

vivo is still under investigation (Skorupski *et al.* 1997; DiRita *et al.*, this volume).

4. SIALYLATION OF GONOCOCCAL LPS BY HOST CMP-NANA AND EFFECT OF LACTATE: A PARADIGM FOR INVESTIGATION OF BEHAVIOUR *IN VIVO*

Sialylation of gonococcal LPS by host-derived cytidine 5'-monophospho-N-acetyl neuraminic acid (CMP-NANA) and lactate has a major influence on many aspects of gonococcal pathogenicity. This was revealed by investigating the cause of a biological property of gonococci in urethral exudates which was lost on subculture *in vitro*. This work shows how bacterial activities *in vivo*, relevant host factors and the metabolism concerned can be identified. References to papers up to 1995 are given in Smith *et al.* (1995).

(a) *Sialylation of LPS by host CMP-NANA affects pathogenicity*

Gonococci in urethral exudates are resistant to complement-mediated killing by fresh human serum. In most cases, resistance is lost on one subculture *in vitro* but it can be restored by incubation with blood cell extracts. Fractionation showed that the resistance inducing activity is due to CMP-NANA. After growing gonococci with CMP-¹⁴CNANA, autoradiography of LPS bands separated by SDS-PAGE showed that some, but not all, LPS components are sialylated. One sialylated component of 4.5 kDa is conserved in many strains and its side chain is Gal β 1-4GlcNac β 1-Gal β 1-4Glc. The sialylated LPS forms an irregular surface coat, which is seen on gonococci in urethral exudates whose LPS was shown to be sialylated. A previously unknown gonococcal sialyltransferase was demonstrated in gonococcal extracts. LPS sialylation is responsible for serum resistance since conversion to resistance accompanies sialylation by CMP-NANA and reversion to sensitivity occurs when sialyl groups are removed by neuraminidase. The crucial importance of LPS sialylation in serum resistance of gonococci is now generally accepted (Vogel & Frosch 1999). Sialylation of LPS also interferes with the following host-defence mechanisms: absorption of complement component C3; ingestion and killing by PMN phagocytes; killing

by antisera against gonococcal proteins; and stimulation of the immune response. On the other hand, sialylation prevents invasion of epithelial cells. Observations on volunteers were consistent with these results. When they were inoculated with a strain whose LPS could not be sialylated, a variant was selected *in vivo* whose LPS could be sialylated (Schneider *et al.* 1991). This variant, recovered from the volunteers, was more virulent than the parent strain provided it was inoculated after being grown in a medium without CMP-NANA (i.e. its LPS was not sialylated so that it could invade epithelial cells) (Schneider *et al.* 1995, 1996). If the inoculum was grown with CMP-NANA, it did not infect volunteers so well, consistent with inhibition by LPS sialylation of the ability to invade epithelial cells (Schneider *et al.* 1996).

A sialyltransferase-deficient mutant, in contrast to its parent strain, did not become serum resistant when incubated with either CMP-NANA or blood cell extracts (Bramley *et al.* 1996). Hence, the latter do not contain a mechanism for sialylating gonococcal LPS, which is independent of CMP-NANA. Also, unlike the wild-type, incubation of the mutant with CMP-NANA did not increase resistance to ingestion and killing by PMN phagocytes, killing by antiserum to porin I and human complement, binding of C3 of complement, and invasion of epithelial cells (Gill *et al.* 1996). The mutant was unable to sialylate any of its LPS components, which were shown by mass spectrometry to be similar to those of the parent strain. They included components sialylatable by the sialyltransferase from the parent strain, such as the 4.5 kDa conserved component mentioned above (Crooke *et al.* 1998). Hence, loss of ability of the mutant to be converted by CMP-NANA to resistance to serum killing, and all the other facets of pathogenicity mentioned above, is attributed to loss of sialyltransferase activity rather than inability to synthesize the LPS substrate for sialylation. Final confirmation would have come from complementation of the mutant by the gene for the sialyltransferase which has been cloned and sequenced (Gilbert *et al.* 1996). This could not be achieved (Crooke *et al.* 1998) so it seems that multiple genetic loci may be essential for LPS sialylation. The mutant has not been examined in human volunteers. A sialyltransferase-negative mutant of gonococcal strain FA 1090 was as infective as the wild-type for volunteers (Cannon *et al.* 1998), but this is not unexpected because this strain is fully resistant to serum killing (Cohen *et al.* 1994) and does not require LPS sialylation to make it so.

(b) Host lactate enhances LPS sialylation, and LPS and protein synthesis

Another blood cell factor, which enhances the ability of CMP-NANA to sialylate gonococcal LPS and to induce serum resistance, has been identified as lactate (Parsons *et al.* 1996a). The enhancement occurs with minute quantities of lactate in a defined medium containing high concentrations of glucose. The action of lactate is separate from that of CMP-NANA because enhanced sialylation occurred when gonococci were pretreated with lactate (Parsons *et al.* 1996b). Lactate did not increase the gonococcal content of sialyltransferase (Gao *et al.* 1998). On the other hand, there was a marked increase in LPS synthesis (10–20%), which could explain the enhancement of sialylation because additional receptors for sialyl groups are provided. The increase in LPS synthesis was paralleled by increases in protein synthesis and ribose content, presumably reflecting additional ribosome production (Gao *et al.* 1998).

(c) Metabolic effects of lactate on gonococci growing in a medium containing glucose, as occurs in vivo

The increases in LPS, protein and ribose synthesis, first noticed under the conditions for detecting enhancement of sialylation by lactate, also occurred when both glucose and lactate concentrations in the defined medium were adjusted to levels akin to those occurring *in vivo* (Gao *et al.* 1998). Hence, there appears to be a general stimulation of gonococcal metabolism when lactate is added to media containing glucose, and there is other evidence. Lactate increased oxygen consumption by gonococci in a solution containing glucose (Britigan *et al.* 1988). Also, in the above-defined medium, growth rate was faster and lactate was metabolized side-by-side with glucose and more rapidly (Regan *et al.* 1999). When gonococci were grown with ^{14}C -labelled lactate in this medium (Yates *et al.* 1999), tricine SDS-PAGE on homogenates showed that lactate is not a general carbon source. Label was concentrated in a low M_r component, LPS and a few proteins. N-terminal sequencing of the three most heavily labelled proteins showed one (M_r ca. 58 kDa) to be the chaperone, GroEl and another (M_r ca. 35 kDa) porin 1B. Nuclear magnetic resonance after ^{13}C labelling, and thin layer chromatography following ^{14}C labelling (Yates *et al.* 1999), showed the low M_r

component to be lipid. Gonococcal membrane lipids consist mainly of phosphatidyl ethanolamine and glycerol esterified to palmitic, myristic, a 16:1 and a 18:1 acid (Sud & Feingold 1975). In the glucose-containing medium, the carbon atoms from the ^{13}C lactate were incorporated specifically into the fatty acid portions, in contrast to both glycerol and fatty acid moieties when ^{13}C glucose was used without lactate present. The location in the LPS of the ^{14}C label from the lactate is not yet known but the fatty acid residues seem likely.

The incorporation of lactate carbon into GroEl is interesting in two respects. GroEl would ensure correct folding of the products of the large increase in protein synthesis (Gao *et al.* 1998). Also, it could contribute to the inflammation seen in gonorrhoea, since it is a potent stimulator of relevant cytokines (Coates & Henderson 1998); and it is significant that patients have high levels of antibody to GroEl (Demarco de Hormaeche *et al.* 1991). Porin 1B plays a major metabolic role, membrane transport, in gonococci (Gotschlich *et al.* 1987) and can contribute to pathogenicity by inserting into host-cell membranes (Bjerknes *et al.* 1995). Stimulation of lipid formation would aid membrane synthesis and therefore metabolism and growth. It is fascinating that gonococci have adapted to use lactate and glucose, which are ubiquitous *in vivo*, to produce a vibrant metabolism and a large content of virulence determinants such as LPS and GroEl. The marked effects of minute amounts of lactate on LPS, protein and ribose synthesis in a medium containing large quantities of glucose suggests that lactate may have a signalling as well as a metabolic role.

(d) *Extension of the work to meningococci*

The work on gonococci stimulated similar investigations on meningococci. Some strains contain LPS components that are endogenously sialylated (serogroups B, C, W and Y) and others have components that can be sialylated exogenously by host CMP-NANA (groups A and 29E) (Smith *et al.* 1995). An LPS sialyltransferase is present (Smith *et al.* 1995). LPS sialylation affects facets of pathogenicity but not as markedly as for gonococci because capsular polysaccharide is the more powerful virulence determinant. It is sometimes difficult to distinguish between their respective roles. LPS sialylation inhibits meningococcal invasion of epithelial cell lines, endothelial cells and mono- and PMN phagocytes (McNeil & Virgi 1997; Virgi *et al.*

1993). Also, it interferes with opsonophagocytosis of some strains (Smith *et al.* 1995). The position regarding serum resistance is equivocal; some papers indicate that LPS sialylation is important (Esterbrook *et al.* 1997; Kahler *et al.* 1998) and others that it is less so (Vogel *et al.* 1997; Vogel & Frosch 1999). In an outbreak of group B meningitis, an immunotype capable of LPS sialylation was associated with invasive disease and an immunotype incapable of LPS sialylation with the carrier state (Smith *et al.* 1995). The effect of lactate on meningococci has not yet been investigated.

5. CONCLUDING REMARKS

I hope this paper has made clear the pertinent questions about the behaviour of bacterial pathogens *in vivo*; and has indicated how they might be answered, despite difficulties in some areas, by a combination of conventional and newly devised methods.

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