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Is Asialo GM_1 Present in Rabbit and Human Corneal Epithelium?

Recently, using solid-phase and thin-layer chromatography (TLC) overlay assays, we have shown that *Pseudomonas aeruginosa* binds to the glycolipid asialo GM_1 and that the lipid is detectable in bovine corneal epithelium (1). Similar results also have been reported by other investigators in nonocular studies (3, 4). In contrast, the recent article by Zhao and Panjwani (5) reported that they could not detect asialo GM_1 in rabbit or human corneal epithelium. The authors also described immunohistochemical studies from this laboratory in which Hazlett et al. detected asialo GM_1 in mouse corneal epithelium (2) and their inability to do the same. They did not indicate that we also used an immunogold labeling procedure, which is highly sensitive and that positive, albeit sparse, labeling at the epithelial wound site also was detected. Nor did they mention binding inhibition assays which showed that incubation of wounded corneas with anti-asialo GM_1 antibody decreased subsequent bacterial binding by approximately 47% when compared with other nonspecific antibodies for which bacterial binding did not differ from control levels (2). The authors also criticized our lack of biochemical data to support these morphological studies. Unfortunately, they overlooked our extensive biochemical study (1) which established the presence of asialo GM_1 in bovine corneal epithelium using an antibody specific for the glycolipid (Wako Chemical, USA Inc., Dallas, Tex.) and an enhanced chemiluminescence technique (ECL; Amersham, Arlington Heights, Ill.). In that study, we also established that pili and lipopolysaccharide of the bacterium served as adhesins and that binding to asialo GM_1 was specific, competitive, and saturable.

Zhao and Panjwani (5) could not detect asialo GM_1 using either histochemical (no immunogold labeling attempted) or biochemical methods. It is difficult to determine why this occurred, but there may be some obvious explanations. Focusing our comments on the lipid isolation, we have used either a total or neutral lipid extract in our lipid separation by high-performance TLC, whereas these authors removed the corneal phospholipids by saponification. With this procedure, it is possible that other lipids, such as asialo GM_1, could have been lost or altered. Secondly, we used a commercial polyclonal antibody, whereas they used a monoclonal antibody. Thirdly, the authors used a less sensitive colorimetric method to detect antibody binding, whereas we used ECL, a technique which comparably is 10 times more sensitive (according to the manufacturer).

Whether the glycolipid is present in rabbit or human corneal epithelium remains hypothetical, but we speculate that it is highly likely and certainly requires further investigation.

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Author's Reply

Multiple studies have independently shown that *P. aeruginosa* binds to asialo GM_1, and we fully concur with this. Our studies were designed to establish whether asialo GM_1 is present in corneal epithelium. Our data suggest that neither rabbit nor human corneal epithelium contains detectable levels of asialo GM_1 even after corneas were scarified and incubated with trypsin to expose potential cryptic sites (4). In contrast, in a recent report, Hazlett et al. (2) have claimed that tissue sections of scarified mouse corneas treated with trypsin reacted positively with anti-asialo GM_1 by immunofluorescence as well as by immunogold staining methods. It is unclear why the authors detected sparse staining with the sensitive immunogold method but rather intense staining with less sensitive immunofluorescence method. With the immunofluorescence technique, even the stromal matrix was found to react positively with the anti-asialo GM_1 antibody, an observation difficult to reconcile with the fact that glycolipids are not usually found in the extracellular matrix. Furthermore, we found that frozen sections of mouse corneas reacted with the secondary antibody, anti-mouse immunoglobulin M (IgM), even when they were not treated with a primary antibody (4). This is to be expected because it is well established that immunoglobulins are present in the cornea.

As stated in our report (4), Hazlett et al. (2) also provided indirect evidence for the presence of asialo GM_1 by studies which indicated that *P. aeruginosa* binding to scarified mouse corneas could be inhibited by anti-asialo GM_1. However, in the absence of the positive identification of the antigen in the tissue, results of such binding inhibition assays are difficult to interpret. It is not clear whether the antibodies used for inhibition studies were Fab′ fragments, purified IgM proteins, or ammonium sulfate precipitates.

We have not overlooked the biochemical data obtained by Gupta et al. using bovine corneal epithelium (1). That study was published in October 1994, after we submitted our manuscript (4) in August 1994, and we have read it with interest. It does not convincingly show the presence of asialo GM_1 in corneal epithelium. It is possible that the *P. aeruginosa* binding to the bovine corneal component detected in that study (1) is not due to asialo GM_1 but is rather due to the presence of *P. aeruginosa*-reactive phospholipids in the corneal epithelial lipid extracts which comigrate on TLC plates with asialo GM_1. Support for such a possibility stems from a number of factors including (i) a discrepancy in the findings of Gupta et al. (1) that, although the immunostaining of the asialo GM_1 standard was much more intense compared with that of the corneal component, which stained only equivocally with the antibody (anti-asialo GM_1), the extent of *P. aeruginosa* binding to the corneal component was markedly greater than to the glycolipid standard; (ii) the corneal epithelial lipid extracts analyzed in the study (1) were not saponified, a process that eliminates phospholipids; and (iii) recent studies from my laboratory indicating that *P. aeruginosa* binds to specific phospholipids (3).

Moreover, the presence of excess lipids in the chromatograms of corneal lipid samples may have caused nonspecific equivocal immunostaining with antiasialo GM_1 detected by Gupta et al. (1).

As regards the concern that asialo GM_1 in our studies could have been lost during saponification, we believe that to be highly unlikely. It is well established that glycolipids are highly resistant to saponification. Regarding the sensitivity of the assays used, we have two comments: (i) the avidin-biotin-peroxidase method we used for detection of antigen-antibody complexes in the tissue sections of corneal epithelium is in general more sensitive than the immunofluorescence method; (ii) the colorimetric assay we used for TLC-immunostaining was sensitive because even as little as 5 ng of standard asialo GM_1 could be detected (4). Therefore, it would appear that even low levels of asialo GM_1, if present in corneal epithelium, would have been detected by the protocols used in our study (4), especially since we used at least 10 times more material than Gupta et al. (1) for TLC-immunostain assays (compare the intensities of the orcinol-stained chromatograms of the two studies) (1, 4). Finally, while it is conceivable that species-related differences may be present with respect to the expression of glycolipids in corneal epithelium, it may be premature to address this issue.

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