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# *Staphylococcus aureus* modifies the cytokine-induced immunoglobulin synthesis and CD23 expression in patients with atopic dermatitis

K. NEUBER, U. STEPHAN, J. FRÄNKEN & W. KÖNIG *Institut für Medizinische Mikrobiologie und Immunologie, Arbeitsgruppe für Infektabwehrmechanismen, Ruhr-Universität Bochum, Germany*

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## SUMMARY

The influence of *Staphylococcus aureus* on peripheral blood lymphocytes (PBL) of patients with atopic dermatitis (AD) was analysed. The parameters studied were spontaneous and interleukin-inducible immunoglobulin (IgA, IgE, IgG) synthesis, as well as CD23 expression. Various heat-killed, clinical isolates of *S. aureus* were analysed. PBL from non-atopic donors served as controls. The time–course of co-cultured PBL with *S. aureus* showed a dose-dependent increase in immunoglobulin (Ig) synthesis from PBL of normal donors, whereas the Ig synthesis of atopic cells was significantly depressed. Additional stimulation with interleukin-4 (IL-4) also led to a pronounced suppression of the IgE and IgA synthesis in normal donor cells, while the effect of *S. aureus* on PBL of atopic donors was not markedly affected by IL-4. Transwell cultures of bacteria separated from PBL by a semi-permeable membrane induced stimulation of IgA and IgE synthesis in patients with AD. The Ig synthesis in the control group was not altered. Co-stimulation of *S. aureus* and IL-4 in this system led to a suppression of IgA with cells of both atopic and normal donors. IgE synthesis from atopic PBL was significantly stimulated. The CD23 expression of atopic PBL was increased by *S. aureus* and IL-4. Our data indicate that *S. aureus* may modulate the cytokine-dependent humoral immunity in patients with AD and that chronic colonization of the skin may be responsible for allergic skin reactions in AD.

## INTRODUCTION

Normal as well as diseased skin of patients with atopic dermatitis (AD) is severely colonized with *Staphylococcus*; [1]staphylococci are also prominent in cultures from nasal and pharyngeal sites of these patients.[2] In contrast to normal individuals, *S. aureus* belongs to the resident flora in patients with AD. Microbiological quantifications have demonstrated that coagulase-negative *S. epidermidis* is also increased in density on the uninvolved skin of patients with AD and may actually predominate over *S. aureus* in these areas.[3]

The underlying mechanism for this chronic bacterial colonization of the skin, as well as the role of *S. aureus* for allergic reactions in AD, are still unclear. Differences in sebaceous and sweat secretions have been suggested.[4] Increased adherence of *S. aureus* to epithelial cells obtained from either the skin or nasal mucosa led to the suggestion that either qualitative or quantitative differences in bacterial receptors (e.g. fibronectin) on keratinized cells of AD skin may predispose to the increased carriage of staphylococci.[5] On the other hand, defective host–defence mechanisms involved in the control of bacterial infections have been suggested.[6] Due to a selective hypo-responsiveness to purified *S. aureus*, cell walls in delayed skin reactivity[7] disorders of T cells in patients with AD were stated to be responsible for the bacterial colonization. Furthermore, disorders in the functional activities of granulocytes and monocytes were suspected, but they were shown to be within the normal range in patients with AD and without concomitant infections.[8]

It is controversial whether the chronic colonization of the skin with *S. aureus* in patients with AD is important for the individual course of the disease. In several studies a reduction in the bacterial load by antibiotic therapy was noted to be associated with clinical improvement.[9] *S. aureus* may also induce a specific immune response in AD patients; increased serum IgE was directed against intact cells, cell wall and soluble antigens of *S. aureus*[10–12]; and basophils of patients with AD respond to *S. aureus* with an IgE-dependent mechanism and release high amounts of histamine.[13]

Few data exist as to the influence of *S. aureus* on the humoral immunity in patients with AD. For this purpose we investigated the effects of heat-killed staphylococci on immunoglobulin synthesis and CD23 expression of peripheral blood lymphocytes (PBL) from patients with AD.

Abbreviations: AD, atopic dermatitis; FcεRII, CD23, low affinity receptor for IgE; FCS, foetal calf serum; IL-4, interleukin-4; mAb, monoclonal antibody; PBL, peripheral blood lymphocytes; PBS, phosphate-buffered saline; RIA, radioimmunoassay.

Correspondence: Professor W. König, Institut für Medizinische Mikrobiologie und Immunologie, AG Infektabwehr, Universitätsstr. 150, 4630 Bochum, Germany.

## MATERIALS AND METHODS

*Isolation of lymphocytes and cell separation*

Lymphocytes were obtained from healthy volunteers and from patients with atopic dermatitis (AD). The AD diagnosis was performed according to the criteria of Hanifin & Rajka;[14] the following four basic features were present: a chronic or chronically relapsing dermatitis, flexural lichenification, pruritis and a personal or family history of atopy (asthma, rhinitis, AD). The serum IgE levels were above 500 ng/ml. The donors did not receive any steroid treatment.

Isolation was performed by centrifugation on a Ficoll–sodium metrizoate (Sigma, München, Germany) gradient according to Böyum.[15] Briefly, heparinized venous blood (200 ml) was layered over Ficoll–sodium metrizoate (density = 1075 g/ml) and centrifuged at 375 *g* for 45 min. Cells at the interface above the Ficoll–metrizoate were removed and washed three times with RPMI-1640. These cells are referred to as 'peripheral blood lymphocytes' (PBL).

*Culture conditions*

The basic culture medium was RPMI-1640 supplemented with 2 mM glutamine, 100 μg/ml streptomycin, 100 IU/ml penicillin, 10 mM HEPES and 20 mM sodium hydrogen carbonate. Medium containing 10% foetal calf serum is referred to as RPMI-1640 with 10% FCS.

Cell suspensions containing $1 \times 10^6$/ml viable cells in RPMI-1640 and 10% FCS were dispensed into each well of 6- and 24-well plates. Stimuli (see results) were added and the cultures were incubated at 37° in a humidified atmosphere of 5% $CO_2$ in air.

PBL and bacteria were cultured in the co-culture system or in Transwells (Costar, Cambridge, MA). The Transwells consist of a lower and an upper compartment which are separated by a polycarbonate-treated membrane with pores of 0·4 μm size and 10 μm thickness. In experiments with the Transwell system bacteria were cultured in the upper and PBL in the lower compartment.

The IgA, IgE and IgG content of culture supernatants were determined by radioimmunoassays.

*Lymphokines*

Freshly prepared PBL were suspended in culture medium with purified recombinant human IL-4 (specific activity: $1 \times 10^8$ U/mg; used quantity: 20 U/ml). IL-4 was obtained from Genzyme (purchased from IC Chemikalien, München, Germany).

*Bacteria*

*Staphylococcus aureus* was isolated from the skin of patients with AD and characterized by API STAPH (Api System S.A., Montalieu, Germany). Additional *S. aureus*, *S. epidermidis* and *Escherichia coli* strains were obtained from clinical isolates cultured in the department of Medical Microbiology, Ruhr-Universität Bochum, Germany.

The bacteria were cultured overnight in BHI medium at 37° and subsequently killed at 100° for 30 min. After centrifugation the heat-killed staphylococci and *E. coli* were dissolved in RPMI-1640.

The quantity of protein was determined according to the method of Bradford.[16]

*Radioimmunoassay for IgA, IgE, IgG*

The IgA, IgE and IgG content of culture supernatants were determined by a solid-phase radioimmunoassay on Day 10, with the exception of time–course experiments. Cell viability was assessed microscopically by trypan blue exclusion analysis. Briefly, purified goat anti-human IgA (100 μg), IgE (100 μg) and IgG (100 μg) antibodies (1·5 mg/ml; Medac, Hamburg, Germany) were labelled with 37 MBq $Na^{125}I$ (specific activity 520·2 MBq/μg of iodine; Amersham, Buchler, Germany), as described by Klinman & Taylor.[17]

Removawell U-bottomed wells (Dynatech, Denkendorf, Germany) were coated with anti-IgA (100 μl, diluted 1:1000 in phosphate-buffered saline supplemented with 0·05% Tween 20) or anti-IgE (100 μl, 1:1000 in PBS, 0·05% Tween 20) or anti-IgG (100 μl, 1:1000 in PBS, 0·05% Tween 20) for 4 hr.

One hundred microlitres of supernatant (IgA and IgG-RIA: 1:10–1:20 dilution) were added and incubated overnight at room temperature. [$^{125}$I]anti-IgA (100,000 c.p.m.), [$^{125}$I]anti-IgE (100,000 c.p.m.) or [$^{125}$I] anti-IgG (100,000 c.p.m.), respectively, were added for an additional 4 hr at 37°. In parallel, standard curves for IgA (0·3–300 ng/ml), IgE (0·2–200 ng/ml) and IgG (0·3–300 ng/ml) were performed.

The detection limits for IgE and IgA or IgG were 0·4 ng/ml and 0·8 ng/ml, respectively.

Specificity of the assays was confirmed by adding Ig of other isotypes to rule out the possibility of cross-reactivity.

All determinations were made in triplicate at least.

*Determination of CD23*

The detection of CD23 on PBL was performed as described previously[18] on Day 4 of the cell culture. In order to prevent a cross-reactivity between the mAb 135 and protein A of *S. aureus* via Fc-receptor, the PBL were preincubated with precipitated (20% polyethyleneglycole; PEG) human serum for 2 hr at room temperature. The monoclonal antibody mAb 135 was a kind gift of G. Delespesse (University of Montreal, Canada). Briefly, $1 \times 10^6$ cells were incubated with $^{125}$I-labelled mAb 135 (50 ng/200 μl, 14·8 kBq) for 1 hr at room temperature. Separation of unbound and bound antibodies was carried out by centrifugation through a 500 μl FCS cushion (500 *g*, 10 min, 4°) in minitubes (Greiner, Nürtingen, Germany). After removal of unbound radioactivity and washing with PBS (0·05% Tween 20), the cell-bound radioactivity was measured with a gamma-counter (Packard Cobra, Packard-Canberra, Frankfurt, Germany). All determinations were made in triplicate at least. The results are indicated as percentage binding of the total of applied $^{125}$I-labelled mAb 135/$1 \times 10^6$ cells.

*Analysis of data*

All experiments were performed three times with different donors. The data were calculated as means ± SD. The significance was evaluated with Student's *t*-test for independent means. $P < 0{\cdot}05$ was considered significant.

## RESULTS

Experiments were carried out to analyse the effects of staphylococci and IL-4 on Ig synthesis and on CD23 expression of PBL from patients with AD. It was our intention to study whether the chronic colonization of the skin in patients with AD is dependent on defined dysfunctions of humoral immunity during

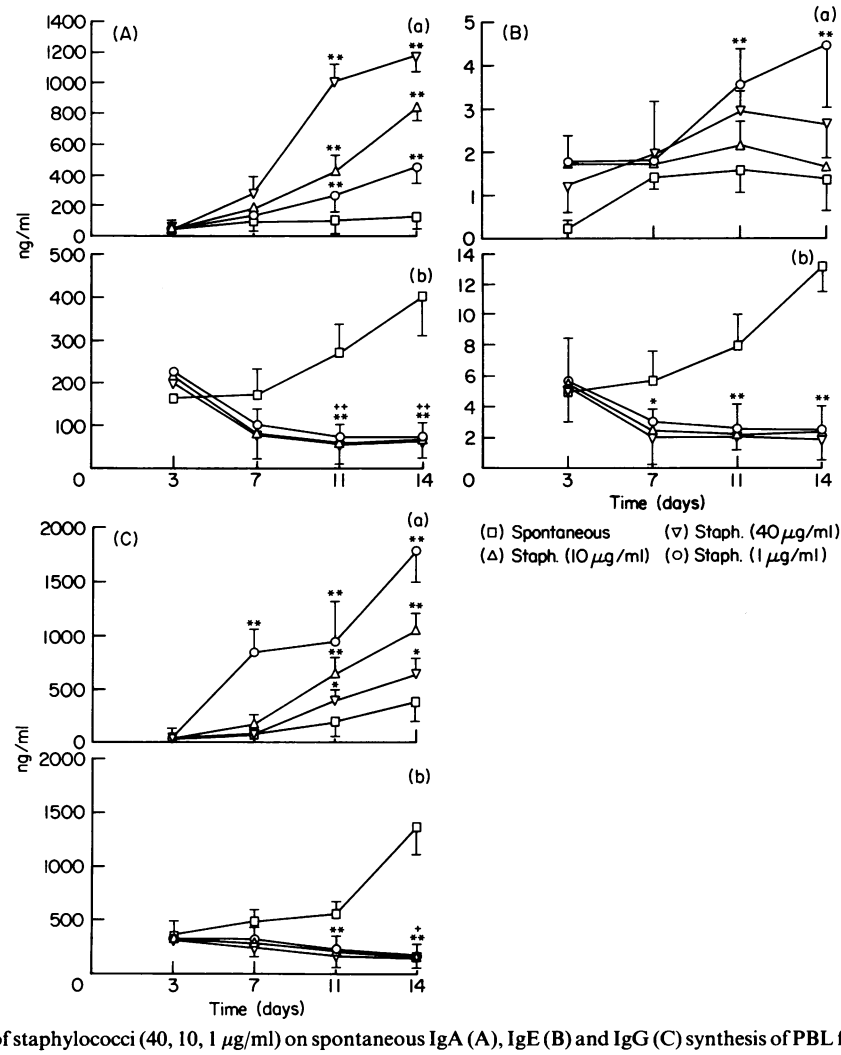


**Figure 1.** Influence of staphylococci (40, 10, 1 μg/ml) on spontaneous IgA (A), IgE (B) and IgG (C) synthesis of PBL from normal donors (a) and patients with AD (b) between Day 3 and Day 14. Means ± SD of three independent experiments in each group with five different strains of *S. aureus* and one strain of *S. epidermidis* are shown. $*P<0.05$ and $**P<0.01$ are significant compared to spontaneous Ig synthesis. †, †† are significant compared to the spontaneous IgA and IgG synthesis of normal PBL.

the disease process. For this purpose PBL from patients with AD were incubated with several strains of coagulase-positive and -negative staphylococci. To investigate additional effects of cytokines, PBL were stimulated with IL-4.

### Staphylococci-induced Ig synthesis

*Time-course of staphylococci-induced Ig synthesis.* In the first series of experiments we investigated the influence of *S. aureus* (five different strains) and *S. epidermidis* (one strain) on the IgA, IgE and IgG synthesis of PBL over 14 days *in vitro*. Figure 1 shows that the spontaneous Ig synthesis of PBL from normal donors was stimulated in a dose-dependent manner by staphylococci. However, the synthesis of all Ig classes by PBL from patients with AD was significantly suppressed from Day 11 on.

The IgA synthesis of normal PBL (Fig. 1Aa) was stimulated significantly ($P<0.01$) by each concentration of *S. aureus* and *S. epidermidis* compared to the spontaneous secretion. The strongest effect was achieved with 40 μg heat-killed staphylococci, which enhanced the concentration of IgA in the supernatant up to 1176·6 ± 54·7 ng/ml. However, the IgA synthesis of PBL from patients with AD was significantly ($P<0.01$) depressed from Day 11 on, whereas the spontaneous IgA synthesis continuously increased up to 399·7 ± 49·2 ng/ml. The staphylococci suppressed the IgA synthesis by 640% on Day 14. Interestingly, this decrease was less dependent on the concentration of staphylococci than the bacteria-induced enhancement of Ig synthesis in normal PBL. The depressed IgA synthesis of atopic PBL was significantly lower than the spontaneous IgA production of non-atopic cells (normal 129·1 ± 15·6 ng/ml; atopic 61 ± 9·1 ng/ml; $P<0.01$).

Figure 1 (Ba, b) shows the effect of staphylococci on the IgE synthesis of normal and atopic PBL. The IgE synthesis of normal PBL was significantly increased ($P<0.01$) by 1 μg staphylococci on Days 11 and 14. On Day 14 it reached a maximum of 4·4 ± 1·3 ng/ml compared to a spontaneous secretion of about 1 ng/ml in the supernatant. In general, the IgE synthesis of normal PBL was less affected by heat-killed staphylococci than the IgA and IgG production. On the other hand, the IgE synthesis of PBL from patients with AD was

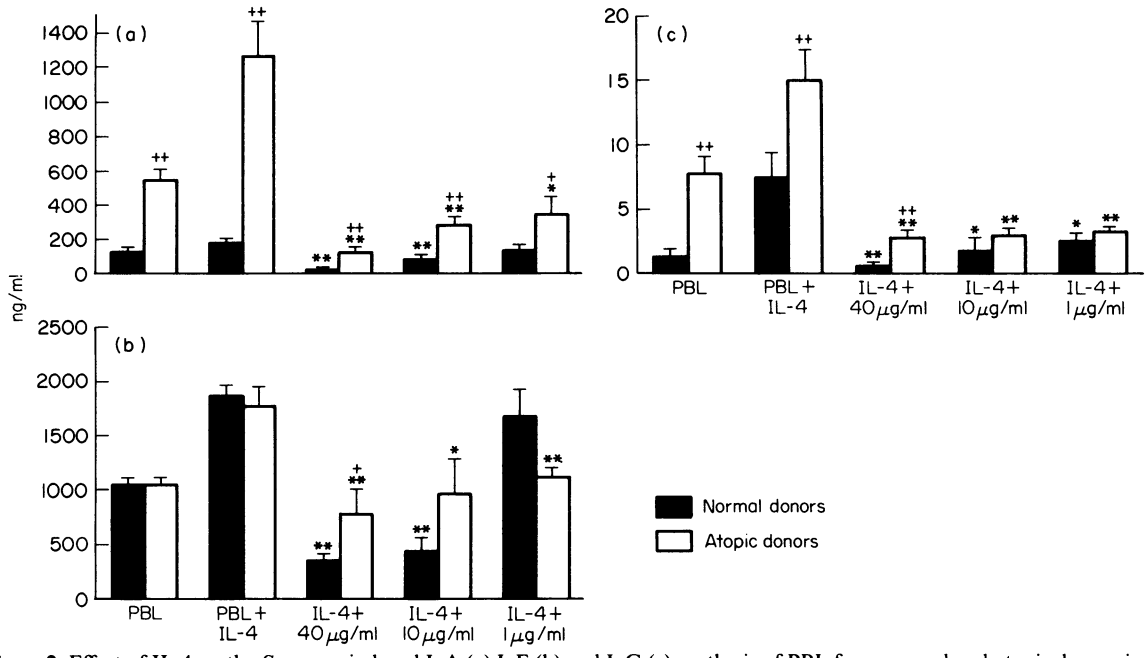


**Figure 2.** Effect of IL-4 on the *S. aureus*-induced IgA (a) IgE (b) and IgG (c) synthesis of PBL from normal and atopic donors in the co-culture system. The data representing the influence of *S. aureus* (40, 10, 1 μg/ml) and IL-4 (20 U/ml) are mean values of three independent experiments in each group ($n=3\pm$SD). $*P<0.05$ and $**P<0.01$ significant modification compared to the control (unstimulated PBL). †, †† are significant compared to the Ig synthesis of PBL from normal donors.

suppressed similar to the IgA and IgG synthesis (Fig. 1Cb). By Day 7 it was suppressed by about 227·2% ($P<0.05$); on Day 14 the highest concentration (40 μg/ml) of staphylococci led to a reduction of 620% ($P<0.01$) compared to an increased spontaneous IgE secretion up to $13.15\pm1.54$ ng/ml.

With regard to the IgG synthesis the observed effects were similar (Fig. 1Ca, b). The cells of normal donors were stimulated in a dose-dependent manner by staphylococci; it can be seen that the highest concentration of bacteria induced a statistically significant ($P<0.05$) enhancement. One microgram of staphylococci stimulated the IgG synthesis by 500% on Day 14 ($P<0.01$). In comparison to the increased spontaneous secretion ($1353.3\pm103.9$ ng/ml on Day 14), the IgG synthesis of atopic PBL was reduced by 1050% ($P<0.01$). The strongest suppression was observed with 40 μg staphylococci ($128.7\pm13.7$ ng/ml). When the spontaneous secretion of IgG by normal PBL was compared with the IgG synthesis of atopic cells, a significant suppression was observed on Day 14.

In none of these experiments were significant differences between *S. aureus* and *S. epidermidis* of their effects on PBL either from normal donors or from patients with AD noticed. Also, the strains of *S. aureus* isolated from the skin of patients with AD did not differ from one strain to another when the Ig synthesis was studied even in the same patient.

In contrast to experiments with *S. aureus*, the *E. coli* strains induced a similar suppression of IgA synthesis in normal as well as in atopic donors. The addition of heat-killed *E. coli* had no significant effects on the IgE synthesis in both donor groups when the cells were studied in the co-culture. With regard to IgG synthesis, control experiments with *E. coli* led to a slight enhancement in normal donors as well as in patients with AD (*E. coli* data are not shown).

PBL from normal and atopic volunteers displayed no remarkable differences as to the viability of the cells.

*Influence of IL-4 on* S. aureus *modulated Ig synthesis.* Further experiments were carried out to investigate the influence of IL-4 on the *S. aureus*-induced effects on Ig synthesis. Figure 2a shows that IL-4 induced a significant increase in IgA synthesis by PBL from patients with AD ($P<0.01$), whereas the IgA synthesis of normal donors was not affected by IL-4. Co-incubation of PBL with *S. aureus* and IL-4 led to a significant suppression of the IgA synthesis by PBL from normal (−600%, 40 μg/ml) as well as from atopic donors (−450%, 40 μg/ml) compared to the spontaneous secretion ($P<0.01$). The effect became less apparent with a decreasing concentration of *S. aureus*.

Interestingly, in all patients with AD the spontaneous IgA synthesis *in vitro* as well as in the serum was about fourfold higher than in the group of non-atopic donors. The suppressive effects of *S. aureus* led to amounts of IgA within the supernatants of atopic PBL that were in the range of the spontaneous secretion from normal cells.

With regard to the IgE synthesis (Fig. 2b) we observed a significant suppression by *S. aureus* and IL-4 compared to the IL-4-induced IgE synthesis of PBL in normal donors (−1190%−−295%; $P<0.05$) and compared to the spontaneous secretion from atopic cells (−250%−−275%; $P<0.01$). Increasing concentrations of *S. aureus* induced a more pronounced suppression.

Figure 2c shows the pattern of IgG synthesis under the influence of *S. aureus* and IL-4. At concentrations of 40 and 10 μg, *S. aureus* in the presence of IL-4 suppressed the IgG synthesis of normal PBL up to $346.5\pm69.8$ ng/ml ($P<0.01$) and $425.5\pm111.3$ ng/ml ($P<0.01$), respectively, compared to the spontaneous IgG secretion ($1043\pm33.5$ ng/ml). *S. aureus* (1 μg/ml) led to an enhanced IgG synthesis even in the presence of IL-4. The IgG synthesis of PBL from patients with AD was not significantly decreased after co-stimulation with *S. aureus* and IL-4 compared to the spontaneous secretion, but was signifi-

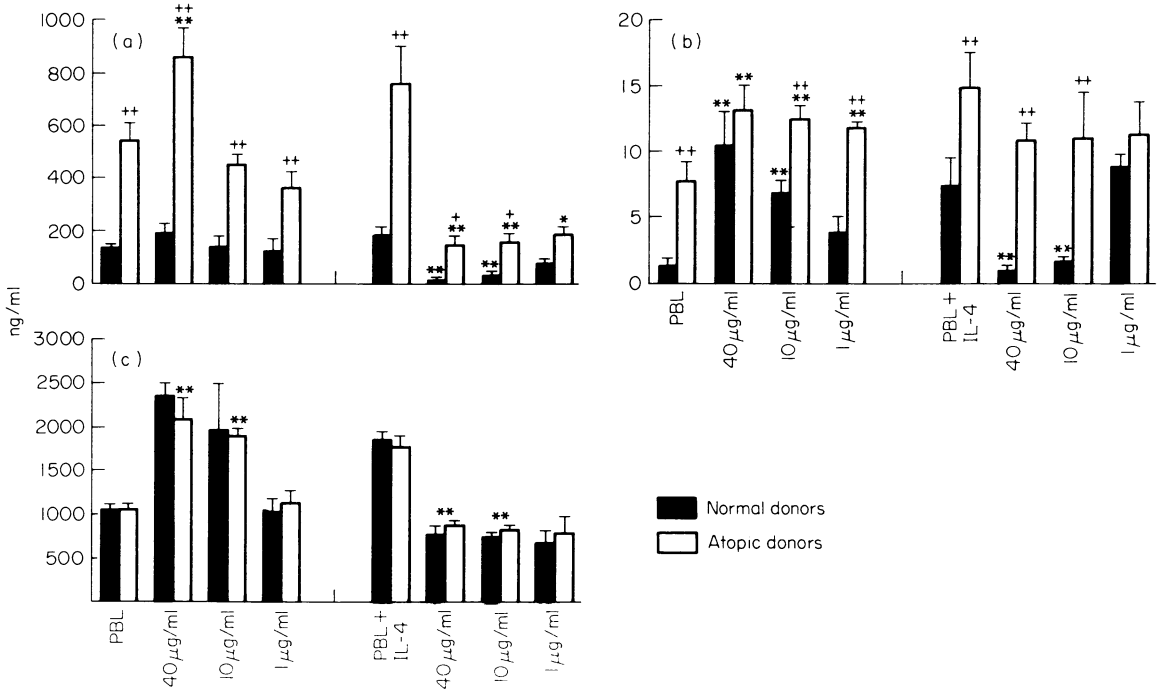


**Figure 3.** Influence of *S. aureus* (40, 10, 1 μg/ml) and IL-4 (20 U/ml) on IgA (a), IgE (b) and IgG (c) synthesis of PBL from normal donors and patients with AD in the Transwell system. Means ± SD of three independent experiments in each group of donors. $*P < 0.05$ and $**P < 0.01$ significant compared to the PBL control. †, †† are significant compared to the Ig synthesis of PBL from normal donors.

cantly ($P < 0.01$) suppressed compared to the IL-4-induced IgG synthesis (PBL control).

In contrast, the addition of IL-4 to the *E. coli* strains did not affect the synthesis of Ig (A, E, G) in both donor groups compared to the results in the absence of IL-4 (*E. coli* data are not shown).

*Experiments in the Transwell system.* In order to investigate whether these effects are dependent on a direct interaction between bacteria and PBL, experiments in the Transwell system were carried out.

Figure 3a shows that in contrast to previous experiments the IgA synthesis of PBL from patients with AD was significantly enhanced by 40 μg *S. aureus* (+158%; $P < 0.05$) and slightly depressed by lower amounts of bacteria. The stimulatory effect on the IgA synthesis of PBL from normal donors was less pronounced than in the co-culture system and not significant. With all concentrations of *S. aureus* the IgA synthesis of PBL from patients with AD was significantly ($P < 0.01$) enhanced compared to the secretion of PBL from normal donors [+450% (40 μg), +320% (10 μg), +296% (1 μg)]. Again, additional IL-4 led to a pronounced suppression of IgA synthesis from PBL in both groups. PBL from non-atopic donors stimulated with *S. aureus* secreted between 191·1 ± 38·4 ng/ml (40 μg) and 123·2 ± 44·8 ng/ml (1 μg) IgA compared to 13·3 ± 19·8 ng/ml (40 μg) and 71·6 ± 28·4 ng/ml (1 μg) IgA after co-stimulation with IL-4. The IgA synthesis of PBL from patients with AD was significantly depressed by 196% (1 μg *S. aureus*; $P < 0.05$) up to 592% (40 μg *S. aureus*; $P < 0.01$). The IL-4-induced IgA suppression was more pronounced in PBL from normal donors ($P < 0.01$).

Control experiments with *E. coli* showed different results: the IgA synthesis of PBL from normal as well as from atopic donors was also suppressed in the Transwell system independently of whether IL-4 was added or not (data not shown). In contrast to the experiments which analysed the direct interaction between bacteria and PBL in the Transwell system, the IgE synthesis (Fig. 3b) was significantly enhanced by *S. aureus* compared to the spontaneous IgE production of PBL from patients with AD (+153%–+170%; $P < 0.01$) and compared to the IgE synthesis of non-atopic PBL (10 μg and 1 μg *S. aureus*; $P < 0.01$).

Stimulation of cells from normal donors in this experimental system led to enhanced amounts of IgE in the culture supernatants (3·76 ± 1·2 ng/ml up to 10·4 ± 2·4 ng/ml), depending on the concentration of *S. aureus*. The addition of IL-4 led to a significant suppression of the IgE synthesis only in PBL from normal donors ($P < 0.01$) which were stimulated by 40 and 10 μg of *S. aureus*. The depression of IgE synthesis in PBL of patients with AD was not significant after co-stimulation with 20 U/ml IL-4. PBL from patients with AD showed enhanced amounts of IgE after stimulation with *S. aureus* and IL-4 in the Transwell system.

*E. coli* slightly enhanced the IgE synthesis of atopic PBL in the Transwell system compared to the control. These results were not significantly affected by a co-stimulation with IL-4. In normal donors no remarkable effects of *E. coli* on the IgE synthesis were observed (data not shown).

Figure 3c shows that stimulation of PBL with *S. aureus* alone as well as with *S. aureus* and IL-4 did not lead to significant differences in IgG synthesis when normal and atopic cells in the

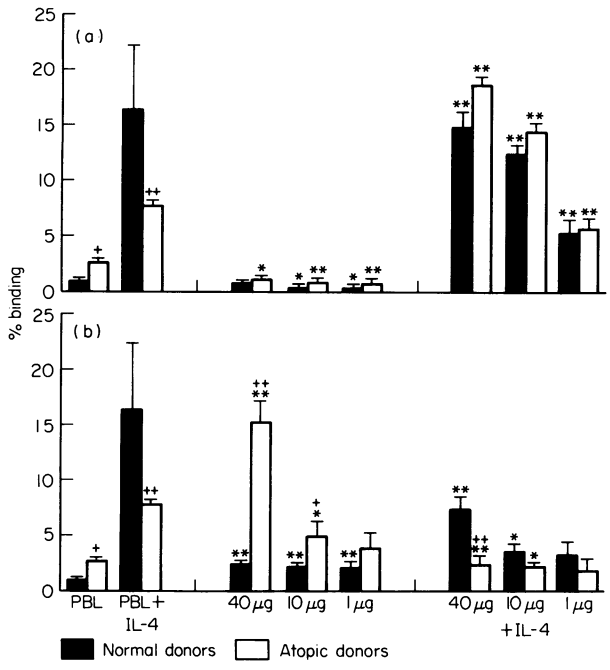


**Figure 4.** Effect of *S. aureus* (40, 10, 1 μg/ml) and IL-4 (20 U/ml) on CD23 expression in the co-culture system (a) and in the Transwell system (b). Means ± SD of three independent experiments in each group of donors. $*P<0.05$ and $**P<0.01$ significant compared to the PBL control. †, †† are significant compared to the CD23 expression of PBL from normal donors.

Transwell system were studied. Co-stimulation with bacteria and IL-4 induced only a slight suppression of IgG synthesis compared to the spontaneous secretion in both groups. Similar results were obtained when normal and atopic PBL were stimulated with *E. coli* (data not shown).

### Staphylococci-induced CD23 expression

In addition to Ig synthesis, the modulation of CD23 expression on PBL from normal and atopic donors by *S. aureus* and IL-4 in co-cultures, as well as in the Transwell system, was studied.

Figure 4a shows the effects of *S. aureus* and IL-4 in co-cultures. CD23 expression of normal and atopic PBL was significantly suppressed (−248%−−322%) by *S. aureus* compared to spontaneous expression. Addition of IL-4 led to a marked increase in CD23 expression up to 14·8 ± 7·4% binding (normal PBL; 40 μg *S. aureus*) and 18·72 ± 3·74% binding (atopic PBL; 40 μg *S. aureus*) compared to spontaneous expression. Although CD23 expression was more enhanced on cells from patients with AD, the differences were not significant.

In the Transwell system (Fig. 4b) *S. aureus* alone significantly enhanced the CD23 expression on PBL from patients with AD up to 15·2 ± 1·84% binding compared to the spontaneous one and compared to the CD23 expression on PBL from normal donors. At concentrations of 10 μg and 1 μg the *S. aureus*-induced CD23 expression was less pronounced. CD23 expression on normal PBL was slightly but significantly increased by *S. aureus*: the spontaneous expression amounted from 0·87 ± 0·19% binding up to 2·35 ± 0·2% binding with 40 μg bacteria ($P<0.01$).

Addition of IL-4 led to an enhancement of CD23 expression on normal PBL of between 368% and 833% compared to the spontaneous expression, whereas the expression of CD23 on PBL from patients with AD was slightly depressed (1·79 ± 0·83% binding). The suppression of CD23 expression by *S. aureus* and IL-4 was marked in both groups compared to that which was induced by IL-4.

The CD23 expression was suppressed by *E. coli* in co-culture experiments when normal as well as atopic donor cells were studied. The addition of IL-4 led to a less pronounced stimulation compared to the experiments with *S. aureus*. In the Transwell system a marked increase of CD23 expression in both groups was observed compared to the controls which were enhanced by IL-4 to amounts which were similar to those obtained after stimulation with *S. aureus* (data not shown).

## DISCUSSION

The high incidence of chronic skin colonization with coagulase-positive staphylococci is a prominent feature in AD. Although many attempts have been made to quantify and characterize the micro-organisms on the skin of patients with AD as well as to detect specific IgE against *S. aureus* in these patients, only few data are available as to the host–defence mechanisms in this disease.

Some investigators stated that dysfunctions in cellular immunity are responsible for the chronic colonization of the skin in patients with AD; a selective hypo-responsiveness to purified *S. aureus* cell walls in delayed skin reactivity was observed.[7,19]

The role of humoral immunity in this regard is still unclear. For this reason we investigated the Ig synthesis and CD23 expression of PBL from patients with AD after stimulation with several strains of *S. aureus*, as well as *S. epidermidis*, and additionally with IL-4 *in vitro*.

The direct interaction between staphylococci and PBL from patients with AD in co-cultures led to a significant suppression of the IgA, IgE and IgG synthesis compared to spontaneous Ig synthesis. The secretion of immunoglobulins after stimulation with staphylococci from normal PBL was, as expected, significantly enhanced. Not only the spontaneous IgE but also IgA and IgG synthesis of PBL from patients with AD were strongly enhanced from Day 7 onwards compared to those of normal cells. This increased spontaneous secretion of immunoglobulins is probably due to the enhanced reactivity of the immune system in patients with AD. The suppression of the IgA and IgG but not IgE synthesis of PBL from patients with AD via staphylococci was significant compared to the spontaneous Ig synthesis of normal lymphocytes.

The fact that we did not observe marked differences among several strains of *S. aureus* and *S. epidermidis* may be due to the fact that heat-inactivated bacterial strains were studied. Heat-labile bacterial enzymes, e.g. coagulase, are inactivated, which may contribute to the suppression of Ig-synthesis in patients with AD. This, however, seems to be improbable because *S. epidermidis* is also increased in lesional sites as well as on the unaffected skin of patients with AD. Furthermore, no significant differences between strains of *S. aureus* isolated from the skin of atopic individuals and those from non-atopic patients were observed. These results indicate that the effects of *S. aureus* on the Ig synthesis of PBL from patients with AD may be

induced by common cell wall components or additional products of staphylococci.

Obviously, immunoglobulins have a protective role within skin gland secretions and on the skin surface.[20] Our results suggest that *S. aureus* may be able to suppress the secretion of IgA and IgG even at the skin site of patients with AD. The reduced concentration of immunoglobulins may support the chronic colonization of the skin with *S. aureus* in these patients.

IL-4 is known as a cytokine that induces IgE synthesis and expression of the low-affinity receptor for IgE (FcεRII, CD23). Therefore, an important role in the pathogenesis of allergic diseases as well as in AD was suggested.[21] In our experiments, co-cultures of PBL, *S. aureus* and IL-4 showed significant modulation of the staphylococci-induced effects on Ig synthesis. Our data demonstrate that IL-4 induces a significant increase in IgA production by PBL from patients with AD but not by non-atopic lymphocytes *in vitro*. The results support the assumption that IgA may be relevant to allergic diseases. This appears reasonable because allergens invade the body via mucous membranes and the skin and IgA is the most important immunoglobulin at these sites.

Interestingly, IL-4 induced suppression of the IgA synthesis and, to a lesser extent, of the IgG synthesis from PBL of normal donors when the cells were stimulated with *S. aureus*. However, the staphylococci-induced suppression of the Ig synthesis of PBL from patients with AD was not affected by IL-4. This indicates that the observed effects of *S. aureus* on the Ig synthesis in patients with AD may be dependent on this cytokine which may play a dual role in the development of AD. One may suggest that a defect in the regulation of the cytokine-induced humoral immune-response in patients with AD may be responsible for the chronic colonization of the skin with *S. aureus*.

In contrast to these experiments which showed a direct interaction between bacteria and lymphocytes, the experiments in the Transwell system led to different results. The suppression of the IgA synthesis after co-stimulation with *S. aureus* and IL-4 was observed in normal and atopic PBL whereas *S. aureus* alone in contrast to previous experiments induced a significant increase in IgA synthesis of PBL from patients with AD. The IgE synthesis of atopic lymphocytes was markedly enhanced by *S. aureus* alone as well as after addition of IL-4; with regard to the IgG synthesis we did not observe differences between cells from normal and atopic donors. These results indicate that distinct mechanisms are responsible for the modulation of humoral immunity by *S. aureus*. Several authors suggested that adhesions of *S. aureus* (e.g. teichoic acid) bind to cell receptors such as fibronectin which may be important for the chronic colonization of the skin in AD.[5] The inability of *S. aureus* to colonize the intact epithelium may be due to the absence of available fibronectin on the skin surface. Patients with dermatitis may, via lesions of the skin surface, express dermal fibronectin receptors which increase the adherence of *S. aureus* to the skin.[22,23] Another possibility is that atopic patients may be colonized by *S. aureus* due to an innate increase in adherence for the organism. Additional adhesions such as protein A[24,25] as well as other receptors (e.g. HLA-DR)[26] could be involved in these processes. Such a direct interaction between cell wall adhesions of *S. aureus* and receptors even on lymphocytes may mediate the suppression of Ig synthesis in patients with AD.[27]

Furthermore, soluble bacterial products (e.g. enterotoxins) or dissolved components of the cell wall (e.g. peptidoglycan) may cause these effects as they were obtained in the Transwell system. These soluble substances may also induce an increase in IgE synthesis[28] and probably CD23 expression. In fact, evidence was obtained that soluble bacterial products, e.g. lipoteichoic acid, peptidoglycan or enterotoxins, exert similar effects as those obtained with heat-killed staphylococci. Thus one may assume that the technical procedure of heat inactivation does not necessarily impair the observed effects. One may also suggest that *S. aureus* mediates, via such bacterial substances, immediate allergic reactions in patients with AD. *S. aureus* may function in a similar way as aeroallergens which penetrate the skin and support allergic skin reactions in AD via binding to IgE molecules on the surface of Langerhans' cells.[29] Obviously, the results are specific for staphylococci, because different data were obtained in control experiments with *E. coli* (data not shown).

Summarizing our data, it is not possible to decide conclusively whether the chronic colonization of the skin in AD is an epiphenomenon or the result of distinct defects in host–defence mechanisms. However, the fact that patients with AD have multiple defects in their immune system makes it probable that the depression of Ig (A, G) synthesis by *S. aureus* and the influence of IL-4 *in vitro* is the result of an altered immune response to bacterial components in this disease. This defect may then lead to a decreased resistance against *S. aureus* on the skin surface. One may also argue that staphylococci reveal an increased binding to components expressed during an inflammatory response, e.g. fibronectin.[5]

The effects on IgE synthesis and CD23 expression may indicate that *S. aureus* supports acute and chronic allergic reactions in these patients.

Future experiments are planned to analyse the nature of the bacterial products and cellular molecules involved in the suppression of Ig synthesis by staphylococci.

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