SHORT COMMUNICATION

Analysis of Surface Receptor Expression on Bacteria Isolated from Patients with Endocarditis

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(Received 4 March 1986)

Fifteen bacterial isolates (12 streptococcal and 3 staphylococcal strains) from patients with bacterial endocarditis were screened for a variety of surface receptors, in an attempt to identify a common feature that might contribute to their ability to attach to and colonize damaged heart tissue. The bacterial receptors screened for, using a dot-blot autoradiographic procedure, included those for the Fc region of human IgG, fibrinogen, fibronectin and human C1q. Bacteria with receptors for each of the probes used could be identified, but no common receptor was present on all endocarditis-causing strains.

INTRODUCTION

The initial event in the pathogenesis of bacterial endocarditis is the attachment of bacteria to damaged heart tissue. Bacterial endocarditis can be caused by a variety of micro-organisms, e.g. streptococci, staphylococci or enterococci that have invaded the bloodstream. It is not clear whether there is some unique property of these different micro-organisms which predisposes them to colonize heart tissue. The relative frequency of a given micro-organism associated with endocarditis appears to vary within different patient populations (Lerner & Weinstein, 1966; Johnson *et al.*, 1975; Phair & Clark, 1979). For example, in individuals developing endocarditis after cardiac surgery, the most prevalent infecting organism is either *Staphylococcus epidermidis* or *Staphylococcus aureus* (Sheagren, 1984). In drug addicts, the major infecting organism is *Staphylococcus aureus* (Tuazon *et al.*, 1975). In contrast, in paediatric populations with bacterial endocarditis, the major infecting organisms are oral streptococci, mainly viridans *Streptococcus* (Kaye, 1976).

We have previously described a sensitive Western blotting approach that enables specific receptors expressed on bacterial surfaces to be detected (Yarnall *et al.*, 1984). This technique was used to study the interaction of four ligands with 15 clinical isolates of bacteria recovered from patients suffering from endocarditis, in an attempt to determine whether all these bacteria share one or more receptors that might be involved in their attachment to heart tissue. Each of the strains was examined for receptors that were specific for fibronectin, fibrinogen, C1q or the Fc region of human IgG. These receptors were chosen for study because they have been reported to be expressed on certain staphylococci or streptococci, and all have been proposed as potential attachment factors for bacteria with cells or sub-cellular organelles (Pinckard *et al.*, 1975; Myhre & Kronvall, 1977; Loos *et al.*, 1978; Runeheggen *et al.*, 1981; Kuusela *et al.*, 1984; Reis *et al.*, 1984).

METHODS

Bacterial strains. All strains tested (12 streptococci and 3 staphylococci) were recovered from patients with endocarditis. The strains were obtained from the Clinical Laboratories at Shands Teaching Hospital, University

Fig. 1. Binding of ^{125}I -labelled fibronectin to endocarditis strains. (a) Photograph of a representative autoradiograph; (b) the c.p.m. associated with each spot of the nitrocellulose blot. The c.p.m. were determined using an LKB autogamma counter and counts bound minus the background are given. In preliminary studies, the reproducibility of samples from well to well has been established not to vary by $> \pm 10\%$. The indicated numbers of bacteria (b) were dotted onto nitrocellulose and probed with ^{125}I -labelled fibronectin, as described in Methods. A, *Streptococcus mitis* 3547496/BL8267; B, *Streptococcus salivarius* 3563169/BL308; C, *Streptococcus intermedius* 3327044/BL5503; D, *Streptococcus salivarius* 2218607/BL18243; E, *Staphylococcus* sp. SES/33; F, *Streptococcus mutans* 2707427/BL17938; G, *Staphylococcus aureus* SES/32; H, non-haemolytic *Streptococcus* SES/100; I, *Staphylococcus aureus* SES/101; J, viridans *Streptococcus* SES/102.

of Florida, College of Medicine, or were a gift from either Dr John Washington, Mayo Clinic, Rochester, Minnesota, or Dr David Durack, Duke University, Raleigh, North Carolina.

Dot-blotting procedure. This was done using the Bio-Rad bio-dot microfiltration apparatus and a modification of the Bio-Rad procedure. A piece of nitrocellulose previously soaked in 25 mM-Tris, 192 mM-glycine, pH 8.3, and 20% (v/v) methanol (wash buffer) was placed in the apparatus. Twofold serial dilutions of the bacteria were pipetted into the wells. The bacteria were diluted in 0.15 M-NaCl containing 0.01 M-phosphate, pH 7.2 (PBS), starting with approximately 1×10^8 bacteria. The concentration of organisms was standardized by measuring the OD₅₅₀. After washing the bacteria in each well with the PBS, the nitrocellulose was removed and washed four times in 0.15 M-veronal-buffered saline (VBS), pH 7.35, containing 0.25% Tween 20 and 0.25% gelatin. For the samples to be probed with fibronectin, gelatin was omitted since non-specific binding between fibronectin and gelatin has been observed. Each wash was done for 15 min using 250 ml buffer. The nitrocellulose was then probed for 3 h in the VBS/Tween/gelatin buffer containing 2×10^5 c.p.m. ml⁻¹ of the appropriate ^{125}I -labelled ligand. After probing, the nitrocellulose was washed four times in 0.01 M-EDTA, 1 M-NaCl, 0.25% gelatin and 0.25% Tween 20 (15 min each wash) and allowed to air dry. All washing and probing steps were done at ambient temperature.

Autoradiographs were prepared from the nitrocellulose blots by exposing them to Kodak XAR-5 film with an intensifying screen for 3–5 d at -70°C before photographic development.

Iodination of ligands. Fibronectin and fibrinogen were obtained from Sigma. Stock human IgG was prepared by chromatography of normal human serum on DEAE-cellulose (Boyle & Langone, 1980) and samples were stored at -70°C . Fab₂ fragments were prepared as described by Reis *et al.* (1983). Human C1q was obtained from Gamma Laboratories. All ligands were iodinated by the mild lactoperoxidase method using enzymebeads (Bio-Rad) (Reis *et al.*, 1983). The labelled proteins were separated from free iodine by passage over a Sephadex G-25 column (PL-10, Pharmacia) and collected in VBS containing 0.1% gelatin or, in the case of fibronectin, VBS containing 0.25% Tween 20. The labelled ligands had specific activities of approximately 9.25 MBq mg⁻¹.

The ^{125}I -labelled human IgG probe was made Fc specific by inclusion of a twofold molar excess of Fab₂ fragments isolated from the same IgG pool as had been used to prepare the labelled ligand. Under these conditions, only interactions between bacteria and the Fc region of human IgG are detected (Reis *et al.*, 1983).

Table 1. *Distribution of surface receptors on bacteria recovered from patients with endocarditis*

The scoring system is based on the minimum number of bacteria required to reveal a detectable dot on an autoradiograph after 5 d exposure at -70°C . Each combination of bacteria and ^{125}I -labelled receptor was run on at least three occasions with similar results. —, No detectable dot on autoradiograph or dot on autoradiograph detectable with: +, 1×10^8 bacteria; ++, 5×10^7 bacteria; + + +, 2.5×10^7 bacteria; + + + +, 1.25×10^7 bacteria; + + + + +, 6.3×10^6 bacteria; + + + + + +, 3.1×10^6 bacteria; + + + + + + +, 7.8×10^5 bacteria.

Endocarditis strain	Presence/absence of receptor for:			
	Human IgG Fc region	Human C1q	Fibro- nectin	Fibrin- ogen
<i>Streptococcus mutans</i> 2707427/BL17938	—	—	+ / —	—
<i>Streptococcus intermedius</i> 3274171/BL10400	—	—	—	—
<i>Streptococcus mitis</i> BL20857	—	—	+ / —	—
<i>Streptococcus salivarius</i> 2721441/BL6747	—	—	+ / —	—
<i>Streptococcus mitis</i> 3547496/BL8267	—	—	—	+ / —
<i>Streptococcus salivarius</i> 3563169/BL308	—	—	+ / —	—
<i>Streptococcus intermedius</i> 3327044/BL5503	—	—	+	—
<i>Streptococcus salivarius</i> 2218607/BL18243	—	—	+ / —	—
<i>Streptococcus mitis</i> 3275539/BL10752	—	—	+ / —	—
<i>Staphylococcus aureus</i> SES/101	+ + + + + +	+ +	+ + + +	+ + +
<i>Staphylococcus aureus</i> SES/32	+ + + + + + + +	+ +	+ + + +	+ +
<i>Staphylococcus</i> sp. SES/33	—	—	+	+ / —
<i>Viridans Streptococcus</i> SES/102	—	+ +	+ +	+ / —
<i>Non-haemolytic Streptococcus</i> SES/100	—	+	+ + +	+ / —
<i>Streptococcus</i> sp. SES/28	—	—	+ / —	—

RESULTS AND DISCUSSION

The results of a typical experiment using ten bacterial isolates probed with ^{125}I -labelled fibronectin are presented in Fig. 1(a) and the corresponding radioactive counts for the individual sample wells in Fig. 1(b). A comparison of these results indicates that the intensity of the spot on the autoradiograph correlates closely with the number of ^{125}I counts bound to the bacteria attached to the nitrocellulose. Binding was dependent on the concentration of bacteria used in the assay; non-specific binding was not significant under the experimental conditions chosen (Fig. 1b). A similar relationship between the intensity of the spot on an autoradiograph and the counts bound to the bacteria was observed with all of the labelled probes tested.

The experiments described above, using ^{125}I -labelled fibronectin as probe, were repeated with three other ^{125}I -labelled ligands, viz. human C1q, fibrinogen and a probe for the Fc region of human IgG. Each of these ligands has been suggested as a potential attachment site for the adherence of bacteria to cells or sub-cellular organelles (Loos *et al.*, 1978; Runeheggen *et al.*, 1981; Pinckard *et al.*, 1975; Kuusela *et al.*, 1984). Receptors for each of the labelled probes tested were detected on some, but not all, of the strains studied. The most frequently detected receptor was the one which bound fibronectin. However, two of the streptococcal isolates did not bind detectable quantities of labelled fibronectin (Table 1). When these two strains were tested they

were not fresh isolates and it is possible that fibronectin receptors had been lost during subculture. We have monitored receptor expression on five of the isolates reported in Table 1 over a period of six to eight months and have not observed loss or acquisition of any of the receptors studied. In other studies, the loss of surface receptors during laboratory subculture has been observed (Myhre & Kronvall, 1977; Yarnall *et al.*, 1984). Stanislawski *et al.* (1985) have suggested that fibronectin can promote adherence of bacteria to cells, not only by providing attachment sites for certain pathogens, e.g. *Streptococcus pyogenes*, but also by inhibiting receptors for other bacteria, e.g. *Escherichia coli*.

The rapid dot-blotting procedure described here has enabled us to compare the expression of a variety of receptors on bacterial isolates from patients with endocarditis. We have been unable to implicate a single surface receptor that correlates with the potential of the bacteria to colonize damaged heart tissue. However, the ability to detect multiple surface receptors that can interact with host immune factors, e.g. C1q or the Fc region of human IgG, or serum proteins, e.g. fibrinogen, may suggest that multiple interaction sites can occur between bacteria and host tissue. Consequently, colonization may occur between a number of different molecular structures exposed on damaged heart tissue and the corresponding receptors on bacteria. The attachment of endocarditis strains to damaged heart tissue may be affected by a yet to be identified receptor(s), or may be due to a cumulative interaction of more than one of the receptors that we have identified on these strains.

The authors would like to thank Dr John Washington and Dr David Durack for providing us with bacterial isolates from endocarditis patients. This work was supported by a grant from the Palm Beach Chapter of the Florida Affiliate of the American Heart Association and NIH grant AI-20445.

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