temperature scanning electron microscopy might be a mucin barrier to bacterial infection. Fresh urothelium stained by dilute Hale's colloidal iron (a mucin stain) and subsequently examined in the frozen hydrated state using low temperature scanning electron microscopy and x-ray microanalysis showed a thin layer of iron over the entire surface of superficial cells with focal accentuation in the extracellular surface layer (unpublished observations). This leads to the hypothesis that the superficial cells secrete a surface layer which is continuously shed and is only visible by low temperature scanning electron microscopy where the shed secretions pool. Furthermore, synthesis and shedding must be coordinated so that a thin surface layer is always present.

The bladder is remarkable in that it has a large epithelial lined surface without a resident bacterial flora. The microbial flora on other surfaces probably survive because of specific bacterial adhesins which combine with epithelial surface antigens. If, however, the bladder sheds its surface layer in the coordinated way that we suggest then any bacteria which adhere to it will be shed and it will remain free of bacterial colonisation.

The physical bond between bacterial adhesin and epithelial surface receptor is equivalent to the bond between antibody and its specific antigen, or lymphocyte surface receptor and target cell antigen. Thus if tumour cells synthesise and shed Ca glycoprotein in the same way as urothelial cells then any tumour specific antibodies, or specifically armed lymphocytes, or macrophages, which adhere to their surface, will be shed, like bacteria in the bladder, before they do harm. Furthermore, the shed antigen will act as decoy molecules and might induce immunological tolerance. Thus tumours that copy the urothelial bacterial clearance mechanism are more likely to avoid immune rejection and produce clinical cancer.

Discussion

The urothelium is special, although not unique, in not having a resident microbial flora. It obviously must have an efficient system for clearing bacteria, and it is our contention that tumour cells copying this mechanism will be particularly effective at avoiding immune attack. There are other body surfaces that are not normally colonised by bacteria, including the alveolar lining cells of the lung and ducted glands. It is therefore of interest that Ca glycoprotein is found on both activated type II pneumocytes and in eccrine and apocrine glands. It is also likely, however, that there are other glycoproteins, which are effective in clearing bacteria in these sites, and some of the tumours, which do not exhibit Ca glycoprotein, might copy these mechanisms. The fallopian tube, which leads directly into the peritoneal cavity, also needs an effective system of bacterial clearance, and interestingly, it too secretes the Ca glycoprotein.

Trophoblast is a foreign tissue, which like tumour cells, avoids immune rejection. The observation that it also produces the Ca glycoprotein provides further support for this hypothesis.

One of the problems with this explanation is that in some tumours there is only focal expression of the Ca antigen. This could be because of the relative insensitivity of immunoperoxidase techniques in formalin fixed tissue failing to detect a thin cell surface layer. It is possible, however, that once immune tolerance develops to shed antigens, clones of cells might arise which lack the mechanism. It is certainly possible that the mechanism is maximally operative when the tumour is first establishing itself in the body.

An important corollary of the idea that shedding of surface glycoprotein protects against immune rejection is the obvious conclusion that in the absence of the mechanism rejection would occur. This implies that there are antigens, perhaps more firmly bound as integral membrane proteins, which are sufficiently tumour specific to allow an immune attack.

JA MORRIS*
K OATES†

*Department of Pathology, Lancaster Moor Hospital, Lancaster LA1 3JR
†Department of Biological Services, University of Lancaster, Lancaster LA1 4YQ
‡Royal Lancaster Infirmary, Department of Urology, Lancaster

Comparison of API-10S strips and tube tests for biotyping Haemophilus influenzae

Biotyping strains of Haemophilus influenzae according to the scheme devised by Kilian has proved useful for epidemiological purposes. Biotypes I and II, for example, are those most commonly reported in meningitis and septicemic illness, although geographical differences are evident. We have previously reported on the value of the technique when applied to isolates from patients with cystic fibrosis.

The procedure has been simplified by the use of API-10S strips for detecting the formation of urease, ornithine decarboxylase and beta-galactosidase (ONPG), and the formation of indole. We have, however, found that the technique recommended by API and based on the report of Mehtar and Aminafshar has very serious limitations. Briefly, this technique entails an inoculum prepared by suspending one colony of H influenzae in 3.0 ml of deionised water (pH 7.0) containing one standard X 2 disc. We compared results using this API-10S strip method with those obtained with a similar inoculum in sterile 3 x 1/4 inch tubes using double strength media in 0.25 ml amounts. We also compared both test systems using a heavy inoculum of 1.25 x 10^6 colony forming units suspended in sterile distilled water. Modified Christensen's medium was used for urease detection and modified Falkow's medium for ornithine decarboxylase. Indole formation was detected with Kovac's reagent.

References

Letters

The Table shows results obtained with 24 strains of ONPG negative *H influenzae*. Using the heavy inoculum, results were identical for tube and API-10S strips, except for one strain that was positive for ornithine decarboxylase in the tube but negative on the strip (strain 8). The Table clearly shows the inadequacy of the smaller inoculum. The numbers of positive tube tests for urease, ornithine decarboxylase, and indole formation were 18, 11, and 16, respectively, for the heavy inoculum compared with 16, 1, and 12 for the smaller inoculum. Inoculum size seems to be especially critical for detection of ornithine decarboxylase. A series of replicate tests showed that reproducible results could be obtained with either tube or API-10S strips with the heavy inoculum, but all three tests gave variable results with smaller inocula. A series of viable counts performed on inocula prepared according to the API recommendations gave values ranging from 1·3 x 10³ to 3·0 x 10⁵ colony forming units, depending on colony size. We found that a minimum inoculum size of 1·25 x 10⁶ colony forming units is required for reliable detection of urease and indole formation and 1·25 x 10⁶ for ornithine decarboxylase production.

Identification of *Legionella pneumophila* with commercially available immunofluorescence test

*Legionella pneumophila* is recognised in the clinical laboratory by its cultural and biochemical characteristics. Confirmation of identity is either by immunofluorescence or slide agglutination with known antisera. In addition, strains may be examined further for fatty acid content, ubiquinone content and, in the case of new serogroups, by DNA homology to confirm their identity. A monoclonal antibody has recently been produced, which reacts with a protein with a molecular weight of 29 000 present in *Legionella pneumophila* but which was not detected in other legionellas.

This antibody does not cross react with a wide range of other bacterial species. A commercially available kit has been produced using this monoclonal antibody conjugated with fluorescein isothiocyanate (Genetic Systems Corporation, Seattle, Washington, United States) that does not require specimens to have special pretreatment with detergent and edetic acid, as described previously.

This antibody can be used to examine either preparations of bacterial antigens or clinical specimens in a direct fluorescence antibody test (DFAT). The Table shows the strains of legionellas and other organisms examined. Legionellas were grown on buffered charcoal yeast extract agar and suspensions in 1% formal saline or, in the case of some legionellas, aqueous suspensions of organisms heated at 100°C for 15–30 minutes were used. A suspension made to the turbidity of McFarland No 1 standard is recommended. One drop of the suspension was placed in a well of a 12 well Teflon coated slide (Hendley, Essex). Suspensions were air dried and then lightly heat fixed. The minimum amount of the fluorescein conjugated monoclonal antibody necessary to cover the well of the slide was applied and the slides incubated at 36°C for 20 minutes in a moist chamber. After rinsing and mounting slides were examined using a × 10 eye piece and a × 50 water immersion objective in a Leitz microscope with a Ploempak incident light fluorescent attachment. Formal saline suspension of *L pneumophila* of all serogroups fluoresced apple green under examination by ultraviolet light, some strains fluorescing more brilliantly than others. Some heated suspensions of *L pneumophila* did not fluoresce, but this may have been due to deterioration of the antigen on

Table Effect of varying inocula in tube tests

<table>
<thead>
<tr>
<th>Strain</th>
<th>Inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1·25 x 10⁶ cfu</td>
</tr>
<tr>
<td></td>
<td>Ur</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
</tr>
</tbody>
</table>

Ur = urease
ODC = ornithine decarboxylase
Ind = indole

References


Strains of organisms examined with monoclonal antibody

- *L anisa*
- *L bozemani* (serogroups 1 and 2)
- *L cherrillii*
- *L demoffii*
- *L erythra*
- *L feelei* (serogroup 1)
- *L gormanii*
- *L hackellae*
- *L jamestowniensis*
- *L jordanis*
- *L longbeachae* (serogroups 1 and 2)
- *L maceachernii*
- *L micdadei*
- *L okridogensis*
- *L parisiensis*
- *L pneumophila* (serogroup 1, 5 strains) (serogroups 2, 3, 6–10, and undesignated strain P183)
- *L rubriucens*
- *L saintelensis*
- *L santivicci*
- *L spiriniensis*
- *L steigerwaltii*
- *L wadsworthii* (undesignated strains 1267, 1466)
- “Species 1” organisms (Los Angeles 1, Dallas IE)
- “Species 2” organisms U7W, U8W and MIC-UB
- *Brucella abortus*
- *Bacteroides fragilis*
- *Pseudomonas aeruginosa*
Comparison of API-10S strips and tube tests for biotyping Haemophilus influenzae.

K C Watson, E J Kerr and M Baillie

doi: 10.1136/jcp.39.6.692

Updated information and services can be found at:
http://jcp.bmj.com/content/39/6/692.citation

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/