An immunofluorescent method for detecting antibodies against viridans streptococci in *Streptococcus viridans* endocarditis

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Streptococcus sanguis, *mitior*, and *mutans* are three species of viridans streptococci that are closely associated with endocarditis (Parker and Ball, 1976). Patients with streptococcal endocarditis develop high serum antibody titres against the infecting organism (Laxdal *et al.*, 1968; Lunn and Bunn, 1965). Serological tests might help to diagnose endocarditis when the blood cultures are negative or yield growth of an organism of uncertain clinical significance, especially after the prior administration of an antibiotic.

We report the results of a study of antibody titres in cases of '*Strep. viridans*' endocarditis using a new immunofluorescent method in which strains of *Strep. sanguis*, *mitior*, and *mutans* were used as the antigens.

Material and methods

Serum was collected one to three months after the onset of symptoms from eight endocarditis cases and from another endocarditis case about three weeks after the onset of symptoms (case 8 in Table). Endocarditis was proven in each case clinically and by numerous sets of blood cultures that yielded growth of viridans streptococci. Three positive standard reference sera, obtained from patients with endocarditis, were included with every batch of tests. The first, second, and third reference sera had high fluorescent antibody titres (1 in 1600) against *Strep. sanguis*, *mitior*, and *mutans* respectively. Serum samples were also collected from 20 control cases with carious teeth who were otherwise healthy. A negative control serum was prepared by pooling the serum samples from six healthy laboratory workers.

Overnight cultures on blood agar of strains of *Strep. sanguis*, *mitior*, and *mutans* were used to prepare fresh whole cell antigen suspensions (for source of strains see Table). A loopful of suspension was spread onto a 4 cm² area on a new glass slide, dried, and flamed.

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Buffer containing 180 g potassium chloride, 8.06 g disodium hydrogen phosphate (Na₂HPO₄), 6.97 g sodium dihydrogen phosphate (NaH₂PO₄), 2 g sodium fluoride, 2 g sodium azide, and 2 g sodium arsenate, in 2 litres of distilled water, as stock solution, was freshly diluted 1 in 10 in distilled water for use at pH 7.2.

Each serum was initially diluted 1 in 50 and then in serial double dilutions up to 1 in 1600 in fresh buffer. Each heat fixed antigen smear was covered with diluted serum and incubated in a moist chamber at 37°C for 45 minutes. The serum was then washed off with buffer and the slide was immersed in fresh buffer for five minutes. Conjugate, fluorescein labelled sheep anti-human immunoglobulin (Burroughs Wellcome Ltd), diluted 1 in 32 in buffer, was added to cover the smear and the slide was incubated in the moist chamber for 45 minutes at 37°C. After incubation the slide was washed in buffer, dried, and mounted in 50% glycerol in buffer. All the slides were examined by incident light fluorescence using a Reichert Zetopan microscope. Negative smears were checked by phase contrast microscopy to confirm the presence of streptococci in the fields examined. The titre was defined as the highest dilution of serum that gave definite fluorescence.

Results

The table summarises the results. Fluorescent antibody titres of 400 or more were demonstrated against at least one of the three streptococcal antigens used in sera from eight out of nine of the endo-

Table Fluorescent antibody titres of sera from endocarditis and dental patients

Case	Patient details	Reciprocal titres against		
		<i>Strep. sanguis</i> *	<i>Strep. mitior</i> †	<i>Strep. mutans</i> ‡
1	<i>Strep. mitior</i> endocarditis	400	1600	<50
2	<i>Strep. sanguis</i> endocarditis	1600	400	<50
3	<i>Strep. viridans</i> endocarditis	400	1600	<50
4	"	400	800	50
5	"	1600	100	<50
6	"	200	800	<50
7	"	400	100	<50
8	"	200	100	<50
9	<i>Strep. mutans</i> endocarditis	200	50	1600
10-11	Dental patients	200	50-100	<50
12-16	"	100-200	<50-100	<50-50
17-29	"	≤50	≤50	≤50
	Pooled normal serum control	50	50	50

Strep. viridans = viridans streptococci that could not be further identified.

**Strep. sanguis* from blood culture of case 2.

†*Strep. mitior* from blood culture of case 1.

‡*Strep. mutans* from blood culture of case 9.

Technical methods

carditis patients. The serum anti-streptococcal antibody titres of 20 dental control patients were always less than 1 in 400 and most frequently the titres were 1 in 50 or less.

Discussion

We have demonstrated high serum fluorescent antibody titres against at least one of the three streptococcal antigens used in eight out of nine of the endocarditis patients studied. In contrast, low antibody titres only were found in the 20 dental control cases. A fluorescent antibody titre in the range of 1 in 400 to 1 in 1600 against *Strep. sanguis*, *mitior*, or *mutans* should be considered as suggestive evidence of endocarditis due to a viridans streptococcus. As low antibody titres were found in one case of endocarditis a low titre does not exclude the possibility of streptococcal endocarditis. This patient (case 8) had serum collected only three weeks after the onset of symptoms and this was possibly too early for a significant antibody titre to have developed. The sensitivity of the immunofluorescent method could probably be improved further by including a wider range of streptococci as antigens.

The estimation of the serum fluorescent streptococcal antibody titres against *Strep. sanguis*, *mitior*, and *mutans* may help to diagnose endocarditis due to viridans streptococci when the blood cultures are negative.

We are grateful to Drs W. Bridgen and A. McDonald for sera from cases of endocarditis at the London Hospital and to Dr J. M. Hardie for identifying the strains of *Strep. sanguis*, *mitior*, and *mutans* used in this study.

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Letters to the Editor

Plasma viscosity as a routine laboratory test

It has been proposed that plasma viscosity (PV) measurement should be introduced as a routine laboratory test giving a non-specific measure of plasma protein changes in disease similar to the erythrocyte sedimentation rate (ESR). In order to find out precisely how the ESR and the PV vary with the plasma protein pattern, we have, in this laboratory, been using a Harkness viscometer to measure PV in parallel with Westergren ESR, total proteins, and differential plasma protein estimations on a series of 50 patients. Fibrinogen levels were measured by the

clot weight method.

It was confirmed that there is a direct correlation between the PV and ESR ($r = 0.75$, $P < 0.001$), and between the PV and fibrinogen level ($r = 0.82$, $P < 0.001$). The ESR also correlates with the fibrinogen level ($r = 0.86$, $P < 0.001$). These close correlations indicate that both PV and ESR reflect a spectrum of changes in the plasma protein pattern in disease, but analysis of the results show that false positives and false negatives occur with both tests. Two patients had raised viscosity but normal ESR in the presence of a normal protein pattern, four had a raised ESR but normal viscosity with normal proteins; there were three false

negative ESRs and four false negative viscosities. In no case was both the ESR and the viscosity incorrect. As it happens, none of the patients had low albumin levels, but we and others (Harkness, 1971; Harris, 1972; Hutchinson and Eastham, 1977) have noted that unexpectedly low PV levels are recorded if the plasma albumin is reduced.

These results are in agreement with the correlations found in a recent paper by Hutchinson and Eastham (1977), and this adds to the evidence that the PV is certainly as useful as the ESR, in some instances more accurate and reliable. Thus it will surely become increasingly accepted