SHORT ARTICLE

Detection of Fc receptor genes from *Staphylococcus aureus* and streptococci by polymerase chain reaction

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A method based upon the polymerase chain reaction (PCR) for detecting genes encoding the Fc receptors of *Staphylococcus aureus* and streptococci is described. Primers were designed from the nucleotide sequences of the five Fc receptor genes encoding protein A, protein G, protein H, FcRA and protein V. Amplification products corresponding in size to the protein A and protein G genes were detected in *S. aureus* strain Cowan 1 and *Streptococcus pyogenes* strain G148, respectively, as expected. *Str. pyogenes* strain AR1 was shown to possess the type H receptor gene. Two clinical isolates of *Str. pyogenes*, strains IP-28 and ES-21L, were shown to possess genes for Fc receptor types FcRA and protein G, respectively. The identification of all these products was confirmed by restriction endonuclease analysis. Amplification of protein H genes from two other clinical isolates of streptococci, MS-4 and MS-38, yielded a product larger than expected and with a different restriction fragment pattern to strain AR1, indicating a new type of Fc receptor gene. This PCR method provides a DNA-based method for the determination of Fc receptor type in *S. aureus* and streptococci.

Introduction

Bacterial cell-surface receptors for the Fc portion of host immunoglobulin G (IgG) have been shown to contribute to virulence [1]. The Fc receptors of *Staphylococcus aureus* and some streptococci have been well documented as immunoglobulin-binding proteins [1, 2]. The Fc receptor of *S. aureus* was designated protein A [3] and that of some Lancefield group C and G streptococci as protein G [4, 5]. Some strains of group A streptococci also produce an Fc receptor called FcRA [6, 7]. These Fc receptors were differentiated and classified on the basis of their binding patterns with the IgG of different mammalian species [2]; this method is still used to determine Fc receptor type.

Four different patterns of receptor distribution on the surface of gram-positive cocci, including *S. aureus* and streptococci, have been identified [8]. A further unique pattern of Fc receptor distribution has been described on the surface of two streptococcal strains

(MS-4 and MS-38), isolated from a single patient [8]. To investigate the genetic basis of the differences in receptor distribution, a method of determining Fc receptor type, and variation within these, is required. The present study aimed to establish a polymerase chain reaction (PCR)-based method to determine Fc receptor type and to use this method to characterise the Fc receptors of strains MS-4 and MS-38.

Materials and methods

Bacterial strains and culture conditions

Seven strains were examined in the present study. *S. aureus* strain Cowan I was supplied by Dr Y. Arai (Saitama Medical School, Saitama, Japan) and *Streptococcus pyogenes* strain AR1 and *Streptococcus* sp. strain G148 (Lancefield group G) were supplied by Dr Y. Minamishima (Miyazaki Medical College, Miyazaki, Japan). The Cowan I strain and the G148 strain produce protein A [3] and protein G [4], respectively. No control strains for proteins FcRA, H or V were available. The AR1 strain is a strong producer of Fc receptors [9], but these have not been typed. Four clinical isolates of streptococci, IP-28, ES-21L, MS-4 and MS-38, which had been confirmed previously to be

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Fc receptor positive by immuno-electron microscopy [8] but not typed, were also used. Strain MS-38 was isolated 4 days after the isolation of strain MS-4 from the same patient. The bacteria were cultured for 17 h at 37°C in 10 ml of Trypto-Soya Broth (TSB; Nissui Pharmaceutical Co., Tokyo, Japan) and washed twice with Dulbecco's phosphate-buffered saline (PBS) before extraction of chromosomal DNA.

Extraction of chromosomal DNA

Extraction of chromosomal DNA from each strain was accomplished with a minor modification of the protocol described by Hudson and Curtiss [10]. Briefly, for streptococci, pelleted, washed cells were suspended in 1 ml of TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA) containing mutanolysin (Sigma) 100 µg/ml. Staphylococcal cells were suspended in the same buffer containing lyostaphin 100 µg/ml. The cell suspension was incubated for 60 min at 37°C, and then pronase K was added to 1 mg/ml, followed by an additional incubation at 37°C for 60 min. After lysis with SDS 1% w/v for 30 min at 37°C, the genomic DNA was extracted with phenol:chloroform:isoamyl alcohol (25:24:1) and precipitated with ethanol.

PCR amplification

The sequences of the primers for amplification of the Fc receptor genes are shown in Table 1. These were selected from the nucleotide sequences of Fc receptor genes, previously reported [6, 11–14], and synthesised with a Biosearch 8750 automated DNA synthesiser.

Target DNA was amplified with the Perkin-Elmer Cetus GeneAmp PCR Reagent Kit and the PCR system 9600 (Takara Shuzo Co. Ltd, Kyoto, Japan), in a total volume of 50 µl containing reaction buffer, 0.2 mM dNTP mixture, 40 pmols each primer, 0.5–1 µg of template DNA and 5U of AmpliTaq DNA polymerase. The amplification conditions consisted of 30 cycles comprising: denaturation for 15 s at 94°C, annealing for 1 min at 55°C and extension for 1 min at 72°C. The PCR products were examined by electrophoresis on agarose gels (1% w/v in TBE buffer) and the bands were detected by staining with ethidium bromide. A negative control, containing all reagents except DNA, was included in each experiment.

Restriction endonuclease analysis of PCR products

Amplification products (10 µl) were digested with at least two of the following restriction endonucleases: *Hinf*I; *Hae*II; *Rsa*I; *Hae*III; *Hgi*AI; *Fnu*4H; *Hinc*II and *Pvu*II; (Table 1) according to the manufacturer's instructions. The expected sizes of restricted products are also shown in Table 1.

Results

PCR amplification

All seven bacterial strains were tested with each of the five pairs of primers. The PCR results are shown in Fig. 1. PCR amplification with the protein A primer set (AF-1 and AR-1) yielded a product of 1.7 kb only for

Table 1. Sequences of PCR primers for the amplification of Fc receptor genes

Gene	Primer	Sequence	Size of expected products (bp)	Restricted by (fragment sizes > 200 bp)
Protein A [11]	AF-1	5'-CCTTTAAATTTAATTATAAATATAGA-3' <i>Dra</i> I	1704	<i>Hinf</i> I (995, 352)
	AR-1	5'-CCTTTAAAGATATCTATCGTTGTGTA-3' <i>Dra</i> I		<i>Hae</i> II (857, 438, 409) <i>Rsa</i> I (1223, 214)
FcRA [6]	XF-1	5'-CCGGATCCTCATCATAGGATTTCAGA-3' <i>Bam</i> HI	1342	<i>Hinf</i> I (408, 388, 367)
	XR-1	5'-CCGGATCCTGTTCTTGTGTTGTGATGGTC-3' <i>Bam</i> HI		<i>Hinc</i> II (471, 449, 422)
Protein H [13]	HF-1	5'-CCGAATTCAGCTGGAGTAGCAGCAGTT-3' <i>Eco</i> RI	1696	<i>Hinf</i> I (1166, 530)
	HR-1	5'-CCGGATCCGTATACGTTTCGGCTGAAAC-3' <i>Bam</i> HI		<i>Hae</i> III (890, 680) <i>Hgi</i> AI (731, 596, 213) <i>Fnu</i> 4H (775, 335, 279)
Protein V [14]	VF-1	5'-CCGAATTCATATTCGTTTCGTTTATAAC-3' <i>Eco</i> RI	2075	
	VR-1	5'-CCGGATCCCTGTTTATTGTCTATTCTC-3' <i>Bam</i> HI		
Protein G [12]	GF-1	5'-CCTTTAAAAAGTCTGTTTCTTAAA-3' <i>Dra</i> I	2147	<i>Hinf</i> I (1192, 272, 269, 225)
	GR-1	5'-CCTTTAAAAATACAAAAAAGAGGAA-3' <i>Dra</i> I		<i>Hinc</i> II (918, 394, 335, 290, 210) <i>Pvu</i> II (1458, 359)

Underlined sequences represent restriction sites to enable cloning and sequencing of products.

Fig. 1. Agarose gel electrophoresis of PCR products amplified with Fc receptor primers. The primer sets shown in Table 1 were used to amplify each Fc receptor gene as indicated: **a**, protein A gene; **b**, FcRA gene; **c**, protein H gene; **d**, protein G gene; **e**, protein V gene. Lanes: **M**, marker (pHY marker, Takara Shuzo); **1**, *S. aureus* strain Cowan I; **2**, streptococcal strain AR1; **3**, streptococcal strain IP-28; **4**, streptococcal strain G148; **5**, streptococcal strain ES-21L; **6**, streptococcal strain MS-4; **7**, streptococcal strain MS-38.

the Cowan I strain of *S. aureus* (Fig. 1a, lane 1). With the XF-1 and XR-1 primers, designed from the FcRA gene, only DNA from the streptococcal IP-28 strain was amplified, as a single product of 1.3 kb, as expected for this gene (Fig. 1b, lane 3). When the HF-1 and HR-1 primers were used to amplify the protein H gene, two bands were detected in three strains (AR1, MS-4 and MS-38) (Fig. 1c). The smaller product (c. 300 bp) was common to these three strains. However, there was a clear difference between the larger bands of strain AR1 and the other two strains, MS-4 and MS-38. Although strain AR1 produced a faint band at 1.7 kb (Fig. 1c, lane 2), which corresponded to the full size of the protein H gene [13], the size of the PCR products from strains MS-4 and MS-38, 3.8 kb, was unexpected (Fig. 1c, lanes 6 and 7). Amplification of the protein G gene with primers GF-1 and GR-1 yielded products of 2.1 kb, as expected for the full length of the protein G gene [12], from strains G148 and ES-21L (Fig. 1d, lanes 4 and 5). No PCR product was detected when the VF-1 and VR-1 primers, for the protein V gene [14], were used (Fig. 1e). No amplification of receptor genes other than those expected, was detected.

Restriction endonuclease analysis of PCR products

With *Hinf*I, *Hae*II and *Rsa*I, the product from the *S. aureus* Cowan I strain showed the cleavage pattern expected from the sequence of the protein A gene, determined by Uhlén *et al.* [11]. The pattern of fragments from the PCR products from the streptococcal strains AR1, IP-28, G148 and ES-21L showed the cleavage patterns expected from the sequences of the protein H gene [13], FcRA gene [6], protein G gene [12] and protein G gene [12], respectively. However, the cleavage patterns of strains MS-4 and MS-38, which were indistinguishable, differed from that expected from the sequence of the protein H gene [13].

Discussion

Five types of Fc receptors of gram-positive cocci have been completely cloned and sequenced. The protein A gene was sequenced by Uhlén *et al.* [11]. Olsson *et al.* [12] cloned the protein G gene of the streptococcal strain G148, which is regarded as the standard protein G producing strain [4]. Fahnestock *et al.* [15] sequenced the Fc receptor from the streptococcal strain GX7809 and showed it to be a protein G variant. The Fc receptor genes, FcRA [6], protein H [13] and protein V [14], have been sequenced also. Based on the nucleotide sequences of these genes encoding Fc receptors, primers were designed for each gene and the results confirmed that these amplified products of appropriate sizes, for the protein A and protein G genes from *S. aureus* Cowan I strain and streptococcal strain G148, respectively. Strains AR1, IP-28 and ES-21L

were shown to possess the protein H gene, FcRA gene and protein G gene, respectively. These results were confirmed by restriction endonuclease analysis of the products. The results also showed that the primers used in the PCR were specific for each of the four Fc receptor genes, protein A, protein G, protein H and FcRA, indicating that the PCR is suitable for the classification of Fc receptors. Furthermore, the present study showed that the Fc receptor genes of strains MS-4 and MS-38 were the same as each other but differed from those of the five known Fc receptors. Thus, a new Fc receptor gene was identified from Lancefield group A streptococci.

Amplification products obtained from the MS-4 and MS-38 strains were approximately twice the size expected for the protein H genes. However, the restricted fragment sizes of the products from these strains indicated that they were not duplications of the protein H gene [6]. These results suggest that strains MS-4 and MS-38 may have a receptor gene which is related to, but different from, the published protein H gene. The primers used in the present study enable products to be cloned or sequenced, or both, because they were designed to contain restriction sites (underlined sequence in the primers, Table 1). Sequencing of the products from strains MS-4 and MS-38 is being done to determine the nature of their Fc receptor genes.

Previously, the distribution of Fc receptors has been determined by the ferritin labelling method and four types of distribution on the surface of organisms have been described [8]. These morphological studies, together with the PCR and restriction endonuclease analysis described in the present study, will enable the relationship between the distribution of Fc receptors on the surface of organisms and the type of Fc receptor genes to be determined.

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