Regulation of expression of complement components, complement regulatory proteins, and chemokines in keratinocytes
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Chapter Seven

Summary and Discussion

The purpose of the work presented in this thesis was to obtain more information than was available on the role of keratinocytes in skin inflammation. As has been postulated by Barker\(^1\), keratinocytes may be considered initiators of cutaneous inflammatory responses. Keratinocytes, the major cell type in epidermis, may be triggered by diverse stimuli to elaborate specific pro-inflammatory molecules, such as cytokines, chemokines, complement proteins and adhesion molecules. The consequences are activation of dermal microvascular endothelial cells and accumulation of inflammatory cells in the dermis and epidermis. Ultraviolet B (UVB) radiation, contact allergens, and thermal injury are well known exogenous stimuli which activate keratinocytes to synthesize cytokines and thereby initiate inflammation. In many inflammatory diseases of the skin, the nature of initial events leading to inflammation are not known but the endogenous stimuli which influence epidermal keratinocytes may be provided by infiltrating cells, e.g., monocytes/macrophages, neutrophils, and T cells. These cells may do this in two possible ways: (1) by releasing mediators such as cytokines which in turn induce keratinocytes to produce additional mediators such as cytokines, chemokines, and complement components and (2) by interacting with keratinocytes in inflammatory environment through some ligands (e.g., CD40L on T cells) and activating them to produce additional inflammatory mediators. Although some information is available on these two modes of participation of keratinocytes in skin inflammation, it is far from complete. In this thesis we present work focused on the production, expression, and regulation of complement proteins and some chemokines in keratinocytes by some endogenous and exogenous stimuli.

Chapter 1 of this thesis presents a brief introduction into current knowledge on mechanisms leading to inflammatory reactions of the skin. This is followed by a comparatively more detailed description of the complement system and cytokines/chemokines network. The discussion on the complement system includes its two pathways and the regulation of their activation by fluid phase and membrane embedded regulators. The discussion on cytokines includes a short description of a number of well known cytokines which are known to be present amongst the inflammatory mediators of mononuclear cells, namely IL-1\(\alpha\), IL-6, TNF-\(\alpha\), IFN-\(\gamma\), and TGF-\(\beta\) and whose influences on pro-inflammatory properties of keratinocytes have been
investigated in subsequent chapters of this thesis. A brief overview of the chemokines is given with special reference to the three chemokines, IL-8, RANTES, and MCP-1, the regulation of whose synthesis in keratinocytes is studied in this thesis in view of their chemotactic properties relevant to the pathogenesis of inflammatory diseases of the skin. Since keratinocytes are the most abundant cells in the epidermal compartment of the skin, this chapter also includes an overview of the production, expression, and functional relevance of complement proteins and chemokines in these cells.

In chapter 2 to 6, the results of a number of in vitro studies on the regulation of synthesis of fluid phase complement proteins and chemokines and the regulation of expression of complement regulatory proteins in keratinocytes are described.

Human keratinocytes have been shown to synthesize two components of complement, namely C3 and factor B\textsuperscript{34,35}. Further studies may show that they synthesize other components as well. They may even be found to produce complete classical and alternative pathway cascades as has been shown in other cell types\textsuperscript{92,96}. As regards constitutive synthesis of C3 and factor B in vitro, it was quite low\textsuperscript{34,35}. From this it may be deduced that in normal skin the synthesis of these components may also be low. The ability of keratinocytes and inflammatory cells to produce a number of cytokines under inflammatory conditions raises the prospects that some of the cytokines may regulate the synthesis of C3 and factor B by keratinocytes. We have investigated if cytokines released from activated mononuclear cells, many of them also produced by keratinocytes, are involved in regulation of C3 and factor B synthesis in keratinocytes. Our data presented in Chapter 2 clearly show that several of these cytokines strongly, but differentially, regulate the synthesis of C3 and factor B from keratinocytes in vitro. IFN-\gamma, and to a lesser extent IL-1\alpha, were found to be inducers for production of both C3 and factor B by keratinocytes, while TNF-\alpha was capable of up-regulating the production of C3 without affecting production of factor B. IL-6 had stimulatory effects on the production of factor B only. The regulatory effects of these cytokines were observed both at protein and mRNA level. Thus, these results clearly demonstrated an important role of cytokines in differentially regulating the complement component production in human keratinocytes.

Complement components produced in high amounts by keratinocytes in response to cytokines, as shown in chapter 2, may potentially damage autologous epidermal cells, since complement activation products, such as C3b, cannot distinguish between invading microbes and self cells. This damage must be prevented by complement regulatory proteins MCP, DAF, and CD59, which inactivate complement activation products deposited on cell membranes. These proteins or most of them should therefore be expressed on the surfaces of keratinocytes, among other cells of the epidermis. Under inflammatory conditions when there is up-regulation of complement synthesis by keratinocytes under the influence of cytokines (see Chapter 2), there should also be up-regulation of the expression of complement regulatory proteins on
keratinocytes to protect them from excessively produced complement. We investigated if cytokines released from activated mononuclear cells, many of them also produced by keratinocytes, are involved in up-regulation of expression of cell surface complement regulatory proteins. Chapter 3 describes the flow cytometric studies on the effects of mixture of cytokines collectively released from activated mononuclear cells and of individual cytokines known to be produced by inflammatory cells and keratinocytes, on the expression of cell surface complement regulatory proteins in keratinocytes. These experiments showed that supernatants of activated mononuclear cells up-regulated the expression of MCP and CD59 but not DAF. TGF-β isoforms, TGF-β1, TGF-β2, and TGF-β3, also increase the membrane expression of MCP and CD59, without affecting the expression of DAF. None of the other investigated cytokines, IL-1α, IL-2, IL-6, TNF-α, and IFN-γ, was able to induce changes in the expression of the three tested complement regulatory proteins. Experiments with supernatants of activated mononuclear cells using monoclonal antibodies to TGF-β showed that, apart from TGF-β isoforms, another unidentified factor(s) can up-regulate MCP and DAF in keratinocytes. These results supported our hypothesis that protection of keratinocytes against complement should be increased under inflammatory conditions.

It was demonstrated in chapter 2 that several cytokines differentially up-regulate the production of C3 and factor B in keratinocytes. Exogenous stimuli, such as UVB exposure, have been shown to increase the production of several cytokines from keratinocytes. UVB exposure also induces infiltration of inflammatory cells in the skin which also release several cytokines. Cytokines released from these cells may be responsible for initiation of cutaneous inflammation in response to UVB. We argued that UVB exposure of the skin may also increase the synthesis of complement components by keratinocytes. UVB could do this either directly or indirectly via some cytokines released from above mentioned cells. If synthesis of components of complement is really increased after UVB exposure as envisaged, increased levels of complement can damage keratinocytes. Therefore, a mechanism(s) must exist to protect keratinocytes from excessively produced complement in response to UVB. This mechanism could be the increased expression of complement regulatory proteins on keratinocyte membrane by UVB either directly or indirectly via some cytokines released from above mentioned cells in response to UVB. We investigated whether UVB exposure of cultured human keratinocytes can directly increase (1) the constitutive release of C3 and factor B and, (2) the surface expression of DAF, MCP and CD59. Chapter 4 describes these in vitro studies. It was concluded that UVB can not directly up-regulate the synthesis of C3 and factor B in vitro, most likely due to excessive dilution of cytokines released from keratinocytes. The possibility of up-regulation of these components in vivo was not ruled out. UVB was shown to up-regulate the expression of complement regulatory proteins MCP, DAF, and CD59. Thus, UVB increases the resistance of keratinocytes against complement attack. This increase in resistance is needed by keratinocytes to protect themselves
from complement produced excessively in response to cytokines of inflammatory cells in UVB induced cutaneous inflammation. **UVB exposure in vivo** has been shown to cause local activation of complement on keratinocytes\(^{359}\).

Keratinocytes have so far been shown to synthesize two complement components, namely C3 and factor B. Production of C3 and factor B suggests that keratinocytes may also be able to produce factor H and factor I to regulate the activity of these components. We investigated the synthesis of factor H by keratinocytes. The role of factor H in suppression of complement at C3/C5-convertase stage demands that, if produced by keratinocytes, its production be up-regulated when there is up-regulation of C3 and factor B production. C3 and factor B synthesis is up-regulated by some pro-inflammatory cytokines differentially as shown in chapter 2\(^{298}\). Thus, if synthesis of factor H occurs in keratinocytes, it should also be enhanced by some pro-inflammatory cytokines to suppress the activation of C3 and factor B and the resulting complement mediated damage of keratinocytes and other epidermal cells. In Chapter 5 we have described our **in vitro** studies on the synthesis of complement factor H in keratinocytes. Keratinocytes were found to produce both the 45-kD and 155-kD isoforms of this protein. Parallel to our previous findings on the regulation of C3 and factor B (chapter 2), factor H production was also demonstrated to be extremely responsive to IFN-\(\gamma\). Other cytokines, UVB, and LPS did not have any effect on the production of factor H. This study shows that keratinocytes are able to produce complement proteins other than C3 and factor B. More research is needed to investigate if keratinocytes, like other cell types\(^{9286}\), are capable of synthesizing other components of the complement cascade and their fluid phase regulators.

Inflammatory diseases of the skin are characterized not only by the presence of cytokines but also by the presence of inflammatory cells (e.g., lymphocytes and neutrophils) in the skin.

Some of these cells bear ligands which can interact with their receptors on keratinocytes and activate them to produce inflammatory mediators. Not much is known about these ligations. One such ligation is the interaction of CD40 with its ligand, CD40L. Keratinocytes are known to express CD40 and activated T cells transiently express CD40L. CD40 activation of keratinocytes by CD40L is known to release IL-8 and up-regulate the pro-inflammatory molecule ICAM-1\(^{147;350}\). We investigated whether CD40 activation of keratinocytes by CD40L can release other chemokines (IL-8, RANTES, and MCP-1) and complement components (C3 and factor B) and alter the expression of complement regulatory proteins (MCP, DAF, and CD59) on keratinocytes. These studies are described in Chapter 6. Using two **in vitro** models of CD40 activation of human keratinocytes we were able to demonstrate that the production of IL-8 and RANTES was strongly up-regulated, whereas the production of MCP-1 was moderately increased. We also showed that CD40 activation of keratinocytes through CD40-CD40L ligation does not affect the release of C3 and factor B nor the expression of complement regulatory proteins MCP, DAF, and
CD59. We therefore conclude that strongly increased chemokine production in some inflammatory conditions may be regulated, in part, by interaction of keratinocytes with T cells through CD40 activation. Factors other than CD40 activation may be responsible for increased production of complement components and decreased expression of MCP and CD59 observed in psoriasis.

In summary, keratinocytes produce a number of inflammatory mediators including chemokines and complement proteins. Their production is tightly and differentially regulated by cytokines, UVB, and direct interaction of keratinocytes with inflammatory cells.