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Pasch, M.C.

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Synthesis of complement components C3 and factor B in human keratinocytes is differentially regulated by cytokines

M.C. Pasch, N.H.A. van den Bosch, M.R. Daha*, J.D. Bos and S.S. Asghar

Department of Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam and
*Department of Nephrology, Academic Hospital, University of Leiden, Leiden, The Netherlands


Abstract

The complement system plays an important role in host defense and inflammation. Locally synthesized complement may perform these functions at tissue and organ level. In the epidermis the keratinocyte is the major cell type known to produce two soluble complement components, C3 and factor B. In the present study we investigated the regulation of synthesis of these components in foreskin keratinocytes by cytokines.

Human keratinocytes were cultured in the presence of supernatant of activated peripheral blood mononuclear cells, IL-1α, IL-2, IL-6, TGF-β1, TNF-α, or IFN-γ. C3 and factor B proteins were measured in culture supernatant by ELISA and C3 and factor B transcripts in harvested cells by reverse-transcriptase polymerase chain reaction.

Cultured keratinocytes constitutively produced C3 and factor B. Supernatant of activated mononuclear cells up-regulated C3 and factor B production by 27- and 15-fold, respectively. IL-1α, IFN-γ, and TNF-α up-regulated C3 synthesis by 7-, 8-, and 22-fold, and IL-1α, IL-6, and IFN-γ up-regulated factor B synthesis by 3-, 3-, and 34-fold, respectively. TNF-α induced production of C3 and IFN-γ induced production of factor B were inhibited by cycloheximide. Cytokine induced up-regulation of C3 and factor B proteins was always associated with the up-regulation of levels of C3 and factor B mRNAs. This indicated that, as expected, cytokine-induced enhancement in C3 and factor B levels was due to increase in synthesis rather than their possible release from intracellular stores.

In conclusion, synthesis of C3 and factor B in keratinocytes is regulated by some cytokines, known to be produced by inflammatory cells and keratinocytes.
Chapter 2

INTRODUCTION

The complement system is comprised of a large number of proteins which include components of classical and alternative pathways\(^3,13\). Complement components C3 and factor B occupy the central position in the alternative pathway as, following activation, they become constituents of the C5-convertase (C3b\(_n\).Bb). C3 is also central to the classical pathway as it provides the catalytic subunit of classical pathway C5-convertase (C4b.C2a.C3b). C5-convertases of both pathways can eventually generate the membrane attack complex (C5b-9; MAC). Generation of MAC on foreign cells, such as microbes, can lead to their killing, but under certain circumstances its generation on self cell can lead to effects such as release of inflammatory mediators and cell-proliferation\(^3,27\).

Although the liver is the primary source of plasma complement, other cells of various organs also produce complement proteins. The list of tissues and cells capable of producing complement components is growing\(^253,258\). Human keratinocytes, the most abundant cell-type in epidermis, have been studied for the biosynthesis of two complement components, C3 and factor B. Biosynthetic labeling and pulse chase studies with human keratinocytes and a human keratinocyte cell line A431 have shown that these cells synthesize a 195-kD pro-C3\(^34\) and a 100-kD pro-factor B\(^35\) molecule. The C3 precursor molecule is processed intracellularly to a mature C3 molecule which consists of disulphide linked 120- and 75-kD C3 alpha and beta chains. The 100-kD factor B precursor molecule is processed intracellularly to a 105-kD extracellular mature factor B. It was also shown by these authors that when mature C3 and factor B were subject to specific cleavage by appropriate enzymes of classical and alternative pathways, respectively, they were cleaved and activated in a manner analogous to that of circulating C3 and factor B. Hence skin epithelial cells were seen as a potential source of biologically active C3 and factor B. Northern blot analysis from human keratinocytes and A431 revealed the presence of a 5.1 kb C3 mRNA and 2.8 kb factor B mRNA in these cells. These results convincingly demonstrated that human keratinocytes are local source of C3 and factor B and of their important cleavage products.

Demonstration of constitutive synthesis of C3 and factor B led to two main questions, (1) are keratinocytes, like some other cell types\(^256,259\), also capable of synthesizing all other components of complement and (2) under what conditions or biological stimuli, synthesis of C3 and factor B (and of other components if produced) in keratinocytes is regulated? Studies aimed at answering these questions are currently underway. As regards the latter question, Terui et al. recently studied a large number of cytokines and growth factors and showed that TNF-\(\alpha\) and IFN-\(\gamma\) are the main cytokines involved in regulation of production of C3 in human keratinocytes\(^36\).
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although IL-1α and IL-1β also up-regulated C3 production to some extent. They also showed that protein kinase C plays a stimulatory role whereas protein tyrosine kinase an inhibitory role in C3 synthesis and release. State of keratinocyte differentiation also influenced constitutive and cytokine mediated release of C3 to some extent; differentiated keratinocytes were more active in producing C3. These workers did not study pretranslational regulation of C3 by the above cytokines. They also did not include the regulation of synthesis of factor B in their studies. In the current study we investigated the cytokine regulation of C3 and factor B production both at protein and mRNA levels.

MATERIALS AND METHODS

Chemicals and reagents

Human recombinant cytokines IFN-γ, IL-1α, IL-2, IL-6, TGF-β1 and TNF-α were purchased from Boehringer Mannheim (Mannheim, Germany). Normal rabbit IgG, neutralizing rabbit antibodies to IFN-γ and cycloheximide were purchased from Sigma (St. Louis, MO, USA). Supernatant of activated mononuclear cells was prepared from stimulated peripheral blood mononuclear cells as described (8). Briefly, peripheral blood mononuclear cells (PBMC; 50 x 10^6/ml) from normal donors were stimulated for 2 h at 37°C with 0.1 μg/ml PMA in Iscove’s Modified Dulbecco’s Modified Eagles Medium (IMDM) (supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin and 100 μg/ml streptomycin). PMA-treated cells were washed extensively and cultured (50 x 10^7/ml) for 48 h with 15 μg/ml Con A in supplemented IMDM. Con A was neutralized by addition of 50 mM α-methyl-mannoside for 30 min at 37°C. Supernatant was obtained by centrifugation. Cytokines, supernatant of activated mononuclear cells and neutralizing antibodies were aliquoted in small portions and stored at -20°C and diluted in keratinocyte serum free medium (keratinocyte SFM; GibcoBRL, Breda, The Netherlands) just before use. The sources of other chemicals and reagents are indicated below.

Keratinocyte culture

Human keratinocytes were isolated from foreskin obtained by circumcision of children (< 5 years). Foreskin was incubated with thermolysin (0.50 mg per ml, Sigma, St. Louis, Mo) at 4°C for 16 h and subsequently trypsinized (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 SFM 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 0.025% trypsin, 1.5 mM EDTA and recultured.

Stimulation of keratinocytes

For stimulation of keratinocytes with cytokines, cells were seeded at a density of 100,000 per well in 2000 μl of medium. When cultures reached 60-80% confluence, medium was removed and monolayers were washed twice with PBS. Keratinocyte SFMs containing different cytokines was added to the cells. The concentrations and ranges
of concentrations of cytokines used in different experiments are mentioned in the section ‘Results’. After stimulation with cytokines for a predetermined suitable period of 72 h (unless indicated otherwise), culture supernatants were collected for C3 and factor B analysis by ELISA. As controls, supernatants were collected from keratinocyte cultured in keratinocyte SFM without cytokines. Cells in representative wells were counted by hemacytometer before the experiment and the cells in all wells were counted after finishing the experiment. Cells in passage 2-5 were used.

In some experiments supernatant of activated mononuclear cells or lipopolysaccharide (LPS), at concentrations indicated in the section ‘Results’, were used instead of cytokines.

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

The concentrations of C3 and factor B in culture supernatant of 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. Standard curves for both ELISA’s were made using Human Complement Calibrator CA1 (ATAB).

Isolation of RNA and semi-quantitative reverse-transcriptase polymerase chain reaction

Total RNA was isolated from human keratinocytes grown in 100 mm Petri dishes in medium alone or medium supplemented with IL-1α (200 U/ml), IL-6 (1000 U/ml), IFN-γ (100 U/ml) or TNF-α (750 U/ml) for 6 h and 24 h using Trizol (Life Technologies, Paisley, UK). The RNA pellet was dissolved in formamide and the amount of RNA was determined by a spectrophotometer at 260 nm and 280 nm.

Reverse-transcriptase polymerase chain reaction (RT-PCR) was carried out as described, with some minor modifications. Briefly, 5 µg of the extracted total cellular RNA was reverse transcribed in a reaction volume of 20 µl and 1 µl of the resulting cDNA solution was used to amplify cDNA by C3-, or factor B-specific PCR. The PCR were performed in 50 µl per well in polyethylene reaction tubes and applying cycles consisting of denaturation step at 94°C for 30 seconds, annealing for 1 min at 59°C, and extension for 1 min at 72°C. The PCR incubation
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mixture, in a total volume of 50 µl, contained 50 mM KCl, 10 mM Tris-HCl pH 8.1, 2.0 mM MgCl2, 0.01% gelatin, 1.25 unit Taq polymerase (Gibco), 250 µM dNTP mix (Pharmacia, Uppsala, Sweden), and 140 ng of the sense and anti-sense primer each. The following specific primer sets were synthesized in our laboratory by an oligo-synthesizer: glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward primer 5'-CGAGATCCCTCCAAAATCAA-3' (nt 298-317); and GAPDH reverse primer 5'-AGGTCAAGGCCTCACCACGTAC-3' (nt 799-808), factor B forward primer 5'-AAACAAAGCCCAAGATCGTC-3' (nt 766-786); and factor B reverse primer 5'-TATCTCCAGGTCGTTCTC-3' (nt 1630-1650), and C3 forward primer 5'-TCGGATGACAAGGTCACCCT-3' (nt 4627-4646); and C3 reverse primer 5'-GACAACCATGCTCCTCGGTGA-3' (nt 5015-5034). 12.5 microliters of each PCR product was mixed with 5 microliters stop layer mix and run on a 1.7% agarose gel in tris/borate/EDTA buffer. After electrophoresis the gel was scanned by an Eagle Eye imager (Stratagene Europe, Amsterdam, The Netherlands) and the signal strength was integrated to obtain a densitometric value for each amplification product.

To enable semi-quantitative analysis, the number of PCR cycles was chosen in such a way that a linear relationship was achieved between PCR product formation (plotted on a log scale) and cycle number (28 to 34 cycles for C3 and factor B and 26 to 32 cycles for GAPDH), without having reached saturation of the product formation.

Statistical analysis

Statistical analysis was performed using the Student's t-test for unpaired samples and a p value of less than 0.05 was considered significant.

RESULTS

Human keratinocytes constitutively produce C3 and factor B

Keratinocytes produce small amounts of C3 and factor B in culture as determined by ELISA (Figure 1). When keratinocytes were cultured up to passage six, and the production of C3 and factor B was monitored in the culture supernatant of each passage, a significant increase of both components with increasing number of passages was observed in cultures derived from three different foreskins (C3 p<0.002, and factor B p<0.02; passage 6 compared to passage 1; Figure 1). Because of these differences we used only cultures from passage 2 to 5 in subsequent studies.

Mediators released from activated mononuclear cells up-regulate the production of C3 and factor B from keratinocytes

Keratinocytes cultured in absence of supernatant of activated mononuclear cells produced 2.1 ± 0.4 ng C3 and 0.4 ± 0.07 ng factor B per 10^6 cells per 24 h. In the presence of 10% supernatant, production of C3 was 56.4 ± 20.7 and of factor B was 6.5 ± 1.4 ng per 10^6 cells per 24 h. In all cultures, supernatant of activated mononuclear cells induced a significant increase in production of both C3 (p<0.005) and factor B (p<0.001) in a dose dependent fashion.
Cytokines differentially regulate the production of C3 and factor B from keratinocytes

The supernatant of activated mononuclear cells is known to contain a number of cytokines, which include IL-1α, IL-2, IL-6, TGF-β, TNF-α and IFN-γ. We tested recombinant forms of these individual cytokines to find if one or more of these mimics the effects seen with supernatant of activated mononuclear cells.

IL-2 (0-1000 U per ml), TGF-β1 (0-10 ng per ml) and IL-6 (0-1000 U per ml) had no effect on the production of C3 (data not shown). IL-1α, IFN-γ and TNF-α showed a dose-dependent up-regulation of C3 production (Figure 2). These cytokines at doses of 100 U per ml, 50 U per ml, and 750 U per ml, respectively, up-regulated C3 production 7-, 8-, and 22-fold, compared to the basal production. Further increase in dose did not cause additional increase in response.

IL-2 (0-1000 U per ml), TNF-α (0-1000 U per ml), and TGF-β1 (0-10 ng per ml) did not show any significant effect on the production of factor B. IL-1α, IL-6, and IFN-γ caused dose-dependent up-regulation of factor B. IL-1α and IL-6 up-regulated factor B production to about three-fold of the basal release at 200 U per ml and 1000 U per ml, respectively, and IFN-γ about 34-fold at 75 U/ml (Figure 2). Further increase in dose did not cause further increase in factor B production.

To confirm that the regulation of C3 or factor B production by normal human keratinocytes was a specific property of the above mentioned cytokines, antibody blocking
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experiments were performed. Keratinocytes were cultured in medium alone or in medium containing optimal concentration of individual cytokines in the presence and absence of neutralizing antibodies or control rabbit or mouse IgG (Figure 2). The results showed that neutralization of IL-1α with anti-IL-1α, IFN-γ with anti-IFN-γ, or TNF-α with anti-TNF-α abolished the induction of C3 production. Neutralization of IL-1α with anti-IL-1α, IFN-γ with anti-IFN-γ, and IL-6 with anti-IL-6, abolished the induction of factor B production.

The batches of FCS, supernatant of activated mononuclear cells, and keratinocyte medium did not show C3 or factor B reactivity in the respective ELISA assays.

Up-regulation of production of C3 and factor B is inhibited by cycloheximide

TNF-α and IFN-γ which were the strongest up-regulators of C3 and factor B production from keratinocytes, respectively, were selected for further studies to find out whether they caused enhanced production by inducing de novo synthesis. This was investigated by observing the effect of cycloheximide on TNF-α and IFN-γ mediated up-regulation of C3 and factor B, respectively. Keratinocytes were grown in medium alone, medium containing IFN-γ (100 U per ml) or TNF-α (750 U per ml) with and without 2.0 μg per ml cycloheximide. Higher concentrations of cycloheximide could not be used because of irreversible toxic effects on the cells. At definite time intervals, supernatants were harvested for C3 and factor B analysis by ELISA. These experiments revealed that cycloheximide significantly inhibited the TNF-α induced C3 and the IFN-γ induced factor B production after 24 h and 48 h (Figure 3). This indicated that increased production of C3 in response to TNF-α, and of factor B in response of IFN-γ were due to increased de novo synthesis. Removal of cycloheximide by washing the cells and again culturing them in the medium containing TNF-α and IFN-γ restored C3 and factor B production, respectively (data not shown).

Cytokines regulate the production of C3 and factor B at pretranslational level

C3 and factor B mRNA transcripts obtained from cytokine treated and untreated cells were analyzed by RT-PCR. Keratinocytes were incubated for 6 h and 24 h in medium containing IFN-γ (100 U per ml), IL-1α (200 U per ml), IL-6 (1000 U per ml) or TNF-α (750 U per ml). After RT-PCR and gel electrophoresis as described in ‘Materials and Methods’ the Eagle Eye analysis revealed 2-, 8-, and 11-fold increase of the ratio C3/GAPDH message after stimulation with IL-1α, IFN-γ and TNF-α, respectively (Figure 4). The ratios of factor B/GAPDH were increased 7-, 4-, and 23-fold, by stimulation of keratinocytes with IL-1α, IL-6, and IFN-γ, respectively (Figure 4). These semi-quantitative data suggest that the up-regulation of C3 synthesis by IFN-γ, IL-1α and TNF-α and of factor B by IL-1α, IL-6 and IFN-γ was at a pretranslational level.
Figure 2. Release of C3 and factor B by keratinocytes is differentially regulated by cytokines. Keratinocytes were cultured for 72 h in the presence of indicated concentrations of cytokines and supernatants were collected and assessed for C3 and factor B concentrations by ELISA. Insets show the effects of cytokine (indicated) specific neutralizing rabbit antibodies on cytokine (indicated) induced C3 and factor B release by keratinocytes. Keratinocytes were cultured with an optimal concentration of indicated cytokine alone, cytokine plus specific neutralizing antibodies, or control IgG. After 72 h, supernatant were collected and assessed for C3 and factor B. The data in main figures and insets are expressed as the mean ± SD of duplicate measurements of three cultures.

Figure 3. Cycloheximide inhibits C3 and factor B synthesis by keratinocytes. Sub-confluent keratinocytes were cultured in medium containing TNF-α (750 U per ml) or IFN-γ (100 U per ml) in the presence and absence of 2 µg cycloheximide per ml. At 24 h and 48 h, culture supernatant were collected and assayed for C3 and factor B. Results obtained with cultures without (●) and with cycloheximide (○) are shown. Values are the mean ± SD for duplicate determinations of triplicate cultures.
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Figure 4. Expression of C3 and factor B in keratinocytes is differentially regulated by cytokines at pretranslational level. RNA was isolated from keratinocytes at 0 h, 6 h, and 24 h after stimulation with indicated cytokines at concentrations given below, and subjected to semi-quantitative C3- or factor B specific RT-PCR as described in the text. After electrophoresis, the densitometric values of the products were determined and corrected for the value of GAPDH, a household gene. The relative densitometric value obtained with untreated keratinocytes (0 h) was arbitrarily set at 1 and were related to those of cytokine-treated keratinocytes (6 h; 24 h).

Inset in the left figure shows up-regulation of C3 transcripts by TNF-α (representative example; effects of other cytokines are not shown). RNA was isolated from keratinocytes after 0 h, 6 h, or 24 h of stimulation and subjected to a C3 RT-PCR. After electrophoresis the products were visualized in ethidium bromide solution. Inset in the right figure shows up-regulation of factor B transcripts by IFN-γ (representative example; effects of other cytokines are not shown) under the conditions identical to those as for C3 (upper figure).

Concentrations of cytokines: IL-1α = 200 U per ml; IL-6 = 1000 U per ml; TNF-α = 750 U per ml; IFN-γ = 100 U per ml

DISCUSSION

The epidermal compartment of skin is known to express immunity. Because the epidermis is in frequent contact with foreign antigens it is expected to possess, besides components of cellular immunity, a complement synthesizing apparatus to form the first line of immunological defense. The most abundant cells in human epidermis, the keratinocytes, indeed have been shown to express C3, factor B, several complement receptors, and complement regulatory proteins, and like some other cell types, may produce all the components of classical and alternative pathways. The complement synthesized in the epidermis can perform some important immunological functions at tissue level as is beginning to be envisaged in other organs.

Keratinocytes are known to synthesize low levels of C3 and factor B, but if local synthesis of complement plays a role in immunological defense and inflammatory conditions of the skin, their production should be amplified when it is most required, e.g. during microbial
invasion and under inflammatory conditions of the skin. This amplification should most likely be mediated by cytokines which are produced by keratinocytes and infiltrating inflammatory cells and which are known to regulate the synthesis of complement-components in many cell types differentially and in tissue specific manner.

In the present study we investigated the regulation of synthesis of C3 and factor B by the supernatant of activated mononuclear cells, a source of mediators derived from inflammatory cells, and by recombinant forms of several cytokines known to be present in this supernatant. Constitutive production of C3 was greatly up-regulated by the supernatant of activated mononuclear cells and TNF-α but also appreciably by IL-1α and IFN-γ. Constitutive production of Factor B was greatly up-regulated by the supernatant of activated mononuclear cells and IFN-γ but also appreciably by IL-1α and IL-6 (Figure 2). IL-2, TNF-α and TGF-β had no effect. These results show differential effects of some cytokines on the production of C3 and factor B by human keratinocytes.

Since several cell types such as neutrophils store large amounts of some complement components and secrete them upon stimulation without stimulating their synthesis, we investigated whether the cytokine induced release of C3 and factor B was due to their export from intracellular reserves or was associated with increase in their synthesis. The possibility of export of intracellular reserves was ruled out by the facts that (1) cycloheximide inhibited cytokine induced enhancement of C3 and factor B production and (2) IL-1α, TNF-α, and IFN-γ mediated enhancement of production of C3 and IL-1α, IL-6, and IFN-γ mediated enhancement of production of factor B was associated with the up-regulation of transcription of C3 and factor B genes as seen by semi-quantitative RT-PCR. LPS was also tested for its ability to induce the synthesis of C3 and factor B by keratinocytes but was found to have no effects at 100 ng per ml, a concentration 10 to 100 times higher than that needed to affect complement synthesis in human fibroblasts.

The up-regulation of C3 synthesis in keratinocytes by IL-1α and IL-6 does not appear to be mediated by autocrine release of TNF-α by keratinocytes in culture medium. Most likely TNF-α released in the medium is diluted to concentrations insufficient to induce the synthesis of C3 and factor B by keratinocytes. The support for this postulation comes from the following observation. In our laboratory, the concentration of TNF-α in culture medium of stimulated keratinocytes was found to be very low (<1 U per ml) whereas that required for up-regulation of C3 in our system in this study was much higher (Figure 2). Similarly, the enhancement of factor B synthesis in response to IL-1α and IL-6 appears not to have been caused by any other cytokine released in autocrine manner from keratinocytes.

IL-1α and IL-6 had comparatively weak stimulating effects on the synthesis of C3 and factor B, respectively, by human keratinocytes. This was in contrast to the findings of Katz et
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...al.93, and Katz and Strunk62 in human skin fibroblasts, who showed that IL-1α and IL-6 play an important role in the regulation of both C3 and factor B synthesis. In glomerular epithelial cells IFN-γ does not increase C3 gene expression271, which is also in sharp contrast to the strong up-regulation of C3 caused by IFN-γ in keratinocytes. Comparison of our results with those obtained with other cell types confirmed the widely held view that regulation of complement synthesis by cytokines is highly cell type specific.

If the observations made in the present study could be extrapolated to in vivo situations, several cytokines produced locally by keratinocytes and infiltrated inflammatory cells, during an inflammatory response, can up-regulate the synthesis of complement components in vivo. Because complement is known to be continuously activated, complement produced by keratinocytes in higher than usual amounts in response to some cytokines may damage keratinocytes. To prevent this there should exist a mechanism(s) which can protect keratinocytes from this autologous complement. One such mechanism could be the differential up-regulation of complement-regulatory molecules, which protect autologous cells from activated complement, by keratinocytes or inflammatory cells derived cytokines. This has indeed been shown in a recent study in which up-regulation of complement regulatory membrane proteins, membrane cofactor protein (CD46) and CD59, by supernatants of activated mononuclear cells and TGF-β has been shown37.

In conclusion, we provide data which confirm that keratinocytes constitutively produce low levels of C3 and factor B. TNF-α, IFN-γ and IL-1α regulate the synthesis of C3 and IFN-γ, IL-1α and IL-6 that of factor B. Local complement protein synthesis may contribute to local immunological defenses. Other possible roles of local synthesis of complement by keratinocytes have yet to be investigated.