with high titres to elements in the core, O-antigen-deficient strains ('rough mutants') have been used. Many studies have been done with antisera to

the *E. coli* rough mutant J5. The original J5 strain was isolated by Elbein & Heath (1965) from a parent culture of *E. coli* serovar O111:B4, as a mutant incapable of fermenting galactose. When grown in the absence of galactose, an incomplete LPS is produced that lacks O-antigen and produces an incomplete core of the Rc chemotype. Addition of galactose to the growth medium reverses this situation. This J5 variant was transferred to the laboratory of the late Dr A. I. Braude (San Diego School of Medicine, University of California, San Diego, USA) where a 'double mutant' (again called J5) was isolated from it that produced mutant LPS even when grown in the presence of galactose (Ziegler *et al.*, 1973). The nature of the additional genetic defect(s) in the double mutant is not known. Cultures of J5 mutants, of both the original one and the double mutant, have been sent to laboratories all over the world, including ours.

Previously we have described that two J5 'substrains', named J5(UK) and J5(a) by us, can be isolated from

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Abbreviations: LPS, lipopolysaccharides; mAb, monoclonal antibody.

cultures originating from the J5 double mutant (Appelmek *et al.*, 1986); the substrains differ from each other with respect to an as yet uncharacterized epitope(s) present on LPS. Thus, at present at least three strains are available in the scientific community that are all referred to as J5, and that differ antigenically in their LPS. J5 strains have been used by many scientists as vaccines to elicit cross-reactive antibodies and results of protection studies performed at various locations have yielded disparate outcomes (Appelmek & Cohen, 1992). The possibility that the choice of J5 strain might influence the outcome of protection studies has already been suggested by us (Appelmek *et al.*, 1986).

In this work we investigated the antigenic make-up of LPS isolated from J5 strains of different origin, i.e. four strains, including the two double mutant substrains mentioned above. We used specific monoclonal antibodies (mAbs) to determine the amounts of incomplete core of the Rc chemotype and the amounts of complete core of the R3 type, a type known to be present in *E. coli* serovar O111 (Schmidt *et al.*, 1970). We also determined whether or not the J5 strains tested produce O-antigen. Finally, we investigated the immune response to the various J5 strains.

Methods

Bacterial strains and growth conditions. *E. coli* serovar O111:B4 was obtained from E. J. Ziegler (San Diego, USA). The origin of the four J5 strains [J5(a), J5(UK), J5(2877) and J5(U)] used in this study is as follows: *E. coli* J5(a) was subcultured by B. J. Appelmek from a slant obtained from E. J. Ziegler. *E. coli* J5(U) was sent by E. J. Ziegler to M. P. Glauser (Lausanne, Switzerland), transferred to J. Verhoef (Utrecht, The Netherlands), then via H. Brade (Borstel, Germany) to B. J. Appelmek. *E. coli* J5(UK) was sent by E. J. Ziegler to L. S. Young (San Francisco, USA), transferred to C. S. Easmon (London, UK) and then to B. J. Appelmek. *E. coli* J5(2877) is from the culture collection of the Max-Planck Institut für Immunobiologie, (Freiburg, Germany). Its origin before that is unclear. It was transferred from Freiburg to H. Brade and then to B. J. Appelmek. Bacteria were stored in lyophilized form, and maintained on agar plates for routine use. Strains J5(a) and J5(UK) were found to be phenotypically stable upon passaging (Appelmek *et al.*, 1986); for the other J5 strains no such controls were performed. For coating of ELISA plates, bacteria were grown overnight in brain heart infusion (BHI); in some experiments (see Results) this was supplemented with 0.25% D-galactose.

LPS. The following LPS preparations were obtained from H. Brade: LPS from *Salmonella minnesota* strains R595 (Re chemotype), R4 (Rd2), R7 (Rd1), R5 (Rc), R345 (Rb2) and R60 (Ra); from *E. coli* strains F470 (R1 core), F576 (R2), F653 (R3), F2513 (R4), strain J5(2877) and J5(U). LPS from strain J5(UK) was a gift from C. S. Easmon (London, UK). LPS from *E. coli* serovar O26:B6 was purchased from List Biologicals; LPS from *E. coli* serovars O111:B4, O55:B5, O127:B8 and O128:B12 were obtained from Sigma. Rough-strain LPS had been purified by the phenol/chloroform/petroleum ether procedure (Galanos *et al.*, 1969), while for smooth LPS the phenol/water method (Westphal & Jann, 1965) had been used. LPS were dispersed in water by sonication, and stored at 1 mg ml⁻¹ at 4 °C.

mAbs. Rc-specific mAb 4 (IgG2a) has been described previously (Appelmek *et al.*, 1988); it was obtained using boiled strain J5(a) cells as a vaccine.

R-core-specific mAbs: five different core types have been described in *E. coli* (Holst & Brade, 1992), viz. R1, R2, R3, R4 and K12. mAbs to various R1, R2 and R3 *E. coli* core types (Gibb *et al.*, 1992) were a kind gift of F. diPadova (Sandoz, Basel, Switzerland); they are coded here as mAbs α -R1, α -R2 and α -R3, respectively. mAb 53 (IgG3) has not been described before and was prepared following vaccination of mice with boiled cells of strain J5(UK). mAb 53 is specific for R3 LPS (see Table 1).

Anti-O mAb: mAb O4-2E, a murine mAb (IgG2a), specific for the O-antigen of *E. coli* serovar O111:B4 has been described by Pollack *et al.* (1989); it was obtained from M. Pollack (Bethesda, USA).

Control mAb: a previously described mAb, Pen 7 (Appelmek *et al.*, 1988), specific for benzylpenicillin was used as a negative control for mAb O4-2E.

mAbs 4 and 53 were available as hybridoma culture supernatant fluids in RPMI containing 10% (v/v) foetal calf serum (FCS). The α -R1, α -R2 and α -R3 mAbs were supplied in purified form at 1 mg ml⁻¹. mAbs Pen 7 and O4-2E were present as murine ascitic fluid. The non-purified mAbs were stored in small portions at -80 °C and thawed only once. The purified mAbs were stored at 4 °C. The procedures used for vaccination, and for hybridoma selection and growth have been described previously (Appelmek *et al.*, 1988).

Determination of antibody concentrations in hybridoma culture supernatant fluid. An ELISA sandwich method was used: for determination of IgG mAbs, plates [96-well polystyrene ELISA plates (Immulon)] were coated (overnight at room temperature) with immunoglobulin purified from goat antiserum to mouse IgG(Fc) (Nordic) at 10 µg ml⁻¹ in PBS. Plates were washed three times with PBS (pH 7.2), containing 0.02% Tween 80 (PBST). mAbs, serially diluted in twofold steps in PBST, were added and incubated overnight at room temperature. Plates were washed three times in PBST and conjugate, diluted 1:1000 in PBST containing 0.5% goat serum, was added [affinity-purified goat anti-mouse IgG, heavy+light chain, labelled with horseradish peroxidase (HRP), American Qualex]. After 3 h at 37 °C, the ELISA was developed for 30 min using conventional procedures with orthophenylene diamine (1 mg ml⁻¹) and hydrogen peroxide (0.015%) in citrate/phosphate buffer, pH 5.5, stopped with 0.5 M-H₂SO₄ and optical density (OD) values were read at 492 nm on an ELISA reader connected to a personal computer, a combination that allowed estimation of OD values higher than 2. For IgM measurements, plates were coated with affinity-purified rabbit antibodies to mouse IgM (μ -chain specific) obtained from Jackson Immunoresearch Labs at 2.4 µg ml⁻¹; as a conjugate, affinity-purified goat anti-mouse IgM heavy+light chain, labelled with HRP (American Qualex) was used for IgM. (Sub)-isotype-matched, purified control mAbs (> 95% pure, as judged by SDS-PAGE), obtained from P. Trown (Xoma Co., Berkeley, CA, USA) were used as controls to calibrate antibody concentration measurements. Both control and test mAbs were titrated in twofold steps, and concentrations were calculated using dedicated software.

Measurement of reactivity of mAbs with LPS and bacteria. LPS was coated overnight at room temperature on ELISA plates at 1 µg ml⁻¹ in PBS unless otherwise indicated. Bacteria grown overnight were washed twice in PBS, adjusted to 5 × 10⁸ c.f.u. ml⁻¹ unless otherwise indicated, boiled for 1 h at 100 °C (a procedure known to expose otherwise hidden core epitopes; Aydinung *et al.*, 1989) and used for coating. mAbs were titrated in twofold steps at a fixed coating concentration of LPS or bacteria, but in some experiments (see Results) checkerboard titrations were done where the LPS or bacterial coating was varied in steps. The conjugates used were the same as described for measurement of mAb concentration. Negative controls were wells 'coated' with PBS and

processed identically to LPS-coated wells. The OD obtained when PBST was substituted for mAb was also measured. Reactivity of mAbs was defined as the concentration of mAb (ng ml^{-1}) that gave an OD value of 0.2 above that of the negative control.

SDS-PAGE and silver staining. LPS were separated on 20% (w/v) polyacrylamide gels (Pharmacia); following separation, gels were either silver-stained (Tsai & Frasch, 1982) or transblotted onto nitrocellulose. Separation and blotting was performed on the Pharmacia Phast system according to instructions of the manufacturer. Amounts of LPS used for silver staining were 9 μg for O111 LPS, 1 μg for J5 and R3 LPS, unless otherwise indicated. Amounts of LPS for blotting were 9 μg O111 LPS, 3 μg for J5 and R3 LPS. The blotted membranes were baked overnight at 70 °C, blocked for 1 h at 37 °C in 5% (w/v) bovine serum albumin, washed three times in PBST and incubated with mAbs for 2 h at 37 °C. Concentrations of mAbs used: mAb 4 and 53, 7 $\mu\text{g ml}^{-1}$ and 3.5 $\mu\text{g ml}^{-1}$, respectively; mAb O4-2E ascites was used at 1:100. Then, blots were washed, incubated with HRP-linked goat anti-murine IgG (see above) for 1 h at 37 °C, washed and developed for 30 min with citrate/phosphate buffer, pH 5.5, containing 0.5 mg 4-chloro-1-naphthol (Sigma) ml^{-1} and hydrogen peroxide (0.015%).

Results

A primary objective of the current investigation was to determine the molecular composition of J5 LPS of various origins, in particular their relative contents of LPS of the Rc chemotype (incomplete core), of complete R3 core and of the amount of O-antigen present. The structure of LPS is shown in Fig. 1. The mAbs used for this purpose are described in Table 1. mAb 4 is truly Rc-specific, i.e. it binds strongly to LPS of this chemotype, and not to longer or shorter LPS chemotypes. At 8 ng ml^{-1} , mAb 4 still displayed binding significantly higher than control values. The OD obtained on control wells 'coated' with PBS (plus mAb, conjugate and substrate) was < 0.2 as was the OD on control wells coated with LPS (or bacteria, see below), but processed with PBST in place of the mAb. Thus, usually, the titre (in ng ml^{-1}) was read at an OD_{492} of 0.4. Binding of mAb 4 to other LPS was not seen up to a concentration of 16 $\mu\text{g ml}^{-1}$; the only exception was R3 LPS where at 500 ng ml^{-1} binding was seen but the binding curve was very flat in this case (data not shown). mAb 53 bound only to complete R3 core (devoid of O-antigen) and not to lower chemotypes or to the complete core when that was linked to O-antigen (see SDS-PAGE and immunoblot). As might be expected, for an anti-O mAb, mAb O4-2E, bound only to the O111 serovar and not to others. The ascites control mAb Pen 7 bound only very weakly to some LPS (titres 1:200 to 1:800). Likewise, mAb 53 binds only to complete core and not to Rc LPS, or to other core chemotypes or O-antigen. In summary, the mAbs described in Table 1 are suitable tools to quantify the molecular components putatively present in J5 LPS, i.e. Rc and R3 LPS and O-antigen.

The mAbs described above were then used to measure the contents of Rc chemotype, R3 complete core and O-

antigen in LPS of three of the four J5 strains under investigation. LPS from strain J5(a) was not available to us.

At a LPS coating concentration of 1 $\mu\text{g ml}^{-1}$, mAb 4 reacted with identical titres (4–8 ng ml^{-1}) with the three J5 LPS, but at 30 ng ml^{-1} this mAb reacted much more strongly with LPS of strain J5(2877) (titre below 16 ng ml^{-1}) compared to LPS of strain J5(UK) (titre 2000 ng ml^{-1}). The reactivity of mAb 4 with LPS of strain J5(U) was comparable to that with LPS of J5(2877) (not shown). These data indicate that LPS of

Fig. 1. Schematic structure of lipopolysaccharide. A–D, sugar residues of the O-antigen. Glc, D-glucose; Gal, D-galactose; GlcNAc, N-acetyl-D-glucosamine; Hep, L-glycero-D-manno-heptose; KDO, 2-keto-3-deoxy-D-manno-octulosonate; AraN, 4-amino-L-arabinose; P, phosphate; EtN, ethanolamine; ~, hydroxy- and non-hydroxy fatty acids. Ra indicates the complete core (for *E. coli*: R1 to R4 and K12), Rb to Re indicate incomplete core structures.

Table 1. Reactivity of chemotype-specific monoclonal antibodies

Reactivity in ELISA is expressed as the lowest amount of mAb (ng ml^{-1} or ascites dilution) that still resulted in a positive reaction. —, Indicates that the mAb does not react at the highest concentration tested, i.e. for mAb 53: 8 $\mu\text{g ml}^{-1}$; for mAb 4: 16 $\mu\text{g ml}^{-1}$; for O4-2E and Pen 7 ascites: 1:200. ND, Not done.

Lipopolysaccharide	Reactivities of mAbs in ELISA			
	4	53	O4-2E	Pen 7
	(ng ml^{-1})	(ng ml^{-1})	(dilution of ascites)	(dilution of ascites)
<i>S. minnesota</i>				
R595 (Re)	—	—	ND	ND
R4 (Rd2)	—	—	ND	ND
R7 (Rd1)	—	—	ND	ND
R5 (Rc)	8	—	ND	ND
R345 (Rb2)	—	—	ND	ND
R60 (Ra)	—	—	ND	ND
<i>E. coli</i>				
F470 (R1)	—	—	—	—
F576 (R2)	—	—	—	—
F653 (R3)	500	8	1:800	1:800
F2513 (R4)	—	—	1:400	1:400
O111	—	ND	1:3 × 10 ⁶	1:400
O55	—	ND	1:200	—
O26	—	ND	—	—
O127	—	ND	—	—
O128	—	ND	1:800	1:200

Fig. 2. Reactivity of mAb 53 with lipopolysaccharide of strain J5(UK) (○) and strain J5(2877) (□). Coating concentration was $1 \mu\text{g ml}^{-1}$.

strains J5(2877) and J5(U) contain similar amounts of Rc LPS, while LPS of strain J5(UK) contains less of it.

mAb 53 did not react with LPS of strain J5(U) (not shown). Binding of mAb 53 with the two other J5 LPS tested is shown in Fig. 2. mAb α -R3 behaved in a similar way to mAb 53 (not shown). Thus, LPS of strain J5(U) does not contain complete R3 core, while R3 LPS is present in LPS of strain J5(2877), and even more so, in that of strain J5(UK).

mAb O4-2E did not react with LPS of strains J5(U) and J5(UK) (titres of 1:200 and 1:800, respectively), but a very high titre (1.4×10^5) was reached with LPS of strain J5(2877), which showed that this mutant makes the same O-antigen as present in its parent. Titres of the control ascites Pen 7 were low (1:200 or less).

Taken altogether, the results obtained with mAbs 4, 53, α -R3 and O4-2E confirm the heterogeneity of the J5 strains we had described previously (Appelmelk *et al.*, 1986) and partly identify the molecular components responsible for these differences.

We then subjected LPS of strains J5(U), J5(UK) and J5(2877), as well as LPS of serovar O111 to SDS-PAGE, a procedure that separates molecules according to their molecular masses (MM) (Fig. 3). The high MM O-antigen in LPS of *E. coli* serovar O111 (Fig. 3a) is readily distinguishable as is the slightly higher MM of J5(UK) LPS compared to those of the other J5 LPS. The MM of J5(UK) LPS is similar to that of R3 LPS. SDS-PAGE-separated LPS were transferred to nitrocellulose, and probed with mAbs 4, 53 and O4-2E. From the immunoblots with mAb 4 (Fig. 3b) it can be concluded that strain J5(UK) contains less Rc LPS compared to the other J5 strains. Evidently, O111 LPS contained some small amount of Rc LPS (Fig. 3b). As expected, the R3-specific mAb 53 bound very well to R3 LPS (Fig. 3c); it also bound well to O111 LPS and to J5(UK) LPS and faintly to J5(2877) LPS but not with J5(U) LPS. Thus, strain J5(UK) contains a higher amount of R3 LPS compared to the amount present in strain J5(2877) LPS.

The O-specific mAb O4-2E detected high MM O-antigen in smooth O111 LPS (Fig. 3d); in LPS of strain J5(2877), O-antigen was of lower MM, i.e., it was less polymerized. We attempted to resolve the Rc and R3 LPS components putatively present in LPS of strain J5(UK) by electrophoresis of much lower amounts of LPS. The results (Fig. 3e) indicate that, although complete separation of the two molecular species was not possible, not even when electrophoresed in different lanes, their presence in LPS of strain J5(UK) can be convincingly demonstrated. By a similar approach we attempted to resolve the three molecular components putatively present in LPS of strain J5(2877), but failed to do so (not shown).

LPS of the original J5 strain (Heath), grown in the presence of galactose is known to contain O-antigen (Elbein & Heath, 1965). The abilities of the various mAbs (O4-2E, α -R3 and mAb 4) for binding in ELISA to whole boiled bacteria grown in the presence or absence of galactose were tested. Titres of mAb O4-2E with strains J5(U), J5(a) and J5(UK), grown in either the presence or the absence of galactose using a coating density of 5×10^8 c.f.u. ml^{-1} were below 1:8000 with very shallow dose-response curves (not shown). At 5×10^8 c.f.u. ml^{-1} , mAb O4-2E reacted with titres of 1:10⁷ with J5(2877) cells grown in either presence or absence of galactose. At 5×10^5 c.f.u. ml^{-1} , reactivity with strain J5(2877) cells grown in the absence of galactose had dropped considerably, whilst the binding of mAb O4-2E to cells grown in the presence of galactose had remained very high (data not shown).

The binding of mAb α -R3 with strain J5(U) or J5(a) cells (coated at 5×10^8 c.f.u. ml^{-1}) was investigated. mAb α -R3 did not react with strain J5(a) or J5(U) when grown without galactose, and only weakly, with a shallow dose-response curve, to cells grown in its presence (not shown). In contrast, at this coating density, mAb α -R3 bound very strongly to strain J5(2877) and J5(UK), in the presence or absence of galactose, displaying steep dose-response curves with OD values > 2 down to a mAb concentration of 32 ng ml^{-1} (not shown). Thus, strains J5(2877) and J5(UK) express more complete core than strains J5(A) or J5(U). For a comparison of strain J5(2877) with strain J5(UK), checkerboard titrations were required. At coating densities of 5×10^5 c.f.u. ml^{-1} of cells grown in the absence of galactose, strains J5(UK) and J5(2877) still behaved similarly (not shown); however, in the presence of galactose, strain J5(UK) produced much more complete core (not shown). mAb 53 behaved in a similar way to mAb α -R3 (not shown). Lastly, the Rc-specific mAb 4 was tested for binding in ELISA by means of checkerboard titrations. At all bacterial coating concentrations tested (5×10^5 – 5×10^8 c.f.u. ml^{-1}) mAb 4 reacted very strongly with strains J5(a), J5(U) and J5(2877). Titres reached at

Fig. 3. (a) Silver stain of LPS separated by SDS-PAGE; (b) immunoblot of gel from (a) developed with anti-Rc mAb 4; (c) blot developed with anti-R3 mAb 53; (d) blot developed with anti-O mAb O4-2E. Lanes: 1, strain J5(UK); 2, strain J5(2877); 3, strain J5(U); 4, strain R3; 5, strain O111:B4. (e) Silver stain. Lanes: 1 and 2, LPS (0.30 μ g) of R3 and strain J5(U), respectively; 3-6, LPS of strain J5(UK), 0.50, 0.30, 0.15 and 0.05 μ g, respectively.

a coating density of 10^5 c.f.u. ml^{-1} were 4 ng ml^{-1} , when cells were grown in both absence or presence of galactose. The six dose-response curves obtained were almost superimposable (not shown). Strain J5(UK) behaved differently: when grown without galactose, binding of mAb 4 was almost the same as for the other J5 strains

tested, but in this case growth in the presence of galactose almost completely suppressed binding of mAb 4 (not shown). Therefore, galactose had little influence on the expression of Rc LPS in strains J5(A), J5(U) and J5(2877), but suppressed almost totally the synthesis of Rc LPS in strain J5(UK).

Table 2. Summary of molecular composition of LPS of J5 strains

Presence of Rc and R3 LPS was quantified with mAbs 4 and α -R3 respectively; mAb O4-2E was used to measure O-antigen.

Strain	Galactose present in growth medium	Presence of LPS antigen*		
		Rc	R3	O-antigen
J5(U)	—	+++	—/+	—
J5(U)	+	+++	+	—
J5(a)	—	+++	—/+	—
J5(a)	+	+++	+	—
J5(UK)	—	++	+++	—
J5(UK)	+	+/-	++++	—
J5(2877)	—	+++	+	+
J5(2877)	+	+++	++	+++

—, Not present; —/+, +/—, +, ++, +++, and +++++, antigen present in increasing amounts.

A summary of the antigenic composition of the four J5 strains is shown in Table 2.

The murine immune response to the various J5 strains was then determined. Our goal was to investigate whether or not strains shown to be antigenically different, would also be immunogenically so, i.e. would induce a different immune response, in particular with regard to their ability to induce antibodies directed against the R3 core. Strain J5(UK) induced high titres of anti-R3 LPS antibodies (1:102000); strains J5(2877) and J5(U) induced titres of at most 1:1600. Preimmune titres were 1:100 or below. The ability of mAb 53, obtained from mice vaccinated with strain J5(UK) and representative of the anti-R3 response to J5 strains expressing a full core, to bind to heterologous smooth LPS was then investigated. mAb 53 bound well to heterologous smooth LPS of the R3 core type (LPS of *E. coli* serovars O111, O26 and O55) but not to LPS expressing other core types.

Discussion

In this paper we provide data showing that four *E. coli* J5 strains of different origins, i.e. strains J5(a), J5(U), J5(UK) and J5(2877), are antigenically different (Table 2). Strains J5(a) and J5(U) express LPS of the Rc-chemotype; J5(UK) expresses both Rc LPS and R3 complete core, whilst strain J5(2877) expressed Rc, R3 and O-antigen. We conclude that strain J5(2877) behaves like the original J5 strain described by Elbein & Heath (1965), whilst the other J5 strains tested behave like the double mutant described by Ziegler *et al.* (1973). Our data also show that the designation 'double mutant' has been given to strains that differ from each other, i.e. strains J5(U) and J5(a) on the one hand, and strain J5(UK) on the other.

Apart from our original observation (Appelmelk *et al.*, 1986), only one study has addressed the problem of J5 heterogeneity (Evans *et al.*, 1992); in the latter study, published after we had finished the experimental work described here, it was concluded that strain J5(UK) contains LPS of the Rc chemotype, whilst the presence of a LPS shorter than Rc was suggested by the authors for strain J5(a). In contrast, the present investigation provides evidence that strain J5(UK) contains complete R3 core in addition to Rc LPS and that strain J5(a) contains LPS of the Rc chemotype. Moreover, strain J5(a) behaved indistinguishably from strain J5(U), a strain for which the Rc chemotype has been proven by chemical means (Kaca *et al.*, 1988).

Our results indicate that the amount of complete R3 LPS present in a given J5 vaccine depends on the actual J5 strain used, as well as on the growth conditions. We have shown that a J5 vaccine containing a large amount of complete core, elicits a strong anti-R3 LPS immune response. mAb 53, representative of this response was shown by us to bind to heterologous smooth LPS expressing the R3 chemotype, but not to those expressing other types. Thus, in this respect anti-J5(UK) antiserum is more cross-reactive, and potentially more cross-protective than antiserum to the other strains tested. Therefore, our results may explain some of the differences in outcome of protection studies with J5 antisera where J5 strains of various origins and culture conditions had been used as vaccines to elicit cross-reactive, cross-protective antisera, and where *E. coli* bacteria or LPS, expressing R3 core or other core types, had been used as challenge.

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