

Detection of group B streptococcal antigen in necropsy specimens using monoclonal antibody and immunoperoxidase staining

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SUMMARY A murine monoclonal antibody, which recognises various serotypes of group B *Streptococcus* in formalin fixed, paraffin embedded tissue, was used to show the organism in necropsy specimens of newborn infant lung by an indirect immunoperoxidase technique. The method seemed to be complementary to that of Gram staining, and may be successfully used to identify group B *Streptococcus* antigen in histopathological material.

Group B *Streptococcus* is a common cause of bacterial sepsis in newborn infants. Disease of early onset, which occurs in the first week of life, produces high mortality, especially if treatment is delayed.¹ Although the clinical presentation is often non-specific, a microbiological diagnosis may be made, usually without difficulty, by culturing blood, cerebrospinal fluid, urine, and tracheal aspirate. More recently, several rapid methods for detecting antigens, including latex agglutination and coagglutination, have become available.^{2,3} Nevertheless, some undiagnosed necropsy specimens such as lung tissue, showing evidence of pneumonia, may be due to group B *Streptococcus*.

We describe an indirect immunoperoxidase staining technique for use on tissue, infected with group B *Streptococcus*, using both monoclonal and polyclonal antibody preparations.

Material and methods

ANTI-GROUP B *STREPTOCOCCUS* MONOCLONAL ANTIBODY

A monoclonal antibody reactive with all types of group B *Streptococcus* was prepared using a method similar to that described by Ruch and Smith.⁴ A BALB/c mouse was immunised with a 0.3 ml suspension of 0.3% formalinised group B *Streptococcus* strain 090R (NCTC 11360), prepared as described by Lancefield,⁵ and injected intraperitoneally. After

seven weeks a single intravenous boost was given three days before the spleen was removed for hybridisation.

Fusion of spleen cells with NS1 plasmacytoma cells was based on the Kohler and Milstein technique.⁶ One millilitre of 50% polyethylene glycol (Sigma) was added to a pellet of combined spleen and NS1 cells for one minute, mixed for one minute, and then 2 ml of Roswell Park Medical Institute 1640 medium (Flow Laboratories) was added over a period of two minutes. After washing the cells were suspended in medium containing 10% fetal calf serum, hypoxanthine, aminopterin, and thymidine. A series of 2 ml wells were filled using a suspension of 5×10^4 cells/well. These were screened for antibody to group B *Streptococcus* on day 16, using the indirect agglutination technique described below. Wells positive for antibody were cloned twice by limiting dilution, once at 10 cells/well and then at a dilution averaging one cell/three wells, after which all supernatants contained anti-group B *Streptococcus* antibody. Supernatant collected after five days of cell growth was used for further screening against previously typed clinical isolates of various serotypes of group B *Streptococcus* (Ia, Ib, Ibc, II/R, and III); streptococci belonging to groups A, D, and G; *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. One antibody was found to react with the representative group B *Streptococcus* serotypes (but not with any of the other organisms tested), by using the immunoperoxidase method on a cytospin preparation of whole formalinised bacteria.

INDIRECT AGGLUTINATION TECHNIQUE

This method was developed to detect antibody to group B *Streptococcus* for use as a rapid screening method in the preparation of the described monoclonal antibody.

Twenty μl of phosphate buffered saline (pH 7.4) was mixed with 10 μl of the test supernatant in a U shaped microtitre well. Subsequently 30 μl of a suspension of group B *Streptococcus* killed in 3% formaldehyde was added. After five minutes of incubation at room temperature 30 μl of a 1/100 dilution of goat antimouse IgG serum (Miles Scientific) was added to the well and incubated for 10 minutes at room temperature. All dilutions were made with phosphate buffered saline. The well was then centrifuged at 1000 rpm at 4°C for five minutes. Agglutination of the group B *Streptococcus* was indicative of the presence of anti-group B *Streptococcus* IgG. Positive screening tests were confirmed by immunofluorescence.⁷

POLYCLONAL SERUM

Commercially available group B *Streptococcus* anti-serum raised in rabbit was used (*Streptococcus* grouping serum group B, Wellcome Diagnostics).

LUNG TISSUE

Lung tissue was removed at necropsy from three newborn infants who had died as a result of overwhelming group B *Streptococcus* septicaemia and pneumonia within 48 hours of birth. In each case the organism had been isolated from blood cultures before death.

Lung tissue was also removed from three newborn infants known to have died from microbial causes other than group B *Streptococcus* for control purposes. The three organisms were *Listeria monocytogenes* and *Staphylococcus aureus*, both associated with pneumonia, and *Pseudomonas aeruginosa*, the cause of a septicaemia in this infant.

IMMUNOPEROXIDASE STAINING

Sections (4 μm thick) of formalin fixed and paraffin embedded lung were stained by the indirect immunoperoxidase technique⁸ with the anti-group B *Streptococcus* monoclonal antibody. Staining was optimal at a dilution of 1 in 10 and at an incubation time of one hour. Serial sections of lung were also stained with the polyclonal antibody by the peroxidase-antiperoxidase (PAP) technique.⁹ The staining with this antibody was optimal at a dilution of 1 in 100 and an incubation time of 30 minutes.

Endogenous peroxidase was inhibited in all sections using 0.5% hydrogen peroxide in methanol for 10 minutes. Control sections of lung in which Tris buffered saline and non-immune serum replaced the primary antibody were run in parallel with the other

immunoperoxidase slides. All immunoperoxidase sections were counterstained with Mayer's haematoxylin.

In addition, sections of the lung were stained with haematoxylin and eosin and Gram's stain for comparison.

Results

Light microscopy of all three lung sections showed the presence of pneumonia; in two cases this was patchy. Hyaline membranes were also present in two cases. The Gram stains of all three cases showed numerous clusters of Gram positive and Gram negative cocci. Most of these cocci were extracellular, but a few intracellular cocci could also be seen (Fig. 1).

The anti-group B *Streptococcus* monoclonal antibody clearly showed both extracellular and intracellular cocci. The extracellular cocci were seen as single organisms lying in tissue spaces. Intracellular cocci were seen within phagocytic cells in greater numbers than had been evident with the Gram stained preparations.

In addition to the discrete staining of intracellular cocci, abundant diffuse staining could be seen in the cytoplasm of some phagocytic cells (Fig. 2). Control sections processed in parallel without the monoclonal antibody confirmed that there was no residual endogenous peroxidase activity in these phagocytic cells.

The staining results obtained with the polyclonal antibody were qualitatively inferior compared with

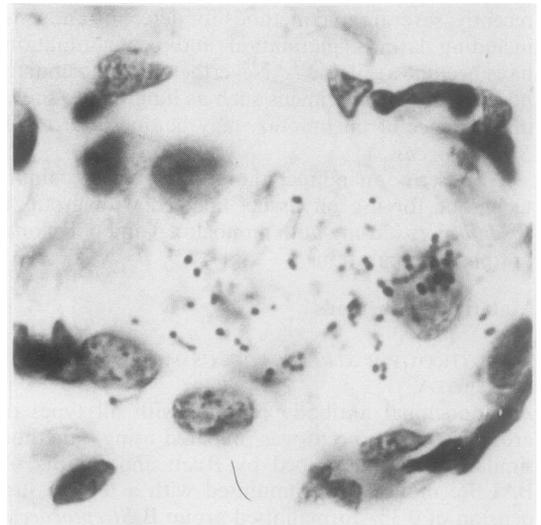


Fig. 1 Section of lung showing extracellular and intracellular Gram positive cocci. Organisms appear lanceolate and some have haloes suggesting the presence of capsules. (Gram stain.) $\times 1000$.

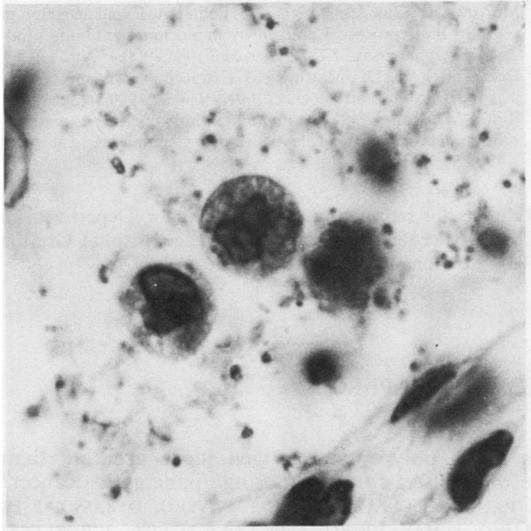


Fig. 2 Section of lung stained with anti-group B *Streptococcus* monoclonal antibody showing discrete cocci and diffuse staining of cytoplasm of phagocytic cells. Some organisms appear as a ring, indicating specific staining of bacterial cell wall. (Immunoperoxidase.) $\times 1000$.

the anti-group B *Streptococcus* monoclonal antibody, although both extracellular and intracellular cocci could be seen.

Immunoperoxidase staining with the monoclonal antibody on the lungs affected by organisms other than group B *Streptococcus* did not show staining of any importance.

Discussion

Infections with group B *Streptococcus* are an important cause of neonatal morbidity and mortality. Although rapid methods for the detection of group B *Streptococcus* antigen in body fluids have been well documented, in cases reaching postmortem examination, differentiating group B *Streptococcus* pneumonia from other bacteriological causes can be difficult because of the similar appearances under light microscopy. Although bacteria can usually be shown by the Gram stain, exact identification is not possible, and non-viable organisms may appear Gram negative.

We have described the use of an anti-group B *Streptococcus* monoclonal antibody in an indirect immunoperoxidase technique that permits the detection and identification of all types of group B *Streptococcus* in formalin fixed paraffin embedded tissues. The specific detection of group B *Streptococcus* in formalin fixed sections should facilitate investigations into the role of this organism in neonatal sepsis.

The superiority of monoclonal antibody over polyclonal antisera with immunohistochemical staining has been well described.¹⁰ The indirect immunoperoxidase technique with the monoclonal antibody seems to be more sensitive for detecting group B *Streptococcus* than either the Gram stain or the PAP technique with the polyclonal antiserum: organisms will be shown, whether or not the bacteria are viable at the time of fixation.

Both group specific and type specific monoclonal antibodies directed against group B *Streptococcus* have been described previously.^{4,11} Type specific monoclonal antibodies may be of use in determining which serotype of group B *Streptococcus* is present in histological specimens.

Group B *Streptococcus* in liquid culture produces excess antigen that dissolves into the medium.¹² The diffuse staining seen in the cytoplasm of many phagocytic cells may represent aggregations of carbohydrate antigen formed in vivo and subsequently taken up by these cells from the extracellular fluid. Alternatively, these aggregations may be the degraded remains of phagocytosed, killed group B *Streptococcus*. Staining of an intracytoplasmic bacterial antigen has been shown previously, using an antibody directed against *Mycobacterium* sp.¹³

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