Extracellular Fibrinogen-binding Protein, Efb, from *Staphylococcus aureus* Blocks Platelet Aggregation Due to Its Binding to the α-Chain*

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Extracellular fibrinogen-binding protein (Efb) secreted by *Staphylococcus aureus* has previously been shown to contribute to pathogenesis in a rat wound infection model. Also antibodies against Efb exhibited a protective effect in a mouse mastitis model. The interaction between Efb and fibrinogen is divalent, with one binding site within the N-terminal repeat region in Efb and one at the C terminus. In this study we show that the distal D domain of fibrinogen contains at least one of the binding domains recognized by Efb. Efb stimulates fibrinogen binding to ADP-activated platelets. Furthermore, Efb inhibits ADP-induced, fibrinogen-dependent platelet aggregation in a concentration-dependent manner. This implies that Efb modifies platelet function by amplifying a non-functional interaction between fibrinogen and platelets. Efb recognizes the αa-chain of the D fragment of fibrinogen. The RGD sequence on the αa-chain is located close to the region recognized by Efb and contains a putative binding site for the platelet integrin GPIIb/IIIa receptor complex involved in platelet aggregation.

Several fibrinogen-binding proteins (FgBPs) from *Staphylococcus aureus* have been described: Clf A (1), Clf B (2), Coagulase (3), FbpA (4), Map (5, 6), or Eap (7), and Efb (previously Eap). In this study, we have investigated the binding ability of the extracellular fibrinogen-binding protein produced by *S. aureus* mainly during the post-exponential phase of the bacterial growth. The protein has been shown to be a potential virulence factor, using an allele replacement mutant, it has been demonstrated that Efb plays an important role in the pathogenesis of *S. aureus* in wound infection (10). In a vaccination study in mice, it was demonstrated that immunization with Efb reduced the number of bacteria colonizing the mammary glands in a mouse mastitis model (11). We have also shown that 49% of sera taken from patients with *S. aureus* infections have raised antibody levels against Efb, implying its expression during infection (12). Taken together, it shows that Efb is a virulence factor that warrants further studies.

Efb has two repeated regions at the N terminus (amino acids 17–38 and 48–69) (8) homologous to the 5–8 repeats in coagulase (3, 13). It has been observed in vitro that the interaction between Efb and Fg is divalent. The combination of equal amounts of these two proteins leads to the precipitation of the Efb-Fg complex (9). This complex is formed as a result of divalent binding between Efb and Fg. Efb has two binding sites for Fg, one located at the two repeat regions of the N-terminal part of Efb and the other at the C-terminal region.

Fg is a 340-kDa plasma glycoprotein that is essential in maintaining normal hemostasis. Fg is not only the precursor of fibrin but also mediates platelet aggregation by binding to the GPIIb/IIIa receptor complex on activated platelets, resulting in the formation of a platelet plug. The symmetrical structure of Fg is arranged into three globular domains, one central E-domain and two distal D-domains (14). Each Fg molecule consists of three pairs of non-identical polypeptide chains (αBβγ)₃ arranged in a manner such that all six N termini are located in the central part of the molecule. The αo-chains consist of 610 residues and has a molecular mass of 67 kDa, the Bβ-chain is a 55-kDa polypeptide composed of 461 residues, and the 48-kDa γ-chain has 411 residues. αo, Bβ, and γ chains are linked together by three interchain disulfide bridges (15).

In this study, we have investigated the binding ability of the two repeats of Efb to Fg. After plasmic cleavage of Fg, we have defined the fragment that is recognized by Efb, a fragment carrying several important biological functions that might be blocked by the interaction with Efb. We have also shown that Efb and coagulase compete for the same binding domain on Fg. We have demonstrated that Efb modulates platelet function, resulting in an increased affinity for Fg and inhibition of platelet aggregation.

### EXPERIMENTAL PROCEDURES

Preparation of αo, Bβ, and γ Chains—The three polypeptide chains of Fg were separated and isolated from each other by preparative SDS-PAGE, continuous-elution electrophoresis using the model 491 prep cell from Bio-Rad. Fg (Sigma) was electrophoresed through a...
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Affinity Chromatography on Efb-Sepharose—The binding ability of purified Fg chains to Efb on Efb-Sepharose was analyzed. Efb-Sepharose was prepared by coupling purified Efb to CNBr-activated Sepharose 4B (Amersham Pharmacia Biotech) using the procedure recommended by the manufacturer. One hundred μl of Efb-Sepharose resin was added to 10 μg of each Fg chain in three Eppendorf tubes and equilibrated with phosphate-buffered saline (PBS; 145 mM NaCl, 10 mM phosphate, pH 7.4). One ml of PBS + 0.05% Tween (PBST) was then added. The tubes with the samples were incubated at room temperature for 1 h. The resin was washed 4 times in PBST, and elution of absorbed material was done by boiling the Efb-Sepharose resin in PAGE-loading buffer (50 mM Tris, pH 7.5, 1% SDS, 1% β-mercaptoethanol). The samples were subsequently analyzed by SDS-PAGE.

Preparation of Fragment D of Fg—Fg was proteolytically digested by plasmin (Sigma). Fg was dissolved in 0.05 M Tris-HCl + 0.1 mM NaCl buffer, pH 7.3. To 3–5 μg of Fg (1 μg/ml) 1 unit of human plasmin was added, and the digestion was carried out at 37 °C for 3 h. Proteinase inhibitor, 200 units/ml aprotinin (Sigma), and 1 mM phenylmethylsulfonyl fluoride was added to stop the enzymatic reaction. To remove the larger or smaller degradation products from fragment D preparation, gel filtration was performed at room temperature on Sephadex G-75 column (20 cm × 90 cm) (Amersham Pharmacia Biotech) containing the fast protein liquid chromatography and equilibrated and eluted with PBS. Protein from the first peak, containing mainly fragment D, was subsequently purified by affinity chromatography on Efb-Sepharose. The isolated D-fragment is shown in Fig. 1B.

6-cm-long cylindrical gel of 7% polyacrylamide. As the three chains migrated through the gel matrix, they were separated by molecular weight as in conventional gel electrophoresis. When individual bands migrated toward the bottom of the gel, they passed directly into the elution chamber for collection. Fig. 1A shows the three chains separated.

Purification of Fg-binding Proteins—The FgBPs used here are Efb, coagulase, Clf, Eap, GST-Fbe, and GST-Coa. Efb and coagulase were purified from S. aureus strain Newman as described in Palma et al. (9). Briefly, 1 liter of S. aureus strain Newman was grown in LB medium overnight at 37 °C. The culture was centrifuged to pellet cells, and the FgBPs located in the supernatant were isolated by affinity chromatography on Fg-Sepharose (Amersham Pharmacia Biotech). Bound proteins were eluted with 0.7% acetic acid and dialyzed against 40 mM phosphate buffer, pH 6.5, and the different FgBPs were separated from each other by fast protein liquid chromatography on a Mono S column. When using a gradient of 0–1 M NaCl, three peaks of proteins were eluted. The first elution peak (0.15–0.25 M NaCl) contained coagulase, and the second one (0.35–0.45 M NaCl) contain Efb. (The third peak was Eap (7)).

GST-RR is a recombinant protein containing the two repeats (amino acids 8–69) from the N-terminal part of Efb fused with GST (9). The GST-RR protein was affinity-purified by glutathione-Sepharose using the procedure recommended by the supplier of the GST system (Amersham Pharmacia Biotech). Clf used in this study was a recombinant molecule fused with six histidines (16). This 42-kDa protein contains residues 221–590 of ClfA, the region containing the Fg binding domain. The His-tagged protein was purified using nickel chelator according to the instruction provided by Qiagen (Bazel, Switzerland). This plasmid was kindly supplied to us by T. J. Foster (Dublin, Ireland). GST-Fbe is a recombinant protein containing the Fg binding region of a Fg-binding protein from Staphylococcus epidermidis fused with GST (17, 18).

Construction of GST-Coa—A 3′ fragment of the coagulase gene encoding the five-repeat region was amplified by polymerase chain reaction using oligonucleotide primers Coa R.U., with the sequence ACCGG-GATCCACTGAAACACAGAG, and Coa R.L., with the sequence AGGCCCGGGGACCTTGACCGTTTGTG (CyberGen AB, Huddinge, Sweden). A previously isolated Coa-containing clone from a GenBankTM was used as a template, and the reaction contained 5 pmol of each primer, 0.2 mM dNTPs, 0.5 units of Taq polymerase. The thermal step program included denaturation at 94 °C for 10 s, annealing at 50 °C for 20 s, and extension at 72 °C for 30 s. The DNA fragment was digested with BamHI and SmaI (Coa R.U. had the BamHI site, and Coa R.L. had the SmaI site incorporated) and ligated into the BamHI-SmaI sites of the vector pGEX (Amersham Pharmacia Biotech). For cloning, Echerichia coli strain XL1-Blue was used, and colonies with a recombinant plasmid containing the five-repeat regions were detected by polymerase chain reaction using the primers Coa R.U. and Coa R.L. For expression of the recombinant protein, GST-Coa, the E. coli strain BL21 was used. GST-Coa protein was affinity-purified by glutathione-Sepharose.

Capture ELISA—Binding of Efb to Fg or to the purified D fragment of plasmin-activated fibrin was analyzed. Microtiter wells (Costar) were coated with Fg, fibrin, or D fragment at concentrations ranging from 0.2–20 μg/ml in PBS at room temperature overnight. The wells were blocked by incubation with 2% bovine serum albumin in PBS for 1 h at 37 °C. After washing 3 times with PBST, native Efb (9) or GST-RR (9) was added, and the digestion was carried out at 37 °C for 3 h. Proteinase inhibitor, 200 units/ml aprotinin (Sigma), and 1 mM phenylmethylsulfonyl fluoride was added to stop the enzymatic reaction. To remove the larger or smaller degradation products from fragment D preparation, gel filtration was performed at room temperature on Sephadex G-75 column (20 cm × 90 cm) (Amersham Pharmacia Biotech) containing the fast protein liquid chromatography and equilibrated and eluted with PBS. Protein from the first peak, containing mainly fragment D, was subsequently purified by affinity chromatography on Efb-Sepharose. The isolated D-fragment is shown in Fig. 1B.

SDS-PAGE and Western Ligand Immunoblotting—SDS-PAGE was run using the Phast System (Pharmacia Biotech). The three different chains of Fg or the D fragment of Fg were separated by a 8–25% gradient Phast gel. The proteins were transferred to a nitrocellulose filter that was then blocked by 2% Tween for 10 min at room temperature. The filter was incubated with 10 μg/ml purified native Efb in PBST for 4 h at room temperature. The bound Efb was detected by rabbit anti-Efb antisera diluted 1:1000 in PBST followed by horse-radish peroxidase (HRP-conjugated swine anti-rabbit immunoglobulin G antibodies and incubated with the filter of 1 h at room temperature. The nitrocellulose filter was washed three times with PBST after every incubation, and the color reaction was developed using 4-chloro-1-naphthol tablets (Sigma).

**Fig. 1.** Isolated polypeptides from Fg. A, chains of Fg separated from each other by preparative SDS-PAGE. Lane 1, molecular mass markers (14, 20, 30, 43, 67, and 94 kDa from bottom to top); lane 2, the three chains of Fg; lane 3, purified Aα-chain; lane 4, purified Bβ-chain; lane 5, γ-chain. B, fragment D of Fg isolated by gel filtration followed affinity chromatography on Efb-Sepharose. Lane 1, Fg; lane 2, plasmically-degraded Fg; lane 3, fragment D.
was added at 1 µg/ml, and the plates were incubated for 2 h at 37 °C. After washing, bound Efb or GST-RR was detected with polyclonal antibodies against Efb or GST-RR (diluted 1000-fold), followed by secondary antibodies conjugated with HRP. The substrate for HRP was orthophenylenediamine tablets (Dakopatts) with H2O2, and the color reaction was measured at 492 nm. Clf from S. aureus and GST-Fbe were used as negative controls for binding to immobilized fragment D and fibrin. Clf was detected with rat antibodies against Clf, and GST-Fbe was detected with rat antibodies against GST.

**Capture Inhibition ELISA**—A 96-well microtiter plate was coated with 100 µl of 2 µg/ml GST-RR or GST-Coa overnight at room temperature. The wells were blocked by incubation with 2% bovine serum albumin in PBS for 1 h at 37 °C. A series of different amounts of inhibitors in 50 µl of PBST (0.01–30 µg) and a constant amount of Fg (10 ng) in 50 µl of PBST were added to the wells. The plate was incubated for 1 h at room temperature. Bound Fg was subsequently detected by HRP-conjugated rabbit immunoglobulins against Fg diluted 1000-fold in PBST. The plate was washed with PBST after each incubation.

**Coagulase Reaction**—To 0.4 ml of Bacto coagulase plasma (Difco) coagulase was added, which is peak 1 after fast protein liquid chromatography purification of fibrinogen-binding proteins, as described above. Serial dilutions were tested, and a dilution giving full clotting after 30 min was used (final concentration 2 µg/ml). The inhibitory activity of Efb, Eap, or ClfA was measured by adding various amounts of these proteins together with a constant amount of coagulase. Clotting of plasma was determined after 30 min.

**Platelet Preparation**—Blood was drawn from healthy members of the staff and collected into vacuum tubes containing citrate, 1:10 (v/v). Centrifugation at 101 g for 10 min produced an upper platelet-rich plasma (PRP), which was removed. The remaining suspension was further centrifuged at 2000 g for 20 min to obtain platelet poor plasma (PPP).

Interaction of Efb with Fg Binding to Human Platelets—Five µl of PRP was mixed with 50 µl of HEPES buffer, pH 7.4, and incubated for 5 min. Five µl of FITC-conjugated chicken anti-human Fg or FITC-conjugated chicken anti-human insulin as an negative control (Biopool, Umeå, Sweden) was then added, and after 2 min, the incubation was stopped by adding 500 µl of 1% paraformaldehyde. Chicken anti-human antibodies were chosen since, in contrast to mammalian IgG immune complexes containing chicken, IgGs do not activate platelets or complement. Incubations were performed with or without 1 µM ADP (Roche Molecular Biochemicals) to activate the platelets and in the presence of either Efb at 0.02, 0.16, or 1.26 µg or GST-RR at 0.013, 0.1, or 0.8 µg (final concentrations). As a negative control, an incubation was also carried out on ADP-activated platelets in the presence of GST-RR and 10 mM EDTA, since EDTA blocks Fg binding to platelets. A FACSCalibur flow cytometer (Becton Dickinson) was used with a gate setting for the normal platelet population. Acquisition and processing of data from 100,000 cells was analyzed in the logarithmic mode.

In a similar experiment, PRP was probed with PercCP-conjugated anti-CD61 (Becton Dickinson). Incubations were performed with or without ADP and in the presence of FITC-conjugated GST-RR, which was conjugated at this laboratory using a FluoroTag™ FITC conjugation kit, FITC-1 (Sigma-Aldrich) at 0, 0.01, 0.05, 0.24, or 1.18 µM (final concentrations).

**Effect of Efb on Platelet Aggregation**—A dual-channel platelet aggregation meter (ChronoLog model 490) was used to assess platelet aggregation by turbidimetry. Platelet concentrations in PRP were adjusted to ~250 × 10⁶ platelets/liter by mixing PRP and PPP. PPP was used in the reference cell to establish a base line of 100% transmission or 0% aggregation. Three hundred µl of PRP was prewarmed to 37 °C in a glass cuvette stirred at 1200 rpm with a magnetic stir bar. Platelets were activated by the addition of 3 µM ADP, and incubations were performed in the presence of 200 µl of GST-RR at 0, 0.11, 0.22, 0.44, 0.88, 1.76, and 3.52 µg (final concentrations). The combination of equal amounts of Efb and Fg led to the precipitation of an Efb-Fg complex that may interfere with the interpretation of results. GST-RR was chosen, since it contains the N-terminal binding region of Efb and is not known to precipitate with Fg. Aggregation in the presence of PBS and GST was performed as negative controls.

**RESULTS**

**Interaction of Aα-chain of Fg and Efb**—Binding of Efb to the Aα-chain was demonstrated by using Efb-Sepharose. The three purified chains of Fg were separately and in equal amounts mixed with Efb-Sepharose. Mainly the Aα-chain was recovered from Efb-Sepharose (Fig. 2A). Binding of Efb to the Aα-chain was confirmed by Western blot. The Aα, Bβ, and γ chains of Fg were separated by SDS-PAGE and subjected to Western affinity blot using Efb as probe, followed by antibodies against Efb. Fig. 2B shows that Efb binds to the Aα-chain.

**Interaction of D Fragment of Fg or Fibrin and Efb**—Binding of Efb to immobilized D fragment was analyzed by a capture ELISA. Fig. 3A shows the data obtained when native Efb and the recombinant GST-RR were allowed to bind to indicated concentrations of immobilized Fg or its fragment D. Binding of the control proteins Clf and GST-Fbe to the D fragment was not detected, indicating that the binding sites for these two latter proteins are not localized in this region.

The ability of Efb and GST-RR to recognize fibrin was also analyzed. Increasing amounts of immobilized fibrin increased the binding of Efb, GST-RR, and the control protein ClfA (Fig. 3B), indicating that the binding site for these proteins is conserved when fibrinogen is converted to fibrin.

**Interaction of Aα-chain of Fragment D with Efb**—The iso-
lated D fragment contained two closely migrating bands of 45 kDa (corresponding to fragments of the B/H9252 and H9253 chains) and a band of 14 kDa (from the A/H9251-chain) as demonstrated by SDS-polyacrylamide gel electrophoresis (Fig. 1B, lane 3). To see which of the polypeptide chains included in the D fragment of Fg is recognized by Efb, a Western blot was performed. The three chains were separated on SDS-PAGE and then transferred to a nitrocellulose membrane. The Efb probe seemed to recognize the A/H9251-chain (Fig. 4). However, it seems that the polyclonal antibodies cross-react with the B/H9252-chain.

Comparison of the Binding between Efb and Coagulase—
Binding of a constant amount of Fg to immobilized GST-RR was measured in the presence of different concentrations of native coagulase. The presence of coagulase decreased the binding of Fg to GST-RR. In contrast, the interaction was not affected by the addition of Clf (Fig. 5A). This was also demonstrated when binding of Fg to immobilized GST-Coa was blocked with an increased concentration of Efb or GST-RR. In contrast, the binding of Fg was not disturbed by the presence of Clf (Fig. 5B). The coagulase activity of coagulase in plasma was also tested in the presence of Efb, Eap, and Clf. Only Efb was able to inhibit the activity of coagulase (Table I). Precipitation of the Efb-Fg complex was observed in the tube with the highest content of Efb, as observed previously (9), although it was distinct from true clotting.

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maximal. Purified GST was used as a negative control and had no inhibitory effect (data not shown).

**DISCUSSION**

*S. aureus* mainly expresses three extracellular proteins that can recognize Fg, namely Efb, coagulase, and Eap (19). Efb and coagulase have partial homology at the repeat regions responsible for Fg binding.

We have demonstrated previously that Efb has two binding sites for Fg, one located at the two repeats of Efb and the other at the C-terminal half of the molecule. In this study we have identified at least one of the two binding domains on Fg recognized by Efb or its two repeats.

In a previous investigation it was demonstrated that Efb recognized the Aα-chain. This was now confirmed here with both Western affinity blotting and affinity chromatography on Efb-Sepharose with separate and purified chains of Fg. Mainly the Aα-chain was enriched by this procedure, indicating that a potential binding domain for Efb is located in this chain.

Plasmic degradation of Fg gives two fragments called D and E, derived from the outer and the central domains of Fg molecule, respectively. We have purified the D fragment of Fg and demonstrated its ability to bind to native Efb and to the two-repeat regions of Efb. This means that Efb recognized some of the polypeptide chains in the D fragment of Fg. The D fragment includes residues 111–197 of the Aα-chain, 134–461 of the Bβ-chain, and 88–406 of the γ-chain (20, 21). Clf did not recognize the D fragment, as expected since it is the C-terminal region of the γ-chain that is recognized by Clf (22), and this part is not included in the D fragment. The recombinant Fbe from *S. epidermidis*, which has been shown to bind to the Bβ-chain (18), did not recognize the D fragment, indicating that the binding site for this molecule is not located between amino acids 88 and 406 of the Bβ-chain.

In addition, we have investigated which of the three polypeptide chains of the D fragment Efb recognizes; Efb interacts in Western affinity blotting to the small polypeptide of the D fragment, corresponding to the Aα-chain. However, the binding appears weak; probably part of the binding domain is destroyed by the protease treatment or as a result of the denaturing conditions on SDS-PAGE.

The exact biological function of Efb is not known but should be related to the ability to bind to Fg, since no other activity of Efb has yet been found. We have hypothesized that binding of Efb to Fg might be beneficial for the bacterium, probably by delaying the wound healing process (10). Binding of Efb to functional sites on Fg could have a blocking effect of important processes where such sites are involved. Such functional amino acid sequences should be located at the part of the Aα-chain on the domain that interacts with Efb.

Several important functions have been found on the part of the Aα-chain included in the D fragment or close to it, functions that could potentially be disturbed by the presence of Efb. For instance (i) the RGD sequence, which is recognized by the platelet integrin GPIIb/IIIa and is involved in platelet activation, is located only 10 residues away from the Aα polypeptide chain in the D fragment (23), (ii) interaction between thrombospondin and Fg takes place at residues 113–126 of Aα-chain and residues 243–252 of the Bβ-chain (24, 25). The interaction between platelet-bound thrombospondin and Fg is thought to facilitate irreversible platelet aggregation, which facilitates wound repair, and (iii) it has been demonstrated that peptides with sequences identical to Aα 148–161 and Aα 149–161 of human Fg play a crucial role in the acceleration of the tissue-type plasminogen activator-catalyzed plasminogen activation (26). All these three are examples of functional domains that in some way are involved in the wound healing. Binding of Efb to
these sites might thus lead to the delaying of the healing process.

Platelet aggregation involves a complex interplay of receptors and factors, and it is initially mediated by Fg binding to an activated GPIIb/IIIa integrin complex. Fg contains three putative binding sites for platelet GPIIb/IIIa, two containing the RGD sequence on the Aα-chain and one on the γ-chain. We have shown here that the repeat region of Efb is a potent inhibitor of platelet aggregation. Since Efb binds to a region of the Aα-chain of Fg close to the RGD sequence, this effect may be related to Efb blocking or causing conformational changes in the RGD sequence recognition site for the platelet GPIIb/IIIa complex or via a direct effect on the receptor. Moreover we investigated the interaction between the repeat region of Efb and platelets using flow cytometry and demonstrated that this region of Efb binds specifically to activated platelets. This binding may occur either directly between Efb and a platelet surface receptor or via Fg bridging, and the exact mechanism involved will be the focus of future investigations.

We have also shown here that the presence of increasing concentrations of Efb or GST-RR increased Fg binding by activated platelets. This suggests that Efb may have a stimulatory effect on platelet function by enhancing Fg binding, and we can speculate that if Efb binds directly to platelets and simultaneously to Fg, then it bridges the platelet Fg interaction and thereby stimulates this interplay. This potential stimulatory effect on platelet function is, however, contradicted by the inhibition of platelet aggregation also observed. We hypothesize that although Efb increases platelet affinity for Fg, this Fg is held in a nonfunctional manner and culminates in a blockade of normal platelet function. Furthermore since platelet aggregation is an in vitro reflection of the ability of platelets to form a stable clot in vivo and thereby maintain hemostasis, this effect may contribute to the retardation of wound healing associated with Efb in S. aureus infection.

In this study we have demonstrated that Efb not only recognized Fg but also fibrin, indicating that the binding domain for Efb does not become hidden when Fg is converted to fibrin. In addition, the two repeats of Efb compete with the five repeats of coagulase for binding to Fg and with coagulase activity. Together with the partial sequence homology between these repeats, it strongly implies identical recognition sites on Fg for
Efb and coagulase. We have demonstrated that Efb enhances a nonfunctional interaction between Fg and platelets and is responsible for repression of fibrinogen-dependent platelet aggregation.

REFERENCES
1. McDevitt, D., Francois, P., Vaudaux, P., and Foster, T. J. (1994) Mol. Microbiol. 11, 237–248
2. N’Eidhin, D., Perkins, S., Francois, P., Vaudaux, P., Hoök, M., and Foster, T. J. (1999) Mol. Microbiol. 36, 245–257
3. Phromnadaeng, P., O’Reilly, M., Nowlan, P., Bramley, A. J., and Foster, T. J. (1996) Mol. Microbiol. 4, 383–404
4. Cheung, A., Projan, S., Edelstein, R. E., and Fischetti, V. A. (1995) Infect. Immun. 63, 1914–1920
5. Palma, M., Nozohoor, S., Schennings, T., Heimdahl, A., and Flock, J.-I. (1996) Infect. Immun. 64, 5284–5289
6. Bacon-Baguley, T., Ogilvie, M. L., Gartner, T. K., and Walz, D. A. (1990) J. Biol. Chem. 265, 2317–2323
7. Voskuilen, M., Veerman, A., Veerman, G. H., van Boom, J. H., Klasen, E. A., Zegers, N. D., and Nieuwenhuizen, W. (1987) J. Biol. Chem. 262, 5944–5946
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