Contact lens wear and its complications

Cheng, K.H.

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CHAPTER III

IMMUNOGLOBULIN A ANTIBODIES AGAINST PSEUDOMONAS AERUGINOSA IN THE TEAR FLUID OF CONTACT LENS WEARERS

Courtesy of Investigative Ophthalmology & Visual Science
ABSTRACT

Purpose.
Pseudomonas aeruginosa is the most important cause of contact lens-associated ulcerative keratitis, especially for those who use extended-wear lenses. Until now, the presence of specific anti-P. aeruginosa immunoglobulin A (IgA) antibodies in the tears of contact lens wearers has not been investigated and is the purpose of the current study.

Methods.
The levels of specific IgA antibodies against P. aeruginosa and total secretory IgA (s-IgA) concentrations were measured in tears of various groups of contact lens and non-contact lens wearers using enzyme-linked immunosorbent assays. Contact lens groups were divided into the following categories: daily-wear rigid gas-permeable lenses (n = 23), daily-wear soft lenses (n = 22), extended-wear soft lenses (n = 17) and non-contact lens wearers (n = 23). As a positive control group, we tested tears obtained from patients with cystic fibrosis (n = 5) because the respiratory tract of these persons often are colonized by P. aeruginosa.

Results.
The percentage of nonresponders (<15 U/ml) varied between 9% in daily-wear rigid gas-permeable contact lens users to 23% in daily-wear soft contact lens users. The percentage of nonresponders in controls was 13%. The frequency of nonresponders was not significantly different among the different groups tested. All patients with cystic fibrosis showed a very high anti-P. aeruginosa IgA response in their tears. When analyzing the mean anti-P. aeruginosa IgA response, a significantly lower level was found in extended-wear contact lens users (38 U/ml) compared to non-contact lens wearers (82 U/ml). Total s-IgA levels in the tears of the various groups tested were not significantly different.

Conclusions.
A substantial number of persons in the population of contact lens wearers tested, lacks detectable IgA antibodies against P. aeruginosa in their tears and may be susceptible to P. aeruginosa keratitis if the physiological condition of their cornea is compromised.
INTRODUCTION

Microbial ulcerative keratitis is the most devastating complication of contact lens wear. *Pseudomonas aeruginosa* is the organism most frequently isolated from these ulcers.¹ The relative risk for and incidence of this disorder is dependent, at least in part, on the lens type (soft or rigid) and the lens-wearing behavior (daily-wear or extended-wear).²-⁴ Several well-controlled studies have shown that overnight wear of contact lenses (including disposable lenses) is the most important risk factor for contact lens-related ulcerative keratitis.²⁻⁷

The pathogenesis of *P. aeruginosa*-induced keratitis is thought to be multifactorial in origin. The first prerequisite is the contamination of the contact lens with the bacterium whereby the inoculum often arises from a contaminated contact lens case or a direct “in eye” adhesion of the bacteria.³⁻⁷⁻⁸⁻⁹ The second factor of importance is the integrity of the corneal epithelium. In vitro¹⁰⁻¹¹ and in vivo¹² studies have demonstrated that binding of *P. aeruginosa* to the corneal epithelium depends on the condition of these cells, whereby corneal hypoxia and epithelial defects enhance binding of the microorganisms to the cells. A third factor that has not received much attention until now involves the role of mucosal immunity in contact lens-associated bacterial keratitis.

Secretory immunoglobulin A (s-IgA) is the predominant class of immunoglobulin in mucosal-associated tissues and external secretions such as tears.¹³ Recently, inhibition of *P. aeruginosa* adherence to the corneal surface by ocular mucin, human tears¹⁴ and s-IgA¹⁵ has been demonstrated in an animal model. It is thought that antiadherence factors present in the tear film bind and entrap this bacterium, which can then be eliminated by tear flow mechanisms. Thus, the binding of tear s-IgA to *P. aeruginosa* may protect the cornea from bacterial adherence, which is an initial step in the infectious process.¹⁶

Although experimental studies suggest a role for s-IgA in the pathogenesis of *P. aeruginosa*-induced keratitis, the presence of specific anti-*P. aeruginosa* IgA in the tears of contact lens wearers has not yet been investigated and is the purpose of the current study.

Our results show that 83% of the contact lens wearers tested have a detectable anti-*P. aeruginosa* IgA response in their tear fluid. This suggests that 17% who have no detectable specific IgA response may be at risk for *P. aeruginosa*-induced keratitis when their lenses are contaminated and their corneal epithelium is compromised.
Materials and Methods

Subjects

Tears were collected from 62 consecutive asymptomatic cosmetic contact lens wearers attending three contact lens clinics for routine ocular examination. The subjects had worn contact lenses for at least 6 months (mean wearing time, 10 years). The contact lens group was divided into the following categories:

1. Daily-wear rigid gas-permeable contact lens users (n = 23; mean age, 35 years).
2. Daily-wear soft contact lens users (n = 22; mean age, 27 years).
3. Extended-wear soft contact lens users (n = 17; mean age, 36 years).

The control group consisted of persons (n = 23; mean age, 32 years) who had never worn lenses before and who attended the clinics to have contact lenses fitted.

As a positive control group, we used tears from five patients with cystic fibrosis; three were colonized by *P. aeruginosa*, as shown by bacteriologic sputum cultures. Slit lamp examination documented that none of the contact lens wearers or controls had ocular disease.

The tenets of the Declaration of Helsinki were followed, and the study was approved by the human experimentation committee of the Medical Faculty of the University of Amsterdam. Informed consent was obtained from subjects before collection of tears.

Tear Collection

Tears were collected by placing a sterile cellulose sponge (Sugi, Kettenbach, Germany) in the conjunctival sac of both eyes as described earlier. Tear collection was performed between 9 AM and 11 AM at the outpatient department of the hospital. Sponges were removed after 1 minute, and tear fluid was separated from the sponges by centrifugation and stored at -20°C. In contact lens wearers, lenses were removed before tear collection.

Strains and Serotyping

*P. aeruginosa* was isolated from the corneal ulcer of a patient with a keratitis (reference strain 474) and serotyped (serotype PI) according to the Sanofi Diagnostics Pasteur (Marnes La Coquette, France) typing scheme, which is identical to the O1 serotype using the International Antigenic Typing Scheme. The O1 serotype is a strain commonly isolated in patients with *P. aeruginosa* keratitis.
**Bacterial Cell Culture**

*P. aeruginosa* was grown overnight on blood agar plates at 37°C. One colony was inoculated in 100 ml of broth medium containing 5% Bacto peptone (Difco Laboratories, Detroit, MI) and 0.25% trypticase soy broth (Difco). The culture was grown on a rotary shaker at 37°C for 18 hours, centrifuged at 7000g for 20 minutes at 4°C, washed three times with phosphate-buffered saline (PBS, 160 mM Na, 1.3 mM $\text{H}_2\text{PO}_4$, 9.2 mM $\text{HPO}_4$ and 140 mM Cl, pH 7.4) and suspended in PBS to a concentration of $10^9$ colony forming units (CFU)/ml. The bacteria were heated at 100°C for 2 hours, divided into 1 ml aliquots, and stored at -20°C before use.

**Bacterial Culture of Contact Lens Containers**

Before the visits, all subjects were not informed about the contamination survey and were not instructed to bring their lens storage cases with them. After tear collection, all contact lens wearers were asked whether they had brought their lens cases. If so, lens cases were exchanged for new cases. Otherwise, they were requested to send their lens containers by mail to the research institute the next day. In total, 56 lens cases (44 of the daily-wear lens users and 12 of the extended-wear lens users) were collected. The presence of *P. aeruginosa* in these storage cases was

![Graph](image-url)  
*Fig. 1. Standard curve of anti-*Pseudomonas aeruginosa* immunoglobulin A. IgA antibodies directed against *P. aeruginosa* were determined by enzyme-linked immunosorbent assay on a pool of tears that had been set arbitrarily to contain 1000 U/ml. Plates either were coated with *P. aeruginosa* (●–●) or with coating buffer (○–○) alone.*
investigated using a *P. aeruginosa*-specific broth (special peptone, casein hydrolysate with "*P. aeruginosa* supplement" consisting of 100 μg/ml cetrimide and 15 μg/ml sodium nalidixate). All media components were purchased from Oxoid (Basingstoke, UK). The fluid in the lens containers was removed, and they were washed with culture medium to remove remaining care solution. The special *P. aeruginosa* bouillon was added to the containers, which were then vigorously vortexed and incubated for 24 hours at 37°C. A sample of the medium was cultured on both cysteine, lactose, electrolyte-deficient plates (Oxoid) and *P. aeruginosa*-specific agar plates (containing cetrimide and sodium nalidixate; Oxoid) for 2 days.

**P. aeruginosa IgA Enzyme-Linked Immunosorbent Assay**

Initial experiments were performed whereby the enzyme-linked immunosorbent assay (ELISA) was compared using heat-killed (60°C for 1 hour and 100°C for 2 hours) versus freshly cultured bacteria. Killing was verified by bacterial culture after heating. Highest absorbance readings were obtained with bacteria that had been pretreated at 100°C for 2 hours. We, therefore, used heat-killed bacteria (100°C for 2 hours), which is similar to protocols used by previous investigators. A dose-response curve of the concentrations of bacteria used for coating the ELISA plates revealed a bell-shaped curve at which 1.25×10⁷ CFU/ml was optimal.

The above-mentioned experiments led to the following protocol. Heat-killed (100°C for 2 hours) *P. aeruginosa* organisms (10⁹ CFU/ml) were diluted 1:80 in 0.05 M carbonate-bicarbonate coating buffer (pH 9.6). ELISA plates (96 wells; #566101; Greiner, Frickenhausen, Germany) were incubated overnight with the organisms (150 μl/well) at room temperature and were washed four times with PBS the next morning. The wells were subsequently blocked with PBS containing 2% (wt/vol) bovine serum albumin (BSA) and 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.3 (blocking buffer, 150 μl/well) during 2 hours at room temperature. Plates were washed and filled with 100 μl/well of serial tear fluid dilutions (1:25 and 1:50 in blocking buffer with 0.1% (vol/vol) Tween 20) and incubated for 1 hour at 37°C. After this incubation, the wells were washed and incubated with a horseradish peroxidase-labelled rabbit antihuman IgA antibody (Dako, Copenhagen, Denmark) diluted 1:500 in PBS containing 0.1% (wt/vol) gelatin, 2% (wt/vol) normal rabbit serum and 0.02% (vol/vol) Tween 20, pH 7.3. After 1-hour incubation at 37°C and a washing procedure, 150 μl/well of substrate buffer was added: 0.1 M acetic acid, 0.01% (wt/vol) tetramethylbenzidine, 1% dimethylsulfoxide, and 0.03% H₂O₂, pH 5.4. The enzyme substrate
reaction was inhibited after 10 minutes by the addition of 50 µl/well of 2 M H₂SO₄. The optical density was measured in a Titertek Multiscan MC (Amstelstad, The Netherlands) at 450 nm. Results were expressed after subtraction of the corresponding optical density of negative controls wells for each sample. Negative controls of the ELISA included the incubation of uncoated wells with tear dilutions, and incubation of *P. aeruginosa* coated wells with buffer instead of tears.

Initial experiments showed that in some persons, their tears contained factors that gave a high background when incubated with the uncoated wells. Addition of normal rabbit serum to the peroxidase-labeled antihuman IgA antibody solved this problem. The ELISA readings were converted to units using a calibration curve obtained by including serial dilutions of a pool of human tears from persons with a high anti-*P. aeruginosa* IgA response. This pool of tears had been set arbitrarily to contain 1000 U of anti-*P. aeruginosa* IgA per ml. Each plate always contained its own standard calibration curve. A dose-response curve of the standard pool of tears is shown in figure 1. A computer program (Logit; Central Laboratory, Dutch Red Cross Blood Transfusion Service, Amsterdam, Netherlands) was used to generate standard curves and to calculate anti-*P. aeruginosa* IgA levels in experimental samples. From the standard curve, it can be seen that reliable measurements could be obtained at tear dilutions of less than 1:1600 corresponding to 0.625 U/ml. Because experimental tear samples were tested at a dilution of 1:25, the sensitivity of our test was 15 U/ml. Persons with ELISA readings less than 15 U/ml were considered nonresponders. Analytical variation of the ELISA, including intraplate (intra-assay) and day-to-day (interassay) variations, was determined with two tear samples (10 determinations for each). The intra-assay and interassay variations of the ELISA were 2% and 7.5%, respectively.

To prove that the response in our *P. aeruginosa* ELISA was associated with s-IgA, the following experiment was performed. Tears were collected from a *P. aeruginosa* responder, and 25 µl were loaded onto an Fast Performance Liquid Chromatography column (SMART System; Pharmacia, Upsalla, Sweden), and tear proteins were fractionated according to their molecular weight. The first peak emerging from the column and known to contain the IgA peak was collected (total volume, 175 µl) and tested in the ELISA, together with the original tear sample; a correction was applied for the dilution (1:7) occurring during the gel filtration procedure. Both the 1:20 and 1:100 dilutions gave similar optical density readings in the ELISA. The 1:20 dilution gave a reading of 2.04 for the original tears compared to 1.97 for the isolated IgA fraction, whereas the 1:100 dilution gave an optical density reading of 1.33 (whole tears) versus 1.18 (IgA fraction).
Table 1. Immunoglobulin A Response Against *Pseudomonas aeruginosa* in Tears of Contact Lens and Non-Contact Lens Wearers (Controls) and Patients With Cystic Fibrosis

<table>
<thead>
<tr>
<th></th>
<th>responders</th>
<th>nonresponders</th>
<th>nonresponders (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>daily-wear RGP</td>
<td>21</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>daily-wear soft</td>
<td>17</td>
<td>5</td>
<td>23</td>
</tr>
<tr>
<td>extended-wear soft</td>
<td>14</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>controls</td>
<td>20</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>cystic fibrosis</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

RGP = rigid gas-permeable.
* responders had an IgA anti-*P. aeruginosa* level above 15 U/ml.

**s-IgA ELISA**

s-IgA in human tear fluid was quantitated using a sandwich ELISA; s-IgA was captured with a monoclonal antisecretory component antibody (Nordic, Immunological Laboratories, Netherlands) and detected with the same peroxidase-labeled rabbit anti-human IgA antibody as described above. ELISA plates (96 wells; #566101, Greiner) were coated overnight with 0.1 mg/ml monoclonal antisecretory component antibody in PBS. After washing, the wells were incubated with serial tear dilutions (1:500, 1:1000, 1:2000) in PBS containing 0.1% (vol/vol) Tween 20. The same tear sample was used as for the detection of the anti-*P. aeruginosa* IgA response. After incubation for 1 hour at 37°C, the wells were washed, and bound s-IgA was detected by incubating (1 hour at 37°C) with a peroxidase-labeled anti-IgA diluted in 1:500 PBS containing 0.1% (vol/vol) Tween 20 and 2% (wt/vol) normal rabbit serum. The ELISA was developed using above-mentioned substrate and quantitated as mg s-IgA/ml using a calibration curve with human colostrum s-IgA (Sigma, St. Louis, MO) as a standard.

**Western Blot Analysis**

For immunoblotting, an outer membrane preparation of the bacteria was made as follows. *P. aeruginosa* was cultured as described above. Bacteria were harvested by centrifugation at 2500g for 10 minutes at 4°C. The pellet was resuspended in 7 ml of 50 mM Tris-HCl (pH 7.8). The suspension was sonicated intermittently for 5 minutes on ice. After centrifugation at 2000g for 20 minutes at 4°C, the supernatant was centrifuged in an ultracentrifuge at 45,000g for 1 hour at 4°C. The supernatant was discarded, and the pellet was resuspended in 200 μl of 2 mM Tris-HCl pH (7.8).

Sodium dodecyl sulfate-polyacrylamide (SDS) gel electrophoresis was
performed on the Phast System (Pharmacia) using precast 10% to 15% gradient minigels. The electrophoresis conditions and silver staining of the minigels were performed according to manufacturer’s instructions. The outer membrane samples were prepared in a nonreducing sample buffer (62 mM Tris, 1 mM EDTA, 2% [wt/v] SDS, 0.005% [wt/vol] bromophenol blue, pH 8.0) and had a protein content of approximately 0.2 µg/µl. After electrophoresis, separated outer membranes were transferred to Immobilon PVDF membranes (pore size 0.45 µm; Millipore, Milford, MA) by electroblotting in a semidry environment. The transfer buffer consisted of 192 mM glycine, 25 mM Tris base, 20% (vol/vol) methanol, pH 8.3, and proved to have a good transfer efficiency as assessed by silver staining of gels before and after electroblotting.

After transfer, blots were incubated for 2 hours, in 2% (wt/vol) BSA in 0.5% (vol/vol) Tween 20, 10 mM EDTA, pH 7.3, in PBS buffer, to block the nonspecific binding sites. Between the blotting steps, the blots were washed in PBS for 15 minutes. Blots were cut into strips. Tears were diluted to a concentration containing 5 to 10 U anti-P. aeruginosa IgA/ml, as previously determined by ELISA. Each immobilon strip was incubated separately with a diluted tear sample for 1 hour at 37°C. This step was followed by incubation with 1:1000 diluted biotinylated rabbit-antihuman-IgA antibody (Dako) in 3% (wt/vol) BSA.

A streptavidin-biotin-HRP complex (StrepABComplex; Dako) was used to detect immunoglobulin-biotin complexes. StreptABComplex, in combination with a biotin-labeled secondary antibody, has a very high sensitivity. The streptavidin-biotin-HRP complex was visualized using diaminobenzidine-metal concentrate (Pierce, Rockford, IL). Negative controls included strips containing bacterial outer membranes; in the first step, buffer alone was used instead of tears.
Chapter III

Statistical Analysis

The level of significance of the data obtained from the various groups investigated was tested using the Mann-Whitney U test.

RESULTS

The ELISA for the detection of IgA antibodies against P. aeruginosa was developed using heat-killed P. aeruginosa obtained from a patient with a corneal ulcer. When analyzing the anti-P. aeruginosa IgA response in various groups of contact lens wearers and controls, the following observations were made. The percentage of nonresponders (ELISA reading less than 15 U/ml) varied between 9% in daily-wear rigid gas-permeable contact lens users to 23% in daily-wear soft contact lens users (table 1). The percentage of nonresponders in controls was 13%. In view of the small amount of individuals tested, these data did not reach statistical significance. When analyzing the mean anti-P. aeruginosa IgA response in the tears of the various groups, a significantly lower response was noted in the group of extended-wear soft contact lens users compared to controls (table 2). Controls had a mean IgA anti-P. aeruginosa response of 82 U/ml, (range, 6.1 to 285 U/ml), whereas users of extended-wear soft contact lenses had 38 U/ml, (range, 5.9 to 102 U/ml) in their tear samples. All patients with cystic fibrosis had a detectable anti-P. aeruginosa response, and those persons chronically infected by the bacterium (sputum culture positive for P. aeruginosa) had extremely high levels (table 3).

Because the low anti-P. aeruginosa IgA response observed in extended-wear soft contact lens users could have resulted from a decrease in the total s-IgA level in the collected tear samples, we measured the s-IgA concentration in the same sample. Although the s-IgA level in extended-wear soft contact lens users appeared to be lower than that seen in controls, no statistically significant differences were found between the

<table>
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<th>Patient Number</th>
<th>age (years)</th>
<th>sputum culture</th>
<th>IgA anti-P. aeruginosa (units/ml)</th>
<th>IgA (mg/ml)</th>
<th>ratio</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>+</td>
<td>2060</td>
<td>0.3</td>
<td>6867</td>
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<tr>
<td>2</td>
<td>18</td>
<td>-</td>
<td>92</td>
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<tr>
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<td>452</td>
<td>1.9</td>
<td>238</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>+</td>
<td>6218</td>
<td>0.6</td>
<td>10363</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>+</td>
<td>28550</td>
<td>1.8</td>
<td>15861</td>
</tr>
</tbody>
</table>
Anti-Pseudomonas response in contact lens wearers

Fig. 2. Western blot of outer membranes from *Pseudomonas aeruginosa* serotype O1. An outer membrane preparation was electrophoresed and transferred to immobilon membrane and subsequently incubated with tears diluted to contain 5 to 10 U of immunoglobulin A anti-*P. aeruginosa*. (Lane 1) Tears from a daily-wear soft contact lens wearer. (Lanes 2, 3) Tears from two different rigid gas-permeable contact lens wearers. M indicates the lane with the biotinylated molecular weight markers. Negative controls in which tears were omitted did not result in detectable bands (data not shown).

groups investigated, including the patients with cystic fibrosis (tables 2, 3).

Because tear flow rate affects the concentration of a number of tear proteins including s-IgA, we measured the volume of tears produced per minute. The volume of collected tears ranged between 35 to 75 µl, and no significant differences in the mean tear volumes were observed among the different groups tested. Moreover, using our method of tear collection, no correlation was observed between the amount of tears collected and the s-IgA concentration.

Subsequently, the specific anti-*P. aeruginosa* IgA response was calculated for each individual sample as ELISA unit anti-*P. aeruginosa* per milligram of total s-IgA. No differences were observed between the contact lens-wearing groups and controls; patients with cystic fibrosis, however, had very high specific IgA responses (tables 2, 3).

Specificity of the anti-*P. aeruginosa* antibodies was demonstrated by immunoblotting. A representative experiment indicates that tears of contact lens wearers react with various bands of an outer membrane
protein preparation of the bacterium (figure 2). Negative controls included strips containing bacterial outer membranes in which tears were omitted in the whole procedure did not result in the appearance of detectable bands (data not shown).

To investigate whether the contact lens containers were contaminated with \textit{P. aeruginosa}, we investigated 56 storage cases using a special \textit{P. aeruginosa} culture medium. None of them contained detectable \textit{P. aeruginosa}.

**DISCUSSION**

This study shows that a substantial number of persons lack detectable IgA antibodies against \textit{P. aeruginosa} in their tears and may be susceptible to \textit{P. aeruginosa} keratitis if they encounter this organism under circumstances in which the physiological condition of the cornea is compromised. Because we only used one isolate (O1 serotype) as a substrate for the detection of IgA antibodies against \textit{P. aeruginosa}, it may not be justified to extrapolate our findings to all strains of \textit{P. aeruginosa}. The O1 serotype, however, is one of the common strains involved in \textit{P. aeruginosa} keratitis.\(^\text{18}\)

\textit{P. aeruginosa} keratitis is the most frequent infectious complication during contact lens wear, and overnight wear appears to be the most important risk factor. Other factors involved in the pathogenesis of \textit{P. aeruginosa} induced keratitis include direct in eye adhesion or contamination of the contact lens care system with the organism, a compromised corneal epithelium, and the absence of adequate specific and non-specific antibacterial factors in the tear film. Recent studies\(^\text{3,4}\) revealed that lens hygiene only has marginal effects on the development of microbial keratitis, which indicates that other factors make some persons wearing certain types of lenses uniquely susceptible to infection. During extended-wear contact lens use, the chance of compromising the cornea is greater\(^\text{23}\) than during daily-wear. An interesting finding in the current study was that the anti-\textit{P. aeruginosa} response was significantly lower in extended-wear users than it was in controls. This suggests that a compromised corneal physiology, in combination with a decreased local specific anti-bacterial immune response, may be the key factors in the pathogenesis of contact lens-associated keratitis.

The specificity of the local ocular IgA response has not received much attention until now, and most studies have dealt with the total IgA levels in tears during contact lens wear. S-IgA plays an important role in the prevention of bacterial adherence to mucosal tissues,\(^\text{24}\) and experimental studies have shown that stimulation of the local ocular immune response
Anti-Pseudomonas response in contact lens wearers can ameliorate *P. aeruginosa* keratitis.\textsuperscript{25,26} Besides a specific immune response, ocular innate immune responses in the tear film—which include mucins, lysozyme and lactoferrin—may also play a role in the defense against microbial infection. In this study, we did not observe significant differences in total tear s-IgA between various groups of contact lens wearers and controls, which is in agreement with our previous studies using a high performance liquid chromatography technique to quantitate tear IgA levels.\textsuperscript{27} However, other studies have reported markedly decreased\textsuperscript{28} and increased\textsuperscript{29} tear IgA levels in contact lens wearers. These discrepancies with our findings may have been caused either by differences in the sampling techniques as well as the method used to quantitate tear s-IgA. Sampling in our study was performed using a standardized technique and was carried out by the same trained observer throughout the study.

Analysis of IgA anti-*P. aeruginosa* antibodies has not yet been reported during contact lens wear. An earlier study\textsuperscript{30} addressed the ocular IgA response against *P. aeruginosa* in patients with cystic fibrosis and reported a much stronger specific IgA response in this patient group than in controls, a finding that could be confirmed by our study. The high anti-*P. aeruginosa* IgA response in patients with cystic fibrosis is attributable to the fact that the respiratory tract of these patients often is colonized by *P. aeruginosa*. In the current study, a huge range response was observed for the IgA anti-*P. aeruginosa* levels in the groups investigated, which may reflect the variability in the mucosal immune response among persons against this bacterium, a finding also reported earlier by others.\textsuperscript{30} We could not detect *Pseudomonas* contamination in the contact lens carrying cases of patients visiting our contact lens clinics. This indicates that this population is not exposed to this antigen on a routine basis, and it may account for their low specific IgA response against *P. aeruginosa*. The mechanisms responsible for the apparent lower anti-*P. aeruginosa* responses we observed in extended-wear contact lens users are not yet clear. One may consider the possibility that these lenses cover the entire cornea and part of the conjunctiva for prolonged periods of time. Mechanical or other barriers can prevent optimal antigen presentation to the dendritic cells of the peripheral cornea, which are thought to be involved in the initiation of the IgA response.

The lower anti-*P. aeruginosa* IgA response in the extended-wear contact lens users could have been caused by differences in total s-IgA in their tears. Although this group did show a trend toward lower s-IgA concentrations than the other groups, the differences were not statistically significant. We also could not detect differences in the tear production among the groups studied, which could account for possible s-IgA concentration differences. No correlation was observed between the
amount of tears, collected during the one minute collection period, and the corresponding s-IgA concentration in the obtained sample. The findings reported in this article suggest a role for a decreased specific IgA response in bacterial keratitis in extended-wear contact lens wearers. More vigorous testing of this hypothesis may be obtained by analyzing the local antibacterial response in a case-control study in patients with bacterial keratitis. If such testing confirms our hypothesis, it may well provide the rationale to implement preventive measures other than improving lens hygiene.
REFERENCES


