SHORT COMMUNICATION

Electron Microscopic Evidence of Antibody Entry into Neutrophils after Phagocytosis of Highly Virulent Group B Streptococci

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An electron microscopic study was undertaken of the entry of specific antibody into neutrophils containing surviving intracellular highly virulent group B streptococci after phagocytosis of the organisms had occurred. Electron micrographs are presented to demonstrate that specific antibody gains access to the ingested bacteria. This antibody binds to the surface of the streptococci, which subsequently permits the neutrophil to kill these organisms.

INTRODUCTION

Group B streptococci (*Streptococcus agalactiae*) are a serious cause of neonatal mortality and the immune status of the host coupled with the pathogenicity of the infecting organism (Smith, 1972) are crucial factors affecting the outcome of an infection. Antibody and complement alone can not kill these organisms, and neutrophils are responsible for killing the bacteria. Efficient phagocytosis of highly virulent streptococci by neutrophils occurs in both the absence and presence of specific antibody, but the organisms can survive within these cells in the presence of normal serum (Cleat & Coid, 1981). The presence of immune serum, however, permits the neutrophils to kill the bacteria. Furthermore, the addition of immune serum to neutrophils containing intracellular streptococci stimulates the phagocytes to kill the ingested organisms (Cleat & Coid, 1982). Previous work (Cleat & Coid, 1984) has demonstrated that activation of Fc receptors on the surface of the neutrophil is not sufficient to activate bactericidal activity, although the neutrophil surface Fc receptors are used by the antibody to gain access to the intracellular bacteria.

Phagosome-lysosome fusion occurs normally in neutrophils containing ingested streptococci in the presence of normal serum, but the organisms are resistant to the microbicidal agents of lysosomes (Cleat & Coid, 1984). However, specific antibody appears to initiate the killing process by gaining access to and 'masking' protective determinants on the surface of the bacteria. The present study was carried out to obtain visual evidence of antibody entry into neutrophils and its 'masking' effect on the surface of the intracellular streptococci.

METHODS

Group B streptococcus. The group B streptococcus used in these experiments was a highly virulent isolate from a case of neonatal meningitis, and had an LD₅₀ for mice (strain CRC/TO) of approximately 10⁴ colony-forming units (c.f.u.) (Coid *et al.*, 1979). Suspensions of this organism were stored at -70 °C, and cultures were grown in Todd-Hewitt broth at 37 °C for 18 h. The bacteria were then washed in Eagles' minimum essential medium (MEM) and resuspended in MEM to a concentration of 2 × 10⁸ c.f.u. ml⁻¹.

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Fig. 1. Specific antibody bound to intracellular highly virulent group B streptococci within neutrophil phagosome, shown by protein A-gold staining. The arrows indicate specific antibody labelled with gold (a). Antibody activity was absent on the surface of intracellular bacteria when absorbed antiserum (b) or normal antibody (c) was used in place of specific antibody. Bar represents 0.5 μm in all micrographs.

Hyperimmune antiserum. Hyperimmune antiserum against the bacterium was raised in New Zealand White rabbits according to the method of Lancefield *et al.* (1975). Serum from non-immunized rabbits of the same strain was used as a control. Both hyperimmune and preimmune sera were heated to 56 °C for 30 min to inactivate crucial components of complement. Absorbed antiserum containing no specific antibodies against the bacterium was prepared by incubating suspensions of bacteria with equal volumes of specific antiserum as described by Lancefield *et al.* (1975).

Preparation of neutrophils containing intracellular bacteria. Polymorphonuclear leucocytes were isolated from the blood of healthy adult human volunteers by the method of Boyum (1968). Permission to obtain blood samples was given by the Harrow District Ethical Committee. The cells were washed in MEM and resuspended in MEM to a concentration of 3 to $4 \times 10^6 \text{ ml}^{-1}$. The cell population consisted of >95% viable neutrophils, as assessed by differential staining and dye exclusion. Neutrophil-bacteria suspensions were prepared as described previously (Cleat & Coid, 1981) and the neutrophils allowed to ingest the streptococci during a 30 min incubation. Extracellular organisms were then removed by differential centrifugation and washing of the neutrophils. Immune antiserum (50 μl of a 1/100 dilution), absorbed antiserum, or normal serum were then added to the neutrophil suspensions followed by a further 30 min incubation before the cells were washed and fixed in 1% (v/v) glutaraldehyde in 0.1 M-phosphate buffer (pH 7.4).

Electron microscopy. Neutrophils were embedded in Lowicryl K4M polar resin (Chemische Werke Lowi, Waldkraiburg, FRG) according to a modification (Fryer *et al.*, 1983) of the method of Roth *et al.* (1981). Ultrathin sections were cut and mounted on nickel grids. Sections were incubated with a 1/20 dilution of goat anti-rabbit IgG for 2 h at room temperature. Immunolabelling with protein A-coated 12 nm colloidal gold was then carried out as described by Roth *et al.* (1981). After staining with uranyl acetate and lead citrate, sections were examined by transmission electron microscopy for the presence of antibody in the neutrophils.

RESULTS AND DISCUSSION

The present investigation was carried out to obtain visual evidence of antibody entry into neutrophils containing intracellular group B streptococci that survive within these cells. By staining with protein A-gold reagent it was shown that specific antibody was bound to or adjacent to the surface of the organisms within the phagocytic vacuoles (Fig. 1*a*). However, no appreciable amounts of normal antibody were detected when absorbed serum (Fig. 1*b*) or normal serum (Fig. 1*c*) were added to the neutrophils. These results showed that specific antibody gained access to bacteria within neutrophils and bound to the surface of the streptococci.

Neutrophils possess surface Fc receptors for antibody, which are used in the phagocytic process. Such receptors could be a useful means for entry of antibody into these cells, and previous work has demonstrated that the surface Fc receptors are used by specific antibody to enter the cell (Cleat & Coid, 1984). It is unlikely, therefore, that this process is unique to specific antibody against group B streptococci but it may well apply to other bacterial infections. However, the observation that specific antibody, but not appreciable amounts of normal antibody, bound to the streptococcal surface suggested that this was not a simple diffusion process through the cell but rather an active process. It is possible that surface antigens of the ingested streptococci may be released into the neutrophil and that specific antibody is attracted to the intracellular bacteria in this way.

These results suggest that specific gammaglobulin preparations may have a role to play in the treatment of those infections where the micro-organism survives within neutrophils by permitting these phagocytes to kill the intracellular organisms.

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