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ATOPIC DERMATITIS

aspects of defence defects

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To Louis and Carl Johan

ABSTRACT

Atopic dermatitis (AD) is an inflammatory skin disease, typically with a chronic relapsing course and a defective skin barrier function. Recently, mutations of the skin barrier gene encoding filaggrin have been reported in a portion of the patients. In this thesis some aspects of defence defects in AD were studied.

In **paper I**, the risk of developing any cancer was increased by 13%. Excess risks were observed for cancers of the esophagus, pancreas, brain, and lung and for lymphoma. There was a nonsignificant 50% excess risk for nonmelanoma skin cancer. Malignant melanoma did not occur more often than expected. The observed risk elevations, all of borderline statistical significance, should be interpreted cautiously. We could not control for possible confounding by cases of cancer caused by smoking, and the combination of multiple significance testing and few observed patients may have generated chance findings.

In **paper II**, the findings suggest that a moisturiser containing both urea and sodium chloride seems somewhat more effective than the same moisturiser without sodium chloride, at least concerning the ability to reverse impedance indices of atopic skin towards normal, an effect ascribed mainly to changes in hydration of the stratum corneum. However, the clinical significance of the impedance measurements is somewhat premature to decide.

In **paper III**, the findings indicate that barrier function and hydration, and certain patterns of electrical impedance of AD skin are abnormal compared with normal skin. Moreover, there was an increase in hydration in patients' skin after treatment and a reversal of certain impedance indices towards normal. The findings demonstrate that the used moisturiser changes some biophysical parameters when applied to atopic skin. In addition, a technique based on electrical impedance seems to give valuable information in atopic skin studies, especially the effects of moisturisers.

In **paper IV**, the distribution of somatostatin receptors (SSTR) 1-5 in skin from patients with AD or psoriasis as well as normal control skin was studied. Normal human skin and lesional skin from patients with psoriasis or atopic dermatitis showed many similarities, but also some differences, as regards SSTR expression. The wide distribution and expression pattern of all five SSTRs in human skin suggest that somatostatin is involved in the interactions between the nervous system and the skin.

LIST OF PUBLICATIONS

This thesis is based on the following four articles, which will be referred to by their Roman numbers:

I. Hagströmer L, Ye W, Nyrén O, Emtestam L. Incidence of cancer among patients with atopic dermatitis. **Arch Dermatol**. 2005;141:1123-1127.

II. Hagströmer L, Nyrén M, Emtestam L. Do urea and sodium chloride together increase the efficacy of moisturisers in atopic dermatitis skin? A comparative, double-blind, randomised study. **Skin Pharmacol Appl Skin Physiol** 2001:14;27-33.

III. Hagströmer L, Kuzmina N, Lapins J, Talme T, Emtestam L. Biophysical assessment of atopic dermatitis skin and effects of a moisturiser. **Clin Exp Dermatol**. 2006:31;272-277.

IV. Hagströmer L, Emtestam L, Stridsberg M, Talme T. Expression pattern of somatostatin receptor subtypes 1 to 5 in human skin: an immunohistochemical study of healthy subjects and patients with psoriasis or atopic dermatitis. **Exp Dermatol**. 2006:15;950-957.

RELATED PUBLICATIONS

- Dou Y, Hagströmer L, Emtestam L, Johansson O. Increased nerve growth factor and its receptors in atopic dermatitis: an immunohistochemical study. **Arch Dermatol Res** 2006:298;31-37.
- Kuzmina N, Hagströmer L, Nyrén M, Emtestam L. Basal electrical impedance in relation to sodium lauryl sulphate-induced skin reactions - a comparison of patients with eczema and healthy controls. **Skin Res Technol**. 2003:9;357-362.
- Kuzmina N, Hagströmer L, Emtestam L. Urea and sodium chloride in moisturisers for skin of elderly: a comparative, double-blind, randomised study. **Skin Pharmacol Appl Skin Physiol** 2002:15;166-174.
- Nyrén M, Hagströmer L, Emtestam L. Instrumental measurement of the Mantoux test: Differential effects of tuberculin and sodium lauryl sulphate on impedance response pattern in human skin. **Dermatology** 2000:201;212-217.
- Nyrén, M, Hagströmer L, Emtestam L. On assessment of skin reactivity using electrical impedance. In: Electrical bioimpedance methods: applications to medicine and biotechnology. Riu PJ, Rosell J, Bragós R, Casas Ó (Eds). **Ann N Y Acad Sci** 1999:873; pp. 214-220.

CONTENTS

LIST OF ABBREVIATIONS

AD	atopic dermatitis
CI	confidence interval
CNS	central nervous system
FFA	free fatty acid
FLG	filaggrin
i.e.	*id est* (latin) that is
IgE	immunoglobulin
IL	interleukin
IMP	impedance
IMIX	imaginary part index
kHz	kilohertz
MHz	megahertz
MIX	magnitude index
NMF	natural moisturising factor
PIX	phase index
P	P-value
PCA	pyrrolidone carboxylic acid
RIX	real part index
SC	stratum corneum
SMR	standardized mortality ratio
SIR	standard incidence ratio
SOM	somatostatin
SP	neuropeptide substance P
SSTR	somatostatin receptor
TEWL	trans epidermal water loss
UV	ultraviolet
VIP	vasoactive intestinal peptide

INTRODUCTION

Atopic dermatitis (AD) is a disease that afflicts 15 - 30 % of all children and 2 – 10% of all adults in the industrialised world, with an increase during the last decades.[1] AD is a chronic recurrent inflammatory skin disease, related to other diseases like allergic rhinitis, allergic conjunctivitis and asthma. Usually, AD first appears before the age of 2, but can also first appear during adulthood. The extent of the lesions varies depending on the age of the patients: on cheeks during infancy, in flexures in adolescence, while in adulthood it will manifest itself in the face, neck, upper part of the thorax and around the wrists, and also in the flexures. This requires treatment during longer periods of time for the patient, which would involve periods with extreme itching, impaired sleep at night and difficulties concentrating which in their turn would affect the patients, or patients family's, quality of life. The eczema of some patients is so serious that they have to be in hospital care. AD is a complex genetic disease, since it develops interactions between different genes and the environment. The disease genes are encoding two major groups of elements: epidermal or other epithelial structural proteins, and important elements of the immune system. [2]

The skin consists of different layers, subcutaneous tissue, dermis and epidermis. Subcutaneous tissue houses larger blood vessels and nerves and is important for the thermoregulation. The thickness of this tissue varies depending on the body site and from person to person. Dermis varies in thickness depending on the location, 0,3 mm on the eyelid and 3,0 mm on the back. Dermis consists of two layers, the papillary and reticular dermis. The papillary layer contains a thin arrangement of collagen fibres and reticular layers of thick collagen fibres, arranged parallelly to the surface of the skin. The specialised cells in dermis are hair follicles, sebaceous, appocrine and eccrine glands. It also contains blood vessels and nerves (transmits sensations of pain, itch and temperature), Meissner´s and Vater –Pacini corpuscles (transmits the sensations of touch and pressure). The thickness of epidermis varies, from 0.5 mm on the eyelids to 1.5 mm on the palms and soles. The epidermis can be divided into four distinct layers: stratum basale, stratum spinosum, stratum granulosum and stratum corneum. 95% of the epidermis consists of keratinocytes. The upper layer of the epidermis, the stratum corneum (horny layer), consisting of corneocytes, acts as a physical barrier towards the surrounding environment but also as a barrier against, for example, viruses, UV radiation, bacteria and fungi which cause a continues loss of material. This layer is created by the differentiation of the keratinocytes when they migrate from the basal membrane through the dermis to the epidermis in order to keep the skin barrier intact. The stratum corneum has an average of 20 corneocyte layers and each corneocyte is approximately 0,030 mm in diameter. The barrier homeostasis is under strict control and seems to be under the influence by TEWL, calcium, sodium, potassium within the epidermis and pH-gradient across the stratum corneum.[3, 4] A corneocyte contains several proteins, including keratin and filaggrin (FLG), inside a protein envelope (cornified envelope) which is highly hydrophilic material and therefore able to bind a lot of water. The keratin serves as a cytoskeleton in the corneocytes. FLG polypeptides contribute to form the keratin by aggregation and binding to the cytoskeleton, which in upper granular cells has been strongly anchored to the cellmembrane by increasing number of desmosomal proteins (Figure 1). FLG collapses the cytoskeleton resulting in flattening of keratinocytes into squames. The condensed protein-lipid package is heavily crosslinked by transglutaminase to form the epidermal barrier.[5]

This complex impedes the entry of allergens, toxic chemicals and infectious organisms. Breakdown products of FLG also contribute to the water binding capacity of the stratum corneum[2] and impedes the TEWL. Decreased level of FLG may lead to decreased ability of the corneocytes to retain water, resulting in their shrinkage. There will be a gap between each corneocyte as they shrink, resulting in a defective barrier, which will be vulnerable to the penetration of allergens and irritants. An intact epidermal function is needed for the skin to act as a physical and chemical barrier. The barrier itself is in the stratum corneum.[6] Barrier damage causes the typically increased transepidermal water loss in AD. Variations in the pH, deficit of the ceramides of the stratum corneum can disturb maturation of lamellar bodies which affects the barrier.[7] Also, changes in the expression of enzymes involved in the balance of epidermal adhesion molecules may contribute to the breakdown of the epidermal barrier in patients with AD.[8, 9] Recent studies have shown the importance of FLG mutations in AD. Several loss-of-function mutations of the gene have been identified in European patients with atopic dermatitis.[10-13] Mutations of FLG occur mainly in early-onset atopic dermatitis and indicate a connection with asthma. FLG mutations are identified in only 30% of European patients with atopic dermatitis, meaning that genetic variants of other epidermal structures may be important.[9, 14] FLG contributes to the keratin cytoskeleton by acting as the template for the assembly of the cornified envelope. Also, breakdown products of FLG contribute to the water-binding capacity of the stratum corneum.[15] Genetic variants of FLG in AD are also likely to contribute to barrier dysfunction.[16] Interestingly, inflammation can change the expression of FLG genes and others that are involved in the barrier function,[17] which may lead to increased transepidermal penetration of environmental allergens[18] and, together with pruritus, favour inflammation and sensitisation.[19, 20] The corneocytes are attached to each other through desmosomes (proteins) which prevent the cells from moving in relation to each other, disrupting the lipid structure in the extracellular space. They also prevent an increased space between the corneocytes due to mechanical influence. The lipids in the extracellular space consist mainly of free fatty acids, cholesterol and ceramides. Theses lipids have a hydrophilic head group and a hydrophobic part. They tend to aggregate and thereby form a bilayer.[21] There are many factors that have an impact of how stable these aggregates are, for example temperature, pH and the length of the carbon chain. The temperature has an influence on the formation of the lipid layer, whether it will be in a crystalline state or in a liquid crystalline state.

A number of investigations have shown the transitions temperature of the skin barrier is around 40 C. The temperature is around 30 C in normal skin and therefore the skin barrier will be essentially impermeable to water. Changes of the pH in stratum corneum can afflict the ceramides and impair the barrier. The stratum corneum is formed like a brick and mortal structure, with keratin filled corneocytes as bricks and the intercellular lipids as mortar.[22] Other cells in epidermis are melanocytes, Langerhan's cells and Merkel's cells.

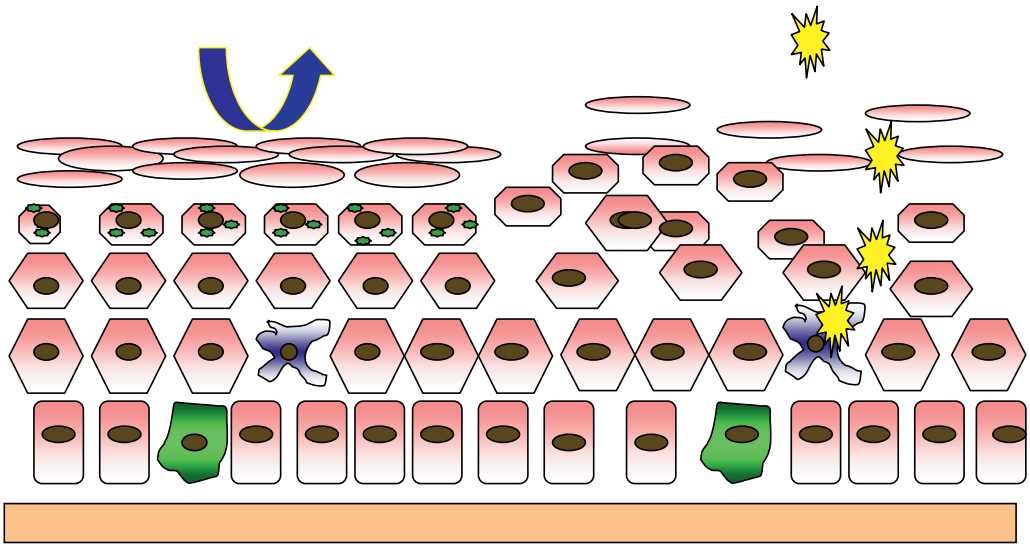

***Figure 1*. Filaggrin: left, normal skin; right, atopic dermatitis skin**

These cells are all specialized. Melanocytes form a close association to 30-40 keratinocytes in the basal layer with their dendrites. This makes it possible to transfer melanin into keratinocytes. It has also been suggested that melanocytes could act as regulators of the skin's immune responses by producing a number of cytokines, including IL-1, IL-6,IL-3 , TNF alfa[23] and NO.[24] Langerhans's cells are located in stratum basale or/and in stratum spinosum and account for 3% to 5% of the cells in the epidermis. These cells originate from the bone marrow and move in and out of the epidermis as required. They also have a dendritic form similar to melanocytes and contain Bierbeck's granula. They are involved in the immune system and present the antigen to immunocompetent T-lymfocytes by phagocytosis. Epidermal dendritic cells in AD bear IgE[25] and express its high-affinity receptor (FcεRI).[26, 27] Langerhans cells are found in normal skin, but inflammatory dendritic epidermal cells appear only when the skin is inflamed. They take up and present allergens to Th1 and Th2 cells. When the allergens are captured, Langerhans cells contribute to Th2 polarisation by an unknown mechanism, and inflammatory dendritic epidermal cells lead to Th1 polarisation. In the atopy patch test, which may be regarded as an experimental model for AD, many inflammatory dendritic epidermal cells invade the epidermis three days after challenge, and FcεRI is upregulated.[28]

Epithelial cells between the skin and the environment are the first line of defense of the innate immune system.[29] This system comprises of different structures, including toll-like receptors (TLR),[30] C-type lectins and peptidoglycan-recognition proteins.[31] They bind to various microbial antigenic structures. TLR-mediated activation of epithelial cells includes the production of defensins and cathelicidins.[29] Lesional and normal appearing skin in AD is colonised by microbes such as *S. aureus* or malassezia. *S. aureus* suppresses the innate immune system of the skin by the inflammation of AD. This explains the colonisation of the skin by *S. aureus* in most patients with AD, which also contributes to allergic sensitisation and inflammation. Scratching increases the binding of *S. aureus* to the skin, and the increased

activity of *S. aureus*–derived ceramidase may worsen the defective skin barrier. *S. aureus* toxins[32] increase the inflammation in AD and provoke the generation of specific IgE, which correlates with the severity of the disease.[33] They also up-regulate the expression of the skin-homing receptor cutaneous lymphocyte-associated antigen on T cells and the production of chemokines that recruit T cells.

Serum samples from patients with severe atopic dermatitis also contain IgE antibodies against proteins from keratinocytes and endothelial cells.[34 35] The serum levels of these autoantibodies correlate well with disease severity. Scratching probably releases intracellular proteins from keratinocytes. These proteins share molecular parts of microbial structures and thus could induce IgE autoantibodies.[36] About 25% of adults with AD have IgE antibodies against self-proteins.[37] In these patients, early-onset atopic dermatitis, intense pruritus, recurrent bacterial skin infections, and high serum IgE levels are typical. IgE antibodies in AD can be induced by environmental allergens, but IgE antibodies against autoantigens in the skin can perpetuate the allergic inflammation. Thus, AD has been proposed to stand between allergy and autoimmunity.[2]

One classification distinguishes an IgE-associated variant of AD (true atopic dermatitis, previously called "extrinsic" AD) from a non–IgE-associated form ("nonatopic" dermatitis, previously called "intrinsic" AD).[38] This would mean that nonatopic dermatitis and AD are two different diseases. However, since dry skin is an important sign of both variants, and the absence of IgE-mediated sensitisation may be only a transient factor, an interesting hypothesis was recently put forward by Bieber.[2] According to his hypothesis, there is a new picture, in which the natural history of atopic dermatitis has three phases. The initial phase is the non-atopic form of dermatitis in early infancy, yet with no sensitisation. Next step, in approximately three quarters of the patients, genetic factors influence the induction of IgE-mediated sensitisation to food and/or other allergens. This would be the transition to true AD. Third, still according to Bieber, scratching damages skin cells, which release autoantigens that induce IgE-autoantibodies in a high proportion of patients with AD. [2]

As for treatment, since the barrier dysfunction of the skin and chronic inflammation are characteristic of AD, prevention is important, individually designed skin care, reduction of bacterial colonisation through local application of antiseptic lotions. The control of inflammation by regular use of topical corticosteroids or topical calcineurin inhibitors in combination with emollients.

Cancer incidence

50.000 persons are diagnosed with cancer each year in Sweden. The National Swedish Cancer Register started in 1958.[39] Between the years 1958 and 2006, 2.1 million tumors were registered and 1.9 million persons were diagnosed with cancer. In 2003 and thereafter, the registration of tumours in Sweden was also to include basal cell carcinoma. Squamous cell carcinoma in skin is one of the most rapidly increasing malignancies arising through mutations in keratinocytes in epidermis in sun exposed areas. UVB-radiation damages the DNA and its repair system and causes mutations in tumor-suppressing genes. The risk of metastasis increases if the mutated

cells penetrate the dermis. Other risk factors for squamous cell carcinoma are suppressed immunosystem, exposure to cyclic aromatic hydrocarbons in tar, or long standing inflammation. Basal cell carcinoma arises from basal keratinocytes in epidermis. UVB is also in this kind of tumour important for the induction of damaging the DNA and its repair system, and also causing mutations in tumour-suppressing genes. Basal cell carcinoma grows by direct extension and does not normally metastasise. Malignant melanoma is a malignancy of melanocytes located predominantly in the skin but is also found in for example eyes, ears, CNS and GI-tract. Malignant melanoma accounts for roughly 4% of skin cancers, but is responsible for more than 74% skin cancer deaths.[40] The melanocyte transformation is poorly understood. It probably involves a multi-step progressive genetic mutation that alter cell proliferation, differentiation and death, and has an impact from the carcinogenic effects of ultraviolet radiation.[41]

Moisturisers

Moisturisers contain different combinations of emollient, occlusives and humectants in order to achieve their beneficial effects. A US study from 2004 found that moisturisers are the third most commonly recommended OTC topical skin product (13.4%) behind hydrocortisone (27.6%) and anti-infectives (23.4%).[42] Moisturisers are a key component of basic skin care, especially when there is an alteration of the epidermal barrier and reduced water content in the epidermis.[43] The stratum corneum must remain hydrated to preserve its function, at least 10% water content in healthy skin. It is of great importance to maintain this balance, in order to maintain flexibility and integrity in the stratum corneum. If the water content is reduced or in the absence of water entirely in the stratum corneum, the skin will become cracked, brittle and rigid. The water content is preserved by three major biophysical mechanisms: lamellar lipids, an intact corneocyte function and natural moisturising factor (NMF). The NMF is found within the corneocytes and is a mix if hygroscopic molecules, for example amino acids, salts including lactates, urea and elctrolytes. Intercellular lipids of the horny layers are provided by lamellar bodies, which are produced by exocytosis from keratinocytes. NMF helps to maintain the hydration in the corneocytes and results in their optimal hydration and swelling. The amino acids derive from the protein filaggrin. The moisturising treatment involves repairing the skin barrier, retaining/increasing water content, reducing TEWL, restoring the lipid barriers' ability to attract, hold and redistribute water, and maintaining skin integrity and appearance.[44] That is performed by acting as humectants, emollients, and occlusives.[45] Emollients consist mainly of lipids and oils and serve to fill the crack between clusters of the desquamating corneocytes. Examples include stearic, linoleic, and oleic acids. Occlusion reduces TEWL by creating a hydrophobic barrier over the skin and contributing to the matrix between the corneocytes. Common substances are for example lanolin acid and propylene glycol. Humectants, urea, for example enhance water absorption from the dermis to epidermis and in humid condition and helps the stratum corneum to absorb water from external environment. The most effective humectant is glycerol which hastens the maturity of corneocytes through the activation of residual transglutaminase activity in stratum corneum.[46]

TEWL

Transepidermal water loss (TEWL) is a well established instrument developed by Gert Nilsson, measuring the passive diffusion of water through the SC.[47, 48] The measurements reflect the water barrier in the stratum corneum without influence from sweat glands, or from follicles (Figure 2). An intact stratum corneum will have a higher water content since it allows water loss only in small amounts, and that in turn forms a physical barrier against surrounding environments. The TEWL is measured after applying the probe for 30-60 s, and is calibrated in grams per square meter per hour. Normal TEWL values are between 2 and 5g/m^2 per hour. The values can reach as high as 90-100 g/m^2 per hour in eczematous skin, depending on the damage in stratum corneum. The ambient temperature and humidity have an impact on TEWL instrument and therefore the measurements need to be performed in a special room with a constant humidity and temperature. These factors mentioned above, including the emotional condition of the person in question, and the season during which the measurements are being performed (differences in summer and winter), have an impact on the measuring subject itself. By these facts an interpretation of the measured values may be difficult.

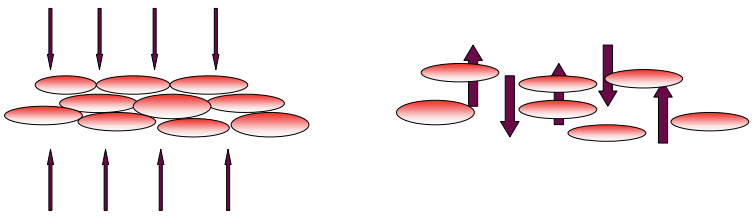

***Figure 2*. Principle of TEWL measurements, left – normal skin, right – AD skin**

Corneometer

Corneometer CM 820 (Courage-Khasaka, Colonge, Germany) is an instrument used since the 1980s to determine the water content in the stratum corneum.[49, 50] The instrument has a probe that is placed in contact with the skin. The instrument is based on the dielectric constant of

material that reflects the electrical properties, mainly of water in the stratum corneum to a depth of about 0.1 mm in order to make sure the measurement are not influenced by capillary blood vessels.[51] The content of keratin and lipids in the stratum corneum is very small compared to water. The technique converts the total capacitance of the skin into arbitrary units (a.u). The dielectric constant for water is 81 a.u. The dielectric constant in normal hydrated skin varies from 60 to 70 a.u. and for very moist skin above 90 a.u. The values can be as low as 30 to 60 a.u. in eczematous skin due to the insufficient barrier in the stratum corneum.

Electrical impedance

The electrical impedance technology has been used for medical purposes since the early 1920s. New instruments and methods have been available for various clinical applications since the past few decades, and methods became available for various clinical applications - e.g., cardiopulmonary tomography,[52] skin hydration,[53] detection of dental decay[54] or of cervical neoplasia[55] and various types of pathological findings in the skin.[56-61] A probe is applied on the skin in order to perform the measurements. The stratum corneum contains intra- and extracellular fluids, which behave as electrical conductors, and cell membranes, that act as imperfect reactive elements.[62] At low frequencies (1kHz), the current passes mainly through extracellular fluids. Higher frequencies (500-800 kHz), penetrates both intra- and extracellular fluids (Figure 3). It records the magnitude and phase spectra of impedance at the 31 logarithmically distributed frequencies mentioned above, and the volume can be adjusted to record spectra stepwise at five skin depths. The data are condensed by using the magnitude and phase values at only two frequencies, 20 kHz and 500 kHz, and expressed as four indices representing changes with frequencies along the for major aspects of electrical impedance space as has been described elsewhere.[59] The four indices are

$$\text{Magnitude index, MIX} = \text{abs}\,(Z_{20\ \text{kHz}})/\text{abs}\,(Z_{500\ \text{kHz}})$$

$$\text{Phase index, PIX} = \arg\,(Z_{20\ \text{kHz}}) - \arg\,(Z_{500\ \text{kHz}})$$

$$\text{Real part index, RIX} = \text{Re}\,(Z_{20\ \text{kHz}})/\text{abs}\,(Z_{500\ \text{kHz}})$$

$$\text{Imaginary part index, IMIX} = \text{Im}\,(Z_{20\ \text{kHz}})/\text{abs}\,(Z_{500\ \text{kHz}})$$

These measurements give information about the electrochemical processes in the tissue, and can be used to characterise the tissue or monitor pathophysiological changes since, for example, ions will contribute to the conduction of electricity through the tissue according to their concentration and mobility. Changes in electrical properties are also found when cells or tissues change from one physiological state to another - e.g. living to dead, dry to moist or normal to pathological.[63, 64] Other factors that will influence the measurement results are for example structural changes, shape, size and packing density on the cellular level, or in pore or duct size as well as body location, age, sex [65] and seasonal variations. [66]

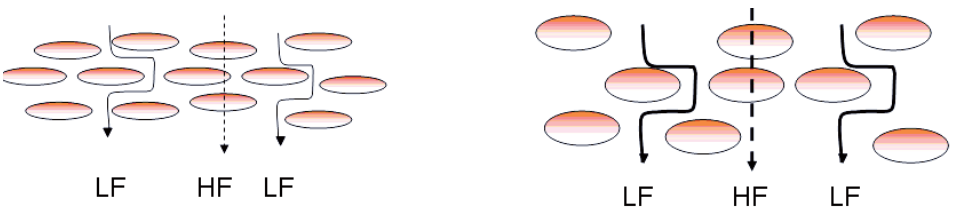

***Figure 3*. Principle of impedance measurements, left – normal skin, right – AD skin**

Neuropeptides

The most important symptom in AD is chronic pruritus, which impairs the patient's quality of life. The lack of effect of antihistamines argues against a role of histamine in causing AD–related pruritus.[67] Neuropeptides, proteases, kinins, and cytokines induce itching. Interleukin-31 is produced by T cells that increases the survival of hematopoietic cells and stimulates the production of inflame cytokines by epithelial cells. Many common system functions, such as hormones, neurotransmitters, and receptors correspond both in skin and CNS.[68] Ectodermal organs such as the lung, gut and the skin are characterised by their dense innervation and close neuro-immune crosstalk.[69-71]

Neuropeptides are found in myelinised A-fibres and the non-myelinised C-fibres, both in sensitive as well as autonomous nerve fibres, which makes the skin organ extensively supplied with various nerve fibres. The sensoric nerves not only conduct afferent nerves from the skin to the CNS but also fulfill efferent neurosecretory functions. Neuropeptides released by sensory nerves in the skin can directly modulate functions of keratinocytes, Langerhans cells, mast cells, dermal microvascular endothelial cells and infiltrating immune cells. Several observations support the idea that an imbalance of cutaneous neuropeptides such as SP, VIP, somatostatin, and neurotensin is one basis for the pathophysiology of itching in AD.[72, 73] Somatostatin is widely distributed in the CNS[74] and in the peripheral tissue.[75, 76] It is known that somatostatin inhibits neurogenic inflammation[77, 78] and nociception. Somatostatin-immunoreactive nerve fibres were decreased in AD patients.[79]

Diagnosis of atopic dermatitis

There are no specific laboratory findings or histologic features to define AD. Elevated IgE levels are found in up to 80 percent of affected patients, but elevated IgE levels are also found in patients with other atopic diseases. The diagnosis of atopic dermatitis is based on the findings of the history and physical examination. The classical Hanifin and Rajka definition was based on 4 major and several minor criteria.[80]

Table 1. Atopic dermatitis definition according to Hanifin and Rajka

Major criteria: *Must have three or more of:*

Pruritus
Typical morphology and distribution
Flexural lichenification or linearity in adults
Facial and extensor involvement in infants and children
Chronic or chronically-relapsing dermatitis
Personal or family history of atopy (asthma, allergic rhinitis, atopic dermatitis)

Minor criteria: *Should have three or more of:*

Xerosis
Ichthyosis, palmar hyperlinearity, or keratosis pilaris
Immediate (type 1) skin-test reactivity
Raised serum IgE
Early age of onset
Tendency toward cutaneous infections (especially S aureus and herpes simplex) or impaired cell-mediated immunity
Tendency toward non-specific hand or foot dermatitis
Nipple eczema
Cheilitis
Recurrent conjunctivitis
Dennie-Morgan infraorbital fold
Keratoconus
Anterior subcapsular cataracts
Orbital darkening
Facial pallor or facial erythema
Pityriasis alba
Anterior neck folds
Itch when sweating
Intolerance to wool and lipid solvents
Perifollicular accentuation
Food intolerance
Course influenced by environmental or emotional factors
White dermographism or delayed blanch

AIMS OF THE THESIS

Several immunological aberrations, both humoral and cellular, are found in patients with AD, and the pathogenesis is not fully understood. Many of the treatments for the disease affect the immunosurveillance of the skin, (for example UV-B, ciclosporin or tar preparations) which might conceivably increase the risk for skin cancer. The epithelium serves as a first line of defence between the body and the environment. Disturbance of the epidermal barrier can favour the penetration of microbes and allergens. Itch is excited on neuropeptide-containing free nerve endings of unmyelinated nociceptor fibres. Several observations support the idea that an imbalance of cutaneous neuropeptides is one basis for the pathophysiology of itching in AD. The general aim of this thesis is to obtain knowledge of some aspects of defence defects in AD.

The specific aims of the individual papers were to:

Paper I: To assess the risk of skin cancer and other cancers among patients with AD.

Paper II: To use several non-invasive methods to characterise skin hydration objectively during treatment in patients with AD.

Paper III: To compare the biophysical properties of the skin of healthy subjects and that of patients with AD as well as measurements of the effects of a new emollient.

Paper IV: To examine the SSTR subtype distribution in skin from healthy subjects and patients with psoriasis vulgaris or AD

MATERIALS AND METHODS

This section is an overview of the materials and methods used in papers I-IV. More detailed descriptions are given in the respective "Materials and Methods" sections of the individual papers.

Subjects

The subjects of **paper I** were all hospitalised patients in Sweden identified in the National Inpatient Register as having discharge diagnoses of AD between January 1, 1965 and December 31, 1999.
The studies of **papers II, III** and **IV** were carried out on patients with AD, defined by the Hanifin and Rajka's criteria (Hanifin and Rajka 1980). For comparison (**papers III** and **IV**), healthy subjects with no history of skin disease, skin sensitivity or allergy, were included. In **paper IV**, comparisons were also made with patients diagnosed clinically as having chronic plaque psoriasis. The studies were approved by the Regional Ethics Committee in Stockholm, Sweden.

Methodology

National Inpatient Register [**I**]	A register of hospitalizations and discharge diagnoses.
National Registration Number [**I**]	An unique personal identifier.
ICD-code [**I**]	International Identification of Disease.
National Swedish Cancer Register [**I**]	Codes close to 98% of all malignant neoplasms.
Total population Register [**I**]	Living persons in Sweden.
Death Register [**I**]	IDs of dead persons and cause of death.
Emigrations Register [**I**]	Persons who have moved from Sweden.
Transepidermal water loss [**II, III**]	The most established barrier function test (TEWL), the passive diffusion of water through the SC.
Electrical capacitance [**II, III**]	Well-established method assumed to measure the water content of SC.
Electrical skin impedance [**II, III**]	Based on the principle that pathophysiological changes are reflected by changes in the electrical impedance spectra.
Immunohistochemistry [**IV**]	After production of the specific antibodies, tissue sections from skin biopsies were stained to localise the five SSTR subtypes.

Statistical analysis [**I, II, III**]

The relative risk of cancer was estimated by the standardized incidence ratio (SIR). The expected number of patients with cancer was calculated by multiplying the number of observed person-years, divided into age- (in 5-year groups), sex-, and calendar year–specific strata, by the corresponding cancer incidence rates. These incidence rates, derived from the relevant strata in the entire Swedish population and aggregated in 5-year periods to avoid instability in rates of rare cancers, were calculated by dividing the number of first primary cancers, excluding those discovered incidentally at autopsy, by person-years at risk (the midyear population of individuals without any previously reported cancer). The 95% confidence interval (CI) of the SIR was calculated. In the main analyses, cancers and person-years accumulated during the first year of follow-up were excluded to minimize the possible impact of selection bias.

In the studies with the moisturisers, the effects of treatment were analysed using a two-way ANOVA with repeated measures of two factors. The significance of differences between patients with AD and healthy controls [**III**] were tested using the Mann-Whitney nonparametric test.

RESULTS AND DISCUSSION

Cancer incidence (Paper I)

On average, patients with AD were followed up for 15.4 years, yielding 241 867 accumulated patient-years at risk, 15 471 of which were during the first year. After excluding this first year of observation, during which 1 case of squamous cell carcinoma of the skin occurred, we ascertained a total of 331 cases of cancer (190 in women, 141 in men). The average age at diagnosis of cancer was 53.0 years for women and 54.9 years for men. The incidence of any cancer (all sites) was increased by 13% (95% CI, 1%-25%), compared with the age- and sexmatched general Swedish population. There was a statistically nonsignificant risk elevation for nonmelanoma skin cancer (SIR, 1.5; 95% CI, 0.8-2.6; 12 patients) but a decreased risk for melanoma (SIR, 0.6; 95% CI, 0.3-1.2; 10 patients). Increased risks were noted for cancer of the esophagus (SIR, 3.5; 95% CI, 1.3-7.7; 6 patients), lung (SIR, 2.0; 95% CI, 1.3-2.8; 31 patients), and brain (SIR, 1.6; 95% CI, 1.1-2.4; 27 patients) and for lymphoma (SIR, 2.0; 95% CI, 1.4-2.9; 29 patients). A 1.9-fold excess risk of pancreatic cancer was of borderline statistical significance (Table 2).

To our knowledge, paper I is the first large-scale follow-up study of patients with AD with cancer as the outcome. There was a statistically significant 13% overall excess risk, driven mainly by excesses of lung and brain cancers and lymphoma. Risk elevations were noted also for esophageal and pancreatic cancers. We also observed a 2-fold elevation of risk for nonmelanoma skin cancer 1 to 35 years after entry into the study among men but not among women and only during the first 10 years of follow-up. With only 12 observed patients with nonmelanoma skin cancer, however, both the overall excess and the sex difference could be a chance finding.

The findings in paper I are in contrast with those of a recent case control study that used a mailed survey and found that 254 patients with a history of AD did not seem to develop nonmelanoma skin cancers more often than patients with other dermatologic conditions.[81] Skin tumours, in contrast to tumours of internal organs, are readily observable. For example, prevalent but undiagnosed skin tumours may be detected in connection with hospital care for AD. However, neither patients with basal cell carcinoma nor those with actinic keratosis are reported in the National Swedish Cancer Register. Thus, the nonmelanoma skin cancer category includes only patients with squamous cell carcinoma and not those with squamous intraepidermal neoplasia or Bowen disease. If a skin tumour was diagnosed during the first hospitalisation and recorded in the Inpatient Register, the affected patient would not be included in our cohort. Consequently, our cohort members had, in effect, been screened for skin tumours before inclusion. Therefore, the rate of skin tumours diagnosed during the ensuing years was probably somewhat lower than in an unscreened population, at least in the older age groups (10 years or older) in whom skin tumours are not exceedingly rare. This hypothetical incidence deficit is expected to be balanced by a bias toward increased detection during follow-up as a result of closer dermatological surveillance linked to the presence of any chronic skin disorder. As the initial screening effect wears

off, the presumed detection bias will dominate the findings. The net effect on overall skin cancer risk is difficult to predict, but because the cohort, as it is aging, is slowly moving from lower to higher absolute risk, any bias will have greater impact during the last years of follow-up.

Table 2. ***Standardized Incidence Ratio (SIR) and 95% Confidence Interval(CI) for Major Cancer Types Among Patients Hospitalized for Atopic Dermatitis from 1965 to 1999 in Sweden***

Cancer Type	ICD-7 Code	No. of patients Observed	SIR (95% CI)
All-site	140-209	331	1.13(1.01-1.25)
Male		141	1.30(1.10-1.54)
Female		190	1.02(0.88-1.88)
Cancer of the skin			
Non melanoma	191	12	1.5(0.8-2.6)
Male		8	2.0(0.9-4.0)
Female		3	1.0(0.3-2.5)
Melanoma	190	10	0.6(0.3-1.2)
Cancers other than skin			
Esophagus	150	6	3.5(1.3-7.7)
Male		3	2.8(0.6-8.1)
Female		3	4.9(1.0-14.3)
Pancreas	157	11	1.9(1.0-3.4)
Lung	162, 163	31	2.0(1.3-2.8)
Male		15	1.8(1.0-2.9)
Female		16	2.2(1.3-3.6)
Brain	193	27	1.6(1.1-2.4)
Male		11	1.5(0.7-2.7)
Female		16	1.7(1.0-2.8)
Lymphoma	200-202	29	2.0(1.4-2.9)
Male		17	2.4(1.4-3.8)
Female		12	1.7(0.9-3.0)

Hence, it is likely that overestimation of the incidence resulting from detection bias will dominate. The shift from underestimation (resulting from initial screening) to overestimation (resulting from detection bias during followup) is expected to lead to

spurious impressions of an increasing relative risk over the follow-up period. Although the small numbers of patients observed hamper the interpretation of our data, the observed decrease in relative risk with increasing follow-up time is probably not the effect of screening or detection bias. Instead, it creates skepticism about the biological relevance of the nonsignificant 50% overall excess risk of nonmelanoma skin cancer in our cohort, seemingly confined to the first 9 years of follow-up.

Because no laboratory marker for AD exists, the diagnosis is based on major and minor clinical criteria, of which the major features are pruritus, typical morphologic traits and distribution of the lesions, chronic relapsing course, and personal and family history of atopic disease (asthma, hay fever, or AD).[80] The pathogenesis is not fully understood, but several immunological aberrations are found in AD, including impaired cellular mediated immunity, elevated serum IgE and eosinophil levels, and IgE-bearing Langerhans cells. Furthermore, colonisation of the more-or-less chronically inflamed lesions by microbes - most important, the yeast *Malassezia* (formerly *Pityrosporum*) *orbiculare* and the bacterium *Staphylococcus aureus* - may contribute to the perpetuation of the lesions. Chronic inflammation and microbial colonisation or infection in combination with the immune impairments (primary infection or adverse effects of treatment) may lead to proliferative epidermal changes; hence, the suspicion of a link to cancer development. Both sexes are affected by AD; among adults, more women than men have the disease.[82] However, among children, 12 years or younger, more boys are affected than girls. The reason for this sex difference is unclear. Because in Sweden the onset of AD begins, on average, earlier among men than among women, the duration of the chronic disease at any given age has generally been longer in men. In children, it also seems as if boys have a more severe disease than girls. Therefore, it is not inconceivable that there might be sex differences in risks of adverse long-term consequences of the disease. The atopic disease triad consists of hay fever, asthma, and AD. Over the life span, an individual with atopic disease may suffer from 1, 2, or all 3 of the manifestations. The connection between AD and lung disease was also manifested in our cohort, where lung disease (mainly asthma) was the most commonly found non-AD diagnosis, followed by other skin diseases and infectious diseases. Although little is known about associations between cancer and hay fever, patients with asthma seem to be at increased risk for lung cancer,[83-86] although contradicting results have been reported.[87-89] Because no risk elevations for esophageal cancer were observed in a cohort of hospitalised patients with asthma in a previous study,[90] the excess of this cancer among patients with AD in our study is unlikely to be an unspecific phenomenon linked to the need for hospitalisation. Among other allergic manifestations investigated for cancer risks, positive epicutaneous tests were reportedly linked to an overall increased risk of cancer within 20 years of follow-up in men but not in women.[91] No risk elevations were found among 1155 patients with chronic urticaria observed for up to 27 years.[92] The excess of lung, esophageal, and pancreatic cancers in our cohort is consistent with confounding by smoking and alcohol consumption. We have no information about these habits among our cohort members. According to epidemiological data, cigarette smoking seems to be associated with many skin diseases, as reported previously.[93] Although smoking is believed to trigger or worsen AD,[94] Mills et al found no significant difference in smoking habits of patients with AD and matched controls.[89] Smoking and alcohol abuse may still have contributed to a biased selection in our cohort of hospitalised patients. Most patients with AD are

treated on an outpatient basis, and those who are hospitalised may have less healthy lifestyles. The major strength of our study is its internal validity; that is, its cohort design has no risk for recall bias, almost no patients lost to follow-up, and virtually complete cancer ascertainment. Our cohort comprised all hospitalised Swedish patients with AD, thus ensuring external validity in relation to all hospitalised patients. The relevance of our results for outpatients with AD is less obvious. The study's restriction to patients admitted to hospitals probably reduced misclassification of AD, in any case. Several limitations should also be noted in the interpretation of our findings. In addition to the possible selection biases associated with the need for hospitalisation (more severe AD is associated with increased comorbidity), scarcity of information regarding potential confounding factors (notably smoking), and the possibility of detection bias, the small numbers of expected cases of some cancers in this cohort make the risk estimates highly sensitive to chance effects. Moreover, the most established criteria for a diagnosis of AD were published in 1980,[80] 15 years after our cohort began to accrue. However, we assume that even before 1980 an experienced dermatologist or pediatrician easily diagnosed AD (such as Besnier prurigo [atopic dermatitis]). In this cohort, dermatologists made the diagnoses in 38% of the cases, and other physicians, most often pediatricians, made the diagnoses in 62% of the cases.

In conclusion, a slight excess of malignant neoplasms was noted among patients with AD. The greatest relative excesses were for cancers of the esophagus, lung, brain, and pancreas and lymphoma. Confounding by smoking and alcohol abuse, however, cannot be excluded. The risk elevations, all of which are of borderline statistical significance, should be interpreted with caution. The combination of multiple significance testing and few observed patients may have generated chance findings. Because (1) the cohort has a high proportion of young patients, who are not yet in the age groups most at risk for developing cancer, and (2) the frequency of occurrence of most types of cancer increases with age, future follow-ups of our cohort would be interesting. Furthermore, this study of a cohort of patients with AD may be of value for future independent evaluation of the relationship between skin cancer development and recent new treatment with two different topical calcineurin inhibitor immunosuppressants.

Urea and sodium chloride in moisturisers (Paper II)

All patients had atopic dermatitis with normal-looking or dry skin, but no active eczema on the part of skin studied. The measurements were made between January and May 1998. No significant changes occurred in the TEWL measurements on days 0, 7 and 14 between the two creams. Thus, the water barrier function, at least according to TEWL measurements, was unchanged (Figure 4). Both creams showed highly significant increases in the capacitance measurements on days 7 and 14, compared to before the study (Figure 5). The measurements using electrical impedance were made at five skin depths, but only the results from depth 2 are represented here – i.e. the optimal depth for studying the stratum corneum (Figure 6). The mean indices MIX and IMIX for the urea-NaCl cream on days 7 and 14, and PIX on day 14 showed significant changes compared to day 0. For the urea- NaCl cream, the mean indices MIX and IMIX, on days 7 and 14, and PIX on day 14, showed significant changes, compared to day 0. For the urea cream, this was true of PIX alone, none of the other indices showing any change following treatment. On day 14, the urea-NaCl cream caused a significantly

higher increase in the indices MIX and IMIX than the urea cream; on day 7, this difference was only significant for the index MIX. No correlation was found between electrical impedance (any index) and the other two methods. We also analysed the patients' evaluations of the creams, on the basis of the questionnaire. No differences were noted between the creams in efficacy, preference and cosmetic appeal, nor were any side-effects or suspected allergies or skin irritations reported. No visual assessment was included in the study design mainly due to paucity of established and reproducible methods on the subject.

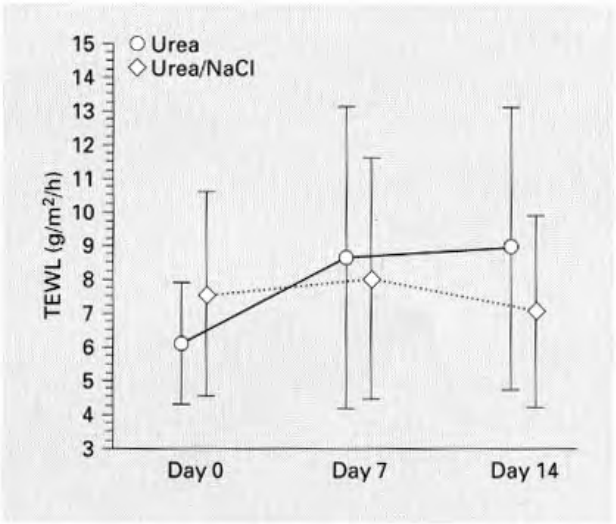

Figure 4. **Mean (and 95% confidence interval) TEWL (g/m2/h) from dry atopic skin in 22 patients before and after treatment with urea-NaCl cream or urea cream for 7 and 14 days.**

The aim of paper II was to examine the effects of moisturisers on the skin barrier properties of the non-eczematous skin of patients with atopic dermatitis. We focused on functional changes related to the addition of urea alone or urea and sodium chloride together by monitoring TEWL, capacitance and electrical impedance spectra. The design of this study does not permit comparison to untreated skin and vehicle-treated skin. In contrast to the findings of Lodén in normal skin,[95] we found an increased skin capacitance after 7 days and this increase persisted after 14 days in the case of the urea containing cream without but the combined product showed a reduction on day 14. Lodén showed a significant difference with her urea containing preparation after 10 days but no difference between control and urea-treated skin after 20 days. This discrepancy might be due to the 2-week wash-out period used in our study but not in the study by Lodén.[96] On the other hand, Lodén *et al*. reported a significant increase in capacitance values in atopic skin with a urea containing moisturiser.[97] In paper II, the increasing capacitance values showed no significant correlation with increases in electrical impedance indices. The increases in the former values were present from day 7 to day 14. The impedance indices MIX and IMIX increased significantly more with the cream containing sodium chloride and urea than that with no sodium chloride.

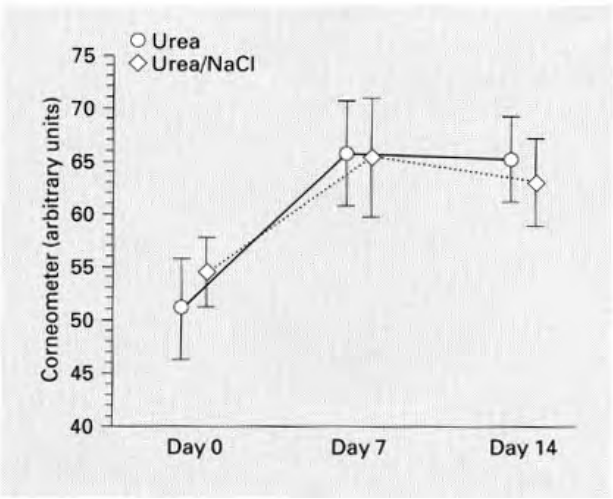

***Figure 5*. Mean (and 95% confidence interval) hydration of dry atopic skin in 22 patients, measured with thecorneometer (arbitrary units), before and after treatment with urea-NaCl cream and urea cream for 7 and 14 days.**

Martinsen and Grimnes have recently developed a new instrument designed for the stratum corneum alone that uses only one very low frequency measurement to assess skin hydration.[64] Yamamoto and Yamamoto have done pioneering work on skin moisture assessment using electrical impedance.[98-102] Impedance spectroscopy permits the study of various experimental skin reactions[61, 103, 104] and subclinical effects of irritants on the skin.[65] The main drawback of the skin electrical impedance method is that the detailed structural tissue correlates for impedance spectra are still unknown. This method is better than others for evaluating the skin barrier because it seems more sensitive and discriminative. It can also detect differences in skin hydration and/or in the amount and mobility of charge carriers at various skin depths, by adding the special depth-selective feature of our device. The impedance of the skin is influenced not only by the state of hydration of the stratum corneum, but it also reflects changes in the lipid content of the normal human stratum corneum, as shown in our laboratory, after lipid extraction.[60, 105] With electrical impedance spectroscopy, we found greater changes in some of the indices after lipid extraction than with determinations of either TEWL or skin moisture content. This suggests that electrical impedance is more sensitive than the other two methods. Electrical impedance indices showed patterns containing additional information, which might become useful in differential diagnosis when more knowledge has been gained about the relation between these patterns and pathohistological conditions. In the present study, the increase in capacitance after long-term treatment with moisturisers was not accompanied by a simultaneous increase in TEWL, indicating that the skin was not hydrated to the extent that its water permeability was affected.

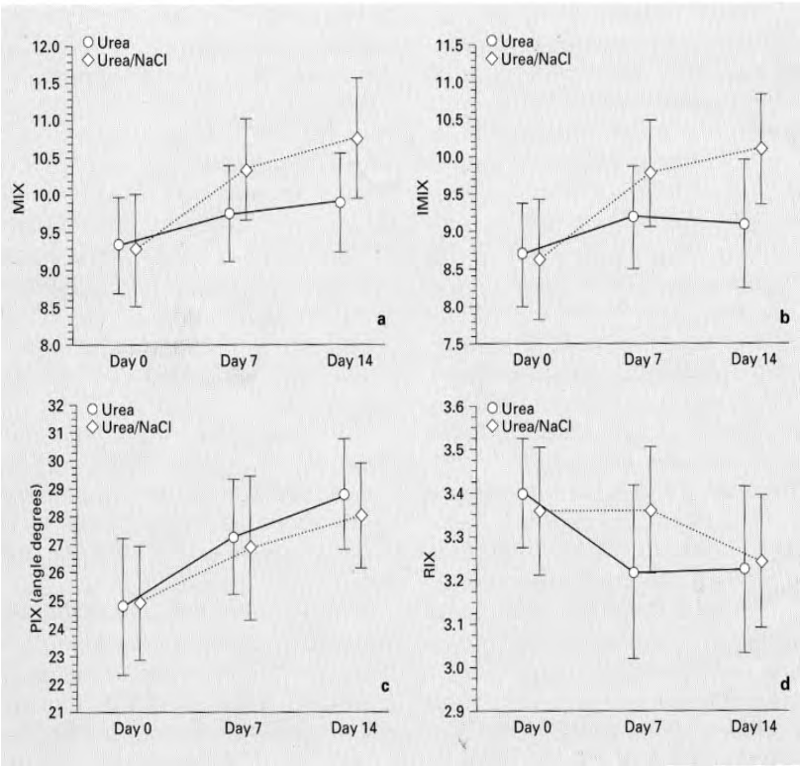

***Figure 6*. Mean (and 95% confidence interval) electrical impedance indices MIX (a), IMIX (b), PIX (c) and RIX (d) for dry atopic skin in 22 patients after treatment with urea-NaCl cream and urea cream for 7 and 14 days.**

These findings differed from those of Lodén, who showed a decrease in TEWL following treatment with urea-containing products after 10 and 20 days of treatment, both in normal skin[96] and atopic skin.[97] In the present study, we found that TEWL increased, but not significantly, after urea cream on days 7 and 14. This is in agreement with our earlier studies where we have observed that TEWL is vulnerable to a number of external factors that are difficult to control and also to the psychological status of the study subjects.[61, 103, 105] No case of irritation, stinging or suspected allergy were reported by the patients. The addition of sodium chloride to the moisturiser seems not to have any drawbacks in the cosmetics nor any adverse effects in the treatment of atopic dermatitis. The key issue of this report is whether urea and sodium chloride together improve efficacy. We have previously shown that the impedance indices MIX and IMIX are correlated with the corneometer readings and, by choosing depth No. 2, our measurements mainly reflect changes in the stratum corneum.[59]

The findings of paper II indicate that a moisturiser containing both urea and sodium chloride seems somewhat more effective than the same moisturiser without sodium chloride, at least concerning the ability to normalise impedance indices of atopic skin.

Major factors accounting for this effect might be first hydration of the stratum corneum by increasing the water binding (urea) and second by absorbing and retaining moisture (hygroscopicity,NaCl). This accords well with the findings of Miettinen et al.,[106] who reported that the reduction in water vapour pressure by sodium chloride and urea was additive. On the basis of chemical studies of an aqueous solution, Miettinen et al. [106]suggested that either sodium chloride or urea is effective in mixtures, but the effect is greater when they are used together. To our knowledge, paper II is the first study utilising the actual electrical impedance technique for the assessment of the effects of moisturisers on human skin and the clinical meaning of the impedance measurements remains to be proven.

Biophysical assessment and a moisturiser (Paper III)

The measurements were made in February-March. The average air temperature in the laboratory was 23.4 (range 22.4°-24.4°)° C, and the relative humidity 20.83 (range 14 - 28)%.

Measurements of the skin of patients with AD and those of healthy subjects before treatment. The mean TEWL measurements were significantly higher in AD skin than in the forearm skin of the healthy controls ($p<0.001$), indicating that the water barrier function was impaired (data not shown). The stratum corneum of AD skin was also significantly less hydrated, when evaluated with the Corneometer ($p<0.05$) (Figure 7). Furthermore, the measurements of impedance showed significant changes in the mean MIX ($p<0.001$) (Figure 8a) and IMIX ($p<0.001$) (Figure 8b) indices, which mainly reflect disturbances in the SC and underlying epidermis.

Evaluation of the efficacy of the foam. The average amount of foam used was 14.2 (range 5-40) g, assessed by weighing the jars that had contained it after the study. No correlation was detected between the amount used and the results of the measurements with any of the non-invasive methods (data not shown).
1. TEWL. No significant changes occurred in the mean TEWL measurements of the two groups during the observation period (data not shown).
2. Capacitance. The treated area showed a significant increase with time on days 10 ($p<0.01$) and 21 ($p<0.01$) of the treatment, compared to day 0, but the untreated area did not. Significant differences were also noted between the mean values of the treated and untreated areas on days 10 ($p<0.001$) and 21 ($p<0.001$) of the treatment, indicating that the product had a moisturising effect (Figure 9).
3. Electrical impedance. Electrical impedance was measured at five different skin depths, but only the findings from depth 2 are given – i.e., the optimal one for studying the SC and underlying parts of the epidermis - because the results of the other four levels were similar in direction and magnitude. No significant changes occurred in the mean MIX and IMIX impedance indices during the study (data not shown). The changes in time were not significant in the control. The mean RIX index showed significant reduction in the treated area on days 10 ($p<0.001$) and 21 ($p<0.001$), compared to day 0. We also found significant differences between the means of the control and treated areas on days 10 ($p<0.001$) and 21 ($p<0.01$) (Figure 10a). For the control area the mean PIX indices significantly increased on day 10

($p<0.05$), but not on day 21 ($p=0.12$), compared to day 0, but in the treated area, it increased significantly either on day 10 ($p<0.001$) or 21 ($p<0.001$), compared to day 0. Moreover, significant differences were also noted between the mean values of the control and treated areas on days 10 ($p<0.01$) and 21 ($p< 0.05$) (Figure 10b).

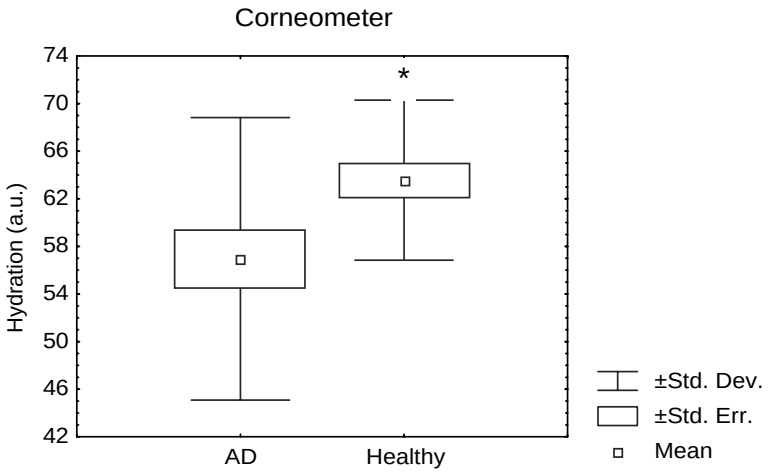

***Figure 7*. Mean (and standard deviation (SD) hydration of the midvolar forearm skin in 22 healthy subjects and 24 patients with atopic dermatitis. The findings were analysed with the Mann-Whitney non-parametric test. The following significance scale was used: * $0.01<p\leq0.05$, ** $0.001<p\leq0.01$ and *** $p\leq0.001$.**

Dry atopic skin showed higher TEWL, lower capacitance and changes in certain impedance indices. Our findings may be due to simultaneous impairment of the mechanisms responsible for skin hydration and barrier function, as regards capacitance and TEWL, which would accord with the findings in comparable studies.[107] In a few other studies dry[95, 108] and clinically normal AD skin[109] have been compared to normal skin. Since both types of AD skin had lower capacitance and higher TEWL values, even clinically normal skin seemed to function abnormally in such patients. These abnormalities may involve other physiological parameters - e.g., increases in trace elements (calcium, zink and iron).[110] Confirming findings in a recent study,[111] we found that certain impedance indices, which depend to some extent on capacitance (IMIX and MIX), were lower in atopic skin, as well as corneometer measurements. This may indicate a higher water content of the stratum corneum in healthy than in AD skin. The electrical characteristics of human skin affected by large variations in factors, such as hydration, lipid content, number of cell layers in the stratum corneum, size of the corneocytes and some properties of deeper skin layers, which may affect the complex nature of the skin barrier. An improvement in the skin condition was reflected by an increase in capacitance and no change in TEWL - i.e., a moisturising effect without an impairment of skin barrier function.

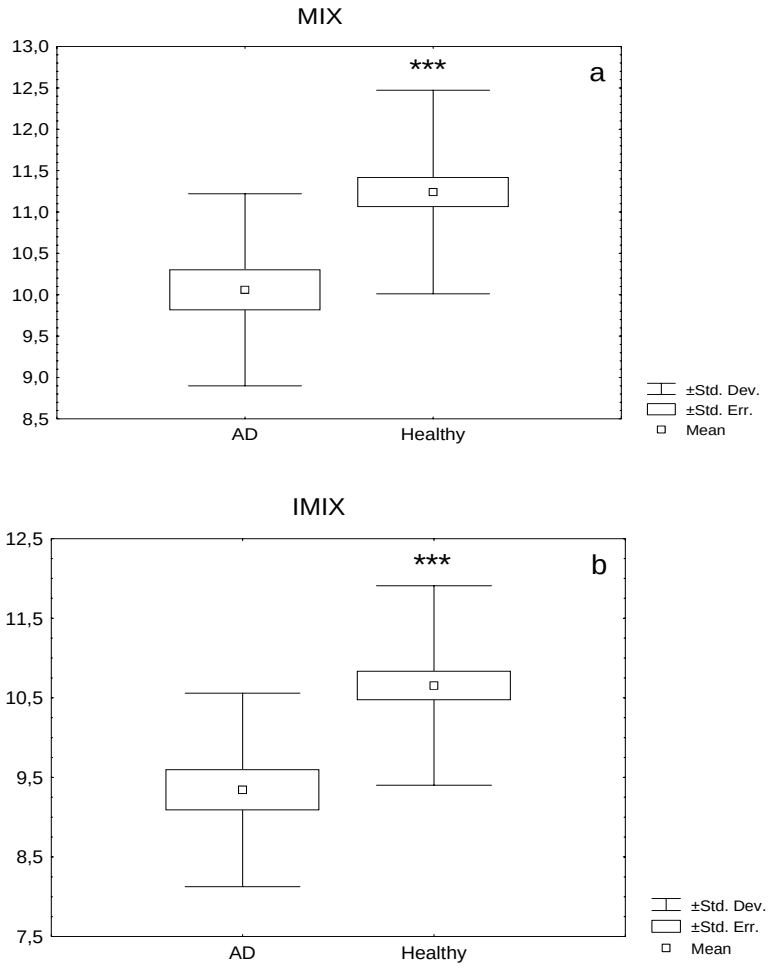

***Figure 8*. Mean and standard deviation (SD) electrical impedance indices MIX (a), IMIX (b) of the midvolar forearm skin in 22 healthy subjects and 24 patients with atopic dermatitis. The findings were analysed with the Mann-Whitney non-parametric test. The following significance scale was used: * $0.01<p\leq0.05$, ** $0.001<p\leq0.01$ and *** $p\leq0.001$.**

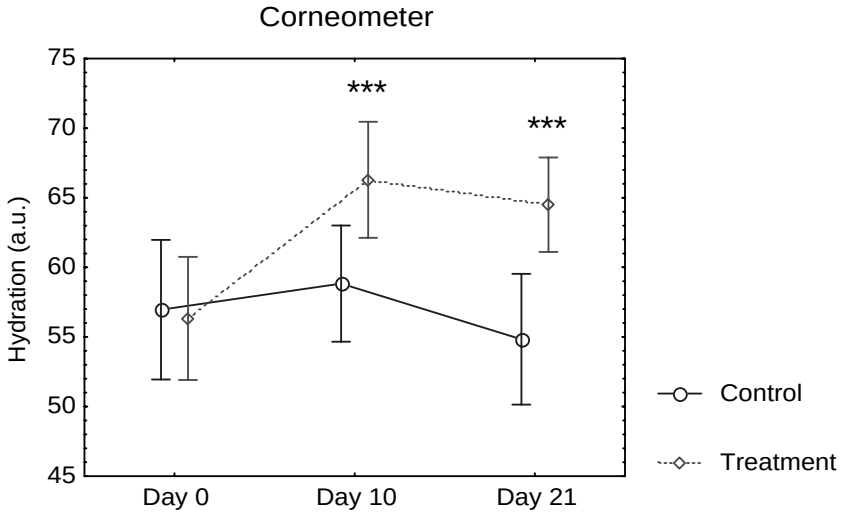

Figure 9. **Mean (and 95% CI) hydration of the midvolar forearm skin in 24 patients with atopic dermatitis. The findings were analysed, using two-way ANOVA with repeated measures. In the comparison of treated and untreated areas, the following p-values were used: * $0.01<p\leq0.05$, ** $0.001<p\leq0.01$ and *** $p\leq0.001$.**

Impedance indices are regarded as reflecting various properties of the skin although the mechanisms responsible for changes in indices are still not understood. However, the improvement in biophysical properties of treated areas was suggested by the tendency to normalisation of certain impedance indices.

TEWL is a well-established method for estimating barrier function of the skin[112] and an increase may indicate irritation or barrier damage caused by topical preparations. Changes in TEWL can reflect a multitude of factors, including lipid synthesis, lipid processing, and lipid organization (for review, see Proksch et al).[6] On the other hand, a decrease is interpreted as a positive effect of the treatment reflecting improvement in skin barrier function. As was shown by Lodén et al, preparations that contain urea reduce TEWL in normal[96] and atopic[97] skin after 10 and 20 days of treatment. In the present study, the moisturising effect of the cream was indicated by higher capacitance values, but this was not accompanied by a simultaneous increase in TEWL, suggesting that the skin was not hydrated sufficiently to affect its water permeability. Moreover, the treatment caused neither clinical (as confirmed by self-assessment) nor subclinical (as confirmed by unchanged TEWL values throughout the study) signs of irritation.

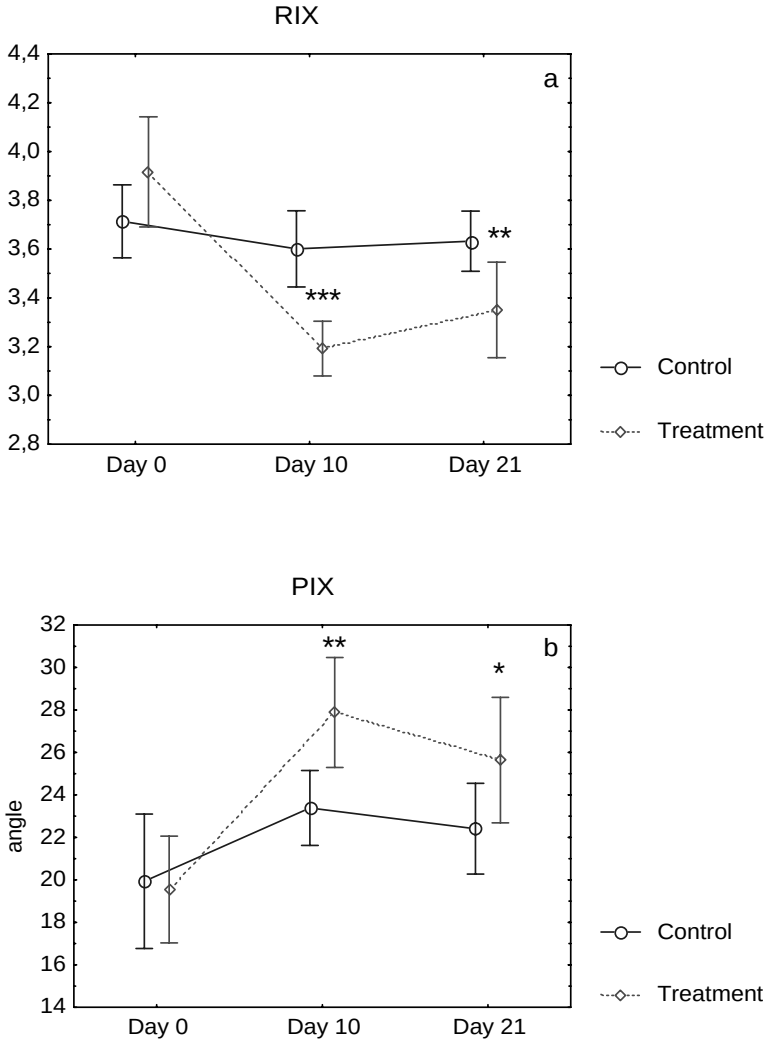

***Figure 10*. Mean (and 95% CI) electrical impedance indices RIX (a) and PIX (b) of the midvolar forearm skin in 24 patients with atopic dermatitis before and on the10th and 21st days of treatment. The findings were analysed using two-way ANOVA with repeated measures. In the comparison of treated and untreated areas, the following p-values were used: * $0.01<p\leq0.05$, ** $0.001<p\leq0.01$ and *** $p\leq0.001$.**

Dry skin is characterised by low electrical capacitance[50] and moisturising creams presumably have an increasing effect.[113] Tagami et al. described a sensitive method for evaluating skin water holding capacity.[114] Lodén et al. reported an increase in skin capacitance in healthy subjects after three applications of urea-containing moisturisers and no change in the effect was noted on the 10th day, but not on the 20th day after treatment.[96] In the present long-term study, the treatment was followed by an increase in the electrical capacitance on the 10th and 20th days of treatment,

which agrees with the findings of Lodén et al. in a study of urea-containing creams in patients with AD.[97]

Unlike the findings in paper II, a double-blind study on AD skin comparing a cream containing urea and sodium chloride and one containing urea alone, we found no increase in the IMIX and MIX indices in paper III, but an increase in PIX and decrease in the RIX indices, that persisted for 10 and 21 days. The skin of patients in paper III was assessed biophysically and compared to healthy skin before treatment. Among the impedance patterns, RIX values tended to be higher and PIX values lower in untreated AD skin. Thus, the changes in RIX and PIX indices after treatment may be ascribed to normalisation of these patterns. This accords with our previous study in which the moisturising effects of two creams containing urea were accompanied by similar changes in RIX and PIX indices (Paper II). This is the third study of the effects of moisturisers on skin during long-term treatment, using this type of electrical impedance instrument, but the findings were not as clear-cut in the second study on the lower legs of a normal geriatric population.[57] However, not surprisingly, the results vary and are affected by several factors - e.g., design, population (especially age), part of the body and the study preparation. Furthermore, it is well known that emulsifiers influence outcome of the data collected using our instruments, including considerable direct skin effects of soaps versus surfactants and physico-chemical interactions. The patients included in this study were in remission, and, to our knowledge information is lacking regarding the effects of AD exacerbation or effects of specific therapy on IMP parameters.

On the whole, paper III showed increases in capacitance with few changes in TEWL measurements and normalisation of certain impedance indices after treatment, indicating that the foam indeed moisturised the skin of patients with atopic dermatitis.

Somatostatin receptor subtypes (Paper IV)

Expression of the five SSTRs in human skin was seen to various extents in all parts of the living epidermis (except in the stratum corneum), dermal dendritic cells, striated muscle fibres, vascular endothelium, hair follicles, sebaceous glands and eccrine sweat glands.The sections without primary antibodies were negative.

Psoriatic skin lesions: All five SSTRs were expressed in the epidermis but the intensity varied considerably between the different layers (Figure 11, Table 3). SSTR1 showed a weak expression in the granular and spinous layers of epidermis, while the expression was moderately strong in the basal layer. SSTR2 was moderately expressed in the granular and spinous layers, but there was only a weak staining of the basal layer. SSTR3 was moderately expressed in the granular and basal layers, but the expression was weak in the spinous cell layer. SSTR4 and 5 were moderately expressed in the granular layer, but weakly expressed in the spinous and basal cell layers. The basal membrane of epidermis showed a strong expression of SSTR3 and 4.

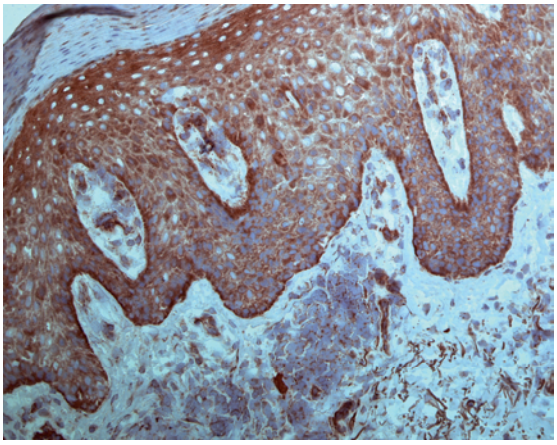

Figure 11. **Expression of SSTR3 in lesional psoriatic skin. SSTR3 is expressed throughout the living epidermis, especially in the granular layer. Note the strong expression of SSTR3 in the basal membrane of the epidermis. Cells having a dendritic appearance, which are mainly located in the papillary and upper reticular dermis, also express SSTR3.**

Cells with a dendritic appearance were seen mainly in the papillary and upper reticular dermis and showed a moderate to strong expression of SSTR1, 3 and 4, but only a weak expression of SSTR2 and 5. Striated muscle fibres showed a very strong expression of SSTR1-4, while the expression of SSTR5 was weak. The vascular endothelium showed a moderate to strong expression of SSTR1-4, but only a weak expression of SSTR5. The eccrine sweat glands expressed all SSTRs moderately to strongly, except for a weak staining of SSTR2. Hair follicles and sebaceous glands showed a moderate expression of SSTR1-3, and a weak expression of SSTR4 and 5.

Table 3. **SSTR-expression of psoriasis – lesional skin**

SSTR	Stratum granulosum	Stratum spinosum	Stratum basale	Dendritic cells	Vessels	Hair follicles	Sebaceous glands	Sweat glands	Muscles
1	+	+	++	+++	++	++	++	+++	+++
2	++	++	+	+	++	++	++	+	+++
3	++	+	++	++	+++	++	++	++	+++
4	++	+	+	++	++	+	+	++	+++
5	++	+	+	+	+	+	-	++	+

AD skin lesions: The intensity of the expression for SSTRs varied between the different epidermal layers (Figures 12-13, Table 4). SSTR1-3 showed a moderately strong expression in the entire epidermis, while SSTR4 and 5 were weakly expressed. The basal membrane of epidermis showed a strong expression of SSTR3 and 4. Dendritic cells located mainly in the upper dermis showed a moderately strong expression of SSTR1-3, but only a weak expression of SSTR4 and 5. Striated muscle fibres showed a very strong expression of SSTR1-4, while the expression of SSTR5 was weak. The vascular endothelium showed a moderate to strong expression of SSTR1-4, but only a weak expression of SSTR5. The eccrine sweat glands expressed all SSTRs moderately

to strongly. Hair follicles and sebaceous glands showed a moderate expression of SSTR1-4, and a weak expression of SSTR5. A weak expression of SSTR1-5 was seen in lymphocytic infiltrates in the dermis.

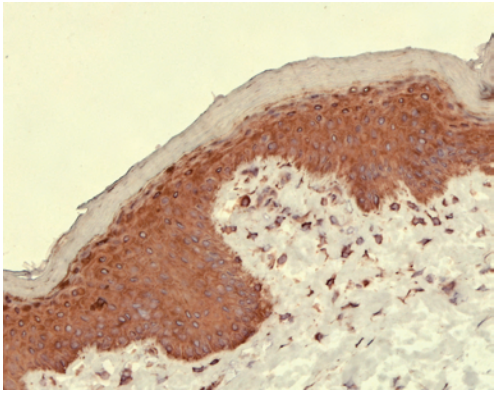

Figure 12. **Expression of SSTR2 in lesional atopic dermatitis skin. SSTR2 is expressed in the entire living epidermis. Note the strong expression of SSTR2 in the cells having a dendritic appearance, which are located in the papillary and upper reticular dermis.**

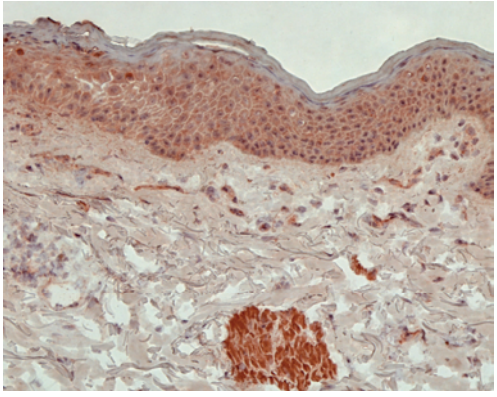

Figure 13. **Expression of SSTR4 in lesional atopic dermatitis skin. SSTR4 is weakly expressed throughout the living epidermis. Note the strong expression of SSTR4 in the striated muscle fibres in the dermis**

Table 4. SSTR-expression of lesional AD skin

SSTR	Stratum granulosum	Stratum spinosum	Stratum basale	Dendritic cells	Vessels	Hair follicles	Sebaceous glands	Sweat glands	Muscles
1	++	++	++	++	++	++	++	+++	+++
2	++	++	++	++	++	++	++	++	+++
3	++	++	++	++	++	++	++	++	+++
4	+	+	+	++	++	+	+	++	+++
5	+	+	+	+	+	+	+	+	+

Normal-looking skin: The intensity of the expression for SSTRs varied between the different epidermal layers also in normal skin of healthy volunteers (Figure 14, Table 5). SSTR1-3 showed a moderate to strong expression in the entire of epidermis, except for SSTR1 in the spinous layer. SSTR4 and 5 were weakly expressed in the epidermis of healthy skin. The basal membrane of epidermis showed a strong expression of SSTR3. Dendritic cells in the upper dermis showed a moderately strong expression of SSTR1-3, but only a weak expression of SSTR4 and 5. Striated muscle fibres showed a very strong expression of SSTR1-4, while the expression of SSTR5 was weak or in some cases absent. The vascular endothelium showed a moderate expression of SSTR1-4, but a weak expression of SSTR5. The eccrine sweat glands expressed all five SSTRs. Hair follicles and sebaceous glands showed a moderate expression of SSTR1-4, and a weak expression of SSTR5. The SSTR expression in healthy skin from patients with atopic dermatitis or psoriasis was similar to that of normal skin from healthy volunteers.

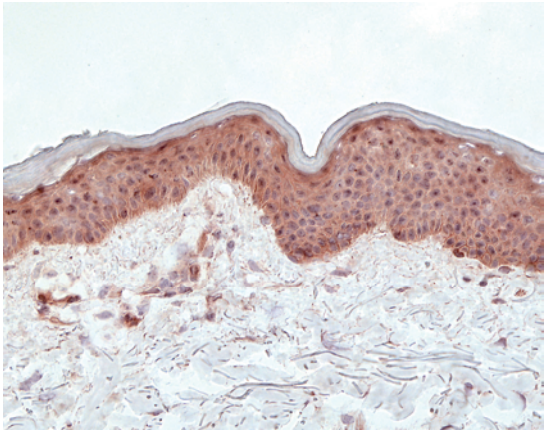

Figure 14. **Expression of SSTR1 in skin of macroscopically normal appearance skin from a patient with atopic dermatitis. SSTR1 is expressed in the entire living epidermis and in cells having a dendritic appearance, which are mainly located in the upper dermis.**

Table 5. **SSTR-expression normal-looking skin**

SSTR	Stratum granulosum	Stratum spinosum	Stratum basale	Dendritic cells	Vessels	Hair follicles	Sebaceous glands	Sweat glands	Muscles
1	++	+	++	++	++	++	++	++	+++
2	+++	+++	+++	++	++	++	++	++	+++
3	++	++	++	++	++	+	++	++	+++
4	(+)	+	+	+	++	+	+	+	+++
5	+	+	+	+	+	+	-	+	+

More attention is now being paid to the interaction between components of the nervous system and target cells in the cutaneous immune system. Inflammatory skin diseases have neurogenic components. Neuropeptides secreted by nerve fibres and various cutaneous cells can directly modify the functions of keratinocytes, Langerhans cells, mast cells, dermal microvascular endothelial cells and infiltrating immune cells.

Among these neuropeptides, substance P, neurokinin A, calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP) and somatostatin effectively modulate skin and immune cell functions, such as cell proliferation, cytokine production or antigen presentation, under physiological or pathophysiological conditions.[115] Expression and regulation of their corresponding receptors, which are expressed on a variety of skin cells, determine the final biological response mediated by these peptides. Therefore, neuropeptides, neuropeptide receptors and neuropeptide-degrading enzymes participate in a complex network that modulates skin inflammation, wound healing and the skin immune system.[116, 117] Pruritus is regarded as the cardinal symptom of atopic dermatitis, and the density of the distribution of cutaneous nerve fibres is much greater in atopic dermatitis than in normal controls.[118] Data suggests that stressful events and local trauma cause the release of neuropeptides, such as substance P, from sensory nerves in the skin, which may initiate the development of psoriasis or atopic dermatitis in predisposed subjects.[119-121] This view is supported by case reports of psoriasis patients in whom cutaneous nerve damage resulted in clearance of their skin lesion at that site, but in its reappearance after the recovery of cutaneous sensation.[119]

To our knowledge, paper IV is the first study concerning the occurrence and distribution of the five different somatostatin receptors in human skin. We evaluated the expression of SSTR subtypes 1-5 in human skin with an immunohistochemical method using specific polyclonal antibodies directed against SSTR1-5. Normal skin from healthy volunteers and skin from patients with psoriasis or atopic dermatitis had many similarities, but also some differences as regards SSTR expression. SSTR1-3 was strongly expressed in the epidermis of normal, psoriatic and atopic dermatitis skin. SSTR4 and 5 was moderately expressed in the epidermis of psoriasis patients, but showed a weaker expression in atopic dermatitis and normal skin of healthy volunteers. The intensity of the staining also varied considerably between the different keratinocyte layers in the epidermis, especially in the acanthotic epidermis of psoriasis patients. Dendritic cells in the papillary and upper reticular dermis showed a strong expression of SSTR1-4, but a weak expression of SSTR5 in all cases. All five SSTRs were expressed in sweat glands, hair follicles and sebaceous glands. Striated muscle fibres showed an intense expression of SSTR1-4, but a weak, or in some cases, even a negative expression of SSTR5.

Somatostatin, a neuropeptide with immmunomodulatory actions, may be important in the pathophysiology of various inflammatory skin diseases. T-lymphocytes play a key role in the pathogenesis of psoriasis and atopic dermatitis and somatostatin affects several fundamental lymphocyte functions. Somatostatin inhibits lymphocyte proliferation induced by mitogens and the production of antibodies.[122, 123] The capacity to migrate and localise in tissues is essential for the protective function of lymphocytes against infectious agents.[124, 125] However, this ability to migrate and infiltrate tissues is one of the main reasons why lymphocytes can cause autoimmunity, allergy and graft rejection and why neoplastic lymphocytes accumulate in tissues. The chemokine stromal cell-derived factor 1α (SDF-1α) is a potent stimulator of T-cell infiltration. Somatostatin selectively inhibits SDF-1α-induced T-lymphocyte infiltration into type I collagen matrices.[126] It also blocks the release of growth factors (IGF1, EGF, PDGF) and cytokines (IL-6, IFN-λ).[122, 127] The wide variation in the distribution of somatostatin receptors between the different skin types in paper IV indicates that somatostatin is an important mediator between the nervous system and skin, and it may

also be involved in the pathogenesis of inflammatory skin diseases by modifying the keratinocytes, the vascular endothelium and the skin's immune system.

Somatostatin has been used in several open-label trials as infusional therapy for psoriasis. In a double-blind placebo-controlled study of 21 patients, Matt *et al*. found a significant clinical improvement in 70% of patients assessed after 15 days with somatostatin treatment.[128] Although the test protocols in these trials are not comparable, the compiled data suggest that somatostatin probably improves psoriasis and psoriatic arthritis, but this therapy has several drawbacks. Its duration of action with a half-life in the circulation of about 3 min requires the use of a continuous intravenous infusion for sustained action. The effect of somatostatin is not selective with rebound hypersecretion of inhibited hormones, hyperglycaemia and gastrointestinal side effects. However, new and more SSTR-selective long-acting somatostatin analogues are under development and may become a therapeutic option for chronic inflammatory diseases.[129]

In conclusion, paper IV showed that all five SSTR are expressed in normal human skin and lesional skin of patients with psoriasis and atopic dermatitis, which suggests that in these diseases the various receptors operate in concert rather than as individual receptors. However, some data suggest a SSTR subtype selectivity. For example, it has been reported that SSTR2 and SSTR5 are the subtypes responsible for somatostatin-mediated inhibition of growth hormone from the pituitary, while SSTR5 mainly inhibits insulin secretion from the pancreas.[130, 131] The data from most clinical trials suggest that somatostatin probably has a positive effect on psoriasis and psoriatic arthritis, and somatostatin binds to all five SSTRs. However, a study of the somatostatin analogue octreotide, which has a high affinity for SSTR2 and 5 and a medium affinity for SSTR3, showed no major improvement inpatients with psoriasis.[132] The marked dermal microvascular expansion in lesional psoriatic skin suggests that psoriasis is angiogenesis-dependent.[133] Proliferating vessels express SSTR2, whereas nonproliferating vessels do not.[134] Moreover, angiogenesis can be inhibited by somatostatin.[135] Future clinical trials with new somatostatin analogues are needed to find a possible new approach for treatment of atopic dermatitis and psoriasis.

CONCLUSIONS AND PERSPECTIVES

The diagnosis of AD is, since there is no laboratory markers, based on major and minor clinical criteria, where the major features are pruritus, typical morphology and distribution of the lesions, chronic relapsing course, and personal and family history of AD. The pathogenesis is not fully understood, but several immunological aberrations are found in AD, including impaired cellular immunity, elevated serum IgE and eosinophil levels and IgE bearing Langerhans cells. Colonisation of the more or less chronically inflamed lesions by microbes may contribute to the perpetuation. The chronic inflammation and microbial colonisation/infection in combination with the immune impairments, primary or due to treatment effects, may lead to proliferative epidermal changes; hence the suspicion of a link to cancer development. In the studied cohort (paper I), there was a statically significant 13% overall excess, driven mainly by excesses of brain cancer and lymphoma. Risk elevations were noted also for esophageal and pancreatic cancer. We also observed a two-fold elevation of risk for non-melanoma skin cancer 1-35 years after entry among males, but not among females, and only during the first 10 years of follow-up. With only 12 observed non-melanoma skin cancer cases, however, both the overall excess and the gender difference could be a chance finding. Cases of basal cells carcinoma were not included since their registrations started in 2003. Thus, the non-melanoma skin cancer category includes only squamous cell carcinoma. The net effect on overall skin cancer risk is difficult to predict since as it aging and is slowly moving from lower to higher absolute risk. The excess of lung, esophageal and pancreatic cancer is consistent with smoking and alcohol. We had no information about these habits among our cohort members. Smoking and/or alcohol over-consumption may still have contributed to a biased selection into our cohort of hospitalised patients. Most patients with AD are managed on an outpatient basis, and those who are hospitalized (as in paper I) may have a less healthy lifestyle. Since the cohort has a high proportion of young patients, who have not yet aged into cancer ages, and the frequency of most cancer types increases with age, it would be of interest to repeat the follow-up when more time has elapsed. The study was performed in computerised inpatient register from January 1,1965 trough December 31,1999. A Danish study on 6275 hospitalised AD patients showed similar results as ours, regarding risks for all cancer among the 2030 adults included (Standard morbibidty ratio (SMR) 1.5 (95% CI: 1.2-1.9).[136] They also showed, among the adults patients, an increased risk of "keratinocyte cancer" (squamous cell cancer and basal cell carcinoma) SMR 2.4 (95% CI:1.2-5.4).[136] Melanomas were not increased. Comparisons with our cohort is somewhat hampered by the fact that basal cells carcinomas were not registered. Synnerstad *et al*. proposed that patients with AD have an decreased risk to develop malignant melanoma,[137] which was not the case in our cohort. Looking at atopy as a whole, Eriksson *et al*. showed no association between atopy or allergic symptoms and cancer, bases on a study of 13811 patients, who had been skin prick tested in 1976-1999.[138]

New treatments for atopic dermatitis, includes one of the latest, calcineurin inhibitors. The topical calcineurin inhibitors tacrolimus and pimecrolimus were approved in the USA for the treatment of AD in 2000 and 2001, respectively.sThey were approved in Sweden shortly thereafter. In 2005, the Pediatric Advisory Committee of the US FDA implemented a 'black box' warning for topical calcineurin inhibitors due to the lack of long-term safety data and the potential risk of the development of malignancies.

Although the elevated cancer risks after topical treatment with calcineurin inhibitors really are suspected, they have not been proven yet. Therefore, a repeated large cohort cancer incidence study of hospitalised Swedish AD patients is in progress.

Moisturising creams are important for treating and preventing AD. They are also used as an adjuvant to local steroids. There are many different kinds and combinations and a big number of formulations are available. It's therefore necessary to gain scientific evidence in additional to clinical experience to increase the understanding of how emollients cause their effects. We focused on functional changes related to addition of urea alone or urea and sodium chloride together by monitoring TEWL, capacitance and electrical impedance spectra in paper II. We found an increased skin capacitance after 7 days and this increased persisted after 14 days in the case of urea containing cream without NaCl, but a combined production showed a reduction on day 14. The increasing capacitance showed no significant correlation with increases in electrical impedance indices. The increases in the former values were present from day 7 to day 14. The impedance indices MIX and IMIX increased significantly more with cream containing sodium chloride and urea than that with no sodium chloride. In this study, the increase in capacitance after long-term treatment with moisturisers was not accompanied by simultaneous increase in TEWL, indicating that the skin was not hydrated to the extent that its water permeability was affected. We observed that TEWL is vulnerable to a number of external factors that are difficult to control and also to the psychological status of the study subjects. No case of irritation, stinging or suspected allergy were reported by the patients. The addition in sodium chloride to the moisturiser seems not to have any drawbacks in the cosmetics nor any adverse affects in the treatment of AD. The impedance indices MIX and IMIX are correlated with the corneometer readings and by, choosing depth No. 2, our measurements mainly reflect changes in the stratum corneum. The findings in this study indicate that moisturiser containing both urea and sodium chloride seems somewhat more effective than the same moisturiser without sodium chloride, at least concerning the ability to normal impedance indices of AD skin.

In paper III, we evaluated the skin of healthy subjects and of patients having AD with an instrument measuring electrical impedance and other non-invasive methods,TEWL and capacitance and studied the effects of an emollient (Proderm). Dry AD skin showed higher TEWL, lower capacitance, and changes in certain impedance indices. Our findings may be due to simultaneous impairment of the mechanism responsible for skin hydration and barrier function with regard to capacitance and TEWL which would accord with the findings in comparable studies. We found that certain impedance indices were lower in AD skin, as well as in corneometer measurements. This may indicate that the water content is higher in healthy skin than in AD skin. An improvement in the skin condition was reflected by an increase in capacitance and no change in TEWL i.e. a moisturizing effect without an impairment of the skin barrier function. Changes in TEWL can reflect a multitude of factors, including lipidsynthesis processing and organization. On the other hand, a decrease is interpreted as a positive effect of the treatment, reflecting improvement in skin barrier function. The results vary and are affected by several factors such as design, population (especially age) part of body and the study preparation. On the whole this study showed increases in capacitance with few changes in TEWL measurements and normalization of certain impedance indices after treatment, indicating that the foam did moisturise the skin of patients with AD.

The electrical characteristics of human skin affected by large variations in factors, such as hydration, lipid content, number of cell layers in the stratum corneum, size of corneocytes and some properties of deeper skin layers, which may affect the complex nature of the skin barrier. Impedance indices are regarded as reflecting various properties of the skin, although the mechanisms responsible for changes in indices are still not understood and the techniques for assessing skin electrical impedance are under development. New instruments may increase their reliability by using the information of the whole impedance spectra in combination with a new micro-invasive electrode system.

Better techniques are needed in order to evaluate the effects of different creams and lotions. To gain more objective methods of investigation, a gradual development is in process from subjective assessments of the results of treatment towards more specific measuring instruments. The methods for measuring skin electrical impedance are still being developed, and new instruments may increase its accuracy – e.g., more effective use of the information inherent in the spectra by the application of sophisticated mathematical tools.[139-141] In spite of the fact that AD is a chronic disease, better methods for the checking and treatment of the negative effects of AD skin will contribute to the preservation of high quality of life. Expectations are therefore high when it comes to the development of new effective preparations and treatments.

The wide variation in the distribution of SSTR between different skin types in Paper IV indicates that somatostatin is important mediator between the nervous system and skin, and it may be involved in the pathogenesis of inflammatory skin diseases by modifying the keratinocytes, the vascular endothelium and the skin system. But does somatostatin have the same inhibitory effect in the skin as the calcitonin gene-related peptide (GCPR)? Has this neuropeptide a specific task or is it secreted together with other neuropetides? Is this peptide involved in the inflammatory reactions or/and in pruritus? Further investigations need to be done in order to find out whether different neuropeptides have a different distribution pattern.

More attention is being paid to the interaction between components of the nervous system and target cells in the cutaneous immune system and inflammatory skin diseases have neurogenic components. Neuropeptides secreted by nerve fibres and various cutaneous cells can directly modify the functions of keratinocytes, Langerhans cells, mast cells, dermal microvascular endothelial cells and infiltrating immune cells. Among these neuropeptides, substance P, neurokinin A, CGRP, vasoactive intestinal peptide (VIP) and somatostatin effectively modulate skin and immune cell functions, such as cell proliferation, cytokine production or antigen presentation, under physiological or pathophysiological conditions.[115] Expression and regulation of their corresponding receptors, which are expressed on a variety of skin cells, determine the final biological response mediated by these peptides. Therefore, neuropeptides, neuropeptide receptors and neuropeptide-degrading enzymes participate in a complex network that modulates skin inflammation, wound healing and the skin immune system.[116, 119, 142] Pruritus is as major criteria of AD, and the density of the distribution of cutaneous nerve fibres is much greater in AD than in normal controls.[118] Data suggests that stressful events and local trauma cause the release of neuropeptides, such as substance P, from sensory nerves in the skin, which may initiate the development of psoriasis or AD in predisposed subjects.[120, 121, 142] This view is supported by case reports of psoriasis patients in whom cutaneous nerve damage resulted in clearance of their skin lesion at that site, but in its reappearance after the recovery of cutaneous sensation.[119]

Somatostatin has been used in several open-label trials as infusional therapy for psoriasis. In a double-blind placebo-controlled study of 21 patients, Matt et al found a significant clinical improvement in 70% of patients assessed after 15 days with somatostatin treatment.[128] Although the test protocols in these trials are not comparable, the compiled data suggest that somatostatin probably improves psoriasis and psoriatic arthritis, but this therapy has several drawbacks. Its duration of action with a half-life in the circulation of about 3 min requires the use of a continuous intravenous infusion for sustained action. The effect of somatostatin is not selective with rebound hypersecretion of inhibited hormones, hyperglycaemia and gastrointestinal side effects. However, new and more SSTR-selective long-acting somatostatin analogues are under development and may become a therapeutic option for chronic inflammatory diseases.[129]

In paper IV it is shown that all five SSTR are expressed in normal human skin and lesional skin of patients with psoriasis and atopic dermatitis, which suggests that in these diseases the various receptors operate in concert rather than as individual receptors. The wide variation in the distribution of somatostatin receptors between the different skin types in paper IV indicates that somatostatin is an important mediator between the nervous system and the skin, and it may be involved in the pathogenesis of inflammatory skin diseases by modifying the keratinocytes, the vascular endothelium and the skin's immune system. But, it is also important to point out that although certain expression patterns of the SSTR are described in paper IV, very little is really known about the *functional* relevance of the findings. Some other data suggest a SSTR subtype selectivity. For example, it has been reported that SSTR2 and SSTR5 are the subtypes responsible for somatostatin-mediated inhibition of growth hormone from the pituitary, while SSTR5 mainly inhibits insulin secretion from the pancreas.[131] The data from most clinical trials suggest that somatostatin probably has a positive effect on psoriasis and psoriatic arthritis, and somatostatin binds to all five SSTRs. However, a study of the somatostatin analogue octreotide, which has a high affinity for SSTR2 and 5 and a medium affinity for SSTR3, showed no major improvement inpatients with psoriasis.[132] The marked dermal microvascular expansion in lesional psoriatic skin suggests that psoriasis is angiogenesis-dependent.[133] Proliferating vessels express SSTR2, whereas nonproliferating vessels do not,[134] Moreover, angiogenesis can be inhibited by somatostatin.[135] Future clinical trials with new somatostatin analogues are needed to find a possible new approach for treatment of AD and psoriasis.

Improved knowledge of the mechanisms that drive the inflammation in AD may lead to a better understanding of this disease and shed light on the critical role of the epidermal-barrier function and the immune system. Both lead to IgE-mediated sensitisation and must be considered as major targets for therapy. Future developments aimed at correcting the molecular defects in the stratum corneum may provide tailor-made possibilities to improve the barrier function. Early treatment and careful management could improve the outcome and quality of life for patients with AD.

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