STUDIES ON A HAEMOLYSIN PRODUCED BY TREPONEMA HYODYSENTERIAE

R.M. LEMCKE, M.R. BURROWS, K.A. KENT AND R.J. LYSONS*
A.R.C. INSTITUTE FOR RESEARCH ON ANIMAL DISEASES
COMPTON, NEAR NEWBURY, BERKSHIRE, ENGLAND

The effect of sodium ribonucleate (Na-RNA) or ribonucleic acid (RNA)-core in increasing the amount of haemolysin detected in supernates or filtrates from broth cultures of T. hyodysenteriae was described by Picard et al. (1979) and Knoop (1981). We have confirmed these observations and demonstrated that haemolytic titres were highest when viable counts were maximal at the end of the logarithmic growth phase (Lemcke and Burrows, in the press). This report describes a study of the conditions necessary for haemolysin production by resting spirochaetes suspended in a defined buffer in the absence of erythrocytes.

Methods

Spirochaetes were grown anaerobically in rabbit serum broth (Lemcke et al., 1979), harvested by centrifugation, and resuspended in an incubation mixture consisting of phosphate buffered saline (PBS) containing 0.001M magnesium sulphate, 0.001M glucose, 0.1% cysteine hydrochloride and a carrier substance. Carriers tested were sodium ribonucleate (0.1, 0.5 or 1.0%), RNA-core Type II-C or RNA-core Type XI-C (0.01, 0.05 or 0.1%), bovine serum albumin fraction V (0.1, 0.5 or 1.0%) or Tween 80 (0.4%). The opacity of the suspensions was adjusted so that a 1 in 10 dilution had an optical density of 0.65 ± 0.05 at a wavelength of 500 nm. Two ml amounts were incubated aerobically at 37°C for 30 min and spirochaetes were removed by centrifugation at 15,000 g for 30 min at 4°C. Supernatants were titrated in two-fold steps against 1% suspensions of thrice-washed erythrocytes. The diluent was PBS containing 0.05% cysteine hydrochloride and the unit volume was 1 ml. Tubes were incubated aerobically for 2 hr at 37°C and left overnight at 4°C. The end point was the highest dilution showing 50% haemolysis. Unless otherwise stated, experiments were carried out with P18A, a virulent strain of T. hyodysenteriae.

Results

Of the carrier substances tested, the preparations of RNA-core at 0.05 and 0.1% gave haemolytic titres at least ten-fold higher (1024-4096) than 1% Na-RNA (128). Bovine serum albumin fraction V and Tween 80 gave much lower titres. No haemolysin was produced in the absence of a carrier. The omission of one or more of the other constituents of the incubation mixture depressed the titre four-fold at most, glucose having the greatest effect. As maximum titres were obtained in the complete incubation mixture containing RNA, this was used in all subsequent experiments. Increasing the concentration of glucose to 0.01 or 0.1M did not increase haemolytic titres, nor did the substitution of maltose for glucose at the same concentrations.

RNA-core haemolysin was produced at titres of 2048-4096 by suspensions incubated at 42°, 37° or 30°C. At 25°C titres fell to 512-1024 and at 18° and 10°C to 128 and 64. Extending the incubation period at 37°C from 30 min to 1, 2 or 4 hr did not increase titres of haemolysin.

Haemolytic titres produced by washed and unwashed spirochaetes differed no more than two-fold. Highest titres were given by spirochaetes harvested in the logarithmic phase between 24 and 46 hr. The amount of haemolysin produced increased with the concentration of spirochaetes in the incubation mixture up to a certain maximum. Thus, suspensions which when diluted 1 in 10 had an OD_{500nm} of 0.2, 0.4, 0.6, 0.8 and 1.0 gave titres of 256, 512, 1024, 2048 and 2048.

If spirochaetes were recovered from the incubation mixture by centrifugation, resuspended and reincubated in fresh incubation mixture, the same organisms could be used three times to produce haemolysin without a significant loss in titre. A fourth resuspension resulted in a four-fold loss. When spirochaetes were stored overnight at 4°C before resuspending and reincubating for a fifth time, no haemolysin was produced.

In titrating the haemolysin, no difference was observed between titrations incubated in air or anaerobically in deoxygenated 10% CO_2 in nitrogen. The treponemal haemolysin lysed sheep, rabbit, guinea pig and pig erythrocytes equally effectively. No loss in activity occurred after filtration of the haemolysin through membranes with average pore diameters of 220 or 450 nm.

Cells harvested from 24, 30 and 46 hr cultures of both the virulent P18A strain and the avirulent FW10 strain of T. hyodysenteriae produced more haemolysin than 70 hr cultures. The maximum titre of P18A haemolysin was 2048 whereas that of FW10 was 512. The weakly haemolytic, non-pathogenic, porcine spirochaete PWS/B, harvested from 24, 30 and 46 hr cultures, gave haemolytic titres of only 32, 256 and 64. Titration of PWS/B haemolysin showed a prozone of incomplete lysis in tubes containing the highest concentrations of haemolysin.

Conclusions

The haemolysin of T. hyodysenteriae resembles streptolysin S (SLS) in requiring a carrier substance for its production from resting cells. RNA-core was the most efficient carrier tested. Like streptococci, the treponemes produced haemolysin only when they were active metabolically. The treponemal haemolysin, like SLS, was oxygen stable. Although the avirulent strain of T. hyodysenteriae produced less haemolysin than the virulent strain, the difference observed may not be significant in regard to virulence. The prozone observed in titrating the haemolysin of a weakly haemolytic spirochaete may indicate that it differs from the haemolysin of T. hyodysenteriae in its affinity for the erythrocyte membrane. The production of the haemolysin of T. hyodysenteriae in a defined buffer should facilitate its purification and further studies on its cytotoxicity.

Selected references: Knoop, F.C.: Infect. Immun. 1981, 31, 193; Lemcke, R.M., Bew, J. Burrows, M.R. and Lysons, R.J.: Res. vet. Sci. 1979, 26, 315; Lemcke, R.M. and Burrows, M.R.: J. med. Microbiol. 1982, in the press; Picard, B., Massicotte, L. and Saheb, S.A.: Experientia 1979, 35, 484.