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Levels of specific antibodies towards the major antigenic determinant of slime-producing *Staphylococcus epidermidis* determined by an enzyme immunoassay and their protective effect in experimental keratitis

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Abstract

Staphylococcus epidermidis is an important cause of bacterial keratitis. Certain *S. epidermidis* strains produce an extracellular slime layer rich in an acidic polysaccharide with a molecular size of 20 kDa (20-kDa PS). We have demonstrated that the level of 20-kDa PS-specific antibodies significantly rises after establishment of slime-producing *S. epidermidis* bacteraemia and, furthermore, that rabbit polyclonal antibodies to 20-kDa PS opsonize cells of slime-producing *S. epidermidis* to a great degree and promote their clearance by polymorphonuclear cells (Arch. Biochem. Biophys. 342 (1997) 389; J. Pharm. Biomed. Anal. 22 (2000) 1029). The purpose of this study was to examine the protective and therapeutic effects both of active immunization, using 20-kDa PS as antigen, and of passive administration of specific antibodies towards the 20-kDa PS in a rabbit keratitis model. For active immunization, 20 rabbits were subcutaneously immunized with 20-kDa PS, whereas for passive immunization specific polyclonal IgG antibodies against 20-kDa PS were administered to 20 rabbits 1 day before induction of infection. Clinical observations were made weekly for 1 month and levels of 20-kDa PS antibodies in serum and aqueous humor in both immunization groups were determined by an enzyme immunoassay. The levels of specific anti-20-kDa PS IgG in serum and aqueous humor following either active or passive immunization were significantly higher as compared with control groups ($P < 0.001$). Although, actively immunized rabbits showed significantly less corneal damage than control animals, passively immunized ones were significantly better protected as compared with both control and those actively immunized. Obtained results suggest that 20-kDa PS plays crucial role in the pathogenesis of *S.*

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epidermidis keratitis and that both types of immunization significantly protect against corneal *S. epidermidis* pathology and damage.

Keywords: *Staphylococcus epidermidis*; Immunoglobulins; Keratitis; Immunization; Polysaccharide; Biofilm; Extracellular matrix

1. Introduction

Staphylococcus epidermidis is among the most prominent organisms responsible for corneal infection that may result in loss of visual acuity and blindness. In fact, in some clinical studies *S. epidermidis* is the leading aetiological microbe of bacterial keratitis and is responsible in about 60% of cases of acute endophthalmitis. [1–6]. In addition, *S. epidermidis* remains the most frequent microorganism in post-surgical cataract extraction and intraocular lens (IOL) implantation. It is a member of the normal ocular and periocular surface flora and can gain entry into the eye through the incision sites at the time of surgery. It can adhere on scleral buckles after retinal detachment surgery, on IOL, and on soft contact lenses, resulting in an increased potential for a hypersensitivity reaction or even infection [7].

S. epidermidis strains can produce a highly aggregating extracellular polysaccharide substance, known as slime. A community of staphylococcal cells encased in an extracellular polysaccharide matrix adhering to biomaterial surfaces is called biofilm. Biofilms have a wide clinical relevance especially with regard to biomaterials, such as urinary catheters, transcutaneous intravenous lines, prosthetic heart valves, etc. [8–10]. Mack et al. [11] have reported that there is a positive association between biofilm production by *S. epidermidis* strains and a specific polysaccharide inter-cellular adhesin, which is a linear $\beta(1,6)$-linked glucosaminoglycan. Studies on the extracellular slime layer produced by reference and clinical *S. epidermidis* strains in our laboratories showed that it is composed of discrete macromolecules [12,13]. More specifically, the major component, which represents approximately 60–65% of the total slime carbohydrate-containing components, is a sulfated 20 kDa acidic polysaccharide (20-kDa PS) [12]. Although, other slime components, such as 80 kDa peptidoglycan and teichoic acid like substances, are highly immunogenic in rabbits, 20-kDa PS has been shown to be the major antigenic determinant of slime [14,15]. Furthermore, specific polyclonal antibodies against 20-kDa PS showed to exert a protective effect against slime-producing *S. epidermidis* strains in IgG-deficient patients and preterm neonates [16,17]. A polydisperse but homogeneously charged acidic 80 kDa peptidoglycan containing a covalently bound polysaccharide, a 12.5 kDa polysaccharide strongly retained by anion-exchange column with a high-charge density, two non-anionic carbohydrate-containing proteins degradable with papain (250 and 125 kDa), a papain-degradable 60 kDa macromolecule bearing acidic carbohydrates covalently bound to protein, and cell wall teichoic acid-like substances constitute the other minor macromolecules of the slime material [12].

Due to the importance of slime-producing *S. epidermidis* as a causative agent of keratitis, we found of interest to examine the protective and therapeutic effects following active immunization, using 20-kDa PS as antigen, as well as passive immunization upon administration of specific antibodies towards the 20-kDa PS in a rabbit keratitis model by monitoring the clinical score and levels of anti-20-kDa PS antibodies in blood and aqueous humor.

2. Experimental

2.1. Bacterial strains

Slime-producing *S. epidermidis* reference strains 35983 and 35984 obtained from the American Type Culture Collection (ATCC, Rockville, MD) were used to isolate the 20-kDa PS, whereas strain 35983 was used to induce keratitis.

2.2. Preparation of 20-kDa PS and its homologous specific antibodies

Extraction of slime material was performed by modified procedures, which have been previously described [12,13]. In brief, extracellular slime material was separated from cells by gentle mechanical shaking with glass beads in 0.15 M NaCl, followed by precipitation of the extract with a mixture of ethanol, sodium acetate and acetic acid at final concentrations of 80% (v/v), 0.25 and 0.05 M, respectively.

Macromolecular substances of slime were isolated as previously described [12,13]. In brief, the anionic substances were first fractionated by ion-exchange chromatography on DEAE-Sephacel using a NaCl linear gradient (10 vol.) from 0.2 to 1.0 M. The major acidic polysaccharide population (0.7 M NaCl, containing the 20-kDa PS) was isolated and re-chromatographed on DEAE-Sephacel with a linear NaCl gradient ranging from 0.4 to 1.0 M. The polysaccharide population was isolated by gel-permeation chromatography on Sepharose CL-6M (Kd = 0.6). Chemical analysis by electrophoresis and high-performance liquid chromatography confirmed the purity of this polysaccharide.

Polyclonal antibodies against 20-kDa PS were elicited in 4–5 months-old New Zealand White rabbits weighing 4–5 kg, as previously described [14,17]. Filter-sterilized (0.2 μm pore size) 20-kDa PS (0.9 mg) was emulsified with equal volume of complete Freund's adjuvant (CFA) for the first injection and incomplete Freund's adjuvant for the following challenges which were performed subcutaneously 2 and 5 weeks later. On the 7th week, animals were sacrificed by cardiac exsanguination under ether anaesthesia.

Enrichment of serum in immunoglobulins was performed with precipitation with one volume of saturated $(NH_4)_2SO_4$ solution, as previously described [14]. One volume of 1 M Tris–HCl, pH 8.0, in ten volumes of serum-$(NH_4)_2SO_4$ mixture was used to maintain constant pH during the precipitation step. After centrifugation ($10\,000 \times g$ for 10 min), the precipitate was washed with a saturated $(NH_4)_2SO_4$ solution and dialyzed against double-distilled water. Ammonium sulfate was removed by buffer-exchange chromatography on Sephadex G-25 (PD-10 prepacked column, Pharmacia, Uppsala, Sweden) eluted with 0.5 M NaCl–10 mM buffered phosphate solution (P_i), pH 7.2, in the presence of 0.1% (v/v) Tween 20. Antibody population eluting in the void volume (V_o) of the column was detected at 280 nm and confirmed by direct dot immunobinding assay. Immunoglobulin containing fractions were collected, dialyzed extensively against double-distilled water, and then freeze-dried. They were ultimately used in buffer solution composed of 0.15 M NaCl–10 mM P_i, pH 7.2. Opsonic activity of highly enriched 20-kDa PS antibodies was determined by measuring their ability to promote phagocytosis of bacteria by human granulocytes, as previously described [17].

2.3. Active and passive immunization of rabbits

Eighty New Zealand White female outbred rabbits (2–3 kg) were used. All animal procedures were maintained in strict accordance with institutional guidelines and the Guide for the Care and Use of Laboratory Animals (Institute of laboratory animal resources 1996, Guide for the care and use of laboratory animals, National Academy Press, Washington, DC).

Two immunization groups were used; one group for active immunization and one for passive immunization. Twenty rabbits were used for active immunization and these were injected subcutaneously with 20-kDa PS mixed with 1:1 (v/v) with CFA for the first injection and with incomplete Freund's adjuvant for booster doses. Injections were made on days 0, 9, 21 and 35 using 0.9, 0.5, 0.5 and 0.3 mg dry weight of 20-kDa PS, respectively. Control rabbits ($n = 20$) for this group were injected with the same volume of a mixture containing normal saline and CFA. In the second group, 4 ml of a 18 mg/ml solution of specific IgG antibodies against 20-kDa PS were administered to 20 rabbits 1 day before induction of experimental infection. Control rabbits ($n = 20$) for this group received the same volume of normal saline.

2.4. Induction of experimental keratitis

Before intrastromal injection, all rabbits were anaesthetised with intramuscular ketamine (33

mg/kg) and xylazine (9 mg/kg). After induction of general anaesthesia, two drops of proparacaine hydrochloride (0.5% Alcaine, Alcon Laboratories, Fort Worth, TX) were applied to the eye.

In order to induce keratitis, the central portion of the right cornea of each rabbit was challenged with a slime-producing *S. epidermidis* strain (ATCC 35983) by intrastromal injections using a sterile 30-gauge needle. The challenge dose was approximately 10^3 viable organisms in 25 μl of 0.9% (w/v) saline. All rabbits were slit lamb examined (SLE) from 10 h post infection (PI) every 12 h for the first 3 days and then at 3 days intervals until time of sacrifice. SLE of rabbit eyes was performed by two masked observers. Corneal erosions were detected using fluoroscein (Fluorets, Smith and Nephew Pharmaceuticals Ltd, Romford, UK). The subsequent course of keratitis was examined for 1 month and the severity of the grossly observable lesions was graded clinically according to a scoring system. In brief experimental keratitis was classified to five different stages (0–4) as previously described [18]: Grade 0, eye macroscopically identical to the uninfected contralateral control eye; inoculation tract, Grade 1, faint opacity partially covering the pupil; $< 1/3$ corneal diameter, Grade 2, dense opacity covering the pupil; extending over an area $> 1/3$ and $< 2/3$ of corneal diameter, Grade 3, dense opacity covering the entire anterior segment extending over an area $> 2/3$ of corneal diameter; and Grade 4, perforation of the cornea, severe bulging or descemetokele.

2.5. Sample treatment and determination of anti-20-kDa PS antibodies

Serum was collected from all actively immunized rabbits (group I) before and during the immunization procedure. All serum samples were monitored for anti-20-kDa PS specific antibodies by an enzyme-linked immunosorbent assay (ELISA), as previously described [17]. Antibody levels were significantly ($P < 0.001$) higher than the preimmune ones after the fourth injection and, therefore, active immunization was considered complete. One week after the last polysaccharide injection, serum and aqueous humor were collected from five rabbits and staphylococcal induction of keratitis took place on the same day (day 0). On days 3, 14 and 30 after infection, serum and aqueous humor samples were collected from five rabbits each time.

For passive immunization experiments, five rabbits were sacrificed on day 0 (1 day after administration of antibodies and before the infection) as well as on days 3, 14, 30 post-infection in order to collect serum and aqueous humor. Rabbits to be killed were graded clinically by gross external examination based on the previously described scoring system. Venous blood was drawn from rabbits' ears and centrifuged at 2000 rpm for 15 min to collect the serum. Paracentisis of 0.1 ml of aqueous humor was performed 1 mm anterior to the limbus using a 30-gauge needle. Aqueous humor samples were stored at -70 °C until analysed. Rabbits were then sacrificed by a marginal ear vein injection of 10–15 ml of pentobarbital sodium 100 mg/ml.

2.6. Enzyme immunoassay to determine 20-kDa PS antibody titers

Specific 20-kDa PS IgG in blood sera and aqueous humor were determined by an ELISA, as previously described [17]. Sterile 96-well round-bottomed microplates were coated with a 5.0 μg/ml solution of 20-kDa PS in a carbonate–bicarbonate buffer, pH 9.6 (100 μl per well), at 4 °C for 16 h. The plates were washed three times with 120 μl per well with PBS containing 1%(v/v) Tween 20, 0.5% (w/v) BSA, and 0.4 M NaCl (PBS-Tween). Non-specific binding was blocked by incubation with a 3% (w/v) solution of BSA in PBS (200 μl per well) at 37 °C for 1 h. After three washings (220 μl per well), microplates were incubated at 37 °C for 1 h with anti-20-kDa PS serum diluted 1:8000, 1:16 000 and 1:32 000 in PBS as well as rabbit blood sera diluted 1:600 in PBS and aqueous humor samples diluted 1:5. Plates were washed with PBS-Tween for three times. Peroxidase H-conjugated goat anti-rabbit IgG (Sigma Chemical Company, St. Louis, MO, USA), diluted 1:2000 were used as detection antibodies. After incubation at 37 °C for 1 h and thorough washings, colour was developed by

adding 100 μl per well of a citrate buffer solution, pH 5.0, containing 0.4 mg/ml *ortho*-phenylenediamine dihydrochloride in 0.1 M trisodium citrate and 0.03% (v/v) H_2O_2. The mixture was incubated for 15 min at room temperature in the absence of light. The reaction was terminated with 1 M H_2SO_4 and the optical density was measured at 490 nm in a Molecular Devices E-max photometer. Calibration and validation of results were performed using the SOFT MAX PRO software (version 1.2.0).

As a control for non-specific binding, specific wells in every microplate were coated only with BSA and incubated with the samples tested. The respective values were always automatically subtracted from those obtained from the incubation of PS-coated wells with samples.

The content of antibodies to *S. epidermidis* polysaccharide in various samples was expressed in relation to reference anti-20-kDa PS sera, as previously described [17]. In brief, a reference curve was constructed by plotting the optical density at 490 nm versus serial dilutions of antisera in a semi-log graph (not shown). For quantification of the amount of specific antibodies towards the 20-kDa PS, it was assumed that the reference serum has 1000 U/ml. This assumption reflects that the value of 1.0 U/ml corresponds to 1/1000 dilution of reference antisera. Reactivity of various IVIG lots was determined by selecting dilutions that generated absorbance values within the linear part [0.031–0.500 U/ml, $y = (4.474 \pm 0.165) + (0.930 \pm 0.038)x$, $R = 0.997$] of the reference curve. The X-intercept of the absorbance values in U/ml was multiplied by the appropriate dilution factor and the titers were expressed in U/ml of the preparation.

2.7. Statistical analysis

Statistical analysis of antibody titers was performed by *t*-test. The non-parametric data derived from clinical score were analysed using the Wilcoxon two-sample test, using commercial software (SAS for WINDOWS, SAS Institute, Cary, NC). $P \leq 0.05$ was considered significant.

3. Results

3.1. Clinical scoring

Ten hours post-infection, marked epithelial defects were visible at the site of infection in all eyes as observed by intense staining with fluorescein. Chemosis and iritis ranged from moderate to severe, and trace amounts of fibrin appeared in the anterior chamber. On day 3, all rabbits showed conjuctival hyperaemia, oedema and scanned exudates. Anterior chamber findings regarded only mild iris hyperaemia. The corneas showed dense white infiltrates, the density and depth of which rapidly increased ranging from partially to entire covering the cornea in control rabbits. By day 7, most of the immunized animals had less severe inflammation of conjunctiva and iris. The cornea opacity was faint, covering partially the pupil in most of the immunized rabbits (grade 1), whereas the rest followed a mild clinical course. Severe clinical changes persisted throughout the duration of 30 days in most of the control rabbits, while 30% of them had loss of red-reflex with perforation of the cornea and phthisis bulbi (grade 4).

3.2. Levels of anti-20-kDa PS antibodies in active immunization and clinical scoring

Active immunization of rabbits with 20-kDa PS resulted in levels of specific anti-20-kDa PS antibodies in serum and aqueous humor on day 0, i.e. the day of keratitis induction, significantly higher ($P < 0.001$) than those in the control group (Table 1). Therefore, the immunization protocol was successful. The serum titers of specific anti-20-kDa PS IgG during all post-infection period were significantly higher than those in control rabbits (Table 1). Mean titers of 20-kDa PS-specific IgG were higher in serum than in aqueous humor, as normally expected. As shown in Table 1, IgG antibodies to 20-kDa PS in aqueous humor of the infected eye, present in trace amounts in preimmune samples and control rabbits, were also significantly increased after immunization. No difference between the infected and fellow eye was observed.

Table 1
Titers of specific anti-20-kDa PS antibodies in blood serum and aqueous humor of infected eyes of rabbits vaccinated with 20-kDa PS (active immunization) and rabbits treated by the same volume of a mixture containing normal saline and CFA (control)

Sample source	Days (post-infection)			
	0	3	14	30
Serum[a]	129.3 ± 30.15*	145.0 ± 25.1	159.4 ± 29.8*	152.4 ± 31.0*
	(36.1 ± 4.8)[b]	(37.3 ± 4.2)	(41.1 ± 5.8)	38.6 ± 3.7)
Aqueous humor	1.3 ± 0.1*	1.2 ± 0.1*	1.2 ± 0.1*	1.2 ± 0.1*
	(0.08 ± 0.01)	(0.06 ± 0.01)	0.06 ± 0.01)	(0.07 ± 0.01)

[a] Analysis in blood sera and aqueous humor were achieved following 1:600 and 1:5 dilutions of the samples in phosphate buffer saline, respectively. Results are expressed in units/ml and represent the average ± S.D.
[b] Values obtained for control rabbits are given in parenthesis.
*, $P < 0.001$.

Clinical scoring of experimental keratitis in the group of actively immunized rabbits showed a marked lower pathology throughout infection. Comparison of average clinical scores 1 week post-infection between vaccinated and control rabbits showed statistically significant ($P < 0.003$) differences (Table 2). Epithelial erosions at 1 week post-infection were significantly smaller in the corneas of immunized rabbits than in the control rabbits.

3.3. Levels of anti-20-kDa PS antibodies in passive immunization and clinical scoring

Polyclonal rabbit antibodies against the 20-kDa PS were tested for specificity to their homologous antigen, as well as for opsonic activity against slime-producing *S. epidermidis*. Results showed that the antibodies exhibit high specificity for 20-kDa PS and high opsonic activity, 65 ± 4% (data not shown). Thus, these antibodies are functional and able to opsonize and promote clearance of slime-producing *S. epidermidis*. Administration of 20-kDa PS-specific rabbit antiserum resulted in levels of specific anti-20-kDa PS antibodies in serum and aqueous humor at a significantly higher rate ($P < 0.001$) than those of the control group on day 0 (Table 3). High antibody levels were observed throughout the infection period, however, as it was expected a significant decrease was observed by day 30 (Table 3). The levels of antibodies to 20-kDa PS in the aqueous humor of the infected eye, not detectable in preimmune samples and significantly lower in control samples, were also significantly increased after immunization, whereas no differences between the infected and fellow eye was observed.

Clinical scoring of experimental keratitis in the group of antibody-treated rabbits showed that antibodies provided significant protection to the cornea, as evidenced by the significantly milder clinical course for the antibody treated group as compared with the control group ($P = 1.08 \times 10^{-6}$) (Table 2). Furthermore, comparison of average clinical scores between antibody-treated and actively-immunized rabbits showed significant dif-

Table 2
Mean clinical scores of corneas exhibiting keratitis in both immunization groups and their respective controls 7 days after the induction of Staphylococcal keratitis

Treatment of rabbits	*n*	Mean clinical score ± SEM[a]
Active immunization	10	1.67 ± 1.05[b,c]
Control	10	2.93 ± 1.03
Passive immunization[b]	10	0.93 ± 0.88[b]
Control	10	2.92 ± 0.88

[a] Mean clinical score is based on a 0–5 grading scale, as described in materials and methods.
[b] Statistically significant ($P < 0.003$) differences between the immunized and the control groups.
[c] Statistically significantly higher ($P < 0.05$) values in the actively immunized group as compared with the antibody-treated group.

Table 3
Titers of specific anti-20-kDa PS antibodies in blood serum and aqueous humor of infected eyes of rabbits that received 20-kDa PS-specific antibodies (passive immunization) and those treated by the same volume of normal saline (control)

Sample Source	Days (Post-infection)			
	0	3	14	30
Serum[a]	414.9 ± 29.9*	427.5 ± 25.0*	312.6 ± 20.0*	194.2 ± 18.9*
	(37.2 ± 4.4)[b]	(37.9 ± 4.5)	(40.2 ± 5.3)	(37.8 ± 4.1)
Aqueous humor	1.1 ± 0.09	1.2 ± 0.1*	1.3 ± 0.1*	1.2 ± 0.1*
	(0.08 ± 0.01)	(0.06 ± 0.01)	(0.06 ± 0.01)	(0.07 ± 0.01)

[a] see Table 1.
[b] see Table 1.

ferences ($P < 0.05$). Specifically, antibody-treated rabbits showed a milder clinical course than the 20-kDa PS-vaccinated rabbits.

4. Discussion

Keratitis is a leading cause of ocular morbidity and blindness worldwide despite early aggressive therapy. *S. epidermidis* has the ability to adhere to smooth surfaces of biomedical materials and form biofilms. Production of a slime layer by certain strains of *S. epidermidis* renders them resistant to phagocytocis and antimicrobials [8–10]. These properties have triggered the interest of researchers in the chemical structure of slime and its biologic properties, with conflicting results thus far. Studies on the chemical organization of slime from our group showed the presence of discrete macromolecules, constituting the extracellular slime layer of both reference and clinical *S. epidermidis* strains [12,13]. Previous immunologic studies have shown that 20-kDa PS is less immunogenic than other slime macromolecules [15]. However, this polysaccharide is the most potent inhibitor of the reaction of slime with its homologous antibodies revealing that 20-kDa PS is the major antigenic determinant [15].

Host immune system has a significant effect on the progression of keratitis [19]. The inflammatory cell response, in particular, is critical to development of disease [19]. The primary immune cells recruited in the initial stages of corneal infection are neutrophils. Although, cornea is normally avascular, immune cells such as polymorphonuclear leukocytes are rapidly recruited from iris and limbal vessels in response to the presence of infecting bacteria. Once activated, specific immunocytes travel hematogenously to the eye where they interact with antigen, mediate the inflammatory response and initiate intraocular antibody production. Complexities of the cornea, the immune system, and their interaction with invading bacteria make the use of animal models of corneal infection critical to developing an understanding of pathogenesis of corneal infection and subsequently devising effective therapies for this potentially blinding disease.

In this study we examined the effects of active and passive immunization on experimentally induced *S. epidermidis* keratitis. Both types of immunization provided protection against corneal melting and opacification. Actively immunized rabbits showed significantly less pathology as assessed by clinical scoring throughout the experimental infection (2–4 weeks post-infection) as compared with non-treated animals. Therefore, active immunization confers protection to the cornea from development of severe tissue damage and inflammation during *S. epidermidis* keratitis. Functional polyclonal antibodies, i.e. able to opsonize in vitro slime-producing *S. epidermidis* and promote its clearance by human polymorphonuclear cells, were administered to rabbits. As it was demonstrated, these antibodies protected rabbits during experimental keratitis. Protection was evidenced by the significantly lower clinical scores obtained for the antibody-treated than for the

control group. The main difference between the two types of immunization is that passive immunization results in milder corneal melting and opacification.

Active and passive immunization of rabbits against 20-kDa PS resulted in significantly higher levels of specific antibodies than in the control ones during the period of study. On day 30 after infection, antibodies in passively immunized group significantly decreased due to physiological catabolism, but they were still higher than those of control. Antibody titers in serum after passive immunization were significantly higher than those in serum of actively immunized rabbits, due to the high amount (~ 70 mg) of 20-kDa PS-specific antiserum infused, a fact that may well be associated with the better clinical outcome.

The ability of administered antibodies to protect against corneal melting opacification was associated with the relatively high IgG titers in aqueous humor. Possible sources of aqueous humor antibody levels to 20-kDa PS include leakage from serum, diffusion from vitreous or local production by plasma cells in the cilliary body and iris [20]. Leakage of serum IgG antibody into aqueous after disruption of the blood-aqueous barrier by inflammation is supported by the findings that the variation in the aqueous humor specific antibody levels follows the same profile of increase and decrease in serum and were always lower than those in the serum. The levels of antibodies in aqueous were similar both in active and passive immunization and therefore the better clinical outcome of passively immunized rabbits is not solely related to aqueous antibodies. Transudation of specific IgG from inflamed conjunctival vessels in the inoculated eye may probably result in high tear specific antibodies and therefore, better corneal protection.

In conclusion, this study reveals the crucial role of 20-kDa PS in the pathogenesis of *S. epidermidis* keratitis. Although, more experiments are needed, our results demonstrate that active immunization of patients at risk could limit corneal damage. Furthermore, passive administration of 20-kDa PS-specific antibodies to infected patients significantly improves the clinical outcome of *S. epidermidis* keratitis.

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