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Citation for published version (APA):
Pasch, M. C. (2000). Regulation of expression of complement components, complement regulatory proteins, and chemokines in keratinocytes

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Chapter Four

Effects of UVB on the synthesis of complement proteins by keratinocytes

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Abstract

UVB-exposure of the skin results in increased production of several cytokines by keratinocytes and infiltration of inflammatory cells. We hypothesized that UVB may increase the expression of complement components and complement regulatory proteins by keratinocytes. In vivo, UVB may up-regulate these proteins by direct effects or via cytokines released by keratinocytes or infiltrating inflammatory cells. In vitro, UVB may up-regulate these proteins only directly, because of dilution of released cytokines in the medium. To test this, we exposed cultured human keratinocytes to UVB (0-64 J per m²) and monitored C3 and factor B release in the medium by enzyme-linked immunosorbent assay, and surface expression of membrane cofactor protein, decay accelerating factor, and CD59 by flow cytometry. Keratinocytes produced small amounts of C3 and factor B, which remained unaffected by UVB. UVB (32 J per m²) caused a transient up-regulation of all three complement regulatory proteins. Decay accelerating factor expression was maximal at 48 h (1.81 ± 0.06-fold increase in mean fluorescence intensity over non exposed cells), membrane cofactor protein at 72 h (2.13 ± 0.09-fold increase in mean fluorescence intensity), and CD59 at 120 h (1.96 ± 0.09-fold increase in mean fluorescence intensity), returning to baseline values within 96, 192 and 192 h, respectively. Exposure to 64 J per m² resulted in significant cell death; cells surviving this dose up to 48 h expressed a higher level of all the three proteins than those surviving 32 J per m².

In conclusion, UVB up-regulated membrane cofactor protein, decay accelerating factor, and CD59 on keratinocytes without affecting the constitutive release of C3 and factor B. Thus, UVB can increase the resistance of keratinocytes against their own complement known to be produced excessively in response to cytokines of inflammatory cells which infiltrate the skin following UVB exposure.
Human keratinocytes, the major cell type in the epidermis, have been recognized as initiators of inflammation. They are immunocompetent cells: they act as accessory cells in T cell responses and synthesize a number of cytokines. They also synthesize complement components C3 and factor B. Recently, it has been shown that some of the cytokines produced by keratinocytes and inflammatory cells can differentially enhance the synthesis of C3 and factor B by keratinocytes. Keratinocytes are also expected to produce other components of complement whose synthesis is also likely to be regulated by cytokines. Therefore, under inflammatory conditions of the epidermis, keratinocytes may be considered a local source of complement.

C3 and factor B produced by keratinocytes are beneficial to the host in that they participate in the first line of defense against invasion by foreign cells such as microbes in the skin. These components can eliminate foreign cells via the alternative pathway of complement by generating the C3/C5-convertases (C3b.Bb)/[(C3b)n.Bb] of the alternative pathway on them. C5-convertase in the presence of C5 through C9, can cause the assembly of the C5b-9 complex, the membrane attack complex, on the cell surface that lyzes the foreign cells.

Although complement produced by keratinocytes is beneficial in host defense against foreign cells, it potentially can damage autologous keratinocytes as well, through the membrane attack complex assembly on them. Keratinocytes have complement regulatory proteins embedded in their membranes to prevent this damage. These include decay accelerating factor (DAF; CD55), membrane cofactor protein (MCP; CD46), and CD59. These proteins inhibit different steps of complement activation on cell surfaces. DAF reversibly interferes with the formation of C3/C5-convertases of both pathways of complement and accelerates their decay. MCP acts as a cofactor for the C3 cleaving enzyme, Factor I, and interferes irreversibly with the assembly of C3/C5-convertases of both pathways. CD59 is incorporated into the membrane attack complex during its assembly on the cell membrane and makes the membrane attack complex noncytolytic. Because DAF, MCP and CD59 are mostly coexpressed, they act synergistically to inhibit complement activation on autologous cells to protect them.

UV light exposure of the skin induces dramatic changes in vivo, among them the release of a large number of cytokines from keratinocytes, including IL-1, IL-3, IL-4, IL-5, IL-6, IL-8, TNF-α, GM-CSF, M-CSF and MCAF. Some of these cytokines can attract other cell types, such as monocytes and T cells. As a result, inflammatory cells infiltrate the epidermis after UVB exposure. These cells secrete cytokines, some of which are not secreted by keratinocytes, e.g., T cells can secrete IL-2 and IFN-γ. Thus, UVB exposure results in accumulation in the skin of different cytokines, which have profound effects on cellular immune responses.

From the foregoing and from other studies showing suppression of delayed
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hypersensitivity it is clear that UVB exposure exerts important effects on cellular immune reactions; however, the effects of UVB exposure on humoral immune system, including the synthesis of complement components, has not yet been studied. We argued that UVB exposure of the skin may increase synthesis of complement components by keratinocytes either directly or indirectly via some cytokines released by keratinocytes or by inflammatory cells infiltrating the skin in response to UVB. If synthesis of components of complement is increased after UVB exposure, increased levels of complement can damage keratinocytes. Therefore, a mechanism(s) must exist to protect keratinocytes from complement, excessively produced by them, in response to UVB. This mechanism could be the increased expression of complement regulatory proteins on keratinocyte, either directly by UVB or indirectly via some cytokines released by keratinocytes or by inflammatory cells infiltrated following exposure to UVB. We investigated whether direct UVB exposure of cultured human keratinocytes can increase (1) the constitutive release of C3 and factor B and, (2) the surface expression of DAF, MCP and CD59.

MATERIALS AND METHODS

Keratinocyte culture

Human keratinocytes were isolated by incubation of foreskin with thermolysin (0.50 mg per ml, Sigma, St. Louis, Mo) at 4°C for 16 h and subsequent trypsinization (0.025%) for 5 min at 37°C. Trypsin (Sigma) was then neutralized by an excess volume of heat inactivated fetal calf serum (GibcoBRL, Breda, The Netherlands). Cells were separated from debris by filtering through a nylon mesh, centrifuged, and resuspended in keratinocyte serum free medium (GibcoBRL) supplemented with penicillin/streptomycin (100 IU per ml, 100 μg per ml; GibcoBRL). The keratinocytes were plated onto 100 mm plastic Petri dishes at a density of 400,000 cells per Petri dish and were incubated at 37°C in humidified, 5% CO₂, tissue culture incubator. Medium was changed every 2-3 d, and at 70% confluence cultures were split after a 5 min exposure to trypsin (0.025%)/ethylenediamine tetraacetic acid (EDTA) (1.5 mM) and recultured. For use in experiments, cells were seeded in 6 well tissue culture plates (Costar) at a density of 100,000 cells per well in 2000 μl of medium. Cells in passage 2-5 were used for experiments as soon as 60-80% confluence was achieved. Cells in representative wells were counted by a hemacytometer before the experiment and cells in all wells were counted after finishing the experiment.

UV exposure and cell viability

Subconfluent cultures (60-80%) of keratinocytes in 6 well culture plates were washed twice with phosphate-buffered saline (PBS), exposed to increasing doses of UVB (0-64 J per m²), brought to 2 ml of culture medium and cultured for 48 or 72 h in two sets of experiments. After incubation, keratinocytes were counted with a hemacytometer and after propidium iodide staining viability of these cells was determined by flow cytometer. Cell survival after UVB exposure was expressed as the percentage of control nonexposed alive cells. Doses that were not lethal to the majority of the exposed keratinocytes at a given time after exposure (48 or 72 h) were used in subsequent experiments.

The UVB source consisted of a bank of two Philips TL-12 lamps (Philips, Eindhoven, The Netherlands).
These lamps emit UV in the range of 250-400 nm, primarily in the UVB region (280-320 nm), with a peak at 315 nm. The UVB output was 1 J per m² per second at a target distance of 40 cm and was monitored by an IL443 radiometer with a SEE 1240 UVB photo detector (International Light Inc., Newburyport, MA, USA).

Enzyme-linked immunosorbent assay (ELISA) for measurement of C3, factor B, and IL-8

The concentrations of C3, factor B, and IL-8 in culture supernatants of UVB exposed and non-exposed keratinocytes were estimated as follows.

For quantification of C3 a previously described sandwich ELISA was used with some modifications. Briefly, wells of 96-well flat-bottom microtiter plates were coated with 0.7 μg polyclonal goat IgG anti-human C3 (Cappel, Boxtel, The Netherlands) per ml in 100 μl carbonate buffer (pH 9.6) overnight at 4°C. After thorough washing with Tween-80 (0.05%) (Sigma) in demineralized water the wells were blocked for 1 h at room temperature with 250 μl PBS containing 2% bovine serum albumin (Sigma) and 1 mM EDTA. Washing was repeated and wells were incubated with 100 μl of sample, diluted in the same buffer that was used for blocking. Plates were incubated for 2 h at 37°C. The wells were then washed and incubated with 100 μl peroxidase labeled goat anti-human C3 IgG (0.05 μg per ml) (Cappel) for 1 h at 37°C. After washing the wells were incubated with 100 μl 3,3',5,5'tetramethylbenzidine (Sigma) in dimethylsulfoxide (Merck, Hohenbrunn, Germany)-citrate buffer for 10 min. Reaction was stopped with 100 μl H₂SO₄ (2 M). Optical density (OD) was measured at 450 nm. The detection limit of this ELISA was 1 ng per ml of C3.

Factor B was assayed by a previously described sandwich ELISA with several modifications. Briefly, wells were coated overnight at 4°C with 3 μg polyclonal goat-anti-human factor B IgG (ATAB, Stillwater, MN, USA) per ml in carbonate buffer. After thorough washing with PBS/Tween-80 (0.05%), wells were blocked with PBS/milk powder (2%) (Nutricia, Zoetermeer, The Netherlands) for 1 h at room temperature, and then washed again. Wells were then incubated for 2 h at 37°C with 100 μl sample, diluted in same buffer that was used for blocking, and washed. They were then incubated with biotinylated goat anti-human factor B IgG (1.25 μg per ml) for 1 h at 37°C. After washing, the wells were incubated for another h at 37°C with peroxidase conjugated poly streptavidin (1:1000; DAKO, Glostrup, Denmark). The wells were then repeatedly washed. Incubation of the wells with the peroxidase substrate, termination of the reaction, and measurement of OD was carried out as described for C3. The detection limit of this ELISA was 100 pg per ml.

For IL-8 ELISA, wells were coated with 1 μg monoclonal mouse-anti-human IL-8 (Biosource, Breda, The Netherlands) per ml in 100 μl carbonate buffer (pH 9.6) overnight at 4°C. After thorough washing with Tween-80 (0.05%) in PBS the wells were blocked for 1 h at 37°C with 200 μl of PBS containing 1% bovine serum albumin. Washing was repeated and wells were incubated with 100 μl of sample, diluted in the same buffer that was used for blocking. Plates were incubated for 2 h at 37°C. The wells were then washed and incubated with 100 μl biotinylated mouse anti-human IL-8 (0.1 μg per ml) (Biosource) for 1 h at 37°C. After washing, the wells were incubated for another h at 37°C with peroxidase conjugated poly streptavidin (1:1000; DAKO) in PBS/Tween (0.05%)/milkpowder (1%). The wells were then repeatedly washed. Incubation of the wells with the peroxidase substrate, termination of the reaction, and measurement of OD was carried out as described for C3. The detection limit of this ELISA was 5 pg per ml.

Standard curves for C3 and factor B ELISA were made using human complement calibrator CA1 (ATAB). Standard curve for IL-8 ELISA was made using IL-8 Calibrator (Biosource).
Flow cytometry

Keratinocytes were detached with the trypsin/EDTA solution for 3-5 min. Trypsin was inactivated by fetal calf serum and detached cells were washed and resuspended in fluorescence-activated cell sorter (FACS) buffer (PBS, 2% fetal calf serum, 0.1% sodium azide). Approximately 10^5 cells were incubated with specific monoclonal antibodies to MCP (clone J4-48, CLB, Amsterdam, The Netherlands), DAF (clone BRIC 110, CLB) or CD59 (1F5) or isotype control (Becton Dickinson, San Jose, CA) for 30 min at 4°C. Cells were washed two times and incubated for 30 min with fluorescein-conjugated F(ab')_2 fragments of goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). Hereafter, cells were washed two times with FACS buffer. Propidium iodide (1 mg per ml) (Sigma) was added to identify dead cells and 20,000 cells were analyzed immediately by FACSCalibur (Becton Dickinson). Dead cells were excluded.

The detachment of keratinocytes with trypsin (0.025%)/EDTA (1.5 mM) at 37°C for 3-5 min did not cause degradation of any of the three complement regulatory proteins, as expression of these molecules on cells detached by this procedure was the same as on those detached with EDTA (2 mM) alone at 42°C for 30 min. The former procedure was selected because it did not cause loss of cell viability, whereas latter procedure caused a high degree of loss of cell viability.

Mean fluorescence intensity (MFI) was calculated with WinMDI software. MFI was corrected for isotype MFI and values of cells not exposed to UVB were taken as 100%. MFI values obtained from cells exposed to UVB were presented in terms of percent of nonexposed cells.

Statistical analysis

Statistical analysis was performed using Wilcoxon signed rank test to determine significance in FACS experiments and a Student t test was used for data from ELISA experiments. A p value of less than 0.05 was considered significant.

RESULTS

Increasing doses of UVB cause progressive loss of cell viability

In order to study the effect of UVB exposure on the expression of complement and complement regulatory proteins by keratinocytes, suitable doses of UVB were determined as described in 'Material and Methods'. Cell survival decreased with increasing doses of UVB (0-64 J per m^2) both 48 h and 72 h cultures (Figure 1). Exposure of keratinocytes to 64 J per m^2 resulted in the death of 45% of the cells in 48 h cultures. The surviving cells were not able to recuperate. Exposure to 32 J per m^2 resulted in about 10% cell death in 48 h and 50% cell death in 72 h. The cells that survived after 72 h (32 J per m^2) were healthy as assessed by the fact that they were able to proliferate (as seen up to 192 h; data not shown). Therefore, a dose range of 0-32 J per m^2 and a culture time of 72 h were used in most experiments. In some experiments a dose of 64 J per m^2 and a culture time of 48 h were used to mimic the situation in vivo in which UV exposure results in keratinocyte death, e.g. sunburn cell formation.
Figure 1. Increasing doses of UVB cause progressive loss of cell viability. Subconfluent keratinocyte monolayers were exposed to UVB (0-64 J per m²) and were cultured for 48 h and 72 h. Cells were counted with the haemacytometer and cell survival was analyzed with the flow cytometer after propidium iodide staining. Cells living after exposure with each dose are presented as a percentage of unexposed living cells. Data are averages ± SD of three experiments.

UVB exposure has no influence on the synthesis of C3 and factor B by keratinocytes

To see if UVB influences the constitutive production of C3 and factor B, keratinocytes were exposed to increasing doses of UVB (4-32 J per m²). Non-exposed cells served as controls. Both exposed and nonexposed cells were cultured for 72 h and supernatants were collected. The concentrations of C3, factor B, and IL-8 were measured by ELISA in the supernatants.

In supernatants harvested from cultures of nonexposed keratinocytes C3, factor B, and IL-8 were found to be present in low concentrations. In supernatants of cultures of keratinocytes exposed to increasing doses of UVB, concentrations of C3 and factor B remained low but the concentration of IL-8 was significantly increased (Figure 2). IL-8 production was highest at 32 J per m². The maximal IL-8 level in the supernatant was 609 pg per ml. To exert biological activity much higher concentrations are required. These results show that UVB activates keratinocytes, as could be concluded from the IL-8 production, but does not directly increase the production of C3 and factor B.

Cultured keratinocytes express DAF, MCP and CD59

Previous immunohistochemical studies in our laboratory have shown that DAF, MCP and CD59 were present in situ on several structures of human skin, including keratinocytes. Flow cytometric analysis of cultured keratinocytes in this study confirmed that all three proteins were expressed on these cells (Figure 3).
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Figure 2. UVB exposure has no influence on the synthesis of C3 and Factor B by human keratinocytes
Subconfluent keratinocyte cultures were exposed to indicated doses of UVB. Supernatants were harvested at 72 h and concentrations of C3 and Factor B determined by ELISA. IL-8 was included as a positive control. The figure shows C3, Factor B and IL-8 protein concentrations in supernatants of three independent experiments. In each experiment three wells were exposed to each dose and the contents of each well were analyzed in quadruplicate. Mean values and SD are shown.

Figure 3. Cultured human keratinocytes express DAF, MCP and CD59
Cultured keratinocytes were washed and 10^4 cells were analyzed for the expression of C regulatory proteins by FACS. Continuous lines show keratinocyte staining of MCP, DAF or CD59. Broken lines show staining with isotype controls. For all parts, the data are shown as cell number versus the log of fluorescence and are representative of three staining experiments each carried out in triplicate.

A single dose of UVB causes a prolonged increase in the surface expression of DAF, MCP and CD59
When cultured keratinocytes were exposed to UVB (32 J per m^2) and surface expression of the complement regulatory proteins was analyzed at several time points (0-192 h), expression of all the three proteins was found to be transiently increased (Figure 4). DAF expression increased rapidly, reaching its maximum at 48 h after UVB exposure (1.81 ± 0.06-fold increase
Figure 4. A single dose of UVB causes prolonged increase in the surface expression of DAF, MCP and CD59. Subconfluent keratinocyte cultures were exposed to 32 J per m² and expression of DAF, MCP and CD59 was monitored by flow cytometer at the indicated time points. MFI, corrected for isotype control, is shown. Data represent one of two independent experiments. In each experiment, three wells were exposed to UVB for each time point and cells in each well were analyzed in duplicate. Mean values and SD are shown.

In MFI over nonexposed cells. Thereafter, DAF expression decreased rapidly, returning to control values within 96 h. MCP expression increased, reaching its maximum at 72 h (2.13 ± 0.09-fold increase in MFI). Expression of MCP gradually returned to control values in 192 h. CD59 expression increased rather slowly, reaching a plateau at 72-120 h (1.74 ± 0.07-fold and 1.96 ± 0.09-fold increase in MFI, respectively). Expression of CD59 returned to almost baseline values in 192 h.

**Increasing doses of UVB cause a dose-dependent increase in surface expression of complement regulatory proteins**

Time-response studies described above showed that with 32 J per m² surface expression of DAF was maximal at 48 h of culture after irradiation and surface expression of MCP and CD59 was maximal at 72 h. Therefore, dose-response studies with a moderate dose of up to 32 J per m² were carried out at 48 h of culture after irradiation for DAF and at 72 h of culture after irradiation for MCP and CD59. In the case of the expression of DAF, there was no effect of UVB doses up to 16 J per m² but the expression was significantly increased with 32 J per m² (Table). In the case of MCP and CD59, expression gradually increased from 8 J per m² onwards and reached its maximum at 32 J per m².

**A high dose of UVB results in a high surface expression of DAF, MCP and CD59**

Further studies were aimed at comparing the effects of moderate (32 J per m²) and high dose of UVB (64 J per m²) on the expression of all the three complement regulatory proteins.
UVB induces complement regulatory proteins

Table. Effect of increasing doses of UVB on the expression of MCP, DAF and CD59 by keratinocytes.

<table>
<thead>
<tr>
<th>dose (J per m²)</th>
<th>DAF</th>
<th>MCP</th>
<th>CD59</th>
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<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>97.4 ± 8.0</td>
<td>123.2 ± 3.7***</td>
<td>114.4 ± 11.7*</td>
</tr>
<tr>
<td>16</td>
<td>102.1 ± 1.7</td>
<td>125.8 ± 3.3***</td>
<td>131.1 ± 7.3**</td>
</tr>
<tr>
<td>32</td>
<td>151.0 ± 29.9**</td>
<td>158.6 ± 18.0**</td>
<td>183.4 ± 18.4***</td>
</tr>
</tbody>
</table>

Subconfluent cultures were exposed to various doses of UVB. After 48 h (DAF) or 72 h (MCP and CD59) membrane expression of complement regulatory proteins on 20,000 cells was determined by FACS analysis. MFI values of unexposed keratinocytes were taken as 100%. All values represent mean ± SD of six experiments; * p < 0.05, ** p < 0.01, *** p < 0.001.

Figure 5. Increasing doses of UVB cause progressive increase in surface expression of DAF, MCP and CD59. Subconfluent keratinocyte cultures were exposed to a moderate dose (32 J per m²) and a high dose (64 J per m²) of UVB. Expression of DAF, MCP and CD59 was analyzed by flow cytometer at 48 h. Single parameter histograms for DAF, MCP and CD59 expression are shown. Thin lines show the expression of DAF, MCP and CD59 on unexposed keratinocytes and bold lines show that on UVB-exposed keratinocytes. Broken lines show the expression obtained with isotype controls. The data are representative of at least two independent experiments carried out in triplicate. MFI after exposure to 64 J per m² was significantly higher for DAF (p<0.01) and CD59 (p< 0.001) but not for MCP (p=0.18) than after exposure to 32 J per m².
These studies were carried out at 48 h of culture because of the low degree of cell survival at 72 h of cells exposed to 64 J per m$^2$ (see above). The results showed that expression of all the three complement regulatory proteins was higher with 64 J per m$^2$ than with 32 J per m$^2$, although the difference in expression of MCP did not reach significant levels (p=0.18) (Figure 5).

**DISCUSSION**

UVB radiation is the mid range portion (290-320 nm) of the ultraviolet radiation spectrum (200-400 nm). UVB is present in biologically significant amounts at the earth's surface and exhibits pleomorphic effects both on the whole organism and on the skin. It penetrates the stratum basale and reaches the keratinocytes. UVB increases production of several cytokines by keratinocytes. Some of these cytokines recruit inflammatory cells and thereby induce inflammation at the site of UVB exposure of the skin$^{303,219,304}$. Alteration in production of some cytokines by UVB induces immunosuppression$^{307}$.

In many inflammatory disorders, like psoriasis, atopic dermatitis, seborrhoeic dermatitis, and lamellar ichthyosis, complement deposits are found in the epidermis without the presence of autoantibodies$^{310}$. In some autoimmune diseases, such as pemphigus, pemphigoid, discoid lupus erythematosus, and photosensitive lupus erythematosus, complement deposits on keratinocytes are seen in lesional skin but can also be induced in nonlesional skin by UVB exposure$^{311,312}$. In some diseases of unknown etiology such as photoallergy, polymorphous light eruptions, solar urticaria, solar eczema, and actinic reticuloid, the disease is exacerbated by light but the role of complement remains unknown. In none of these diseases is the origin of complement in the skin known. And, in none of these diseases, except aforementioned autoimmune diseases, has the effect of UVB on the development of complement deposits been studied. In spite of these gaps in knowledge, studies on the effects of UV exposure on the expression of complement and complement regulatory proteins by keratinocytes isolated from normal individuals and from patients has not been carried out.

We argued that UVB exposure can probably increase the constitutive production of complement components by keratinocytes. In evolutionary terms, this may be to compensate for local immunosuppression induced by UVB. We also argued that UVB exposure can increase the surface expression of complement regulatory proteins on keratinocytes to protect them from their own complement. We raised the possibility that UVB can up-regulate the synthesis of both complement and complement regulatory proteins, either directly or indirectly via cytokines released by keratinocytes or infiltrated inflammatory cells.

We observed that keratinocytes constitutively produced small amounts of C3 and factor B. This was in agreement with previous observations$^{34,35}$. Exposure to UVB caused stimulation
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of keratinocytes, as evidenced by the production of IL-8, but did not increase production of C3 and factor B (Figure 2). This proved that direct stimulation of keratinocytes by UVB could not induce an increase in production of C3 and factor B. This also raised the question why those C3 and factor B up-regulating cytokines that are released from keratinocytes upon UVB exposure do not increase the production of C3 and factor B in our system. One of the possible explanations is that they may be released in the medium in concentrations not high enough to stimulate the keratinocytes. The concentrations of TNF-α needed to up-regulate C3 in our system were 50-1000 U per ml. But the concentrations of TNF-α in culture medium of UVB exposed keratinocytes (at 72 h) was found to be very low (<1 U per ml; data not shown here). The same appears to be true for IL-8, whose effects on the expression of C3, factor B, and complement regulatory proteins is not known. The level of IL-8 in the culture medium of UVB exposed keratinocytes was always lower than 610 pg per ml (Figure 2). Biologically effective IL-8 concentrations vary from 5 to 50 ng per ml. These results indicate that cytokines released from keratinocytes in culture medium after UVB exposure probably become too diluted to be effective. It remains to be seen if cytokines released from keratinocytes upon UVB exposure can up-regulate C3 and factor B in vivo.

DAF, MCP and CD59 are present on keratinocytes in the human skin as seen in situ in previous immunohistochemical studies. This study shows surface expression of DAF, MCP and CD59 on cultured keratinocytes. The coexpression of DAF, MCP and CD59 on human keratinocytes suggests that these molecules collectively offer a high degree of protection to keratinocytes against complement attack. Indeed, human keratinocytes and a keratinocyte-derived squamous carcinoma cell line, SCC-12F, are remarkably resistant to complement mediated lysis. In addition, cell death is not a prominent feature in diseases like pemphigus in which strong complement attack against keratinocytes occurs. Thus, keratinocytes in vivo are strongly protected against autologous complement. Keratinocytes do need strong protection against complement attack under physiologic conditions, because the epidermis is under a continuous threat of exposure to microbes and other agents that can activate complement and cause bystander complement attack on keratinocytes.

This study also shows that the expression of DAF, MCP and CD59 on keratinocytes is increased for several days by UVB exposure in a dose-dependent manner. Because these molecules act synergistically, increases in all three implies that UVB exposure results in a prolonged and a high degree of protection against autologous complement. We did not try to correlate increased expression with increased protection against complement attack because increased expression of DAF, MCP and CD59 in response to UVB exposure could be accompanied by increased or decreased expression of other complement regulatory proteins, such as complement receptor 1, C1q receptor and homologous restriction factor. Possible changes in expression of these latter complement regulatory proteins would have made results difficult to
interpret. For example, an increase in the expression of CD59 in the EA.hy926 cell line caused by inducers of protein kinase complement and protein kinase A could not be correlated with the increase in resistance to complement mediated lysis.\textsuperscript{313}

The increased expression of DAF, MCP and CD59 seen \textit{in vitro} could have been due either to a direct effect of UVB on keratinocytes or an indirect effect in response to mediators released by keratinocytes. The latter possibility appears to be less likely because the concentrations of cytokines released from UVB-exposed keratinocytes into the culture medium do not appear to be high enough to be able to up-regulate complement regulatory proteins significantly.

If these findings may be extrapolated to the \textit{in vivo} situation, keratinocytes in UVB exposed areas may be better protected against complement mediated lysis than keratinocytes in nonexposed areas. The situation \textit{in vitro}, however, may differ from the situation \textit{in vivo} in which infiltrating cells may also influence the expression of complement regulatory proteins through the release of their cytokines.

UVB is known to activate protein kinase complement in keratinocytes.\textsuperscript{314} Increase in expression of DAF, MCP and CD59 by activators of protein kinase C, phorbol myristate acetate, and calcium ionophore A23187, and an activator of protein kinase A, butyryl-cAMP, has been shown in our laboratory (data not presented here). Protein kinase complement and protein kinase A signalling may perhaps be involved in the up-regulation of DAF, MCP and CD59 by UVB.

In conclusion, this study demonstrates that cultured human keratinocytes constitutively release low amounts of C3 and factor B, which remains unaffected by UVB. They also express DAF, MCP and CD59. Expression of these complement regulatory proteins is increased by UVB. Because UVB may increase complement production \textit{in vivo} through cytokines of keratinocytes and infiltrated cells, this increase in complement regulatory proteins may be important for protecting keratinocytes from becoming bystander victims of complement during UVB-mediated inflammation.