Letter to the Editor

Lipopolysaccharide Gel Profiles of *Haemophilus influenzae* Type b for Epidemiologic Analysis

Recently, Tolan et al. (4) reported that analysis of *Haemophilus influenzae* type b lipopolysaccharide (LPS) gel profiles was not a valid epidemiologic tool, because the apparent molecular weight of the LPSs of 5 of 14 isolates changed following passage in vitro or in vivo. Although the results were quite interesting, I was concerned because the gels published were very difficult to interpret. The LPS profiles (individual bands) were not visible, and the authors based their conclusions on apparent molecular weights of the LPSs. After discussing the problem with Dr. Granoff, I was sent 20 isolates of *H. influenzae* type b under code from the published study. The LPSs were extracted, and the profiles were analyzed as previously described (2). My results agreed with those reported by Tolan et al., indicating that some strains of *H. influenzae* type b may change the structure of their LPSs. I do not agree, however, that analysis of LPS profiles cannot be useful for epidemiologic analysis. In contrast, the work by Tolan et al. indicates that analysis of LPS profiles is a very sensitive method for identifying identical strains of *H. influenzae* type b during an outbreak. If the LPS profiles of different isolates of *H. influenzae* type b from a local outbreak are the same, it is very possible the isolates are of the same strain.

Other methods of subtyping *H. influenzae* type b include analysis of outer membrane protein profiles and electrophoretic typing. However, 44% of type b isolates studied have been classified into one outer membrane protein type (1), and 47% have been classified into one electrophoretic type (3). Therefore, these methods may not be sensitive enough to differentiate all genotypes. Since only about one-third of the isolates in the study by Tolan et al. changed their LPS profiles, a difference in the profiles of different isolates may still be relevant but should be interpreted with caution. I recommend that a combination of epidemiologic markers of *H. influenzae* type b be examined for careful epidemiologic studies and that LPS profiles can still provide useful epidemiologic information.

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Author's Reply

Among *Haemophilus influenzae* type b isolates, outer membrane protein subtypes (1) and electrophoretic types (4) have proven to be stable characteristics of a strain. Therefore, if two isolates differ in either of these characters, it can be concluded with a high degree of certainty that the isolates are not epidemiologically related. In contrast, as indicated in the above letter, Dr. Inzana has confirmed our findings that lipopolysaccharide (LPS) gel profiles are not stable features of some *H. influenzae* type b strains (5) and that two isolates derived from a single strain can show distinctive differences in LPS gel mobility. Thus, the conclusions in several previous epidemiologic studies (2, 3) of strain differences being present based on differences in LPS gel mobilities may be erroneous.

Dr. Inzana also suggests that if two isolates have identical LPS gel profiles, the organisms are likely to represent a single strain. However, the inference that two isolates are epidemiologically related because they share certain chemical or molecular markers is basically a statistical conclusion, and the validity of the analysis is dependent on the prevalence of the markers being measured among randomly obtained isolates. Unfortunately, we lack data on the prevalence of different LPS gel patterns among randomly obtained type b isolates or on the reproducibility of the gel patterns measured in different laboratories. Therefore, any conclusions with respect to applying this technique to determination of strain similarities must await further data.

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