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Immunoglobulin G-binding protein of *Streptococcus suis*

Siti Isrina Oktavia Salasia[1]

1) Department of Clinical Pathology, Faculty of Veterinary Medicine, Gadjah Mada University, Sekip Unit II, Yogyakarta 55281, Indonesia

Abstract

Immunoglubolin G (IgG) binding proteins on the surface of Streptococcus suis *could be solubilized by heat treatment of the bacteria at an acid pH and also by mutanolysin treatment. The IgG binding proteins could be detected by Western blot using human IgG and peroxidase-labelled anti-human, and autoradiography analysis using 125I-labelled human IgG. Western blot and autoradiography analysis revealed numerous protein bands with IgG binding activities. In binding studies using Western blot and autoradipgraphy,* S. suis *P43,* S. suis *P143 and* S. suis *2872, but none of the other* S. suis *isolates, showed a significant binding of the protein. The IgG binding proteins were also released into the culture supernatant of the bacteria. This could be detected for 52 of the 76* S. suis *isolates.*

Keywords: Streptococcus suis -- *immunoglobulin G - binding protein*

Introduction

Bacterial proteins capable of reacting with the constant (Fc) region of immunoglobulin G have been detected in a number of staphylococcal and streptococcal strains (Forsgren and Sjoquist, 1966; Kronvall, 1973; Chhatwall *et al.*, 1985). Among streptococci, protein G. a group C and group G streptococcal binding protein which preferentially interacts with IgG has been intensively characterized and widely used as an immunological reagent (Bjorck and Kronvall, 1984; Akerstrom *et al.*, 1985; Bjorck and Blomberg, 1987).

Comparable IgG binding proteins were described for *S.dysgalactiae*, *S. canis* and streptococci of serological group L, all isolated from animals (Lammler *et al.*, 1988; Lammler and Frede, 1989; Sippel and Lammler, 1995) and more recently also for the important swine pathogen *S.suis* (Serhir *et al.*, 1993; 1995). According to Serhir *et al.* (1993) IgG binding to *S. suis* appears to be related to a common protein with an approximate molecular mass of 52 kDa. However, using electronmicroscopy, this binding proteins were best observed with unencapsulated *S. suis* indicating that the capsular layer of this bacteria might mask the binding sites (Serhir *et al.*, 1993).

The present study was performed to further characterize IgG binding property of *S. suis*.

Materials and Methods

Bacterial cultures

A total of 76 *S. suis* isolates from various animals and humans sources were used in this study. The cultures had been characterized biochemically and serologically as described (Estoepangestie and Lammler, 1993; Salasia *el al.*, 1994; Salasia and Lammler, 1995).

Isolation and solubilization of IgG binding proteins

The solubilization of IgG binding proteins was performed by heat extraction (52°C, 2 h) of the *S. suis* at an acid pH and in parallel by treatment of the bacteria with mutanolysin (Sippel and Lammler, 1995). For mutanolysin extraction, *S. suis* were cultivated in 250 ml Todd-Hewitt broth (THB, Diagnostic Pasteur, Marnes-la-Coquette, France), centrifuged, resuspended in 1 ml PBS in the presence of 0.1 U mutanolysin (Sigma), incubated for 1 h at 37°C under shaking and centrifuged. Both crude extract were subsequently applied to sodium dodecyl sulphate polyacrylamide gel electrophoresis (11 %, SDS-PAGE) according to Laemmli (1970) followed by immunoblotting (Burnette, 1980). The nitrocellulose membranes were treated for 1 h at room temperature with peroxidase-labelled rabbit-anti-human IgG (Dakopatts, Hamburg, Germany) and diluted 1:500 in PBS. The filters were washed and developed with a freshly prepared solution containing 5 ml 4-chloro-1 naphtol (Merck, Darmstadt, Germany) 3 mg ml methanol, 100 µl 30% H_20_2 and 25 ml PBS.

IgG binding study with ^{125}I-labelled IgG using autoradiography

In a parallel experiment, the IgG binding assay was performed with autoradiography as described by Lammler *el al.* (1988). After immunobloting of both mutanolysin and heat-acid extracts, the nitrocellulose membranes were incubated for 2 h at room temperature in 10 ml PBS pH 7.5 containing of 20 µl ^{125}I-labelled plasma proteins (ca. 5 x 10^4 cpm) and washed with PBS. The nitrocellulose membrane was applied to rontgen film for 10 days at room temperature. A positive reaction was indicated by a dark colouration of rontgen film.

Detection of IgG binding proteins in the culture supernatant

For this purpose, the bacteria were cultivated in 100 ml THB for 24 h at 37°C, centrifuged, then the culture supernatant was precipitated with ammonium sulphate (472g/1) for 24 h at 4°C, resuspended in 1 ml 0.5 mol/l phosphate buffer pH 7.5, and dialysed against distilled water for 48 h at 4°C. The concentrated supernatants (100 µl) were filtrated with a microfiltration apparatus (Hybri Dot Manifold, Bethesda Res. Lab., USA) containing the nitrocellulose membrane (Lammler *et al.*, 1986). In a parallel experiment, the supernatants were subsequently applied to SDS-PAGE followed by immunoblotting. The nitrocellulose membranes were treated as described above.

Results

The IgG binding proteins could be solubilized from the streptococcal surface by heat treatment of the bacteria at an acid pH or by mutanolysin treatment. SDS-PAGE and immunoblotting of the solubilized crude protein extracts revealed numerous protein bands with IgG binding activities. This was demonstrated on nitrocellulose by developing the membranes with human IgG and peroxidase-labelled antibodies against human IgG. Extracts from *S. suis* P178 with

no detectable IgG binding protein on nitrocellulose membranes showed no IgG binding activity (Fig. 1). Binding studies with ^{125}I-IgG using autoradiography of the solubilized crude mutanolysin and heat-acid extracts showed more protein bands than in immunoblotting assay, indicated that autoradiography method appeared to be more sensitive to detect the IgG binding of *S. suis* (Fig. 2).

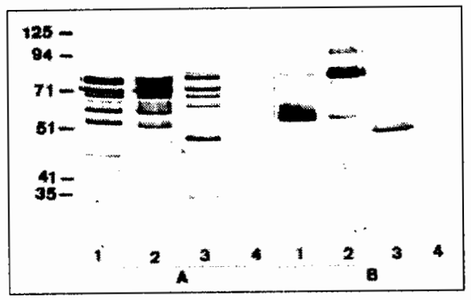

Figure 1. Demonstration of IgG binding properties of *S. suis* P43 (1), P143 (2), 2872 (3), and P178 (4).The proteins were solubilized by heat treatment of the bacteria at acid pH (A) or by mutanolysin treatment (B); The nitrocellulose membrane was developed with human IgG and peroxidase labelled anti-human IgG.

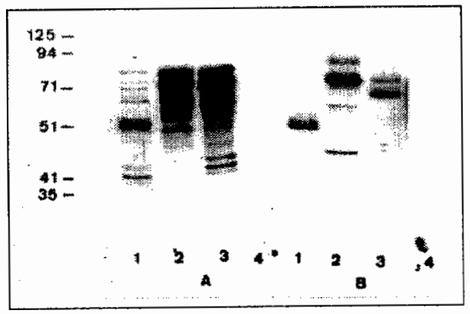

Figure 2. Demonstration of IgG binding properties of *S. suis* P43 (1), P143 (2), 2872 (3), and P178 (4) using autoradiography. The proteins were solubilized by heat treatment of the bacteria at acid pH (A) or by mutanolysin treatment (B); The nitrocellulose membrane was developed with ^{125}I-labelled human IgG.

Using the microtiltration assay, the ammonium sulphate precipitate obtained from culture supernatant of 16 of the 76 *S. suis* isolates tested showed strong (++) colouration of the membranes, 36 cultures a weak (+) colouration of the nitrocellulose, indicating that the IgG binding proteins were secreted into the culture supematant. The culture supernatant of the remaining cultures showed no comparable binding activities (Fig. 3). The significant results are showed in the western blot of culture supernatant of *S. suis* cultures (Fig. 4).

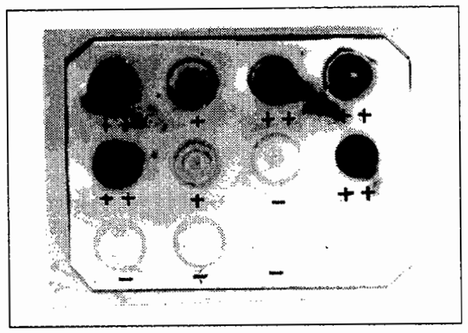

Figure 3. Typical reaction of ammonium sulphate precipitates of various *S. suis* cultures on nitrocellulose membranes detected by a microfiltration assay; ++ (strong), + (weak), - (no colouration of the membranes).

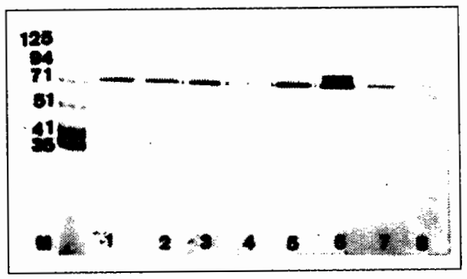

Figure 4. Demonstration of IgG binding properties of supernatants of *S. suis* P43 (1), P143 (2), 2872 (3), P178 (4), P179 (5), P180 (6), P181 (7) and P182 (8), in Western blot. The nitrocellulose membrane was developed with human IgG and peroxidase labelled anti-human IgG.

Discussion

The IgG binding proteins of *S. suis* could be solubilized by heat treatment at acid pH or by mutanolysin treatment of the bacteria. Both extraction procedures proved to be useful for solubilization of protein A and protein G and for isolation of IgG binding proteins of group L streptococci (Sting *et al.*, 1990; Sippel and Lammler, 1995). It was of interest that only 3 of the 76 *S. suis* isolates, namely *S. suis* P43, P 143 and 2872, displayed detectable IgG binding proteins. To further characterize the binding activity of *S. suis*, additional study was performed with 125I-labelled human IgG using autoradiography. Comparable to the Western blot analysis, only three *S. suis* cultures P43, P143 and 2872 displayed significant IgG binding activities. Binding study with 125I-labelled IgG proved to be sensitive and repeatable. This technique has been used to characterize numerous bacterial-protein interaction (Lammler *et al.*, 1986; Lammler *et al.*, 1988; Sippel and Lammler, 1995).

The *S. suis* culture P43 has already been shown to be unencapsulated and non typeable when cultivated in unsupplemented fluid media. After cultivation of this strain in fluid media supplemented with 10% fetal calf serum, the *S. suis* culture appeared to be encapsulated and typeable. Extracts of the later reacted with type 1 specific antiserum (Wibawan and Lammler, 1994). IgG binding to this unencapsulated strain corresponded to the finding of Serhir *et al.* (1993). This author proposed that the capsular layer might mask the binding proteins. However, IgG binding studies with two previously characterized unencapsulated mutants of *S. suis* revealed no comparable binding activities (Salasia *et al.*, 1995).

Depending on the extraction procedure, the IgG binding proteins of *S. suis* appeared in numerous bands with IgG binding activity. This corresponded to the *S. suis* binding proteins described by Serhir *et al. (* 1993) and to IgG binding proteins group L streptococci (Sippel and Lammler, 1995). However, Serhir *et al.* (1993, 1995) described a major IgG binding protein of *S. suis* with a molecular mass of 52 Kda. Comparable to Serhir *et al. (* 1993, 1995) the IgG binding proteins of *S. suis* cultures of the present investigation could also be observed in the culture supernatant of the bacteria. This could be demonstrated with a microfiltration assay which proved to be useful for demonstration of IgG binding proteins from various staphylococcal and streptococcal species (Lammler *et al.*, 1986). Secreted IgG binding proteins could be demonstrated with most of the *S. suis* cultures tested, independent to their degree of encapsulation. However, *S. suis* P43, P143 and 2872 also showed a strong IgG binding activity in the microfiltration assay and Western blot analysis. These results again indicated that the IgG binding proteins appear cell wall-associated and in a secreted form in the culture supernatant.

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