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The immunochemistry of neisserial LOS

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Abstract. The outer membrane glycolipids of *Neisseria* lack long polysaccharides and are properly termed lipooligosaccharides (LOS). A *Neisseria* strain makes from two to six LOS of M_r 3150–7100. Different species commonly make LOS of identical M_r and epitope content. Oligosaccharide (OS) differences account for physical heterogeneity. OS consist of a conserved triantenary basal oligosaccharide, two linear segments of (n) hexose residues that determine OS mass, and terminal sequences similar to those of glycosphyngolipids. Epitope expression is linked to physical heterogeneity and conditioned by the molecular environment of the outer membrane. Serotype epitopes are expressed on M_r-restricted LOS. LOS regulate complement activation onto the bacterial surface and, hence, immune lysis.

Narrative

An historic perspective

Investigations of the immunochemistry of gonococcal endotoxins by Prof. J. A. Maeland (1966, 1969) demonstrated their biologic importance and stimulated us to study them. When Kasper et al. (1973), reported that meningococcal lytic antibody in human sera was specific for antigens other than capsular polysaccharides or denominated outer membrane proteins, we began studies of the analogous outer membrane glycolipids of *Neisseria meningitidis*. These efforts quickly led to serotyping systems for both species (Apicella 1976; Mandrell & Zollinger 1977), a radioactive antigen-binding assay of meningococcal lipooligosaccharide (LOS) antibodies (Bertram et al. 1976), chromatographic techniques for separating LOS into its component molecules and estimating their molecular mass (Schneider et al. 1978), and analyses of partial composition (Apicella 1976; Schneider et al. 1978).

At the time of the first of these biennial Symposia, we knew that each strain of *Neisseria* makes several glycolipids, of M_r 3000 to 7000 (Schneider et al. 1978),

and with structures disordered by intercatenary interposition of galactose (Perry et al. 1978). We also knew that LOS of *N. meningitidis* share antigens with those of *Neisseria gonorrhoeae* (Tramont et al. 1976), and that each strain expresses many antigenic determinants because each makes many different LOS molecules (Bertram et al. 1976). The antigenic diversity within LOS was thus linked to their physical heterogeneity.

Bactericidal, or lytic, antibodies were known to protect the bloodstream from neisserial dissemination, and the specificities of such antibodies were of great interest (Griffiss & Bertram 1977). LOS antigens could be shown to bind lytic antibodies (Stead et al. 1975; Rice & Kasper 1977), but our methods were inadequate to the task of unravelling the maddening physical diversity of these amphipathic and amphoteric molecules, and our efforts to more precisely define potentially protective epitopes were frustrated.

The development of a simple technique for visualizing sodium dodecyl sulfate (SDS)-disaggregated LOS in polyacrylamide gels (PAGE) (Tsai & Frasch 1982), the resolution of unique LOS molecules by refined SDS-PAGE separation (Schneider et al. 1984), and the antigenic precision of monoclonal antibodies (Apicella et al. 1981) have combined to provide us with powerful new tools. I will summarize our renewed progress in this report.

Physical structure of LOS

Because of their size, neisserial outer membrane glycolipids are most properly termed lipooligosaccharides, or LOS (Schneider et al. 1984). This term also serves to distinguish their disordered structure from that of the regularly ordered outer membrane lipopolysaccharides, or LPS, of enteric bacteria.

In Figure 1 the LOS of each neisseria are shown to be comprised of two to six individual molecules. The six LOS of strain PID_2 are of M_r 3150 to 5880 (Schneider et al. 1984); the mass of their monophosphoryl lipoidal moieties are 1463 to 1667, depending on the degree of acyloxyacylation and hydroxylation of substituent laurate residues (Takayama et al. 1986). Each LOS bears an unique glycose moiety, or oligosaccharide (OS). Mass differences among the OS of a stain's LOS are primarily responsible for the physical heterogeneity of the intact glycolipids (Griffiss et al. 1987).

Chemical structure of LOS

Neisserial LOS structures more resemble glycosphingolipids than enteric LPS. Their lipoidal moieties are analogous to those of enteric LPS, but their glycose moieties are triantenary (sometimes biantenary) structures that branch at basal heptose residues (Griffiss et al. 1988). Lipid moiety glucosamines are esterified with laurate, rather than myristate, and both subunits bear normal fatty acids

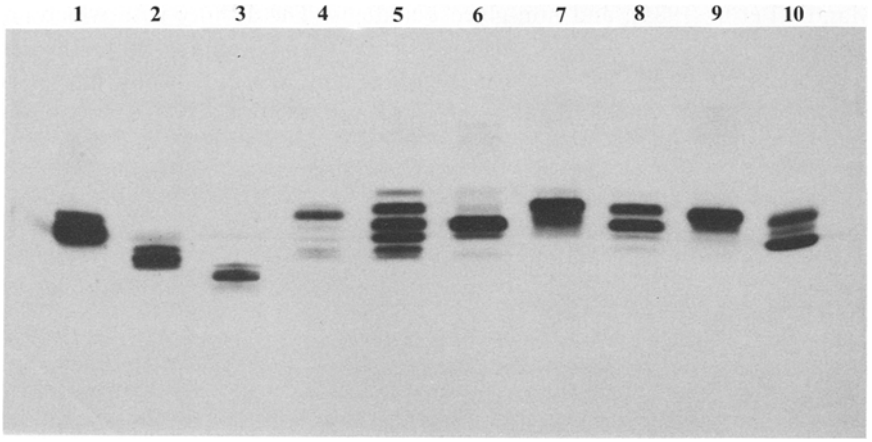

Fig. 1. Silver stain of SDS-PAGE-separated LOS of the Apicella-schema prototype *N. gonorrhoeae* serotype strains *(lanes 5–8)* and *N. meningitidis* group Y strain 8032 *(lane 4)*. The gonococcal serotypes are, respectively: 5 (PID_2), 2 (1291), 1 (1342), 6 (3893), 3 (4505), and 4 (8551). LPS of *Salmonella minnesota* Ra, RcP-, and Re isogenic rough mutants was co-electrophoresed as M_r markers in *lanes 1–3*, respectively. The six LOS made by meningococcal strain 8032 (*lane 4*; M_r from slowest to fastest migrating component: 5430, 5070, 4540, 4020, 3580, 3200) and gonococcal strain PID_2 (*lane 5*; M_r: 5880, 5280, 4640, 4070, 3490, 3140) can be used as M_r markers. Note the absence of repeating oligosaccharides and the heterogeneity and multiplicity of LOS made by each neisserial strain.

(Schneider et al. 1982; Takayama et al. 1986). Laurate residues are variously hydroxylated.

The main OS branch terminates in a tri- or tetrasaccharide similar to the terminal sequences of human glycosphingolipids; several meningococcal LOS terminate in Paragloboside of ABH blood group substances. The mass of the OS, and hence of the LOS, is determined by the length of the main branch and one of the tertiary branches; the various OS produced by a strain therefore differ in chemical composition (Griffiss et al. 1987). The main branch is elongated by interposition into an internal segment or by the elongation of this segment prior to "capping" with the terminal sequence. This maintains the terminal sequence in an "immunodominant" position. The tertiary branch is sequentially elongated. The secondary basal heptose is variously substituted with phosphoethanolamine.

Antigenic (serotypic) structure of LOS

The epitope structure of neisserial LOS is extremely complex (Mandrell et al. 1986). Epitopes are conformed of the oligosaccharide moiety, but their expression is also dependent on membrane integration, divalent cation-bridging between electronegative cavities of adjacent molecules to form "box-aggregates"

(Mandrell et al. 1985), and non-glycose adducts. The affinity with which an epitope binds an antibody is affected by its chemical environment including the presence of the lipoidal moiety which contributes *ca.* $2X10^{-1}$ of affinity, the particular membrane into which it is integrated, and the composition of flanking segments (Griffiss et al. 1988).

Each LOS bears several epitopes. As physical and chemical differences account for antigenic heterogeneity (Mandrell et al. 1986), the different OS of a strain's LOS have different antigenic compositions (Griffiss et al. 1987). We have ordered antigenic complexity by dividing LOS epitopes into four classes based on immunophysical characteristics (Griffiss et al. 1988).

- *Restricted epitopes* are found only on LOS of a certain M_r. Expression of the epitope denotes the presence of the M_r-restricted LOS. Serotypy is based on restricted epitopes; the SDS-PAGE profile of a strain's LOS therefore shows its serotype repertoire (Fig. 1). The meningococcal L8 serotype that is borne on a 3.6 kDa LOS is a restricted epitope (Schneider et al. 1985).
- *Common epitopes* are expressed on at least one LOS of most strains. The M_r of LOS bearing these epitopes varies; therefore the presence of the epitope gives no information about LOS repertoire.
- *Partially conserved epitopes* are common epitopes that are expressed on more than one, but not all, LOS of a strain.
- *Conserved epitopes* are common epitopes that are expressed on all LOS. The epitope may be occluded on LOS of high M_r.

LOS are not species-restricted. Immunophysically identical LOS are made by *Neisseria lactamica, N. meningitidis,* and *N. gonorrhoeae;* common epitopes are common to all three species (Griffiss et al. 1988). This accounts for our preference for the generic term, "neisserial LOS", and suggests ways of generating "natural" immunity.

Complement activation-regulation by LOS

Normal human serum IgM initiates immune lysis of serum sensitive (ser^s) *N. gonorrhoeae* by binding to an hexosamine-rich epitope on an high M_r LOS (Schneider et al. 1982; Griffiss et al. 1985; Griffiss et al. 1987); strains without this LOS are serum resistant (ser^r). The lytic effectiveness of bound IgM is a function of strain-permissive alternative complement pathway (ACP) augmentation of classical complement pathway (CP) activity (Griffiss et al. 1985). Differences in ACP augmentation of the same IgM-initiated CP signal reflects differences in a strain's LOS repertoire. Certain LOS also down-regulate immune lysis. Expression of the 3.6 kDa LOS that bears L8 and L11 serotype antigens results in a stain's becoming serum resistant (Schneider et al. 1985).

Biologic relevance of LOS

LOS serotyping can provide useful epidemiologic information, but its utility has not been adequately assessed (Griffiss 1982). LOS epitopes are present on neisserial surfaces in vivo. Patients respond immunologically to them during disseminated disease (Griffiss et al. 1984), and lytic antibody specific for LOS antigens commonly circulates in human sera. As the content of this Symposium amply demonstrates, the continued scrutiny of the complex immunochemistry of LOS should clarify the nature and generation of neisserial immunity and make possible the development of immunoprophylactics.

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