

No apparent association between periocular and ocular microcolonization and the degree of inflammation in patients with atopic keratoconjunctivitis

E. Nivenius*, P. G. Montan*, E. Chryssanthou†, K. Jung†, M. van Hage-Hamsten‡ and I. van der Ploeg*

*Department of Clinical Sciences, Karolinska Institutet, Division of Ophthalmology, St Erik's Eye Hospital, †Microbiology and Tumor Biology Center, Division of Clinical Microbiology and ‡Department of Medicine, Division of Clinical Immunology and Allergy, Karolinska Institutet and Hospital, Stockholm, Sweden

Summary

Background The cause of the chronic inflammation in atopic keratoconjunctivitis (AKC), the ocular manifestation of atopic eczema/dermatitis syndrome, is largely unknown.

Objective To investigate the possibility that microorganisms may be important in the inflammatory activity in AKC.

Methods Fifteen patients with AKC participated in the study. The presence of aerobic bacteria and fungi was related to the severity of clinical signs, the numbers of inflammatory cells in tears and conjunctival biopsies, and the concentration of various cytokines in tears. In addition, serological evidence for IgE sensitization to *Staphylococcus aureus* B antigen and *Malassezia sympodialis* antigen was investigated. Twelve healthy subjects were included for control purposes.

Results The patients exhibited moderate clinical signs of AKC. No relation was found between the severity of AKC and the presence of microorganisms, despite the fact that *S. aureus* was frequently isolated. AKC patients showed significantly higher levels of IFN- γ , TNF- α (tumour necrosis factor- α), IL-2, IL-4, IL-5 and IL-10 than controls. An association was found between conjunctival signs and the levels of all cytokines except IL-5.

Conclusion We found no evidence to suggest that periocular and ocular microcolonization are related to inflammatory parameters in AKC. However, confirmation of the present results in a longitudinal study with repeated clinical examinations and samplings in the same individual is required before the contribution of *S. aureus* to on-going inflammation in AKC can be dismissed.

Keywords atopic dermatitis/eczema syndrome, atopic keratoconjunctivitis, cytokine, eye, IgE, *Malassezia*, *Staphylococcus*, tear

Submitted 30 June 2002; revised 20 September 2003; accepted 29 December 2003

Introduction

Adult patients suffering from atopic eczema/dermatitis syndrome (AEDS) [1] are prone to develop atopic keratoconjunctivitis (AKC), i.e. various ocular inflammatory surface manifestations such as blepharitis, conjunctivitis and keratitis often in conjunction with eyelid eczema [2]. The cellular and cytokine response in AEDS and AKC is that of a Th1/Th2-influenced disease [3–5], suggesting that T cells are important in the disease process. However, the underlying factors responsible for the triggering and maintenance of the disorder are largely unknown. Since microorganisms have been implicated in eczema exacerbations in AEDS, substantiated by the observed clinical benefit of antibacterial and antimycotic treatment [6, 7], we wanted to investigate whether the presence of periocular and ocular surface bacteria and fungi has any relation with ocular surface inflammatory

parameters in AKC. As a secondary objective, various biological correlates of the clinical manifestations of the disease were sought.

Materials and Methods

Subjects and study design

Fifteen patients (eight women and seven men; 25–68 years old; median 37 years) known to one of the investigators (PM) participated in the study. They all presented typical signs and symptoms of AKC including eyelid eczema. All but one had at least a 6-year disease history. Seven patients used topical steroids, ointment or eye drops regularly before the washout period. Topical or systemic anti-inflammatory or anti-infective treatment was not allowed during the two weeks preceding the study. The examinations and samplings were performed on one occasion outside the pollen season. Only the most inflamed eye according to the score system, *vide infra*, was sampled. Thirteen of the 15 patients showed serologic evidence of IgE-mediated allergy screened with Phadiatop[®], Pharmacia CAP System Specific IgE FEIA

Correspondence: Ingeborg van der Ploeg, Division of Ophthalmology, St Erik's Hospital, Polhemsgatan 50, SE-112 82 Stockholm, Sweden.

E-mail: ingplo@ste.ki.se

(Pharmacia Diagnostics, Uppsala, Sweden) that detects reactivity against common aeroallergens.

Twelve healthy subjects (five men and seven women; 29–58 years old; median 43 years) participated in the study to provide control data on tear cytokines and tear cytology, and periorcular and ocular microcolonization, and accordingly underwent the below-described samplings in an arbitrarily chosen eye. Exclusion criteria for controls were contact lens wear, a positive history for allergic manifestation, serologic evidence of IgE-mediated allergy screened with Phadiatop[®], a recent history of any ocular inflammation or current antibiotic or anti-inflammatory treatment.

Clinical signs

Both eyes of the patients were examined by the two ophthalmologists (PM and EN). With a 3-grade score system (0 corresponding to no signs at all; 1 to mild and 2 to severe signs) the degree of lid eczema, blepharitis, conjunctivitis and keratitis was determined. Lid eczema, 0: no eczema; 1: mild eczema, mild oedema and erythema; 2: eczema with indurations and oozing. Blepharitis, 0: no blepharitis; 1: mild plugging of Meibomian glands, moderate oedema, thickening and keratinization; 2: excessive thickening, redness and keratinization. Conjunctivitis, 0: no changes; 1: conjunctival injection and sub tarsal fibrosis; 2: conjunctival and limbal injection and conjunctival scarring. Keratitis, 0: no corneal affection; 1: superficial punctuate keratitis; 2: corneal ulcers and/or opacities and corneal neovascularization.

Microorganisms

Presence of bacteria, *Malassezia* and *Candida* species was investigated in culture material sampled from cilia, lid margin and inferior palpebral conjunctiva. Three cilia were taken from the inferior lid margin using sterile pincers. One was placed on a hematin agar plate for isolation of aerobic bacteria, one on a Leeming & Notman agar (LNA) plate [8] for isolation of *Malassezia* species, and one on a CHROMagar *Candida* plate (CHROMagar Co., Paris, France) for isolation of *Candida* species. Samples for aerobic bacteria were taken from the inferior and superior eyelid margins with a sterile cotton tipped swab moistened with 0.9% saline and from the lower and superior conjunctival fornices with an unmoistened swab (Copan, Brescia, Italy) which were placed in transport tubes (Copan). Samples for *Candida* species from lid margins and conjunctival fornices were taken with sterile unmoistened wooden cotton tipped applicators and were carefully spread on a CHROMagar plate. For the lid margin sampling of *Malassezia*, the cotton tips were dipped in autoclaved 80% glycerol that had been found to enhance culture sensitivity in a preliminary experiment (data not shown). For isolation of *Malassezia*, the applicators were then spread on an LNA plate. All transport tubes and plates were transported at room temperature to the Department of microbiology within 3 h after sampling.

Swabs with bacterial samples and cilia were inoculated on hematin agar plates aerobically in 5% CO₂ and were incubated at 36 °C for 48 h. A qualitative assessment of the cultures was done in a blinded fashion and species identification was done according to standard criteria [9].

The LNA plates and CHROMagar plates were incubated at 30 °C for 7 days. *Malassezia* species were identified by microscopic appearance of the yeast cells and by the ability to use Tween 20, 40, 60 and 80 [10]. *M. furfur*, *M. slooffiae* and *M. sympodialis* strains (a kind gift from Dr Gillian Midgley, St John's Institute of Dermatology, London, UK) were used as controls.

Tear cytology and cytokines

A tear volume of at least 32 µL was collected from the external canthus with a glass blood capillary tube (50 µL) and then transferred to Eppendorff tubes. Two microlitres were directly fixed with 2 µL of 4% formaldehyde and stored overnight at room temperature for cytology analysis. This sample was placed on a glass microscope slide and dried prior staining with May Grünwald (BDH, VWR, Stockholm, Sweden)/Giemsa (BDH). Eosinophil, neutrophil, macrophage and lymphocyte numbers were determined by one masked observer (IP). The remaining fluid was frozen on dry ice and stored for less than 4 months at –80 °C for cytokine analysis. The concentrations of IFN-γ, TNF-α (tumour necrosis factor-α), IL-2, IL-4, IL-5 and IL-10 were simultaneously investigated with the Becton Dickinson Cytometric Bead Array (Becton Dickinson, Stockholm, Sweden) using three-colour flow cytometric analysis in a FACScan flow cytometer Becton Dickinson Immunocytometry Systems (BDIS). The measurements were performed essentially as previously described [11]. Briefly, 25 µL tear fluid was thawed and added to a 50 µL mixture containing each capture antibody-bead reagent and 50 µL detector antibody-phycoerythrin (Ab-PE) reagent. The mixture was subsequently incubated for 3 h at room temperature, and washed to remove unbound detector Ab-PE reagent before flow cytometry. Data were acquired and analysed using Becton Dickinson (BD) Cytometric Bead Array (CBA) software that calculates the cytokine concentration based on the standard curves and a four-parameter logistic curve-fitting model. The lowest cytokine concentration measured is 2–10 pg/mL.

Conjunctival histology

A biopsy specimen was excised from the patients with scissors under local anaesthesia from the lower, mid-tarsal conjunctiva area. The tissue, having a minimum surface of 2 × 3 mm and a depth of 2 mm, was split and placed in 4% formaldehyde or snap frozen and stored at –80 °C pending further processing.

The paraffin embedded tissue was cut at 4 µm, deparaffinized, rehydrated, and stained with the avidin–biotin complex (ABC) technique. The primary antibodies were EG2 (1 : 20; specific for eosinophil cationic protein and secreted forms of eosinophil protein X; Pharmacia Diagnostics), elastase (1 : 50; specific for neutrophils, Dako, Copenhagen, Denmark) and AA1 (1 : 50; which stains mast cell tryptase, Dako). With the AA1 antibody, pre-treatment with pronase 0.05% was performed. Seven-µm-thick acetone fixed cryostat sections were stained with the primary antibodies anti-Leu-4 (1 : 32, reacting with CD3, 'pan T cells', Becton-Dickinson, San José, CA, USA) and anti-IgE (clone B3102E8, 1 : 1000 ImmunKemi, Järfälla, Sweden/Southern Biotechnology Associates,

Birmingham, AL, USA). The ABC method was used and counterstaining was performed with Mayer's hematoxylin. Cell numbers were counted/mm length of the conjunctival surface by one masked observer (EN) [12].

IgE against *S. aureus* B antigen (SEB) and *M. sympodialis*

Specific serum IgE against SEB and *M. sympodialis* was assessed with the Pharmacia CAP System (Pharmacia Diagnostics AB, Uppsala, Sweden) according to the instructions of the manufacturer.

Ethics

The sampling procedure followed the tenet of the Declaration of Helsinki. Informed consent was obtained from the concerned subjects. The local ethics committee approved the project.

Statistical analysis

The Spearman rank correlation coefficient was used to explore associations between colonization and inflammation parameters in AKC patients. The Mann-Whitney *U*-test was used to analyse variable differences between AKC subjects and controls. Data were computed with STATISTICA 5.1 (1997) (Statsoft®, Tulsa, OK, USA). A probability level of <0.05 was considered to be statistically significant. No corrections for multiple comparisons were made.

Results

Clinical signs

Overall, the disease activity in the study group was moderate, since patients with severe AKC were unable to halt topical anti-inflammatory treatment and accordingly could not meet the inclusion criteria. All patients included had some degree of eczema, blepharitis and conjunctivitis, while only seven showed corneal signs (five patients had superficial punctuate keratitis and two patients had corneal opacities and corneal neovascularization).

Occurrence of ocular and peri-ocular microorganisms

S. aureus was isolated from 12 of 15 patients, but not from the controls, while coagulase negative *Staphylococci* were isolated from 7 of 15 AKC patients and from 10 of 12 controls (Table 1). Other bacteria and *Malassezia* species were rarely found (Table 1). No association was found between the use of

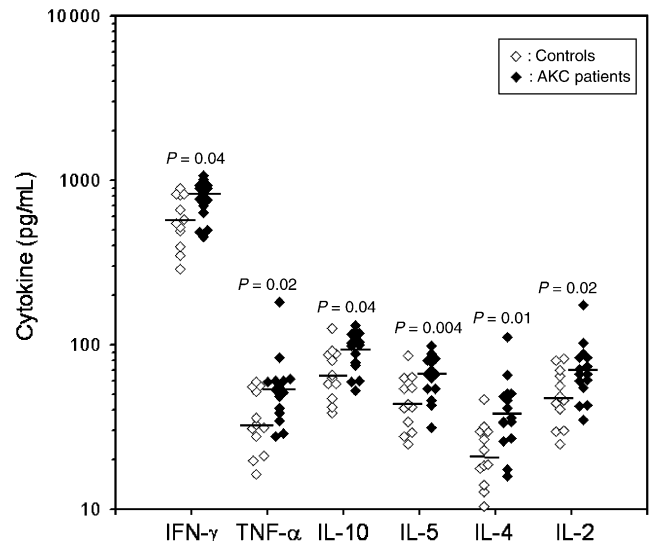


Fig. 1. Cytokine levels in tears of AKC patients and control subjects. The concentrations of IFN- γ , TNF- α , IL-2, IL-4, IL-5 and IL-10 in tears (25 μ L) were simultaneously investigated in a multiplex assay with the Becton Dickinson Cytometric Bead Array using a three-colour flow cytometric analysis in a FACScan flow cytometer. Indicated are medians.

anti-inflammatory treatment regularly before the washout period and the presence of microorganisms.

Objective parameters of inflammation

All six cytokines examined were detectable in tears from every participating subject. All cytokine levels were statistically significantly higher in AKC patients than in controls (Fig. 1). The highest cytokine levels were observed for IFN- γ (Fig. 1). The amount of inflammatory cells found in tears and conjunctival biopsies of the AKC patients is given in Table 2. No eosinophils were detected in tears of the controls, whereas five of the controls showed neutrophils and macrophages, although generally low in numbers (data not shown). Two of fifteen AKC patients showed IgE-antibodies against SEB, whereas 12 of 15 patients exhibited IgE-antibodies against *M. sympodialis* (median 2.9 kU/L, range 0.41–10 kU/L). The control sera were all negative in these analyses.

Colonization and induced IgE responses in relation to parameters of inflammation

No significant association between the presence of microorganisms and clinical signs or inflammatory cells in conjunctival biopsies or cells and cytokines in tears was observed in the patients. Some associations explored are given in Table 3. There was no significant relationship between

Table 1. Number of individuals positive for microorganisms in or near the eye

	<i>S. aureus</i>			Coagulase negative <i>Staphylococci</i>			Other bacteria			<i>Candida</i>			<i>Malassezia</i>		
	Conjunctiva	Margo	Cilia	Conjunctiva	Margo	Cilia	Conjunctiva	Margo	Cilia	Conjunctiva	Margo	Cilia	Conjunctiva	Margo	Cilia
Patients (<i>n</i> = 15)	9	11	9	4	3	5	1*	0	0	0	0	0	0	3†	0
Controls (<i>n</i> = 12)	0	0	0	2	10	7	0	0	0	0	0	0	0	2†	0

**H. influenza*. †*M. sympodialis*.

Table 2. Enumeration of positive cells in tears and biopsy specimens of patients with AKC

Cells*/marker†	Tear/biopsy	Median	Range
Neutrophils	Tear	141	2–2599
Eosinophils	Tear	6	0–330
Macrophages	Tear	5	0–79
Elastase ⁺	Biopsy	5	1–28
EG2 ⁺	Biopsy	2	0–17
AA1 ⁺	Biopsy	26	19–55
CD3 ⁺	Biopsy	23	3–133
IgE ⁺	Biopsy	34	3–96

*The total amount of each cell type in 2 µL tear fluid was calculated.†Cell numbers were counted/mm length of the conjunctival epithelium. AKC, atopic keratoconjunctivitis.

specific IgE production for *S. aureus* or *M. sympodialis* and inflammatory parameters. It is of note, though, that the two patients showing IgE-antibodies against SEB had the worst clinical signs, including keratitis.

Association between clinical signs and objective parameters of inflammation

As a secondary objective of the study, various biochemical correlates of the clinical manifestations of the disease were sought. The severity of the conjunctival signs was significantly associated with TNF- α , IFN- γ , IL-2, IL-4 and IL-10 levels (Table 4) as well as with tear neutrophils ($R_s = 0.60$; $P < 0.05$) and eosinophils ($R_s = 0.54$; $P < 0.05$). We found no further correlation between clinical signs of eyelid eczema, blepharitis or keratitis and objective parameters of inflammation.

Discussion

An impact of microorganisms on the disease process of AEDS has been suggested in studies demonstrating an association between microcolonization or immune responses to the microorganisms on the one hand and disease severity on the other [13–15]. The pathogenic role of colonizers in AEDS seems further confirmed in studies showing eczema improvement after medication with antibiotics [6] or anti-fungals [7], but the literature in this field is contradictory [16, 17]. As for AKC, there is, to the best of our knowledge, no paper that has proved that colonizers influence the sympto-

Table 3. Relationship between colonization with *S. aureus* and clinical signs ($n = 15$) and inflammatory parameters ($n = 14$)

Presence of <i>S. aureus</i> on	Related to	R_s	P
Cilia	Degree of eczema	0.49	0.06
Both margo and cilia	Degree of eczema	0.42	0.12
The conjunctiva	Degree of conjunctivitis	0.11	0.69
Margo, cilia and conjunctiva	Degree of conjunctivitis	0.06	0.80
Margo, cilia and conjunctiva	Degree of keratitis	0.19	0.50
Margo, cilia and conjunctiva	Total clinical score	0.27	0.33
Both margo and cilia	Neutrophils in tears	0.003	0.99
Both margo and cilia	Eosinophils in tears	0.12	0.69
The conjunctiva	Neutrophils in tears	-0.018	0.95
The conjunctiva	Eosinophils in tears	0.06	0.85

Table 4. Relationship between the score of conjunctival signs and cytokine levels in tears from AKC patients ($n = 15$)

Cytokine	R_s	P
TNF- α	0.85	<0.0001
IFN- γ	0.85	<0.0001
IL-2	0.57	<0.05
IL-4	0.71	<0.01
IL-5	0.50	= 0.055
IL-10	0.79	<0.001

AKC, atopic keratoconjunctivitis; TNF- α , tumour necrosis factor- α .

matology, although a strikingly high percentage of AKC patients with ulcerative blepharitis were reported to be colonized or to be infected with *Candida* species [18].

The novel feature of this study was the objective to relate the microcolonizers and the immune response elicited by relevant microorganisms to both subjective and objective inflammatory parameters of the patients. We found a markedly high occurrence of *S. aureus* on the lids and in the conjunctiva of the AKC patients. This has been described earlier in AKC in the same proportion of patients as we observed currently [18–20]. This indeed shows that the colonization pattern of *S. aureus* is highly abnormal and is most likely due to deficiencies of naturally occurring antibacterial agents in lesional skin described in AEDS patients [21, 22]. Interestingly, there was a near significant relationship between the degree of eczema and presence of *S. aureus* found on cilia and lid margins in our study, perhaps indicating a relevant interplay between inflammation and bacteria at this site. In contrast, we believe that the conjunctival *S. aureus* colonies are not essential to the ocular disease process in AKC and merely represent a spread from the adjacent skin. Nonetheless, longitudinal studies rather than the present cross-sectional design and data from a cohort of patients with more severe symptoms are needed before *S. aureus* colonization can be ruled out as an important factor behind ocular surface inflammation in AKC. Furthermore, toxins secreted by *S. aureus* may intensify inflammation in AKC as has been evinced in AEDS [23], but this so-called superantigen issue was not addressed in our investigation. As for fungal involvement, we could not confirm the importance of *Candida* in moderate AKC. In fact, no single colony-forming unit was grown. Perhaps the finding presented by Huber-Spitzy et al. [18] of *Candida* on the lid margins as a common feature in AKC patients with ulcerative blepharitis owes to differences in patient populations between our studies, since this kind of lid margin affection was not observed in our subjects. *Malassezia* species, finally, now recognized as an important treatment target in AEDS patients [7] were not recovered at all in the conjunctiva and rarely on adjacent skin, probably because eczematous lesions and the ocular surface do not present an ideal lipophilic environment for yeasts. Interestingly, in a recent quantitative culture study the yield of *Malassezia* was reported to be even less in AEDS patients than in healthy controls [24].

Another way that colonizers may contribute to disease activity in AEDS is by evoking immune responses, which in their turn may trigger disease manifestations. This has been shown for IgE production to *S. aureus* enterotoxin B [14, 25, 26], but only two of 15 patients in the current work exhibited

such antibodies. Incidentally, these latter two subjects had the highest clinical scores; so additional samples were collected from four AKC patients with ongoing inflammatory keratitis who were not part of the original study cohort. It is of note that two of these also presented IgE against SEB, implicating that this immunological response merits further investigation in a subgroup of AKC patients with severe manifestations. In contrast, *Malassezia*-specific IgE was common in our study group, but considering our culture results and the lack of correlation of these specific IgE levels to ocular inflammation, it is unlikely that the positive effect of antimycotic treatment in AEDS would be observed in the studied AKC population [7, 27].

A secondary aim of the study was to assess whether any of the objective parameters examined in the AKC patients was related to the severity of the clinical signs and as such would serve as a valid marker of ocular surface inflammation in future investigations. Indeed, most tear cytokines were found to correlate to conjunctival signs. The strongest correlations were found for IFN- γ , the pro-inflammatory cytokine TNF- α and the anti-inflammatory cytokine IL-10. Weaker but still significant associations were detected for IL-4 and IL-2, but not for IL-5. This supports the notion that AEDS and AKC are not typical Th2 mediated diseases [3]. We propose that the currently used cytokine multiplex analysis is a promising non-invasive tool for evaluating ocular surface inflammation in AKC. To some extent, also tear neutrophils and eosinophils appeared to be associated with the degree of conjunctivitis and should be further evaluated while biopsy data do not seem to be important in this respect.

In conclusion, we did not find evidence to suggest that periocular and ocular microcolonization are related to clinical signs. However, we found associations between severity of signs in the conjunctiva and cytokine levels in tear fluid, reflecting a Th1/Th2-influenced disease. Confirmation of the present results in a longitudinal study with multiple examinations and samplings in the same individual is required before the contribution of *S. aureus* to on-going inflammation in AKC can be ruled out.

Acknowledgements

The authors would like to thank Dr Catharina Johansson for valuable scientific advice and Berit Spångberg and Margareta Oscarsson for valuable technical support. This work was supported by grants from Crown Princess Margareta's Foundation for the Visually Impaired (KMA), Stiftelsen Synfrämjandets Forskningsfond, the Swedish Research Council, the Swedish Asthma and Allergy Association, the Swedish Foundation for Health Care Sciences and Allergy Research, the Hesselman Foundation and Karolinska Institutet.

References

- Johansson SG, Hourihane JO, Bousquet J et al. A revised nomenclature for allergy: an EAACI Position Statement from the EAACI Nomenclature Task Force. *Allergy* 2001; 56:813–24.
- Garritty JA, Liesegang TJ. Ocular complications of atopic dermatitis. *Can J Ophthalmol* 1984; 19:21–4.
- Thepen T, Langeveld-Wildschut EG, Bihari IC et al. Biphasic response against aeroallergen in atopic dermatitis showing a switch from an initial TH2 response to a TH1 response *in situ*: an immunocytochemical study. *J Allergy Clin Immunol* 1996; 97:828–37.
- Metz DP, Hingorani M, Calder VL, Buckley RJ, Lightman SL. T-cell cytokines in chronic allergic eye disease. *J Allergy Clin Immunol* 1997; 100:817–24.
- Uchio E, Ono SY, Ikezawa Z, Ohno S. Tear levels of interferon-gamma, interleukin (IL) -2, IL-4 and IL-5 in patients with vernal keratoconjunctivitis, atopic keratoconjunctivitis and allergic conjunctivitis. *Clin Exp Allergy* 2000; 30:103–9.
- Breuer K, Häussler S, Kapp A, Werfel T. *Staphylococcus aureus*: colonizing features and influence of an antibacterial treatment in adults with atopic dermatitis. *Br J Dermatol* 2002; 147:55–61.
- Lintu P, Savolainen J, Kortekangas-Sovolainen O, Kalimo K. Systemic ketokonazole is an effective treatment of atopic dermatitis with IgE-mediated hypersensitivity to yeasts. *Allergy* 2001; 56: 512–7.
- Leeming JP, Notman FH. Improved methods for isolation and enumeration of *Malassezia furfur* from human skin. *J Clin Microbiol* 1987; 10:2017–9.
- Murray PR. *Manual of clinical microbiology*, 7th Edn. Washington DC: American Society for Microbiology, 1999.
- Nakabayashi A, Sei Y, Guillot J. Identification of *Malassezia* species isolated from patients with seborrhoeic dermatitis, atopic dermatitis, pityriasis versicolor and normal subjects. *Med Mycol* 2000; 38:337–41.
- Cook EB, Stahl JL, Lowe L et al. Simultaneous measurement of six cytokines in a single sample of human tears using microparticle-based flow cytometry: allergies vs. non-allergies. *J Immunol Methods* 2001; 254:109–18.
- Montan PG, Scheynius A, van der Ploeg I. Similar T helper Th2-like cytokine mRNA expression in vernal keratoconjunctivitis regardless of atopic constitution. *Allergy* 2002; 57:436–41.
- Higaki S, Morohashi M, Yamagishi T, Hasegawa Y. Comparative study of staphylococci from the skin of atopic dermatitis patients and from healthy subjects. *Int J Dermatol* 1999; 38:265–9.
- Bunikowski R, Mielke M, Skarabis H et al. Prevalence and role of serum IgE antibodies to the *Staphylococcus aureus*-derived superantigens SEA and SEB in children with atopic dermatitis. *J Allergy Clin Immunol* 1999; 103:119–24.
- Nordvall SL, Lindgren L, Johansson SG, Johansson S, Petrini B. IgE antibodies to *Pityrosporum orbiculare* and *Staphylococcus aureus* in patients with very high serum total IgE. *Clin Exp Allergy* 1992; 22:756–61.
- Broberg A, Faergemann J. Topical antimycotic treatment of atopic dermatitis in the head/neck area. A double-blind randomised study. *Acta Derm Venereol* 1995; 75:46–9.
- Ewing CI, Ashcroft C, Gibbs AC, Jones GA, Connor PJ, David TJ. Flucloxacillin in the treatment of atopic dermatitis. *Br J Dermatol* 1998; 138:1022–9.
- Huber-Spitzy V, Böhler-Sommeregger K, Arock-Mettinger E, Grabner G. Ulcerative blepharitis in atopic patients – is *Candida* species the causative agent? *Br J Ophthalmol* 1992; 76:272–4.
- Tuft SJ, Kemeny DM, Dart JK, Buckley RJ. Clinical features of atopic keratoconjunctivitis. *Ophthalmology* 1991; 98:150–8.
- Nakata K, Inoue Y, Harada J et al. A high incidence of *Staphylococcus aureus* colonization in the external eyes of patients with atopic dermatitis. *Ophthalmology* 2000; 107:2167–71.
- Arikawa J, Ishibashi M, Kawashima M, Takagi Y, Ichikawa Y, Imokawa G. Decreased levels of sphingosine, a natural antimicrobial agent, may be associated with vulnerability of the stratum

- corneum from patients with atopic dermatitis to colonization by *Staphylococcus aureus*. *J Invest Dermatol* 2002; 119:433–9.
- 22 Ong PY, Ohtake T, Brandt C et al. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N Engl J Med* 2002; 347:1151–60.
- 23 Campbell DE, Kemp AS. Proliferation and production of interferon-gamma (IFN-gamma) and IL-4 in response to *Staphylococcus aureus* and staphylococcal superantigen in childhood atopic dermatitis. *Clin Exp Immunol* 1997; 107:392–7.
- 24 Gupta AK, Kohli Y, Summerbell RC, Faergemann J. Quantitative culture of *Malassezia* species from different body sites of individuals with or without dermatoses. *Med Mycol* 2001; 39:243–51.
- 25 Nomura I, Tanaka K, Tomita H et al. Evaluation of the staphylococcal exotoxins and their specific IgE in childhood atopic dermatitis. *J Allergy Clin Immunol* 1999; 104:441–6.
- 26 Breuer K, Wittmann M, Börsche B, Kapp A, Werfel T. Severe atopic dermatitis is associated with sensitization to staphylococcal enterotoxin B (SEB). *Allergy* 2000; 55:551–5.
- 27 Bäck O, Bartosik J. Systemic ketokonazole for yeast allergic patients with atopic dermatitis. *J Eur Acad Dermatol Venereol* 2001; 15:34–8.