

high concentrations of spleen type alone. Of the patients with high serum ferritin concentrations (473–762 $\mu\text{g/l}$), two had increases in both heart and spleen type ferritin and two of spleen type ferritin alone.

Patients with idiopathic sideroblastic anaemia have considerably increased intracytoplasmic ferritin concentrations in addition to mitochondrial iron deposition.^{3,4} This study shows that although high concentrations of erythrocyte ferritin are common in patients with myelodysplastic syndromes, these are often associated with transfusional iron overload and in such cases it is difficult to determine whether the increase in ferritin is due solely to an intracorpuscular abnormality. Among the non-transfused patients, however, high erythrocyte ferritin concentrations were found in all four patients with idiopathic macrocytosis, seven of nine patients with sideroblastic anaemia, one of seven patients with refractory anaemia, and one of three patients with chronic myelomonocytic leukaemia.

The reason for this increase in red cell ferritin content is not clear. It does, however, indicate that an abnormality of erythroid iron metabolism is common in this condition and is not confined to patients with sideroblastic anaemia, thus supporting the suggestion that sideroblastic anaemia is not a discrete entity but merely signifies a more prominent abnormality in some patients with myelodysplastic syndromes.^{2,5}

SW PETERS

SJ MAY

A JACOBS

Department of Haematology,
University of Wales College of Medicine,
Heath Park,
Cardiff

References

- Bennett JM, Catovsky D, Daniel MT, *et al*. Proposal for the classification of the myelodysplastic syndromes. *Br J Haematol* 1982;51:189–99.
- May SJ, Smith SA, Jacobs A, Williams A, Bailey-Wood R. The myelodysplastic syndrome: analysis of laboratory characteristics in relation to the FAB classification. *Br J Haematol* (in press).
- Peters SW, Jacobs A, Fitzsimons E. Erythrocyte ferritin in normal subjects and patients with abnormal iron metabolism. *Br J Haematol* 1983;53:211–6.
- May A, De Souza P, Barnes K, Kaaba S, Jacobs A. Erythroblast iron metabolism in sideroblastic marrows. *Br J Haematol* 1982;52:611–21.
- Rosenthal DS, Moloney WC. Refractory dysmyelopoietic anaemia and acute leukaemia. *Blood* 1984;63:314–8.

Opsonophagocytosis of group B streptococci: the role of sialic acid

Dr Hindocha *et al*¹ have shown that serotypes Ib and II of group B streptococci are resistant to opsonophagocytosis as measured by neutrophil chemiluminescence. The resistant strains were unaffected by neuraminidase treatment, while trypsin was able to induce susceptibility within the same strains.

We have studied serotypes Ia, Ib, Ic, II, and III group B streptococci by chemiluminescence before and after neuraminidase treatment. Human neutrophils were separated by dextran sedimentation (60 min at room temperature) and Ficoll-Paque centrifugation (30 min at 500 g) to eliminate mononuclear cells. Reference group B streptococci (supplied by the Public Health Laboratory Service, Colindale, London) were cultured overnight in Todd-Hewitt broth with or without neuraminidase (0.43 U/ml, Sigma), washed three times in phosphate buffered saline (PBS) and the suspension adjusted to an optical density of 0.8 at 620 nm ($3 \times 10^8 - 10^9$ CFU/ml). Opsonisation of group B streptococci was performed by rotating bacteria with human pooled serum at 37° for 30 min. Forty group B streptococci wild strains isolated from neonates and asymptomatic mothers were also studied with the same technique. Two hundred microlitres of bacteria at 3×10^7 CFU/ml were mixed with 2×10^5 neutrophils in PBS Ca, Mg, and with 200 μl of 2×10^{-5} M Luminol (Lumac) in the counting chamber of a Picolite luminometer.² Sialic acid measurement was performed with the thiobarbituric acid assay.³

No correlation was found between the absolute sialic acid content of the five group B streptococci serotypes or of the 40 wild strains and chemiluminescence (data not shown). Serotypes Ib and Ic were particularly resistant to opsonophagocytosis (Fig. 1). The removal of sialic acid (more than 60% of the initial amount was lost after enzyme treatment) caused an increase of chemiluminescence in all serotypes except Ia (Fig. 2). The extracellular production of neuraminidase in the five group B streptococci serotypes has been investigated previously⁴ but no correlation was found with chemiluminescence results. Our experiments suggest that sialic acid influences interactions between bacteria and neutrophils, although intrastrain differences in sialic acid content could influence opsonisation.⁵

The role of contaminating proteolytic

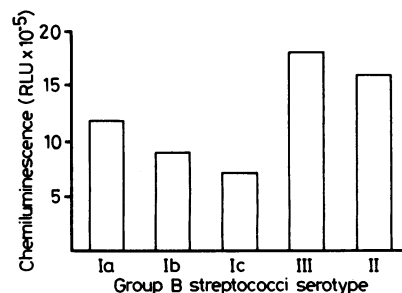


Fig. 1 Chemiluminescence (expressed as relative light units (RLU)) of the five group B streptococci serotypes.

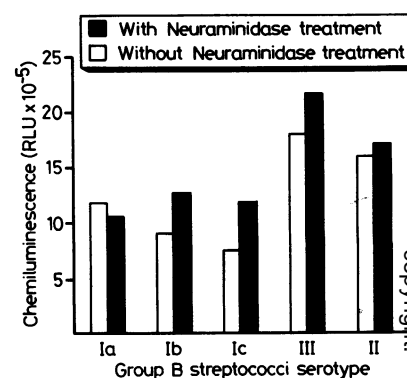


Fig. 2 Chemiluminescence (RLU) of the five group B streptococci before and after neuraminidase treatment.

enzymes could not be excluded from our experiments; however, the inability of high neuraminidase doses (we used 0.43 U/ml) used by Dr Hindocha¹ to influence chemiluminescence seems to indicate that proteolytic contamination, if present, is negligible. The experiments of Jennings^{6,7} have indicated that sialic acid associates with the backbone of the native streptococcal antigen (type III); these and our results from five group B streptococci serotypes and 40 wild strains suggest that sialic acid plays a role in the interactions between bacteria and neutrophils by modulating an articulated antigenic complex.

The differences between Dr Hindocha's work and our findings are difficult to explain. Technical reasons, such as separation of the blood cells, heat killing of the group B streptococci, or opsonic capacity of the sera, could be implicated. In our opinion, however, these discrepancies

Letters to the Editor

point out the great biological variations that characterise interactions between bacteria and immune cells and further stimulate other studies on this interesting topic with special attention to clinical applications.

P DE PAOLI
G MUCIGNAT
G OREFICI*
G SANTINI

*Servizio di Microbiologia Immunologia,
Ospedale Civile, Pordenone, e
Istituto Superiore di Sanità,
Roma, Italy*

References

- Hindocha P, Hill R, Wood C, Patel U, Hunt G. Impaired opsonophagocytosis of serotypes Ib and II of GBS as compared with serotypes Ia and III: role of the alternative pathway of complement in opsonization of serotype II of GBS. *J Clin Pathol* 1984;37:790-5.
- Cecchin E, De Marchi S, De Paoli P, et al. Valutazione della funzionalità granulocitaria in pazienti in dialisi peritoneale. *Min Nefrol* 1983;30:105-11.
- Warren L. The thiobarbituric acid assay of sialic acid. *J Biol Chem* 1959;234:1971-5.
- Orefici G, De Stasio A, Guarino M, et al. Group B Streptococci: extracellular neuraminidase production and virulence in mouse. *Microbiologica* 1984;7:75-8.
- Shigeoka AO, Rote NS, Santos JI, Hill HR. Assessment of the virulence of GBS: correlation with sialic acid content. *J Infect Dis* 1983;147:857-63.
- Jennings HJ, Rosell KG, Kasper DL. Structure and serology of the native polysaccharide antigen of type Ia GBS. *Proc Natl Acad Sci USA* 1980;77:2931-5.
- Jennings HJ, Lugowskj C, Kasper DL. Conformational aspects critical to the immunospecificity of the type III GBS polysaccharide. *Biochemistry* 1981;20:4511-6.

Adherence of neomycin to the tubing of a plate pouring machine

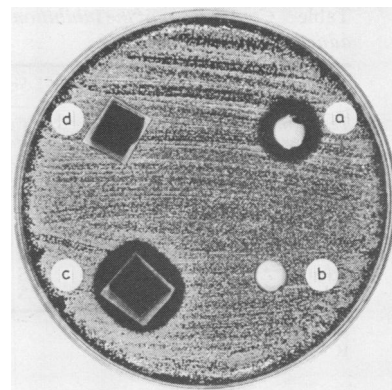
For the past two years in our laboratory, selective and non-selective media have been made using two Herdial Jouan media preparators. These are small autoclaving units capable of sterilising up to 5 litres of agar medium. Agar is dispensed from the stainless steel sterilising chamber by being pumped through silicone rubber tubing

into petri dishes via a plate pouring machine, which controls the operation. The stainless steel container is relatively easy to clean. The tubing is flushed through with hot water and autoclaved before reuse.

In June 1983 some batches of diagnostic sensitivity test agar (DST, Oxoid CM 261) inhibited the growth of certain strains of staphylococci. The effect was intermittent, and most noticeable on plates poured at the beginning of an affected batch. Blocks of agar cut from these plates were inhibitory when placed on a lawn of the sensitive staphylococci (see Figure, block labelled c). The tubing of the machine had been in use for some time and was discoloured. This suggested that chemicals from a previous pouring might adhere to the tubing, and, despite washing and autoclaving, leach out into the next medium poured. To test this hypothesis the tubes were filled with water and sonicated for 30 min. The washings from inside the tubes inhibited the staphylococci (Figure, well labelled a).

Eight inhibitory compounds were added to media poured by the machine. Solutions of these were sterilised in the usual way and then autoclaved at 115°C for 10 min to mimic the treatment of the tubing. The activities of these substances were compared with the activity of the inhibitory washings and plugs of inhibitory DST on plates lawned with different organisms. Preliminary screening of the additives was done on plates lawned with coagulase negative staphylococci and a salmonella. The washings and the plug of DST gave 15-19 mm zones on the staphylococcal lawns but did not inhibit the salmonella. Comparison of the pattern of inhibition led to four compounds being excluded from further tests, leaving neomycin, vancomycin, and solutions A and B (used in making deoxycholate citrate agar). After finding the dilution of each solution which produced a 15-19 mm zone of inhibition on the lawns of staphylococci, we tested these four compounds against a range of organisms. The results are summarised in the Table.

The washings and the DST plug gave zones with the coagulase negative staphylococci only, and neomycin was the only substance to give a similar pattern. The concentration of neomycin which produced a comparable zone to the washings and the DST plug was 0.04 mg/l. This concentration was too low to be detected by other methods, so the identity of the inhibitory substance could not be absolutely confirmed. Two other observations, however, support neomycin as the active



Effect of washings from a tube used to pour neomycin plates (a and b) and of inhibitory blocks of DST (c and d) on a strain of Staphylococcus epidermidis. (a) Washings filtered through medium Postlip paper before being used to fill the well. (b) Washings filtered through phosphocellulose paper before being used to fill the well. (c) Block standing on medium Postlip paper. (d) Block standing on phosphocellulose paper.

agent. Firstly, when the indicator strains were tested for sensitivity to a 10 µg neomycin disc, the coagulase negative staphylococci gave zones of 25-29 mm, but the remaining indicator strains gave zones of 19 mm or less. Secondly, phosphocellulose impregnated paper (which absorbs out aminoglycosides¹) removed the inhibitory effect of the washings, the DST block (see Figure), and the diluted neomycin, though not that caused by the other substances.

We have been unable to find a previous reference to difficulties caused by neomycin adhering to equipment. The final concentration in our neomycin agar is 50 mg/l and it was a surprise that sufficient remained behind in the tubing to affect the next medium poured by the machine. The effect was detected with the DST only, presumably because coagulase negative staphylococci were not looked for on the other media poured by machine. For a short time an attempt was made to keep one set of new tubing for pouring neomycin agar. After two weeks use, washings from this tube were tested and found to give 11-14 mm zones of inhibition. It is probably therefore desirable to avoid preparing neomycin agar in machines using tubing of