THE ANTIGENIC STRUCTURE OF THE LIPOOLIGOSACCHARIDES
OF NEISSERIA GONORRHOEAE

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INTRODUCTION

The importance of lipooligosaccharide (LOS) (this term which will be
used in this chapter in place of "lipopolysaccharide" was suggested by
Schneider et al. (29) for the lipid A containing glycolipids of pathogenic
Neisseria because of the oligosaccharide nature of the saccharide moiety in
these organisms) in the pathogenesis and immunobiology of Neisseria
gonorrhoeae is unquestioned. Studies by Ward et al. (32), Rice and Kasper
(27) and Schneider and co-workers (28) indicate the LOS is a key target on
the gonococcal cell surface for human bactericidal antibody. Thus, an under­
standing of the physicochemical and antigenic structure of gonococcal LOS
as well as its characteristics in vivo and in vitro will be important in
comprehending the immunobiology of the gonococcus.

Isolation of gonococcal LOS was first published by Tauber and Garson
in 1959 using the phenol-water method (31). Little was known about this
material until Maeland and associates (13-20) published a series of studies
concerning the chemical and antigenic nature of gonococcal LOS. He has
described two major antigenic determinants in various crude endotoxin prepara­
tions and has designated these the alpha and beta antigens. Maeland be­
lieved that these antigens were components of the lipid A carbohydrate com­
plex of gonococcal endotoxin and proteins from the outer membrane. His
studies indicate that the beta antigen was a protein. The alpha determinant
is a carbohydrate and has been shown to lose antigenicity after oxidation
with sodium metaperiodate. This antigen is unaffected by pronase digestion.
Maeland had assumed that the alpha antigen may be analogous to the O somatic
antigen of the enterobacterial lipopolysaccharides. Using a hemagglutination
inhibition system with cross absorption, he has demonstrated that there may
be as many as six alpha antigen factors and has revealed a potential tool
for serotyping the gonococcus.

Perry et al. (23), Stead et al. (30), and Wiseman and Caird (33) studied
the chemical composition of gonoccal LOS isolated by phenol-water extraction.
These investigators found glucose, galactose, glucosamine heptose and KDO
present as the major components. Stead et al. were the first to recognize
that gonococcal LOS lacked the repeating O-side chains of the enterobacteria­
aceae and clearly showed that colonial type had no bearing on LOS chemical
structure.
The studies of Maeland et al. (13-20) are the foundations upon which more recent efforts in studying gonococcal LOS have been based. Apicella and co-workers confirmed and extended his observations and have isolated a series of six immunologically distinct acidic polysaccharides from the phenol-water LOS (2-9). These antigens are the carbohydrate component of gonococcal LOS and are isolated from the phenol-water extract of gonococci after NaOH digestion of the phenol-water extract followed by pronase digestion and ion exchange chromatography (2-4). The resulting product is a series of oligosaccharides linked by the amide linked lipid which retains the antigenic properties of the native LOS. It contains less than one percent protein or fatty acid and less than 0.5% nucleic acids (6). Studies have indicated that the antigenic structure of gonococcal LOS is complex with each LOS containing a common determinant (6). An additional antigen, termed the variable antigen, is present on three (types 1,3, and 4) of the six LOS serotypes. These studies have been confirmed in part by the work of Rappuoli who demonstrated two distinctive "core" antigens on one gonococcal LOS (25-26). A second gonococcal LOS was shown to contain only a single "core" antigen. In addition, our own studies have shown that each LOS contains its respective serotype antigen (6,22).

The terminology "rough and smooth" has been applied to Neisserial LOS by many investigators (6,25,26). Such terminology probably has little meaning when considered in the same context as enterobacterial LPS. Unlike enterobacterial LPS, gonococcal LOS has an intricate antigen structure without a series of repeating O side chains (10-12). Sizing of gonococcal LOS oligosaccharide chains indicates chain length no greater than the enterobacterial Ra mutant by both SDA-PAGE (11) and molecular sieve chromatography (28). Thus, while gonococcal LOS has specific serotype determinants similar to enterobacterial O-antigens, it lacks the corresponding chain length of these enterobacterial LPS. A different mechanism for expression of these antigens must be operative and probably is a steric arrangement of a limited repertoire of sugars. Studies by Allen and co-workers (1) and those of others (24) have indicated that phenol-water extracted gonococcal LOS contains glucose, galactose, glucosamine, galactosamine, and ethanolamine. The hexosamine content of the six serotype LOS preparations is quantitatively similar (1) but qualitatively different, with molar ratios of glucosamine to galactosamine varying from 1:1 (LOS type 1) to 4:1 (LOS types 4 and 5). Studies in a number of laboratories indicated that the O-D-galactopyranosyl (1-4)-D-glucopyranose (lactose) moiety may be an important constituent of the gonococcal LOS antigen structure (8,15). Using a monoclonal antibody, 3F11, which recognizes a region in the oligosaccharide portion of all six gonococcal LOS serotypes, Apicella and co-workers have identified its specificity for a D-galactosamine-O-D galactopyranosyl-(4-4)-glucopyranose moiety. In five of the six gonococcal LOS serotypes studied, isolation of the pyocin resistant variant resulted in a strain which lacked serotype antigen expression and also lacked the ability to bind 3F11 monoclonal antibody (8,21). This would suggest that presence or expression of this trisaccharide moiety while common to all gonococcal LOS studied, is related in some way to the serotype antigen expression.

The antigenic structure of gonococcal LOS is important immunobiologically, is complex and regulated by a number of diverse factors. The following results outline recent studies with this intriguing macromolecule.

Utilizing the serum resistant strain JW31 and its serum sensitive pyocin resistant variant, JW31R, bactericidal studies were undertaken to determine the role of the different human immunoglobulin types in the bactericidal response. The LOS of these strains differ since JW31 LOS contains the Gc, serotype antigen, the variable antigen and the common antigen, while JW31R LOS contains only the LOS common antigen (21). Thus, these bactericidal studies allow analysis of the effect of changes in the