

Pitfalls of Proteomics

Lindholt et al¹ used purified serum antibodies to *C pneumoniae* outer membrane protein (OMP) to probe protein extracts of abdominal aortic aneurysms separated by 1D and 2D electrophoresis. Although no specific signal for OMP was detected, strong staining was obtained for a 50-kDa protein, which subsequently was identified as immunoglobulin heavy chain through the use of mass spectrometry. The authors conclude that OMP antibodies cross-react with human immunoglobulins and that *C pneumoniae* might trigger an autoimmune reaction.

We agree that autoimmune reactions are a possible link between infections and atherosclerosis.^{2,3} However, additional controls are essential for supporting their conclusion, ie, a negative control probing the blots with horseradish peroxidase-conjugated secondary antibody only. It is well established that immunoglobulin deposits accumulate during progression of atherosclerosis.⁴ We frequently have observed strong positive staining for immunoglobulin chains in protein extracts of human vessels when the blot was probed with anti-human secondary antibody only. Hence, it is possible that their secondary antibody has recognized immunoglobulins in protein extracts of abdominal aortic aneurysms irrespective of the primary antibody, and according to their mass spectrometry data, the conjugate might have been specific for gamma (heavy) chains.

Furthermore, with regard to their experimental design: Silver staining only visualizes the most abundant proteins (detection limit 10^{-9} g) and more than one protein can be present in a single spot, which complicates data interpretation. Nevertheless, if mass spectrometry data suggest that one protein predominates in a particular spot on 2D gels, then it is likely that this protein also dominates the silver-staining pattern. In contrast, immunoblotting combined with enhanced chemiluminescence (ECL) detection amplifies the signal (detection limit 10^{-12} g). Consequently, ECL will visualize proteins at concentrations well below the limit of detection for silver staining and mass spectrometry. Because of this difference in sensitivity, one has to be extremely cautious to assume that spots detected with the ECL method correspond to a protein stained by silver and identified by mass spectrometry. The results might be accurate only if the protein of interest happens to be present in high abundance, as demonstrated for immunoglobulin heavy chains. For less abundant proteins, eg, *C pneumoniae* OMP, this approach is very likely to give wrong identifications. Thus, any potential cross-reactivity must be, at least, confirmed by independent methods.

We appreciate that the use of proteomic techniques is a timely one,⁵ but we hope that our comments can increase the awareness for this important methodological limitation.

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Response

We thank Mayr et al for commenting on our recent article.¹ We demonstrated by enhanced chemiluminescence (ECL) that an affinity-purified human antibody fails to detect outer membrane protein in extracts of abdominal aortic aneurysms in 16 of 17 patient samples. This conclusion was reached by using 1D and 2D gel electrophoresis with subsequent blotting where no specific reaction was seen according to the molecular mass and pI of outer membrane protein. However, we found that a strong reaction was obtained by ECL at 50 to 60 kDa and pI approximately 10. Similarly localized silver-stained spots were identified as immunoglobulin heavy chain by mass spectrometry.

Mayr et al have now drawn our attention to the fact that they have frequently observed strong positive staining for immunoglobulin chains in protein extracts of human vessels when blots were probed with anti-human secondary antibody. Thus, they suggest that it might be that the secondary antibody has recognized immunoglobulins in the protein extracts irrespective of the presence of the primary antibody. Because we have used a human primary antibody and a secondary anti-human antibody, we must agree with Mayr et al that we cannot rule out such a possibility.

Finally, Mayr et al have some general comments on the use of proteomic techniques with regard to detection levels. We agree that with 2D gel electrophoresis there is always a possibility that 2 or more proteins comigrate² and that this may present a problem because the detection levels are very different, about 10^{-9} g for silver-staining and mass spectrometry and about 10^{-12} g for immunoblotting combined with ECL. A high-abundance protein detected by silver-staining and identified with mass spectrometry could thereby comigrate with a different low-abundance protein easily detected with the ECL technique. Thus, we fully agree with Mayr et al that one should be cautious when claiming correspondence between 2 proteins identified with either technique when the protein is less abundant.

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