Detection of antibodies to Staphylococcus epidermidis in infected total hip replacements by an enzyme linked immunosorbent assay

Infection is the most serious complication of total hip replacement operations, with reported rates of between 0.77% and 11%. Coagulase negative staphylococci are often implicated in these prosthetic infections, but as they tend to be chronic and low grade, a positive diagnosis may, in some cases, be difficult. In these cases, the ability to detect the infection by showing increased concentrations of antibodies to the organism would be a useful adjunct to the diagnosis. The use of an agglutination test to detect antibodies to Staphylococcus epidermidis in patients with infected cerebrospinal fluid shunts has been described. Although a good discrimination between results from infected and uninfected patients was obtained in this generally younger population, this technique has proved less successful in cases of infected total hip replacements, where most patients are over the age of 60.

The use of cell wall teichoic acids from Staphylococcus aureus to detect the serological response to infections by these organisms has been successful, and we thought that a similar cell wall antigen from a coagulase negative staphylococcus might be used for the detection of prosthetic infection. Staphylococcus epidermidis sensu stricto, which contains the cell wall polysaccharide B, is the most common biotype of coagulase negative staphylococcus isolated from clinical sources. An antigen was therefore prepared from an organism known to contain cell wall polysaccharide B, and its potential for use in the serological diagnosis of infection of total hip replacements was investigated.

Material and methods

**ANTIGEN PREPARATION**

The organism used was isolated from an infected total hip replacement. It was biotyped as *Staphylococcus epidermidis* using API Staph. (API Laboratories Ltd) and was shown to contain cell wall polysaccharide B. The antigen was prepared as described previously. Briefly, this consisted of extracting 20 g of organisms with 0.07M phosphate buffer at pH 6.5; precipitating with ethanol; and, after dialysing against distilled water, lyophilising the resulting polysaccharide.

**ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)**

An indirect ELISA described previously was used and the method was based on that detailed for viral antigens with the following exceptions: the serum was incubated for 90 min at 37°C and the substrate, at a concentration of 0.25 g/l, was incubated for 1 h at room temperature. The antigen was used at a dilution of 10 mg/l and the samples of serum were diluted 1/400 for use in the test.

The antibody concentration in each sample of serum was expressed as a fraction of the optical density obtained with a positive control serum tested on the same microtitre plate.

Serum samples from 88 patients about to undergo surgery at the Royal National Orthopaedic Hospital were tested using the method described to assess the concentration of antibody in an uninfected population. The ages of the patients ranged from 4 to 84 years with average age of 56.9 years. In 95% of cases the serum from these patients gave an ELISA result of less than 0.6, and this was therefore taken to be the upper limit of normal.

**Case report**

A 73 year old woman underwent a right total hip replacement for osteoarthritis in May 1976. Her immediate postoperative course was uneventful, but later in the same year she noticed that her hip was clicking when she walked. In May 1977 she complained of pain in her hip and a radiograph showed changes that were thought to indicate infection. At this time, she had an erythrocyte sedimentation rate of 48 mm in the first hour (Westergren). From this stage onwards the hip began to deteriorate clinically, and it was removed in May 1979. At operation, there was no pus present, although much granulation tissue was seen. Both the acetabular and the femoral components were loose. Immediately after removal the prosthesis was sent to the laboratory, where it was examined as described previously. A heavy growth of coagulase negative staphylococci was obtained from the broth used to wash the prosthesis, and this was defined by API Staph as *Staphylococcus epidermidis* (API code 6606113). The Table shows the results of sequential serological studies on this patient.

**Discussion**

The increase in erythrocyte sedimentation rate in patients with infected total hip replacements has been described previously. Although this test on its own is simple to perform and a fairly reliable indication of infection, it is non-specific with regard to the causative organism. Therefore, the availability of an additional test such as the ELISA described here, which allows the detection of specific antibody concentrations, may offer an advantage. In this particular case, the antibody concentrations were considerably higher than the

**Table Results of sequential serological studies on patient described in case report**

<table>
<thead>
<tr>
<th>Months after total hip replacement</th>
<th>ELISA (OD)</th>
<th>Erythrocyte sedimentation rate (mm in the first hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.205</td>
<td>4</td>
</tr>
<tr>
<td>12</td>
<td>1.19</td>
<td>48</td>
</tr>
<tr>
<td>13</td>
<td>1.19</td>
<td>50</td>
</tr>
<tr>
<td>14</td>
<td>1.01</td>
<td>42</td>
</tr>
<tr>
<td>16</td>
<td>1.14</td>
<td>23</td>
</tr>
<tr>
<td>18</td>
<td>1.4</td>
<td>55</td>
</tr>
<tr>
<td>22</td>
<td>1.11</td>
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<tr>
<td>26</td>
<td>1.37</td>
<td>49</td>
</tr>
<tr>
<td>38</td>
<td>0.82</td>
<td>15</td>
</tr>
<tr>
<td>41</td>
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</tr>
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<td>43</td>
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</tr>
<tr>
<td>49</td>
<td>0.45</td>
<td>3</td>
</tr>
<tr>
<td>61</td>
<td>0.37</td>
<td>8</td>
</tr>
</tbody>
</table>

The prosthesis was removed in month 36.
normal level of up to 0·6 even only one year after insertion, the erythrocyte sedimentation rate was also raised. In some cases however, the antibody response may not be quite so pronounced, and here a preoperative serum sample should be examined, so that the two values may be compared. Had samples of serum been taken more frequently in the immediate postoperative period an earlier diagnosis could perhaps have been made. In cases of infection the detection of increased antibody concentrations as soon as possible after the total hip replacement operation may mean that appropriate treatment can be started, thereby increasing the likelihood of successfully salvaging the prosthesis.

We wish to thank the surgeons at the Royal National Orthopaedic Hospital whose patients were included in this study.

S BARSHAM
R BAYSTON
Department of Paediatric Surgery,
Institute of Child Health,
London WC1

SY ALI
Department of Experimental Pathology,
Institute of Orthopaedics,
Royal National Orthopaedic Hospital,
Stanmore, Middlesex

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Cryopreservation with glycerol during cryostat sectioning for localisation of lymphocytes and accessory cell phenotypic subsets in tissue biopsies

The phenotypic antigens of lymphocytes and accessory cells recognised by monoclonal antibodies are readily destroyed by traditional methods of histological prepara-
tion1 and so the immunoperoxidase method is usually applied to cryostat sec-
tions; the sections are often fixed briefly in acetone before staining. We have recently shown that an adaptation of the freeze sub-
stitution method2 may be applied to the immunocytochemical localisation of lymphocytes and accessory cells in tissue biop-
sies treated with small quantities of acetone before cryostat sections are cut to improve the contrast of the immunoperoxidase staining without loss of specificity.

Preliminary experiments were made on samples of human tonsil. Small blocks were snap frozen by immersion in liquid nit-
rogen or an acetone and dry ice slurry. Many of the latter group were accidentally contaminated with acetone; some of the former group were subsequently intention-
taneously contaminated with small amounts of the solvent. The acetone free blocks were readily sectioned in the cryostat, but the acetone treated blocks could not be cut properly. Two types of cryoprotectant solu-
tions were tried: 5, 10, and 20% glycerol and 5% dimethylsulphoxide, all precooled to 0°C. Acetone contaminated blocks were immersed in these cryoprotectant solutions for 15 to 30 min, placed on metal chucks, refrozen with Cryo Jet spray (Raymond Lamb, London NW10 6JL), and sectioned at 6 μm in a cryostat. Of the cryoprotec-
tants studied, only 5% glycerol consistently allowed the cutting of serial sections; the blocks that had had a greater exposure to acetone required longer immersion in glycerol and sometimes had to be refrozen repeatedly with Cryo Jet spray while in the cryostat. Haematoxylin and eosin stained sections of this tissue did not show any evi-
dence of ice crystal damage and fairly intense immunoperoxidase staining was obtained with monoclonal antibodies to lymphocyte subsets.

A normal volunteer was given an intra-
dermal injection of 0·1 ml of 1/1000 (10 units) purified protein derivative from Mycobacterium tuberculosis (Evans Medical Ltd, Middlesex, England) on the volar aspect of the forearm; 48 h later a biopsy was taken from the centre of the delayed hypersensitivity reaction with a 4 mm skin punch (Stiefel Laboratories Ltd, Slough, England). This was bisected, and the halves were frozen as in the pilot experiments. The acetone treated block was exposed to 5% glycerol cryopreservant and refrozen. Serial 6 μm sections were cut from each block. The immunocytochemical staining experiments were made on contiguous groups of sections from the acetone treated and un uncontaminated blocks. After treat-
ment with the monoclonal antibody, the section was then treated successively with the Vectastain kit containing biotinylated antimouse immunoglobulin and the third stage reagents, avidin/biotinylated peroxi-
dase (Sera Lab Ltd, Crawley Down, Sussex, UK), and finally a histochemical method was used for generation of the brown reaction product.3 Preliminary examination suggested that the immunocytochemical staining was similar in both sets of sections and so detailed histometric measurements2 were made to determine whether the extra procedures influenced the sensitivity and accuracy of the immunoperoxidase localisation of the two major subpopulations of lymphocytes.

The patterns of staining in acetone and cryopreservant treated sections and the controls stained in the same batch were closely similar; the intensity of staining of T8 cells (Leu 2a, Becton Dickinson) was similar in both blocks, but T4 cells (Leu 3a) were stained more intensely and more crisply in sections from the cryoprotectant treated block. The Table summarises the
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S Barsham, R Bayston and S Y Ali

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