

Formation of Active IL-1 β from Pro-IL-1 β Catalyzed by Stratum Corneum Chymotryptic Enzyme *In vitro*

ELISABET NYLANDER-LUNDQVIST and TORBJÖRN EGELRUD

Department of Dermatology, Umeå University, Umeå, Sweden

Interleukin 1 β (IL-1 β) is produced as a biologically inactive 31 kD precursor, which is converted to the active 18 kD form by proteolytic processing. Keratinocytes express IL-1 β but not the active form of the specific IL-1 β converting enzyme (ICE). We have recently presented evidence that IL-1 β activation in human epidermis occurs via an alternative mechanism involving hitherto unknown proteases. We asked whether stratum corneum chymotryptic enzyme (SCCE), which is a serine protease specifically expressed in keratinizing squamous epithelia, can act as an IL-1 β activator *in vitro*. Recombinant human pro-IL-1 β was incubated with recombinant SCCE, and the reaction products were characterized as regards molecular size and ability to induce expression of E-Selectin in human umbilical cord endothelial cells. SCCE caused degradation of pro-IL-1 β and the accumulation of a component with electrophoretic mobility slightly lower than recombinant mature IL-1 β . Whereas incubation mixtures with pro-IL-1 β , which had been incubated in the absence of SCCE, or with SCCE, which had been incubated in the absence of pro-IL-1 β , did not induce expression above baseline levels of E-Selectin, pro-IL-1 β which had been incubated with SCCE induced a significant increase in E-Selectin expression. This effect could be abolished by neutralizing antibodies to IL-1 β , but not by antibodies to IL-1 α . In addition to IL-1 β activation, SCCE also appeared to be able to catalyze a further degradation of IL-1 β , leading to a loss of biological activity. We conclude that SCCE is a potential candidate for being responsible for IL-1 β activation in human epidermis.

(Accepted November 29, 1996.)

Acta Derm Venereol (Stockh) 1997; 77: 203–206.

T. Egelrud, Department of Dermatology, University Hospital, S-901 85 Umeå, Sweden. E-mail: torbjorn.egelerud@dermven.umu.se

The pro-inflammatory cytokine interleukin 1 β (IL-1 β) is produced by proteolytic cleavage of its biologically inactive precursor (1–4). In cells secreting active IL-1 β the activation is catalyzed by a highly specific cysteine proteinase, ICE, which cleaves the 31 kD precursor C-terminal of Asp 116 (5–9). The resulting 18 kD C-terminal fragment is the fully active IL-1 β ("native" or "mature" IL-1 β). Active ICE is not, however, present in all cells which can produce pro-IL-1 β . An example of this is the keratinocyte (10–13). Human keratinocytes express ICE in an apparently inactive form (14). Cultured human keratinocytes have been shown to express mRNA for pro-IL-1 β (13), but the only IL-1 β secreted is the inactive precursor (15).

An alternative means of IL-1 β activation is proteolytic cleavage of the precursor at sites N-terminal of Asp 116. *In vitro* IL-1 β , with nearly full biological activity, is produced when pro-IL-1 β is treated with proteolytic enzymes such as chymotrypsin and cathepsin G, which cleave at Tyr 113, and leukocyte elastase, which cleaves at Ile 103 (16). It has

therefore been suggested that the pro-IL-1 β produced by keratinocytes may act as an enhancer of inflammatory responses after being activated extracellularly by proteolytic enzymes produced by invading inflammatory cells (17).

We have recently shown that plantar stratum corneum from individuals with no inflammatory skin disease contains high amounts of biologically active IL-1 β , with biochemical properties compatible with an activation site approximately 10 amino acid residues N-terminal of Asp 116 (18). This suggests that epidermis contains an alternative mechanism for IL-1 β activation. The nature of the enzymes involved is not known. We have described a protease, SCCE (19–22), which appears to be specific for keratinizing epithelia. SCCE is secreted to the stratum corneum intercellular space, where its function is believed to be the degradation of desmosomes as part of the events leading to desquamation (23–25). SCCE has a primary substrate specificity similar to chymotrypsin and cathepsin G (26). Since chymotrypsin and cathepsin G can transform pro-IL-1 β to biologically active IL-1 β *in vitro*, it seemed that this conversion can be catalyzed also by SCCE. In order to elucidate whether SCCE is a potential IL-1 β activator in the epidermis, we studied the effect of recombinant SCCE on pro-IL-1 β as regards degradation and biological IL-1 activity. Our results suggest that SCCE is a potential candidate for being responsible for IL-1 β activation in the epidermis.

MATERIALS AND METHODS

Recombinant human IL-1 β precursor was obtained from Cistron, Pine Brook NJ; recombinant human IL-1 β , corresponding to mature IL-1 β , was from Genzyme, Cambridge, MA. The IL-1 β specific mouse monoclonal antibody 11E (moab 11E) was from HyTest, Turku, Finland. Neutralizing polyclonal rabbit antibodies to IL-1 α and IL-1 β were from Genzyme, Cambridge, MA. Recombinant human SCCE, prepared by tryptic cleavage of recombinant pro-SCCE (19), was a gift from Symbicom AB, Umeå, Sweden. Bovine chymotrypsin was purchased from Boehringer, Mannheim, Germany.

Electrophoresis and immunoblotting

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE) under denaturing conditions in 12.5% gels was carried out according to Laemmli (27). After electrophoresis separated proteins were electrophoretically transferred to polyvinylidene difluoride membranes (BioRad, Solna, Sweden). The membranes were blocked with bovine milk proteins and immunostained with moab 11E followed by alkaline phosphatase conjugated goat anti-mouse IgG (Dakopatts, Älvsjö, Sweden). Alkaline phosphatase was detected according to Blake et al. (28) with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium as chromogenic substrate (BioRad). Prestained molecular weight standards were from BioRad.

Measurement of biological IL-1 activity

The assay was based on the ability of IL-1 to induce expression of E-Selectin in human umbilical cord vein endothelial cells (HUVEC) (29–31). The details of the method are given elsewhere (18). In brief, HUVEC (32) were incubated with samples of pro-IL-1 β , which had

been subjected to various activating conditions, or with a standard prepared from recombinant mature human IL-1 β . Dilutions of samples and IL-1 β standard were carried out in HUVEC growth medium without calf serum. In some experiments neutralizing antibodies were added to diluted samples, which were then incubated at 4°C overnight before being analyzed. Expressed E-Selectin was quantified with a two-step enzyme linked immunosorbent assay (ELISA) procedure with alkaline phosphatase-conjugated secondary antibodies, and p-nitrophenylphosphate as phosphatase substrate. A standard solution of recombinant IL-1 β was prepared assuming a specific activity of 10⁷ U/mg.

Protease treatment of IL-1 β

To 8 μ l of a mixture containing 0.05 M Tris-HCl pH 7.0, bovine serum albumin 0.1 mg/ml, and 9 ng of pro-IL-1 β , 1 μ l of SCCE or bovine chymotrypsin, appropriately diluted in 0.05 M Tris-HCl pH 7.0, was added. After incubation for 4 h at 37°C, the reactions were stopped by means of addition of either 2.5 μ l of a four times concentrated electrophoresis sample buffer containing mercaptoethanol (27), or 25 μ l of HUVEC culture medium with 10% fetal calf serum. The electrophoresis samples were heated to 90°C before being applied to the gel; samples in culture medium were stored at -20°C until analyzed for biological activity. Incubation mixtures in which either the protease or pro-IL-1 β , or both, had been substituted with 0.05 M Tris-HCl, pH 7.0, served as controls.

RESULTS AND DISCUSSION

In Fig. 1 are shown pro-IL-1 β and its degradation products, as recognized by moab 11E, after incubation with SCCE or bovine chymotrypsin. Recombinant mature IL-1 β was included for comparison. At the highest concentration of SCCE (36 μ g/ml) and with chymotrypsin at 0.6 μ g/ml one major component with an electrophoretic mobility slightly lower than mature IL-1 β was found. The staining intensity of

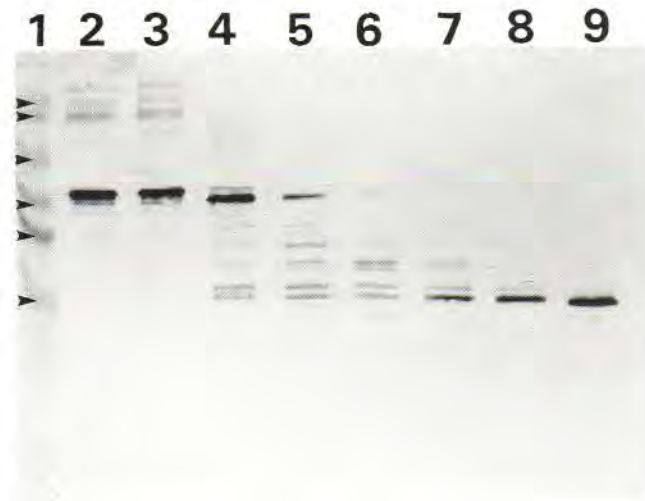


Fig. 1. Digestion of IL-1 β precursor with SCCE and chymotrypsin. Immunoblot stained with moab 11E. Lane 1: Prestained molecular weight markers; arrow heads from bottom to top: 106, 80, 49.5, 32.5, 27.5 and 18.5 kD, respectively. Lanes 2-8: Pro-IL-1 β , 9 ng in a total volume of 10 μ l was incubated at 37°C with no enzyme, 0 h (lane 2), no enzyme, 4 h (lane 3), SCCE 6 ng, 4 h (lane 4), SCCE 23 ng, 4 h (lane 5), SCCE 90 ng, 4 h (lane 6), SCCE 360 ng, 4 h (lane 7), chymotrypsin 6 ng, 4 h (lane 8). Lane 9: Mature recombinant IL-1 β , 4 ng. The high molecular weight components seen in lanes 2 and 3 probably represent aggregates of pro-IL-1 β . They were seen also in samples without albumin (not shown).

this component, after incubation with SCCE as well as with chymotrypsin, was lower than that of the major 31 kD component in samples containing pro-IL-1 β incubated in the absence of proteases. This difference could not be accounted for by components with molecular weights higher than the major component. If it is assumed that moab 11E has the same affinity for its epitope in pro-IL-1 β as in the detected degradation products, the explanation of this decrease in staining intensity may be that both enzymes catalyzed a further degradation of a fraction of the IL-1 β -precursor beyond a state where the products could be detected, either due to destruction of the epitope recognized by the antibody on immunoblots, or the degradation products being too small to be detected. The 18 kD component formed by chymotrypsin has previously been shown to have around 40% of the biological activity of mature IL-1 β (16). A component which had the same electrophoretic mobility as the component formed by chymotrypsin was found in samples incubated with SCCE at concentrations varying between 0.6 and 36 μ g/ml. It therefore seemed possible that SCCE could act as a pro-IL-1 β activator in the same way as chymotrypsin, albeit with lower efficiency. In addition to the major 18 kD component obtained with SCCE at 36 μ g/ml and chymotrypsin at 0.6 μ g/ml, components with apparent molecular weights intermediate between those of pro-IL-1 β and the 18 kD component were produced. This was most clearly seen at lower enzyme concentrations (Fig. 1; results for chymotrypsin at lower concentrations not shown). This suggests that both SCCE and chymotrypsin catalyze the cleavage of the IL-1 β precursor at several sites N-terminal of Asp 116.

The electrophoretic analyses thus suggested that active IL-1 β could be produced from pro-IL-1 β in a proteolytic reaction catalyzed by SCCE. To further investigate this possibility, the ability of pro-IL-1 β , which had been incubated with various concentrations of SCCE, to induce expression of E-Selectin in HUVEC was investigated. We used an ELISA method in which the increase in absorbance at 405 nm is a function of the amount of E-Selectin expressed by the cells. As shown in Fig. 2A, pro-IL-1 β which had been incubated in the absence of SCCE did not induce more expression of E-Selectin than culture medium without additions (cf "0 ng SCCE" and "Control 1" in Fig. 2A). Pro-IL-1 β which had been incubated with SCCE, on the other hand, caused a marked stimulation of E-Selectin expression. Incubation mixtures with SCCE but no pro-IL-1 β caused no increase in E-Selectin expression (Fig. 2A, "Control 2"). The stimulation of E-Selectin expression after incubation of pro-IL-1 β with SCCE was inhibited by antibodies to IL-1 β , but not by antibodies to IL-1 α (Fig. 3). It could thus be concluded that incubation of pro-IL-1 β with recombinant SCCE resulted in the production of components with IL-1 β -like biological activity.

The results presented in Fig. 1 gave evidence that SCCE as well as chymotrypsin, in addition to processing biologically inactive pro-IL-1 β to components with IL-1 β activity, catalyzed a further degradation of active IL-1 β . In order to get further information on this point we calculated the expected maximum yield of biologically active IL-1 β from the amount of pro-IL-1 β in the incubation mixtures and compared these values with the amounts of biologically active IL-1 β obtained after incubation of the precursor with the two enzymes. For this purpose a standard curve for the assay of biologically active IL-1 with mature recombinant human IL-1 β was con-

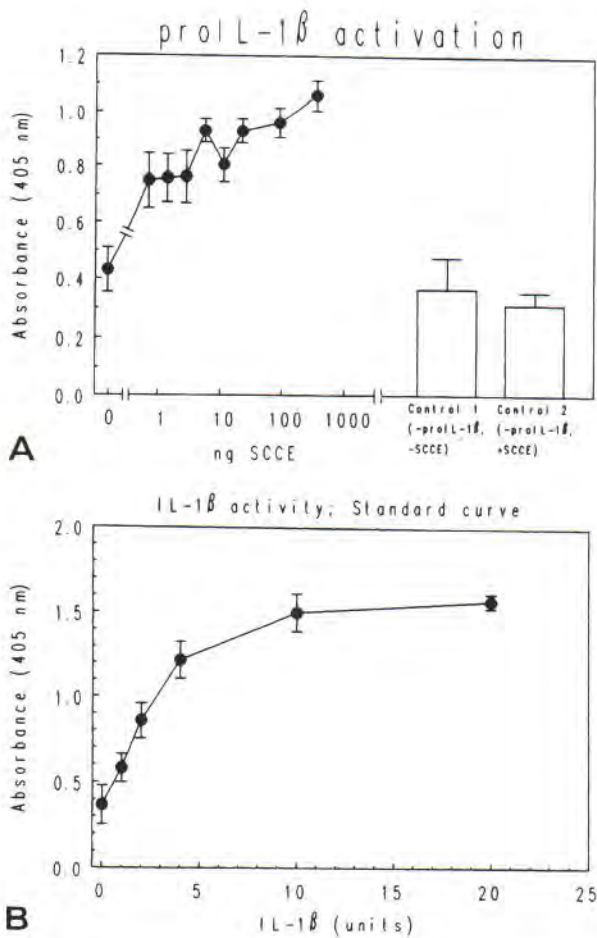


Fig. 2. (A) Biological activity of IL-1 β precursor digested with SCCE. Absorbance at 405 nm is a measure of E-Selectin expression in HUVEC. Pro-IL-1 β , 9 ng, in a total volume of 10 μ l, was incubated at 37°C for 4 h with various amounts of SCCE as indicated prior to the assay. In control no. 2 the amount of SCCE in the incubation mixture was 360 ng. (B) Standard curve for biological IL-1 β activity. Absorbance at 405 nm is a measure of E-Selectin expression in HUVEC induced by various amounts of recombinant mature IL-1 β as indicated. The standard solution of IL-1 β was prepared assuming a specific activity of 10 units/ng. Error bars = standard deviation, $n=8$.

structed. The results, Fig. 2B and Table I, show that the yield of biologically active IL-1 β was at most only around 6% and 15% of the calculated maximum yield for SCCE and chymotrypsin respectively. Even if the fact that IL-1 β activated with enzymes other than ICE may have a specific activity that is only 20–40% of the specific activity of mature IL-1 β is taken into account (16), these results together with the results presented in Fig. 1 suggest that SCCE and chymotrypsin can not only produce active IL-1 β from pro-IL-1 β , but also degrade active IL-1 β , thereby making it biologically inactive. This was supported by results showing that incubation of pro-IL-1 β with 6 ng of SCCE produced almost the same amount of biological IL-1 activity as incubation with 360 ng of SCCE (Table I). This was in spite of the fact that most of the material reacting with the IL-1 β antibody still had a molecular weight close to that of the intact IL-1 β precursor after incubation at the lower enzyme concentration (Fig. 1, lane 4). Whether or not the inactivation involves C-terminal cleavage cannot be decided at present.

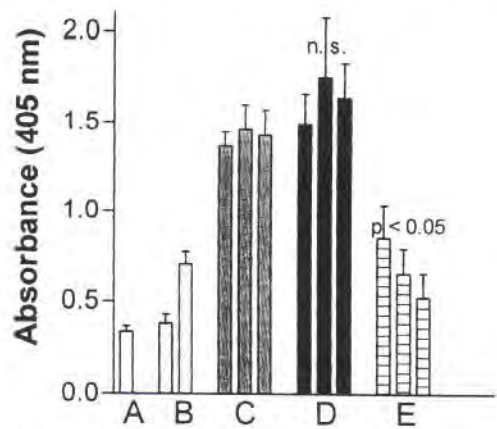


Fig. 3. Effect of neutralizing antibodies on the stimulation of E-Selectin expression in HUVEC, measured as absorbance at 405 nm, of pro-IL-1 β treated with SCCE. Pro-IL-1 β , 9 ng in a total volume of 10 μ l was incubated at 37°C with 90 ng SCCE for 4 h. After the reactions had been stopped the samples were diluted in growth medium with 200 μ g/ml of antibodies and incubated at 4°C over night before being added to HUVEC. Each bar represents one incubation mixture. A: Negative control (HUVEC incubated in medium only), B: Incubations were carried out with pro-IL-1 β but no SCCE., C: Incubations were carried out with pro-IL-1 β and SCCE; no antibodies were added, D: As C, but rabbit anti-IL-1 α was added, E: As C, but rabbit anti-IL-1 β was added. Error bars = standard deviation, $n=6-8$. D and E were compared with C by means of Student's *t*-test.

Table I. Amounts of biologically active IL-1 β formed from 9 ng of IL-1 β precursor after incubation with various amounts of SCCE and chymotrypsin in a total volume of 10 μ l, as described under Materials and Methods

Enzyme	IL-1 (Units)	Yield (%)*
SCCE 360 ng	3.0	5.6
SCCE 11 ng	2.0	3.7
SCCE 6 ng	2.5	4.6
SCCE 0.7 ng	1.5	2.8
Chymotrypsin 6 ng	8.3	15.4
Chymotrypsin 0.6 ng	4.8	8.9

*The theoretical maximal yield of active IL-1 β from 9 ng of precursor, corresponding to 5.4 ng mature IL-1 β , is 54 units.

Keratinocytes constitutively express mRNA for pro-IL-1 β as well as IL-1 α , (10, 11, 13) and this expression can be upregulated by a number of stimuli (33, 34). In some instances the upregulation of pro-IL-1 β exceeds that of IL-1 α (34). When the potential role of IL-1 produced by keratinocytes in host defence and pathological conditions is considered, the means by which pro-IL-1 β is activated in the epidermis must be taken into account. Activation catalyzed by enzymes normally present in the epidermis on one hand, and activation by enzymes released from invading inflammatory cells on the other (17), will most likely differ as regards physiological and pathophysiological consequences. We have recently presented evidence of IL-1 β activation in non-inflamed epidermis (18). The enzymes involved may be potential targets for pharmacological interventions. In this work we have presented evidence that SCCE, i. e. an enzyme generally present in the epidermis, can act as an IL-1 β activating enzyme in vitro. Whether SCCE has this capacity also in vivo, remains to be elucidated as well

as the true nature of the mechanisms responsible for IL-1 β activation in the epidermis.

ACKNOWLEDGEMENTS

The technical assistance by Astrid Lundgren is gratefully acknowledged. This work was supported by the Swedish Medical Research Council (grant no. B95-12X-11206-01A), the Edvard Welander Foundation, the Finsen Foundation, the Swedish Psoriasis Association, the Medical Faculty, Umeå University, and Astra-Hässle AB.

REFERENCES

- Dinarelo CA. Interleukin-1. *Rev Infect Dis* 1984; 6: 51-95.
- Oppenheim JJ, Kovacs EJ, Matsushima K, Durum SK. There is more than one interleukin-1. *Immunol Today* 1986; 2: 45-56.
- Dinarelo CA, Savage N. Interleukin-1 and its receptor. *Crit Rev Immunol* 1989; 9: 1-20.
- Schmidt JA. Purification and partial biochemical characterization of normal human interleukin 1. *J Exp Med* 1984; 160: 772-787.
- Black RA, Kronheim SR, Sleath PR. Activation of interleukin-1 beta by a co-induced protease. *FEBS Lett* 1989; 247: 386-390.
- Sleath PR, Hendrickson RC, Kronheim SR, March CJ, Black RA. Substrate specificity of the protease that processes human interleukin-1 beta. *J Biol Chem* 1990; 265: 14526-14528.
- Kronheim SR, Mumma A, Greenstreet T, Glackin PJ, Van Ness K, March CJ, et al. Purification of interleukin-1 beta converting enzyme, the protease that cleaves the interleukin-1 beta precursor. *Arch Biochem Biophys* 1992; 296: 698-703.
- Cerretti DP, Kozlosky CJ, Mosley B, Nelson N, Van Ness K, Greenstreet TA, et al. Molecular cloning of the interleukin-1 beta converting enzyme. *Science* 1992; 256: 97-100.
- Thornberry NA. Interleukin-1 beta converting enzyme. *Meth Enzymol* 1994; 244: 615-631.
- McKenzie RC, Sauder DN. The role of keratinocyte cytokines in inflammation and immunity. *J Invest Dermatol* 1990; 95: 105S-107S.
- Bell TV, Harley CB, Stetsko D, Sauder DN. Expression of mRNA homologous to interleukin 1 in human epidermal cells. *J Invest Dermatol* 1987; 88: 375-379.
- Barker JN, Mitra RS, Griffiths CE, Dixit VM, Nickoloff BJ. Keratinocytes as initiators of inflammation. *Lancet* 1991; 337: 211-214.
- Kupper TS, Ballard DW, Chua AO, McGuire JS, Flood PM, Horowitz MC, et al. Human keratinocytes contain mRNA indistinguishable from monocyte interleukin 1 alpha and beta mRNA. Keratinocyte epidermal cell-derived thymocyte-activating factor is identical to interleukin 1. *J Exp Med* 1986; 164: 2095-2100.
- Groves RJ, Allen H, Kupper TS. Production of IL-1-beta convertase (ICE) without detectable pro-IL-1-beta processing by human keratinocytes. *J Invest Dermatol* 1996; 106: 814 (abstr.).
- Mizutani H, Black R, Kupper TS. Human keratinocytes produce but do not process pro-interleukin-1 (IL-1) beta. Different strategies of IL-1 production and processing in monocytes and keratinocytes. *J Clin Invest* 1991; 87: 1066-1071.
- Hazuda DJ, Strickler J, Simon P, Young PR. Structure-function mapping of interleukin 1 precursors. Cleavage leads to a conformational change in the mature protein. *J Biol Chem* 1991; 266: 7081-7086.
- Kupper TS. Immune and inflammatory processes in cutaneous tissues. Mechanisms and speculations. *J Clin Invest* 1990; 86: 1783-1789.
- Nylander-Lundqvist E, Bäck O, Egelrud T. IL-1-beta activation in human epidermis. *J Immunol* 1996; 157: 1699-1704.
- Hansson L, Strömqvist M, Bäckman A, Wallbrandt P, Carlstein A, Egelrud T. Cloning, expression, and characterization of stratum corneum chymotryptic enzyme. A skin-specific human serine proteinase. *J Biol Chem* 1994; 269: 19420-19426.
- Egelrud T. Purification and preliminary characterization of stratum corneum chymotryptic enzyme: a proteinase that may be involved in desquamation. *J Invest Dermatol* 1993; 101: 200-204.
- Egelrud T, Lundström A. A chymotrypsin-like proteinase that may be involved in desquamation in plantar stratum corneum. *Arch Dermatol Res* 1991; 283: 108-112.
- Lundström A, Egelrud T. Stratum corneum chymotryptic enzyme: a proteinase which may be generally present in the stratum corneum and with a possible involvement in desquamation. *Acta Derm Venereol (Stockh)* 1991; 71: 471-474.
- Sondell B, Thornell LE, Egelrud T. Evidence that stratum corneum chymotryptic enzyme is transported to the stratum corneum extracellular space via lamellar bodies. *J Invest Dermatol* 1995; 104: 819-823.
- Lundström A, Egelrud T. Evidence that cell shedding from plantar stratum corneum in vitro involves endogenous proteolysis of the desmosomal protein desmoglein I. *J Invest Dermatol* 1990; 94: 216-220.
- Lundström A, Egelrud T. Cell shedding from human plantar skin in vitro: evidence of its dependence on endogenous proteolysis. *J Invest Dermatol* 1988; 91: 340-343.
- Skytt A, Strömqvist M, Egelrud T. Primary substrate specificity of recombinant human stratum corneum chymotryptic enzyme. *Biochem Biophys Res Commun* 1995; 211: 586-589.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; 227: 680-685.
- Blake MS, Johnston KH, Russell Jones GJ, Gotschlich EC. A rapid, sensitive method for detection of alkaline phosphatase-conjugated anti-antibody on Western blots. *Anal Biochem* 1984; 136: 175-179.
- Bevilacqua MP, Stengelin S, Gimbrone Jr MA, Seed B. Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science* 1989; 243: 1160-1165.
- Graber N, Gopal TV, Wilson D, Beall LD, Polte T, Newman W. T cells bind to cytokine-activated endothelial cells via a novel, inducible sialoglycoprotein and endothelial leukocyte adhesion molecule-1. *J Immunol* 1990; 145: 819-830.
- Kuijpers TW, Hakkert BC, Hoogerwerf M, Leeuwenberg JF, Roos D. Role of endothelial leukocyte adhesion molecule-1 and platelet-activating factor in neutrophil adherence to IL-1-prestimulated endothelial cells. Endothelial leukocyte adhesion molecule-1-mediated CD18 activation. *J Immunol* 1991; 147: 1369-1376.
- Nylander-Lundqvist E, Bäck O, Nilsson TK, Rantapää Dahlqvist S. Prevalence of anti-endothelial cell antibodies in patients with autoimmune diseases. *Clin Rheumatol* 1992; 11: 248-253.
- Luger TA, Schwarz T. Evidence for an epidermal cytokine network. *J Invest Dermatol* 1990; 95: 100S-104S.
- Wood LC, Jackson SM, Elias PM, Grunfeld C, Feingold KR. Cutaneous barrier perturbation stimulates cytokine production in the epidermis of mice. *J Clin Invest* 1992; 90: 482-487.