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# **Study of Pruritus in Psoriasis Vulgaris: Role of Tachykinins**

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*Dedicated to the memory of my belated father*

## ABSTRACT

Pruritus has not been considered as the most important symptom of psoriasis. In the present thesis various methodologies have been used to investigate this symptom in psoriasis.

The focus groups created a proper atmosphere for discussion on different aspects of pruritus in psoriasis. Pruritus was most common on the lower back and legs. Stress, cold weather and skin dryness were considered as the most common worsening factors for the condition. Sunbath and application of emollients with or without corticosteroids and calcipotriol cream were suggested as factors that relieved pruritus. Quality of life was affected in some patients.

A comprehensive questionnaire was used to investigate in detail about pruritus in psoriasis. The frequency and intensity of pruritus were higher in women. Lower leg and scalp were reported as being the most commonly affected sites. Major aggravating factors were stress and dryness of skin. Sun, sleep and vacation could relieve to the condition. The most common anti-pruritic treatments used by the patients were topical glucocorticoids, vitamin D and emollients, while antihistamines were used by a small number of patients. Mood, concentration and sleep were negatively affected by pruritus.

Substance P, neurokinins A (NKA), B (NKB) and NK-2 receptor (R), reactive nerves and substance P, NKA, NKB and their respective receptors NK-1, NK-2 NK-3 reactive inflammatory cells were more numerous in lesional than non-lesional psoriasis and healthy control skin, respectively. The pruritus intensity and number of NK-2R positive cells in lesional psoriatic skin were significantly correlated.

Intradermally injected substance P induced pruritus, flare and wheal in psoriasis patients. Substance P ( $10^{-5}$  mol/L) induced a tendency to larger intensity of pruritus in lesional than non-lesional psoriatic skin. Histamine produced a shorter itch latency period in lesional and smaller wheal in non-lesional psoriasis skin compared to healthy individuals.

In conclusion, pruritus is one of the common symptoms of psoriasis. Members of the tachykinin family might play an important role in the pathogenesis of pruritus in this disease.

*Keywords:* Itch, pruritus, psoriasis, tachykinin, questionnaire, focus group, intradermal injection.

## LIST OF PUBLICATIONS

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- III. **Amatya B, El-Nour H, Holst M, Theodorsson E, Nordlind K.** Expression of tachykinins and their receptors in plaque psoriasis with pruritus. Manuscript
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## LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropin releasing hormone
BDI	Beck's Depression Inventory
CGRP	Calcitonin gene-related peptide
CNS	Central nervous system
CRH	Corticotrophin releasing hormone
HPA	Hypothalamus-pituitary-adrenal axis
IL	Interleukin
NGF	Nerve growth factor
NKA	Neurokinin-A
NKB	Neurokinin-B
NK-R	Neurokinin receptor
NPY	Neuropeptide Y
PASI	Psoriasis Area and Severity Index
RIA	Radioimmunoassay
TAC	Tachykinin gene
UV	Ultraviolet
VAP-1	Vascular adhesion protein-1
VAS	Visual Analogue Scale
VIP	Vasoactive intestinal polypeptide

# **1 INTRODUCTION**

## **1.1 PSORIASIS**

### **1.1.1 Epidemiology**

Psoriasis is a common chronic inflammatory skin disease. It is a multifactorial disease with genetic and environmental interactions (Raychaudhuri and Farber, 2000) affecting about 0.5 to 4.6% of world population with rates varying between countries and ethnic groups. It tends to be more common in higher latitudes and in Caucasians populations (Lebwohl, 2003). It may appear at any age and is almost equally prevalent in males and females (Lebwohl, 2003).

### **1.1.2 Clinical features**

Depending upon the inheritance and environmental factors, severity of the disease varies. Some patients may present as isolated erythematous scaly plaque on elbows, knees or scalp, whereas others can have up to 100% cutaneous surface affected. Psoriasis lesions are usually distributed bilaterally symmetrical. Women are more likely to rate their psoriasis more severe than men (Koo, 1996). The most common form of psoriasis is plaque-type psoriasis occurring in more than 80% of the cases, the guttate form occurring in about 10% and erythrodermic and pustular types occurring in less than 3% (de Rie et al., 2004).

### **1.1.3 Pathogenesis**

Psoriasis is characterized by an epidermal hyperproliferation and abnormal cell differentiation, together with a pronounced infiltrate of inflammatory cells (mast cells and lymphocytes) as well as dilated capillaries.

In psoriatic skin, an increased number of mast cells and nerve-mast cell contacts are evident compared to non-involved (Michaelsson et al., 1995) and control (Naukkarinen et al., 1991, Petersen et al., 1998) skin. The lifespan of the mast cells may be of importance in psoriasis, as in other chronic inflammatory diseases. A delayed apoptosis may lead to

chronic inflammation. Mast cells are also increased in itching compared to non-itching psoriasis and also have extended dendrites (Nakamura et al., 2003).

Another cell that plays an important role in the pathogenesis are the lymphocytes, which infiltrate the dermal tissue. Among the lymphocytes, T helper and suppressor/cytotoxic lymphocytes (Lebwohl, 2003) and natural killer cells (Cameron et al., 2002) have been speculated to be of importance. The migration of these cells from the circulation to the peripheral tissue is mediated by several adhesion molecules on the endothelial cells and leukocytes. Significant over-expression of vascular adhesion protein (VAP-1) was found in both lesional and non-lesional skin psoriatic skin and this protein was evident at a higher serum level in psoriatic patients compared to healthy controls (Madej et al., 2007).

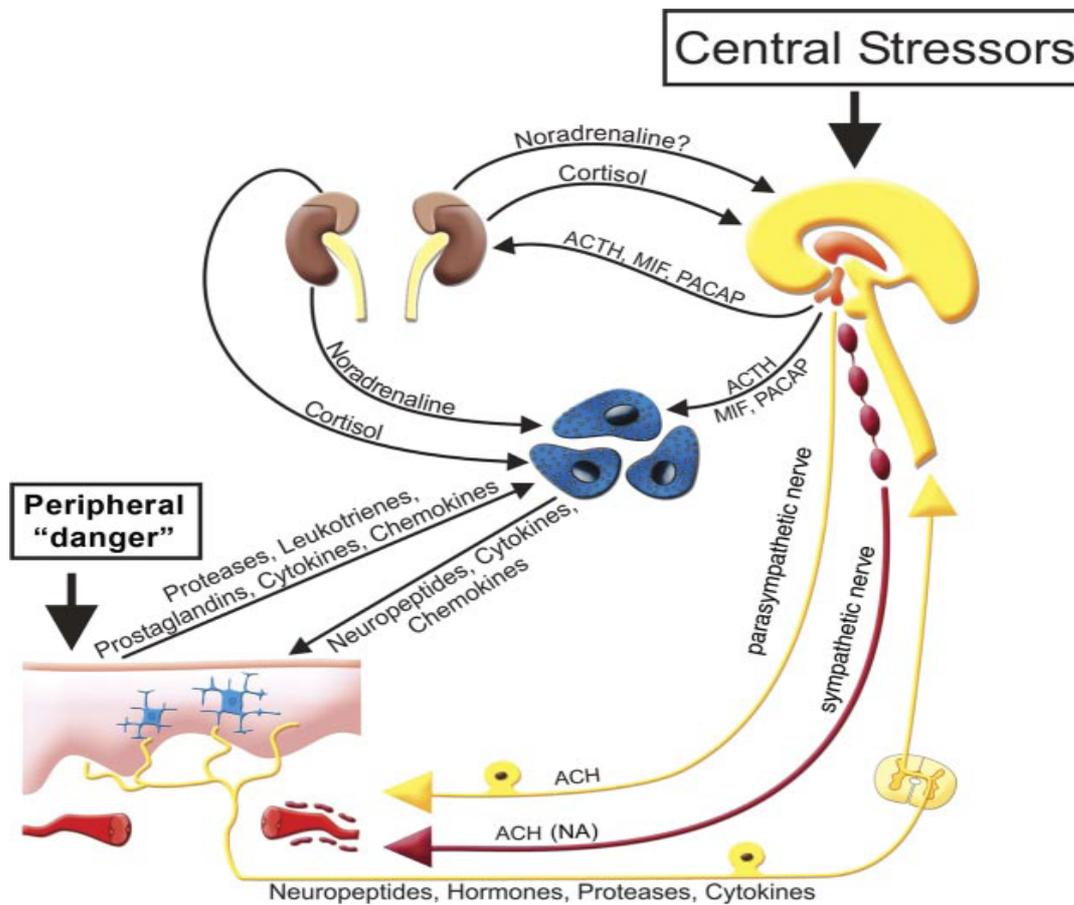
## **1.2 INNERVATION OF SKIN**

The skin is innervated with a dense network of highly specialized afferent sensory and efferent autonomic nerve fibers. The neurocutaneous interacting signalling substances influence a variety of physiological and pathophysiological functions including cell growth, wound healing, inflammation pain and pruritus.

The sensory nerves can be divided into epidermal and dermal skin nerve organs. The epidermal skin nerve organ consists of “free” nerve endings or hederiform nerve organs (Merkel cells) and in the dermal part, free nerve endings, the hair nervous network (Pinkus discs) and the encapsulated endings (Ruffini, Meissner, Krause, Vater-Pacini) (Roosterman et al., 2006). The afferent somatic nerve fibers derive from dorsal root ganglia with fine unmyelinated (C-) or myelinated (A $\delta$ -) fibers. Both types of fibers respond to a physiological stimulation such as physical stimuli (e.g. heat, cold) and low intensity mechanical stimulations. The network of sensory nerves releases a series of neuromediators upon stimulation that activate specific receptors on many target cells in the skin, thereby modulating inflammation, cell growth and immune responses.

Autonomic nerve fibers are functionally divided into adrenergic, cholinergic and non-cholinergic subtypes, which are not strictly anatomically separated. Adrenergic and cholinergic nerves which release norepinephrine and acetylcholine, respectively, represent only a minority of cutaneous nerves innervating eccrine, apocrine, sebaceous glands and blood vessels. Autonomic nerves also produce neuropeptides such as calcitonin gene-related peptide (CGRP), vasoactive intestinal polypeptide (VIP), and neuropeptide Y (NPY) (Roosterman et al., 2006).

The central nervous system (CNS) is directly (via efferent nerves or CNS-derived mediators) or indirectly (via adrenal glands or immune cells) connected to skin functions (Fig .1).



**Fig 1.** Skin as a neuroimmunoendocrine organ (Roosterman et al., 2006) (With permission from the authors).

### **1.3 HYPOTHALAMIC-PITUITARY-ADRENAL AXIS**

The hypothalamus-pituitary-adrenal axis (HPA) is of major importance with regard to an organism's response to physical or psychosocial stimulation. It is characterized by a robust circadian rhythm with cortisol level peaking in the early morning hours around the time of awakening and being lowest in the midnight in subjects with a normal sleep-awake cycle. Stimulation of the HPA axis in the morning results in higher HPA hormone responses compared to comparable stimulation in the evening (Federenko et al., 2004). When a stimulus is perceived as a stressor, the activation of HPA axis occurs slowly within minutes or hours with the release of corticotrophin releasing hormone (CRH) from the hypothalamus. CRH stimulates the pituitary to release adrenocorticotropin releasing hormone (ACTH) into the systemic circulation, which in turn stimulates adrenal cortex to release a glucocorticoid, cortisol. In humans, cortisol is the primary glucocorticoid. Normally, cortisol is synthesized and secreted in the adrenal cortex at the rate of about 10 mg daily in humans. Under stressful condition, cortisol level can increase at least 10 folds. An increased level of cortisol acts as a negative feedback signal to suppress further CRH and ACTH release at the level of the hypothalamus and pituitary, respectively (King and Hegadoren, 2002).

Stress hormones can be measured to assess the stress response at different level of the HPA axis. Cortisol is an easily accessible peripheral parameter that provides a reliable indication of HPA axis dysfunction. It can be measured in saliva, plasma and urine. A major advantage of salivary sampling is that cortisol in saliva is 100% unbound and biologically active. Besides that, salivary sampling is non-invasive, painless, less stressful and more easily performed. It is also less expensive, raises lesser ethical concerns than more invasive procedures and has a high rate of good compliance (Baum, 1997).

A special susceptibility of the skin to acute and or/chronic psychological stress has been described in a wide range of skin diseases, including psoriasis, atopic dermatitis, acne

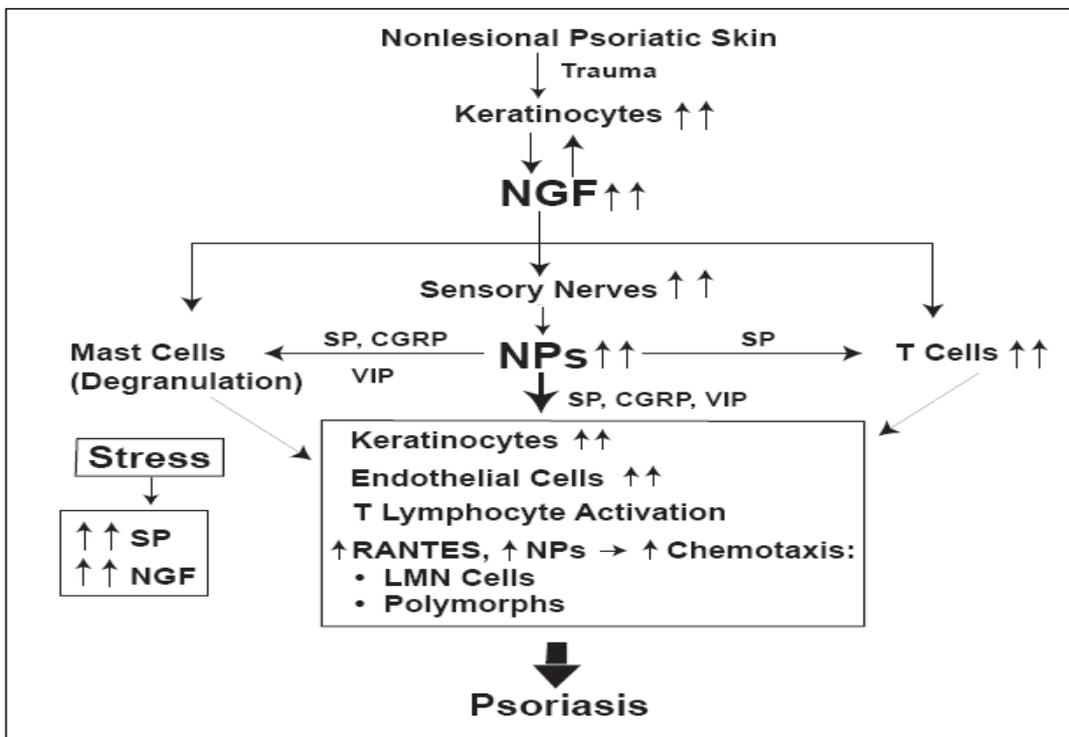
vulgaris etc. (Arck et al., 2006). Moreover pruritus may worsen by stress in skin diseases (Schneider et al., 2006, Verhoeven et al., 2008).

#### **1.4 NEUROIMMUNE INTERACTION IN PSORIASIS**

It has been known for a long time that there is an association between the nervous system and psoriasis. After accidental sectioning of spinal nerves, psoriasis declines in the anaesthetic skin (Dewing, 1971). Treatment with capsaicin, which gives a release of neuropeptides from unmyelinated sensory neurons, may give an improvement of the disease (Bernstein et al., 1986). It has been reported an increased length (Naukkarinen et al., 1991) and an increased number (Chan et al., 1997, Steinhoff et al., 2003) of substance P immunoreactive intraepidermal nerve fibers in psoriatic lesions. Moreover, using suction blister fluid or extract from psoriatic skin, an increased level of substance P has been found by radioimmunoassay (RIA) (Anand et al., 1991, Eedy et al., 1991, Wallengren et al., 1986).

Stress has been reported to worsen psoriasis (Farber and Nall, 1993, Kimyai-Asadi and Usman, 2001, Zachariae et al., 2004b). It is not known which neuromediators that are involved in the crosstalk between the psoriatic skin and the CNS. Nerve growth factor (NGF) plays an important role in regulating innervation and upregulating neuropeptides in psoriasis (Fig.2) (Raychaudhuri and Farber, 2000). Recently, NGF is recognized as the crucial mediator of stress response (Arck et al., 2006, Peters et al., 2004). Studies have reported that psychological stress events could increase the level of NGF in blood as well as NGF messenger RNA synthesis in the hypothalamus. Levels of NGF might also increase during stressful condition in psoriasis (Raychaudhuri and Farber, 2000).

Stressful events might also alter the levels of substance P in the CNS and the periphery (Raychaudhuri and Farber, 2000). In an animal model, it has been observed that stress can increase levels of substance P in the adrenal glands by activating descending autonomic nerve fibers (Vaupel et al., 1988).



**Fig 2.** Neurogenic inflammation in the pathogenesis of psoriasis. ↑ Indicates increase in amount;

↑↑ Increase in number LMN, lymphomononuclear cells; NPs neuropeptides (Raychaudhuri et al., 2000) (With permission from the authors).

## 1.5 TACHYKININS

Tachykinins are a family of closely related peptides which are widely distributed within the mammalian peripheral and the CNS. Members of the tachykinin family include substance P, neurokinin A (NKA) and neurokinin B (NKB) which are present in neuronal and non-neuronal cells such as endothelial cells, Leydig cells and immune cells. The tachykinins share a common C-terminal sequence of Phe-X-Gly-Leu-Met-NH<sub>2</sub> and have different amino-terminal sequences that are receptor specific.

*Substance P* is present in the CNS and in primary afferent sensory neurons (Pennefather et al., 2004). It is encoded by the tachykinin 1 (TAC1) gene (Pennefather et al., 2004). Substance P is thought to cause degranulation of mast cells through histamine-dependent and possibly-independent pathways (Hagermark et al., 1978). It also has numerous proinflammatory actions such as acting as a chemotactic factor for neutrophils,

monocytes, T cells and eosinophils. Substance P induces the expression of adhesion molecules by post-capillary venules of the skin. It stimulates the secretion of proinflammatory cytokines from monocytes and keratinocytes (Marsella and Nicklin, 2001) and may induce pruritus mediators such as by acting on epidermal keratinocytes to release leukotrienes B<sub>4</sub> which causes itch-associated responses in mice (Andoh et al., 2001).

*NKA* is present in the CNS and in primary afferent sensory neurons supplying a number of peripheral tissues. It has been detected in human peripheral blood monocytes (Ho et al., 1997), lymphocytes and fetal microglia and also in bone marrow-derived dendritic cells (Lambrecht et al., 1999). Similar to substance P, *NKA* is also capable of activating keratinocytes resulting in the release of proinflammatory cytokines (Scholzen et al., 1998). *NKA* is also encoded by the *TAC1* gene (Pennefather et al., 2004).

*NKB* is present in the CNS and the spinal cord. The role of *NKB* in neurogenic inflammation has been controversial in earlier studies (Brodin et al., 1986, Campos and Calixto, 2000). *NKB* recently has been reported to be expressed in human immune cells (Klassert et al., 2008). The *TAC3* gene produces the peptide *NKB* in human (Pennefather et al., 2004).

There are three mammalian tachykinin receptors, neurokinin-1 receptor (NK-1R), NK-2R and NK-3R. Substance P has an affinity for NK-1R, and *NKA* and *NKB* tachykinins have affinities for NK-2R and NK-3R, respectively (Nelson and Bost, 2004). Among the tachykinins, besides *NKA*, other members such as *NKB* and substance P can also behave as agonists of NK-2R (Lecci et al., 2004).

The NK-1R is widely expressed at both the central and the peripheral level and is present in neurons, vascular endothelial cells, muscle and different types of immune cells (Ho et al., 1997, Lai et al., 1998, Patacchini and Maggi, 2001, Stewart-Lee and Burnstock, 1989, Tsuchida et al., 1990). It recognizes the C terminal sequence of the substance P peptide. NK-1R is upregulated in unmyelinated afferent axons during inflammation in rats

(Carlton and Coggeshall, 2002) and is involved in the itch-associated response to substance P (Andoh et al., 1998).

In contrast, the NK-2R is mainly expressed in the periphery with the exception of specific brain nuclei in the CNS. Conversely NK-3R, is mainly expressed in the CNS and has only been detected in certain peripheral tissues such as the human uterus, skeletal muscle, lung and liver and certain enteric neurons from the gut of different species (Pennefather et al., 2004).

In human skin a dense innervation with tachykinin immunoreactive nerves in the upper and lower dermis, as well as the epithelium, supports the capacity for these neuropeptides to participate in sensory transmission as well as to facilitate interaction with epidermal and dermal target cells (Roosterman et al., 2006). The tachykinin immunoreactive sensory nerves are often associated with dermal blood vessels, mast cells, hair follicles or epidermal cells such as keratinocytes, Langerhans cells and melanocytes (Roosterman et al., 2006).

Intradermal injections of tachykinin members cause pruritus in human skin. Of the tachykinins, substance P is a well known inducer of itch (Hagermark et al., 1978), while NKA elicited pruritus in normal but not in inflamed skin, and NKB elicited pruritus neither normal nor in inflamed skin (Thomsen et al., 2002).

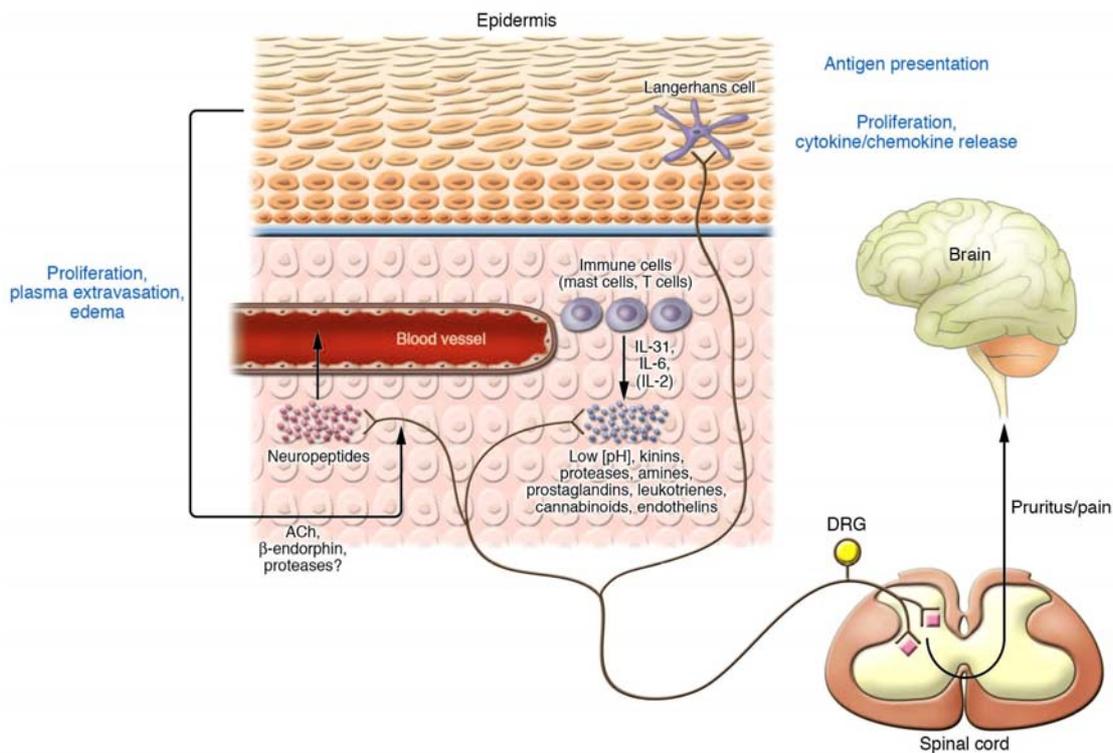
## **1.6 PRURITUS**

Pruritus is one of the most common symptoms of many dermatological diseases and systemic disorders such as hepatic and chronic renal diseases. Chronic pruritus is problematic for patients as well as for physicians. It has been considered to be one of the most distressing physical sensations that can lead to sleep deprivation, psychological disturbances and may also be associated with anxiety and depression (Zachariae et al., 2004a).

There is no internationally accepted clinical classification of itch (Bernhard, 2005, Twycross et al., 2003). A neuropathophysiologically based classification was proposed in

2003, which classified itch as i) pruritoceptive; ii) neuropathic; iii) neurogenic; or iv) psychogenic (Twycross et al., 2003). Recently, the *International Forum for the Study of Itch* (IFSI) formulated the clinical classification of itch based on the origin and clinical manifestation of itch in diseased and normal skin (Stander et al., 2007). According to clinical classification it was grouped as i) pruritus on primarily diseased, inflamed skin; ii) pruritus on primarily normal, non-inflamed skin; and iii) pruritus with chronic secondary scratch lesions.

A wide range of peripheral itch-inducing stimuli generated within or administered to the skin can trigger pruritus. There are various known itch-associated mediators including amines (e.g. histamine, serotonin etc.), proteases (e.g. tryptases, chymases, etc), neuropeptides (e.g. substance P; CGRP; VIP; somatostatin etc.), opioids, eicosanoids (leukotrienes and prostaglandins), growth factors, cytokines (interleukin (IL); interferon- $\gamma$ ; tumour necrosis factor e.g.), eosinophil products and platelet activating factor (Nakamura et al., 2003). Mediators act either directly on free nerve endings or cells or indirectly by inducing the release of cell content or by potentiating the effect of other mediators (Yosipovitch and Papoiu, 2008). Once released the exogenous and endogenous mediators stimulate unmyelinated C-fibers. High affinity receptors for pruritogenic mediators transmit the stimulus via intracellular signalling from the periphery to dorsal root ganglion and spinal cord. In the spinal cord, a specific area of the dorsal horn (at Lamina I) transmits the signal via the lateral spinothalamic tract to the CNS after crossing to the contralateral side (Fig. 3, Paus et al. 2006). Activation of certain areas in the CNS such as the anterior cingulate and dorsal insular cortex, supplementary motor, premotor area and inferior parietal lobe results in the perception of itch and the scratch response (Paus et al., 2006, Reich and Szepietowski, 2007).



**Fig 3.** Neurophysiological pathway of pruritus (Paus et al., 2006) (With permission from the authors).

## 1.7 PSORIASIS AND PRURITUS

Although the word “psora” derived from the Greek, means “itch”(Glickman, 1986), pruritus has never been considered as the important symptom in psoriasis.

Prevalence of pruritus in psoriasis has been reported from different parts of the world, ranging from 64-84 % (Chang et al., 2007, Sampogna et al., 2004, Szepietowski et al., 2004, Yosipovitch et al., 2000).

Among the clinical variants of psoriasis, pruritus is more common in plaque-type psoriasis compared to other variants such as guttate, pustular or erythrodermic (Sampogna et al., 2004, Yosipovitch et al., 2000). The characteristic of pruritus in psoriasis is not well known.

The mechanism of pruritus and the involvement of mediators in psoriasis are still not clear. Better understanding of such mechanisms might contribute to the development of new treatments compared to the traditional systemic and topical therapies for pruritic psoriasis patients available at present, which often have limited effect.

Currently available effective treatment modalities of pruritus in psoriasis are limited, the treatment options being similar to the treatment of psoriasis *per se*. Antipruritic therapies that are used for treating psoriasis itch are coal tar products, topical corticosteroids, salicylates, menthol, pramoxine, capsaicin, vitamin D analogues and topical immunomodulators (Dawn and Yosipovitch, 2006). Oral sedating antihistamines, mirtazapine, methotrexate and biologicals are other treatment options for pruritus in psoriasis. Recent studies have demonstrated that among the biologicals, efalizumab is effective in reducing itch (Gordon et al., 2003, Menter et al., 2005). Phototherapy has been considered as an effective treatment of pruritus in psoriasis (Gupta et al., 1999, Lebwohl, 1994, Samson Yashar et al., 2003). However, other studies have indicated that it is less effective (Amatya et al., 2008, Yosipovitch et al., 2000)

### **1.8 PSORIASIS, PRURITUS AND TACHYKININS**

Neuropeptides may contribute to the induction and maintenance of the inflammatory process during psoriasis (Chang et al., 2007). Interaction between the stratum corneum, keratinocytes, mast cells, immune cells, neuropeptides and nerves during the neurogenic inflammation induce itch in many inflammatory dermatoses (Reich and Szepietowski, 2007).

Among the neuropeptides, substance P (Chang et al., 2007, Nakamura et al., 2003, Reich et al., 2007b), CGRP, VIP and NPY (Reich et al., 2007b, Reich and Szepietowski, 2007) are thought to play a role in the pathogenesis of pruritus in psoriasis. When comparing non-itching and itching psoriasis, there was an increase of substance P containing nerve fibers in the perivascular area in the latter skin and moreover, a downregulation of neutral endopeptidase (responsible for the degradation of substance P) basally in the epidermis and within the endothelium, as well as many degranulating mast cells (Nakamura et al., 2003). In addition, there was a correlation between the pruritus intensity and the number of intraepidermal nerve fibers. In contrast, a study by Remröd *et al.* (2007) could not confirm a

correlation between substance P positive nerve fibers or cells with pruritus intensity in psoriasis.

In another study, keratinocytes in the psoriatic plaques of patients with pruritus showed consistently displayed increased expression of substance P receptor (NK-1), high affinity nerve growth factor receptor (TrkA) and CGRP receptor (CGRPR) and these markers were also related to the severity of psoriasis (Chang et al., 2007).

## **1.9 FOCUS GROUP**

Several techniques can be used to collect and analyze qualitative data for different diseases, such as in-depth interviews, focus groups, case studies and observational studies.

A focus group contains approximately four-to-ten patients (depending on the disease) who have experience of a topic of common interest and who gather together to discuss their understanding of and perspectives on the subject. This allows groups rather than individuals to explore topics and to express opinions, but not necessarily to reach a consensus in an open-ended manner (McNally et al., 1998). In addition, the focus group method is well suited to the study of attitudes and experiences (Kitzinger, 1994) and has considerable potential in health research. The main stages in a focus group study include sample selection, facilitation of contributions from group members, transcription, analysis of text and, finally, interpretation. The groups meet in relaxed surroundings, the aim being to encourage conversation. A moderator aids this by asking open-ended questions. The data generated by focus group discussions is transcribed and themes within the data are coded and used to build up theories. Informal design of the focus groups may stimulate new ideas and insights from the participants and can provide new and valuable information for the particular topic that the researcher may not have considered (Ramirez and Shepperd, 1988).

In dermatology, focus group discussion has considerable potential, particularly in studies of patients' perspectives and attitudes to skin diseases, their causes and treatments. It has been used in a study of the experiences of parents of children with atopic eczema,

regarding the disease cause, triggering factors and its emotional effects (Chamlin et al., 2004). In psoriasis, focus group discussion has been used to determine patients' perceptions of the advantages and disadvantages of different topical psoriasis therapies (Housman et al., 2002) and to assess the impact on quality of life (McKenna et al., 2003).

## 2 AIMS AND OBJECTIVES

- To study the characteristics of pruritus in psoriasis using focus groups. (Study I)
- To study the prevalence and characteristics of pruritus in psoriasis using a questionnaire. (Study II)
- To investigate the expression in psoriasis of members of the tachykinin family, substance P, NKA, NKB and NK-1, NK-2 and NK-3 receptors in plaque psoriasis and to correlate with pruritus. (Study III)
- To study the cutaneous effects of an intradermally injected member of the tachykinin family, substance P, in psoriasis, compared to healthy control skin. (Study IV)

### **3 PATIENTS AND METHODS**

Various methodologies had been used to study the different aspects of pruritus in psoriasis.

#### **3.1 GENERAL CRITERIA OF PATIENTS (STUDY I-IV)**

Psoriatic patients with a complaint of pruritus and with the following inclusion criterias i) age range >20 years to <60 years; ii) with stable plaque psoriasis; iii) equal male-to-female ratio; iv) who had not received topical treatment (except emollients) for at least 2 weeks, were recruited (Study III, IV). Patients with other types of psoriasis than plaque psoriasis, who were receiving methotrexate, psoralen-UVA (PUVA), retinoid or biological therapies or anti-histamines (Study III, IV), having pruritus due to other dermatological or systemic diseases and who are psychologically unstable, alcoholics or pregnant were excluded from the study.

#### **3.2 PSORIASIS AREA AND SEVERITY INDEX (PASI) (STUDY I, III, IV)**

Each patient was investigated clinically and the severity of psoriasis was scored using the Psoriasis Area and Severity Index (PASI) (Fredriksson and Pettersson, 1978), which was assessed by the same individual (BA).

#### **3.3 PRURITUS INTENSITY (STUDY I-IV)**

Patients were asked to mark their current clinical pruritus intensity using a 10 cm Visual Analogue Scale (VAS) (Study I-III). The scale of left and right ends was marked “no pruritus” (0 cm) and “maximum pruritus” (10 cm), respectively. Experimentally-induced pruritus for study IV was measured using the computerized VAS (Somedic, Hörby, Sweden) (details of experimental pruritus given below).

#### **3.4 FOCUS GROUP (STUDY I)**

The focus group sessions took place between September 2006 and April 2007. Twenty patients (13 females and 7 males; age range 30-55 years) with a diagnosis of plaque psoriasis with pruritus intensity more than 4-to-10 cm measured by VAS, were selected from the outpatient clinical records of the Department of Dermatology, Karolinska University Hospital, Stockholm, Sweden. They were divided into five groups in order to ensure good

interaction. At the beginning of the discussion, the moderator pointed out the themes that needed to be discussed and invited each of the participants to share their perceptions and experiences related to pruritus in psoriasis. The patients discussed the area of involvement and extent of pruritus, its characteristics, worsening and relieving factors, treatment modalities and the effects of pruritus on quality of life.

According to the information gathered from the focus group discussions, relevant questions were added to the questionnaire (Study II).

### **3.5 PRURITUS QUESTIONNAIRE (STUDY II)**

A comprehensive questionnaire was sent out to 109 patients with plaque psoriasis, who had visited the Department of Dermatology, at Karolinska University Hospital, Solna, or the Department of Dermatology, Utsikten Medical Center, Stockholm, Sweden between January 2006 and January 2007. These patients, who were diagnosed with psoriasis vulgaris of the plaque variant, were selected from the clinical registries of the respective clinics.

This questionnaire was a modification of the McGill pain questionnaire (Melzack, 1987). The modification involved adding certain parameters such as those related to effects of dryness of skin, clothing, food, bathing, and the effects of smoking and alcohol on pruritus. Additionally, the questionnaire assessed frequency and intensity of pruritus, its characteristics as well as worsening and improving factors, treatment modalities and their effects, and impact on quality of life.

The area of involvement of psoriasis and total body area of itching were marked by the patients in the diagrams present in the questionnaire. Then the total area of involvement of psoriasis and itch was then calculated using the rule of nine as in burn assessment (Goodwin, 1994).

The pruritus VAS score of 0-10 cm was categorized into three groups:  $<5$  = mild, 5-7 = moderate and  $>7-10$  = severe pruritus. This categorization of VAS scale was based on our own experience.

### **3.6 IMMUNOHISTOCHEMISTRY AND RADIOIMMUNOASSAY (STUDY III)**

Twenty-eight patients (10 males and 18 females; mean age, 46.1 years; age range 20 to 60 years) with stable plaque psoriasis on the back and who attended the outpatient department of Karolinska University Hospital, Solna, and the Swedish Psoriasis Association treatment units at Sundbyberg, Enskede and Kungsholmen, Stockholm, Sweden, were recruited in the study through March 2007 to March 2008. Ten healthy controls (5 men and 5 women; mean age, 57 years) were also recruited. The study protocol was explained to each patient and informed consent was also taken before commencement of the study.

#### **3.6.1 Salivary cortisol test**

Twenty-six out of the 28 psoriasis patients took part in the salivary cortisol test. The salivary cortisol samples were collected in plastic vials on 3 consecutive days at 8 a.m. At 10 p.m. on the last day each patient was asked to administer 0.25 mg of dexamethasone orally, which was followed by a cortisol test the following morning. These samples were stored at -20°C until analyzed. The cortisol concentration was determined using a RIA kit (Spectria Cortisol, Orion Diagnostica, Espoo, Finland). A salivary cortisol quotient was determined dividing the mean of the 3 consecutive cortisol values with the last cortisol value following the dexamethasone challenge. A low ratio is an indicator of chronic stress (Rosmond et al., 1998).

#### **3.6.2 Beck's Depression Inventory**

The level of depression of each psoriasis patient was assessed using Beck's Depression Inventory (BDI), which consists of 21 questions and in which a score of 21 or over that represents depression (Exton et al., 2003).

#### **3.6.3 Processing of specimens for immunohistochemical staining**

Punch biopsies of 3 mm were taken from the edge of the lesional and non-lesional skin (at least 2 cm away from plaque) from the back (lumbar area). The biopsies were also taken from the normal healthy controls at the same site.

The specimens were immersed in phosphate-buffered 4 % formaldehyde containing 0.17 % picric acid for 1 h and thereafter rinsed in 0.1 M Sørensen's phosphate-buffered saline (PBS) supplemented with 10 % sucrose for at least 24 h. The samples were then frozen rapidly on dry ice and stored at -70°C until further processing. While still frozen the biopsy material was placed in a tissue holder; embedded in Tissue Tek OCT Compound (Sakuro Fientek, Zoeterwoude, Netherlands); sliced into 14-µm thick sections with a Microm HM500 cryostat (Heidelberg, Germany); and finally mounted onto SuperFrost slides for immunohistochemical staining using a streptavidin-biotin procedure.

#### **3.6.4 Immunohistochemical staining**

Rabbit polyclonal antibodies against substance P (T-4107; 1:10000), NKA (T-4445; 1:5000), NKB (T-4449; 1:5,000) all from Bachem (SanCarlos, California, USA), NK-1R (ab13133;1:5000, Abcam, Cambridgeshire, UK), NK-2R (SP4506P; 1:2000; Acris Antibodies, Herford, Germany) and NK-3R (NB300-102; 1:2000; Novus Biologicals, Littleton, CO, USA), were used. The sections were incubated with the respective antibodies at 4°C overnight. The slides were then incubated with secondary biotinylated goat anti-rabbit IgG (BA-1000, 1:200; Vector Laboratories, Burlingame, CA, USA). The fluorochrome CyTM 2-labelled streptavidin (PA 42001; 1:2000; Amersham Pharmacia Biotec, Uppsala Sweden) was finally used. All antibody solutions were diluted in PBS containing 1% bovine serum albumin (BSA) (A9418, Sigma-Aldrich, Stockholm, Sweden) prior to use.

In the case of substance P, NKA and NKB, preadsorption with the respective antigens (Bachem) at  $10^{-6}$  mol/L negated the staining. For NK-1, NK-2 and NK-3 receptors, control staining was performed by omitting the primary antibodies or replacing them with normal rabbit immunoglobulins (X0936; Dako, Glostrup, Denmark), at the same dilutions, and this generally resulted in negative immunostaining.

### **3.6.5 Double immunofluorescence labelling**

The sections were incubated with polyclonal primary antibodies as described above, followed by biotinylated goat anti-rabbit or secondary antibody and by Texas Red (SA-5006; 1:2000; Vector). Sections were further stained with mouse monoclonal antibodies, fluorescein isothiocyanate (FITC)-conjugated anti-CD3 (555916; 1:50; Beckton-Dickson, San Jose, CA, USA), anti-tryptase (MAB1222; 1:1000; Chemicon Temecula, CA, USA) or anti-CD68 (M0876; 1:25; Dako), or anti-elastase (M0752; 1:200; Dako). Finally, a FITC-conjugated rabbit anti-mouse antibody (F0261; 1:40; Dako) was added in order to detect tryptase, CD68 or elastase positive cells respectively.

### **3.6.6 Microscopy**

The sections were cover slipped using with glycerol/gelatin (Merck, Darmstadt, Germany) and then examined using an immunofluorescence Zeiss Axioskop microscope. Colored images were taken using a digital camera system attached to the microscope and connected to PC.

Tachykinin expressing nerves and inflammatory cells were counted in the epidermis and papillary dermis. For each biopsy, the mean number of nerves and cells in four different sections was determined and these values used to calculate the mean  $\pm$ SD value for all of the specimens in lesional, non-lesional and normal healthy skin. All the immunoreactive nerve fibers and cells were counted manually by one observer (BA).

### **3.6.7 Radioimmunoassay**

A biopsy of 3 mm from the lesional skin was also taken from the same individuals for the RIA. The biopsies were extracted in 2 mL of boiling 1 mol/L acetic acid (MERCK, Darmstadt, Germany). After boiling for 10 min the tissues were homogenized using a polytron (CAT X520D, Zipperer, Staufen, Germany), and centrifuged at 1,500 x g in 4°C for 10 min. The supernatants were lyophilized and stored at -70°C.

*Substance P* (SP-LI) was analyzed using antiserum SP2 (Brodin et al., 1986) which reacts with substance P and substance P sulfoxide, but not with other tachykinins. The detection limit was 10 pmol/L. Intra- and inter-assay coefficients of variation were 7 and 11%, respectively. NKA was analyzed using EIA kit EK-046-15 from Phoenix Pharmaceuticals (Burlingame, CA, USA), which cross-reacts 35% with substance P and 57% with NKB. NKB was analyzed using EIA kit EK-046-26 from Phoenix Pharmaceuticals.

### **3.7 INTRADERMAL INJECTIONS (STUDY IV)**

Fifteen patients (8 females, 7 males; mean age ( $\pm$ SD) 41.2 $\pm$ 12.3 years) with pruritic chronic plaque psoriasis, located at least on the arms, were enrolled in the study. The current complaint of pruritus (at least on two preceding days) in the psoriasis lesion should be present. An equal number of sex-matched healthy controls with the mean age of 39.5 $\pm$ 11.2 years were also enrolled in the study.

#### **3.7.1 Substances**

Substance P (Bachem, San Carlos, California, USA) at concentrations of  $10^{-5}$  and  $10^{-6}$  mol/L, histamine dihydrochloride 10  $\mu$ g/ml (Sigma-Aldrich), as positive control, and Sørensen's buffered saline (see below) as negative control, were injected intradermally in volumes of 20  $\mu$ l. Injections were given into the different lesional and non-lesional psoriatic areas (at least 5 cm away from the psoriasis plaques) on the lateral part of arms in psoriasis patients and also in the normal healthy controls. All substances were dissolved in sterile, pyrogen-free, physiological saline containing 10% (v/v) Sørensen phosphate buffer ( $\text{Na}_2\text{HPO}_4+\text{KH}_2\text{PO}_4$  67mM), pH 7.4, and were coded to the investigators and subjects in vials by a technician. Uncoding was performed by the technician following completion of the whole study. Thus the experiment was carried out under double-blinded condition. To maintain experimental consistency, injections and recordings were done by the same individual (BA).

### **3.7.2 Recordings of experimental pruritus**

The duration and intensity of pruritus were both recorded simultaneously with the VAS attached to a computer (Somedic, Hörby, Sweden), whereas the area of the flare and wheal reactions were measured manually after each injection. Subjects were asked to rate their itch by moving a knob on the 100 mm VAS, which was graduated from “no itch” (0 mm) and “maximum itch” (100 mm). The time intervals between each injection and initiation and termination of pruritus perceived were recorded. Thus calculations of pruritus latency (sec), pruritus duration (sec), maximum pruritus intensity (mm) and area under the curve (mm x sec) were automatically downloaded in the computer. Such methods for measuring experimental and clinical pruritus have been used extensively and were validated in previous studies (Wahlgren et al., 1995, Wahlgren et al., 1989, Wahlgren and Ekblom, 1991). The areas of flare and wheal reactions were recorded after 5 minutes and outlined on transparent plastic film from which the maximum perpendicular diameters were measured and used for the calculation of area (mm<sup>2</sup>) (Dreborg, 1989). If the injected substance did not produce pruritus within 10 minutes, we proceeded to another vial.

### **3.8 STATISTICAL ANALYSIS**

Statistical analyses were performed using Epi Info 3.3.2 versions (study II), SAS 8.02 (study II and III) and Stata version 8 (study IV). A descriptive statistics and graphical methods were used to characterize the data. A *P* value <0.05 (two-tailed) was considered as being statistically significant

For study II, the frequencies of various factors were tabulated. Associations between factors were determined using chi-square test with odds ratio (OR) with 95% confidence interval (CI) whenever appropriate. Pearson’s correlation test was used to show the correlation of different variables with pruritus intensity.

As for study III and IV, to detect the difference between variables, the Wilcoxon Signed Ranks test for dependent samples and the Mann-Whitney *U* test for independent samples

were used. The Spearman rank-order test was used to determine the correlation between the variables.

## 4 RESULTS

### 4.1 QUALITATIVE AND EPIDEMIOLOGICAL FINDINGS (STUDY I AND II)

A qualitative method, *focus group* was used to format the questionnaire to investigate about itch in psoriasis. Five groups met at different time points to discuss about their perspective of pruritus in psoriasis. Additional questions were added to the McGill pain questionnaire pertaining to the important subjects indicated during discussion on pruritus in psoriasis.

Among the 109 *questionnaires* sent, 80 (74%) responses (41 males and 39 females; mean age of  $44.2 \pm 13.6$  ( $\pm$ SD) years) were received. Eighty percent of the psoriasis patients reported that of having pruritus during the course of their disease. Among these, more female patients reported having pruritus than did males (OR, 3.8; 95% CI 2-15;  $p < 0.05$ ). Pruritus intensity using the verbal four-point intensity scale and VAS measured was  $1.7 \pm 0.7$  ( $\pm$ SD) and  $5.2 \pm 2.6$  respectively. Female patients reported a higher pruritus intensity ( $r = 0.5$ ,  $p = 0.07$ ) of moderate (63%) and severe (55%) degrees, compared to men (37% and 45% respectively).

During focus group discussions and from the questionnaires, it was evident that the most common sites for pruritus reported were the lower back and the lower legs followed by the scalp with very few reporting generalized itch. The descriptions of pruritus included “painful”, “like pins and needles”, “pain could be handled more easily than pruritus” and “the more attention you pay to itch, the more it itches”. Some patients associated itch with the severity of psoriasis, “the more inflammation in the lesions, the more it itches”. In the questionnaire patients reported itch as stinging, tickling and crawling sensations, with a higher level in women (17%, 16% and 12% respectively) than in men (7%, 6% and 9%).

Stress was considered as a major aggravating factor for itch. The greater the stress, the greater was the pruritus intensity ( $r = 0.8$ ,  $p < 0.05$ ). Additionally, other aggravating factors were dry and cold weather, hot shower, chlorinated public bath, clothes such as woolen and

synthetic textiles, foods (chocolate, dairy products, nuts) and irregular meals. Several methods were used by the patients to relieve pruritus such as scratching until it bleeds, using a wet towel or pinching the area where it itched. Sunbathing, cold shower, vacation and climate journeys could relieve most patients.

The most common anti-pruritic treatments used were topical glucocorticoids (44%), followed by topical vitamin D (26%), emollients (20%), ultraviolet (UV) light therapy (17%), and antihistamines (2%). Few patients used antihistamines for pruritus and all reported a short-term effect. A majority of patients reported that pruritus affected their quality of life. Mood (60%), concentration (47%), sleep (35%), sexual desire (21%), and appetite (11%) were negatively affected by pruritus.

## **4.2 EXPERIMENTAL FINDINGS**

### **4.2.1 Tachykinin expression (Study III)**

The mean ( $\pm$  SD) values of PASI and pruritus intensity measured were  $17.5\pm 9.9$  and  $4.7\pm 2.8$  cm, respectively. There were three patients who did not have current pruritus. The mean ( $\pm$  SD) value of the cortisol ratio was  $3.3\pm 3.0$  and the BDI score was  $8.5\pm 8.4$ .

Immunohistochemical staining revealed tachykinin expression in nerves and inflammatory cells

#### *4.2.1.1 Expression in nerves*

The number of substance P immunoreactive nerve fibers was higher ( $p < 0.001$ ) in lesional ( $5.3\pm 2.1$  fibers) than in non-lesional ( $2.0\pm 1.3$ ) and normal healthy control ( $1.0\pm 0.4$ ) skin. There was also a marginal difference ( $p = 0.05$ ) of substance P positive nerve fibers between non-lesional psoriasis compared to normal healthy skin. Similarly, NKA immunoreactive nerve fibers were higher in number in lesional ( $3.2\pm 2.8$ ) compared to non-lesional ( $0.5\pm 0.7$ ;  $p < 0.001$ ) or healthy control ( $0.4\pm 0.3$ ;  $p < 0.01$ ) skin. There were also a higher number of nerve fibers stained for NKB in lesional ( $2.2\pm 1.4$ ) than in non-lesional ( $0.4\pm 0.6$ ;  $p < 0.001$ ) or healthy ( $0.8\pm 0.5$ ;  $p < 0.01$ ) skin. NK-2R immunoreactive nerves were more prevalent ( $p$

<0.001) in lesional skin ( $2.8 \pm 1.6$ ) than in non-lesional ( $0.4 \pm 0.4$ ) or healthy control ( $0 \pm 1$ ) skin. Neither NK-1R nor NK-3R positive nerves could be detected. There was a strong tendency ( $r=0.36$ ;  $p=0.06$ ) to a correlation of pruritus intensity with the number of substance P immunoreactive nerve fibers in the lesional skin.

#### 4.2.1.2 Expression in inflammatory cells

The mean value for substance P positive inflammatory cells was higher ( $p < 0.001$ ) in lesional skin ( $52.9 \pm 12.6$ ) compared to non-lesional ( $15.8 \pm 5.5$ ) and normal healthy control ( $17.0 \pm 9.0$ ) skin. NKA immunoreactive cells were also higher in number ( $p < 0.001$ ) in lesional ( $15.3 \pm 3.8$ ) than non-lesional ( $6.3 \pm 2.3$ ) and healthy ( $0.9 \pm 0.9$ ) skin. In addition there was a significant difference ( $p < 0.001$ ) of NKA reactive cells between non-lesional and healthy skin. NKB immunoreactive cells were expressed more in lesional ( $18.5 \pm 5.2$ ) than in non-lesional ( $2.2 \pm 1.6$ ;  $p < 0.001$ ) and healthy control ( $3.7 \pm 1.9$ ;  $p < 0.001$ ) skin. Similarly NK-1R, NK-2R and NK-3R positive cells were expressed more in lesional ( $54.6 \pm 20.4$ ), ( $11.6 \pm 5.4$ ), ( $11.4 \pm 4.2$ ) than in non-lesional ( $17.1 \pm 11.3$ ;  $p < 0.001$ ), ( $5.4 \pm 3.1$ ;  $p < 0.001$ ), ( $2.9 \pm 1.5$ ;  $p < 0.001$ ) and healthy control ( $2.4 \pm 1.6$ ;  $p < 0.001$ ), ( $2.2 \pm 1.5$ ;  $p < 0.01$ ) ( $0.8 \pm 0.7$ ;  $p < 0.001$ ) skin, respectively.

The studied different types of inflammatory cells had the ability to express the ligands or receptors as assessed by double staining.

There was a correlation ( $r=0.42$ ;  $p < 0.05$ ) between the pruritus intensity and number of NK-2R positive cells in lesional skin. The BDI was positively correlated ( $r=0.61$ ;  $p < 0.001$ ) with the number of substance P positive cells in non-lesional skin and there was a strong tendency for NK-1R ( $r=0.35$ ;  $p=0.07$ ) and NK-2R immunoreactive cells ( $r=0.36$ ;  $p=0.06$ ) in lesional skin. Further more, an inverse correlation ( $r=-0.46$ ;  $p < 0.05$ ) was demonstrated for the cortisol ratio and number of NK-1R positive cells in lesional skin and a strong tendency ( $r=0.37$ ;  $p=0.07$ ) for NK-1R immunoreactive cells in non-lesional skin.

### 4.2.2 Intradermal injections (Study IV)

Twelve psoriasis patients out of 15 had experimentally-induced pruritus with the injected substances, and among them all had pruritus in both the lesional and non-lesional psoriasis areas except for one who had only perceived itch in the non-lesional areas. Thirteen healthy controls reported pruritus upon experimental injections.

The PASI measured was  $9.4 \pm 2.6$  (mean ( $\pm$ SD)) and the clinical pruritus intensity measured using the VAS was  $61 \pm 23$  mm.

#### 4.2.2.1 Substance P

Substance P ( $10^{-5}$  mol/L) evoked itch more in psoriasis patients ( $n=9$ ) than in healthy controls ( $n=5$ ), while this was less obvious with substance P ( $10^{-6}$  mol/L) (psoriasis patients  $n=5$ ; healthy controls  $n=4$ ).

Among the injected substances, both the concentrations ( $10^{-5}$  and  $10^{-6}$  mol/L) of substance P induced an itch latency period in psoriasis patients that did not significantly differ from the healthy controls. Substance P ( $10^{-5}$  mol/L) induced a tendency to a larger intensity of pruritus in lesional than in non-lesional skin ( $p = 0.08$ ). The areas under the curve of pruritus induced by the injected substances in psoriatic skin and in healthy controls did not differ significantly.

A positive correlation was observed between clinical itch and maximum pruritus intensity with substance P ( $10^{-5}$  mol/L) in lesional psoriasis skin ( $r=0.51$ ,  $p < 0.05$ ).

#### 4.2.2.2 Histamine

Fewer psoriasis patients ( $n=10$ ) reported pruritus with histamine than did healthy controls ( $n=13$ ). It produced shorter ( $p < 0.05$ ) itch latency period in lesional psoriasis skin ( $22.6 \pm 32.6$  sec) than in healthy controls ( $58.9 \pm 60.9$  sec). The maximum intensity of pruritus produced by histamine was lower ( $p = 0.05$ ) in lesional psoriasis than in healthy control skin. The clinical pruritus was positively correlated with the histamine-induced pruritus area under the curve ( $r=0.52$ ,  $p < 0.05$ ) and the duration of pruritus in lesional skin of psoriasis patients ( $r=0.54$ ,  $p < 0.05$ ).

#### 4.2.2.3 *Flare and wheal*

The flare area produced by histamine or substance P ( $10^{-5}$  mol/L) was larger in non-lesional psoriasis areas than in healthy controls, however, no statistical significance was observed. In contrast, wheal area produced by histamine was significantly smaller ( $p < 0.05$ ), in non-lesional psoriasis skin than in healthy controls, while with substance P ( $10^{-6}$  mol/L) there was tendency ( $p = 0.07$ ) for this difference to be significant.

## **5 DISCUSSION**

Pruritus has not been considered as one of the major symptom of psoriasis. Pruritus is a distressing physical sensation that can lead to sleep deprivation, psychological disturbances and also associated with anxiety, depression and suicidal thoughts. Few studies have been conducted to examine the prevalence as well as on the characteristics of pruritus in psoriasis (Sampogna et al., 2004, Szepietowski et al., 2004, Yosipovitch et al., 2000). It seems essential to further investigate the epidemiology and mechanisms of pruritus in psoriasis and this might also contribute to a greater knowledge of anti-pruritic drug activity in this disease.

### **5.1 METHODOLOGIES**

A qualitative method such as a focus group plays an important role in the field of dermatology to know patient's perspective and attitudes to certain diseases. In previous studies it has been used to study the effectiveness of therapies and impact on quality of life of psoriasis patients (Housman et al., 2002, McKenna et al., 2003). In our study, focus groups were used to let patients discuss about their perspective of pruritus in psoriasis.

A modified McGill pain questionnaire (Melzack, 1987) helped us to study in the detail about characteristic, aggravating and relieving factors and effect of quality of life of pruritus in psoriasis. Such questionnaire had also been used in a previous study to evaluate pruritus in psoriasis (Yosipovitch et al., 2000). Seventy-four percent of the questionnaires were replied out of 109 sent. We could speculate that those patients who did not reply to the questionnaire might not have experienced the complaint of itch.

The VAS was used to measure clinical and experimental itch and had been validated in previous studies (Wahlgren et al., 1989, Wahlgren and Ekblom, 1991, Wahlgren et al., 1995).

### **5.2 QUALITATIVE AND EPIDEMIOLOGICAL FINDINGS**

The focus group and the comprehensive questionnaire were used to study the prevalence and characteristic of pruritus in psoriasis.

The prevalence of pruritus in psoriasis was high, which is similar to the findings of other studies (Chang et al., 2007, Sampogna et al., 2004, Yosipovitch et al., 2000). The mean pruritus intensity score, however, was lower in patients with plaque psoriasis as compared to other diseases such as atopic dermatitis (Yosipovitch et al., 2002) and uremic pruritus (Yosipovitch et al., 2001).

The description of pruritus reported by the patients was consistent with the itch sensations described as burning and tickling in the previous study (Yosipovitch et al., 2000). Our patients suggested that perception of pruritus is at a “higher brain level”, since they could obtain relief through pinching the skin. Thus, patients regarded themselves as being able to discriminate between pruritus and pain.

Occurrence of higher pruritus intensity, as well as the symptoms such as heat sensations, pain, stinging, tickling and crawling, were more common in women than in men, which is consistent with the findings of Sampogna *et al.* (2004). However, before confirming the higher frequency of pruritus and its intensity in women, other confounding factors such as ethnic groups, age, and psychological factors have to be considered, as has been reported for pain perception (Sheffield et al., 2000). It would be interesting to further study further the gender differences with respect to other factors such as aggravating and relieving factors and treatment aspects. Previous studies have demonstrated gender differences with respect to patients’ view of care and treatment of psoriasis (Osika et al., 2005, Waernulf et al., 2008).

In our study the most common sites of itching reported during the focus group discussions and the questionnaire were the lower legs and back, which was similarly reported in other studies (Chang et al., 2007, Szepietowski et al., 2002, Yosipovitch et al., 2000). Additionally, our patients reported scalp as one of the common sites for itch. A study by Yosipovitch et al. (2000) reported that only 15% patients had pruritus in the scalp. We speculate that our patients wear a cap/hat for a substantial part of a year, the pressure of which might affect the perception of pruritus in the scalp. A generalized itch experienced in

only 5% of patients in our study, while 28.7% in the study by Szepietowski *et al.* (2002). Variation in the density of nerve innervation of the skin in different parts of the body could have affected the perception of pruritus, including the distribution of various pruritic mediators (Naukkarinen *et al.*, 1989, Ozawa *et al.*, 2006).

Sweating and physical exercises were reported as aggravating factors in 35% and 10%, respectively, in our patients. In a study from Singapore, sweating and physical exercise were reported as being aggravating factors for most of the patients (Yosipovitch *et al.*, 2000). Perhaps heat and humidity are of importance in the aggravation of pruritus in a tropical climate. Stress was regarded as an important aggravating factor for pruritus, in our study which is consistent with the findings of other studies (Chang *et al.*, 2007, Gupta *et al.*, 1988, Reich, 2003). During winter months, itching was aggravated in our patients. We could speculate that extreme dryness during winter could have contributed to the pruritus.

Interestingly, pruritus was aggravated with certain foods, which was somewhat an unexpected finding. Vegetarian diets and diets rich in omega-3 polyunsaturated fatty acids from fish oil have been correlated with psoriasis *per se* in many studies (Wolters, 2005).

Patients tried to relieve itch by taking cold showers. Physiological stimuli can affect the pruritus, which can be inhibited by substituting the temperature of skin to another sensation to interfere with the steps in evolvment of scratching. Such a substitution may be cooling the skin by using cold compresses or ice cubes, for example (Hercogova, 2005).

Almost all patients were dissatisfied with the available treatment modalities for pruritus in psoriasis, especially with systemic oral treatments. Topical applications such as glucocorticoids and vitamin D analogue were reported to have a better effect than oral antihistamines. In contrast to the findings of our study, the use of antihistamines for pruritus in psoriasis seems to be more common in other studies (Szepietowski *et al.*, 2002, Yosipovitch *et al.*, 2000). The insufficient effect of antihistamines for pruritus of psoriasis

might be explained by the fact that various pruritic mediators, other than or together with histamine, play a role in the pathogenic mechanisms of pruritus in this disease.

In our study, among the patients who were treated using phototherapy, half of them found it beneficial, while the other half reported no effect in the long-term. Phototherapy is considered to be one of the effective treatments for pruritus in inflammatory dermatoses (Samson Yashar et al., 2003, Gupta et al., 1999), while other studies have reported an insufficient effect (Yosipovitch et al., 2000, Dawn and Yosipovitch, 2006).

Patients with higher ratings of pruritus have noticed more psychosocial stress caused by the disease affecting their quality of life. Mood, concentration, and sleep were negatively affected by pruritus in many of our patients, and sexual desire and appetite were affected in a few. Similar findings have been reported in an earlier study (Yosipovitch et al., 2000, Chang et al., 2007) except for the effect on sexual desire which was relatively higher (40%) (Yosipovitch et al., 2000).

### **5.3 EXPERIMENTAL FINDINGS**

#### **5.3.1 Tachykinin expression**

Immunohistochemical staining of biopsy sections of pruritic psoriasis patients revealed a higher expression of substance P, NKA, NKB and NK-2R reactive nerves and substance P, NKA, NKB and their receptors NK-1, NK-2 and NK-3 in inflammatory cells compared to the non-involved psoriatic and control skin. The reason for the difference also between non-lesional and healthy control skin may be due to the inflammatory process in psoriasis. There is a certain degree of inflammation also in non-lesional psoriatic skin.

We found a correlation between pruritus and NK-2R positive cells in lesional skin. This indicates a role for NKA as a pruritogenic mediator in psoriasis. An earlier study (Thomsen et al., 2002) showed that NKA evoked itch upon intradermal injection into normal human skin.

Earlier findings by Nakamura *et al.* (2003) suggested that there was a correlation between intraepidermal substance P positive fibers in the epidermis and the degree of pruritus. A strong tendency to a correlation between pruritus intensity and number of substance P positive nerves in lesional skin was observed in our study.

Our RIA results indicated that there were higher values for NKA and NKB compared to substance P. The reason for this might be a higher turnover rate for substance P compared to the other tachykinins, being due to enzymatic activity. There was in our study also a correlation between the NKA and NKB concentrations, measured by RIA, as well as between the number of NKA and NKB immunoreactive nerves in lesional skin.

NKB is spliced from another precursor and also encoded from another gene than substance P and NKA. There might be an impact on the RIA results due to a crossreactivity between the NKA and NKB antibodies. Thus, these results must be interpreted with caution. There should be of importance to confirm the presence of these tachykinins by, e.g., performing high pressure liquid chromatography (HPLC).

NKA expression has to our knowledge not been reported in psoriasis. However, NKA has earlier been described in sensory nerve fibers in normal human skin (Johansson *et al.*, 1999, Karanth *et al.*, 1991, Schulze *et al.*, 1997). In contrast Pincelli *et al.* (1990) were not able to detect NKA in inflamed skin of patients with atopic dermatitis.

In the present investigation NKB immunoreactivity was expressed in a few peripheral nerves, as well as on inflammatory cells. NKB has earlier not been detected in capsaicin sensory neurons in the guinea pig (Hua *et al.*, 1985). However, sensory nerves in the airway mucosa have been reported to contain NKB (Widdicombe, 1991). It has been reported that rat sensory neurons express functional NK-3 receptors (Brechenmacher *et al.*, 1998).

The role of NKB in neurogenic inflammation has been questioned earlier (Campos and Calixto, 2000). Beding-Barnekow and Brodin (1989) suggested that NKA but not NKB might play a role in neurogenic inflammation in the rabbit eye. Thomsen *et al.* (2002)

determined that NKB could neither elicit itch in normal nor in inflamed skin. Inoue *et al.* (1995) reported that NK-1, NK-2 and NK-3 receptors might participate in the oedema response to local NKA and NKB in the ear skin. Klassert *et al.* (2008) recently reported expression of NKB in human immune cells by using RT-PCR and immunohistochemistry.

The NK-2R was also expressed also on nerve fibers. An expression of NK-2R has earlier been reported on nerve fibers in the intestine of guinea pigs (Vannucchi *et al.*, 2000) and in the human colon (Jaafari *et al.*, 2008). In the guinea pig the same fibers expressed substance P, which might indicate that the NK-2R was an autoreceptor modifying nerve signal transmission. Even if we in the present study could not find a correlation between number of NK-2R positive nerves and pruritus, there may still be a possibility that these nerves have some role for pruritus in psoriasis.

The cortisol ratio was inversely correlated with NK-1R positive cells in lesional skin. This might suggest that substance P modulates pruritus in stressed psoriasis patients. A previous study has reported that substance P released during stress may indirectly induce mast cell degranulation and this could be due to activation of NK-1R (Erin *et al.*, 2004).

There was also a correlation of BDI score with a number of substance P expressing cells and strong tendency with NK-1 and NK-2 receptor positive cells. Depression has been associated with pruritus in psoriasis (Conrad *et al.*, 2008, Gupta *et al.*, 1994). Interestingly, it was recently reported that depressive symptomatology strongly correlated with substance P in sweat patches and plasma in patients with major depressive disorder in remission (Cizza *et al.*, 2008).

### **5.3.2 Intradermal injections**

Cutaneous reaction of intradermally injected substance P in psoriasis has not been studied till date. Experimentally-induced pruritus by injected substances was observed in psoriasis patients and healthy controls. Substance P and histamine induced more pruritus in psoriasis patients and healthy controls as compared to saline, which is consistent with a previous study

performed in experimentally (sodium lauryl sulphate-induced) inflamed skin (Thomsen et al., 2002).

There were no statistically significant differences between the latency period, duration, area under the curve and maximum intensity of pruritus evoked by substance P ( $10^{-5}$  and  $10^{-6}$  mol/L) between psoriasis and healthy control skin. Our finding is similar with the previous findings in atopic dermatitis (Giannetti and Girolomoni, 1989) and experimentally induced inflamed skin (Thomsen et al., 2002) in which no difference of itch in involved skin and healthy controls was evident following intradermally injection of substance P. However, in another study of intradermal injection with substance P in atopic dermatitis (Heyer et al., 1991, Heyer, 1992), there was a weaker itch response in atopic dermatitis patients compared to healthy controls.

Substance P ( $10^{-5}$  mol/L) induced a tendency to higher, maximum intensity of pruritus in lesional than in non-lesional psoriatic skin. A positive correlation was also observed with clinical itch and maximum pruritus intensity with substance P ( $10^{-5}$  mol/L). These findings might indicate the role of substance P as a mediator of itch in psoriasis.

The role of substance P as an inflammatory mediator in many inflammatory diseases such as atopic dermatitis (Salomon and Baran, 2008) and in psoriasis (Reich et al., 2007a, Saraceno et al., 2006) is well established. Several studies have studied a possible role of substance P in pruritic psoriasis skin (Nakamura et al., 2003, Reich et al., 2007b, Remrod et al., 2007). Substance P is thought to act as a pruritic mediator directly and also indirectly, by degranulating mast cells through histamine-dependent pathways (Hagermark et al., 1978).

With histamine the maximum intensity of pruritus was lower in lesional psoriasis than in healthy control skin. This result is consistent with previous studies performed in atopic dermatitis (Heyer et al., 1989, Marsella and Nicklin, 2001, Uehara, 1982). The decreased sensation of pruritus with injected histamine in psoriatic patients could be due to an increased concentration of histamine in psoriasis plaques (Krogstad et al., 1997) leading to

downregulation of histamine receptors. It also indicates that histamine might not be the major pruritic mediator in the pathogenesis of pruritus in psoriasis. This could be one reason why antihistamines are less effective for treatment of pruritus in psoriasis (Szepietowski et al., 2002, Yosipovitch et al., 2000).

The wheal area produced by histamine was smaller in psoriasis patients than in healthy individuals. Our result contrasts with the findings reported in previous studies performed in atopic dermatitis (Coulson and Holden, 1990, Giannetti and Girolomoni, 1989) and experimentally induced irritant contact dermatitis (Thomsen et al., 2002). A downregulation of histamine receptors in the dermal blood vessels in psoriasis skin may explain the smaller wheal response. However, we could not discern any difference in flare area induced with injected substances, which is similar to previous studies of atopic dermatitis (Coulson and Holden, 1990, Giannetti and Girolomoni, 1989).

The subjective description of experimentally-induced itch reported by patients was similar to that in the previous studies (Amatya et al., 2008, Sampogna et al., 2004). The perception of pruritus might vary with regard to gender aspect (Amatya et al., 2008). However we did not observe a gender differences in perception of clinical or experimentally-induced pruritus in psoriasis patients in our study.

## 6 CONCLUSION

Pruritus is a common complaint among psoriasis patients. Most of our psoriatic patients seem to have some form of pruritus. Focus group discussions on pruritus in psoriatic patients provided information on patients' perspectives of their psoriasis symptoms. The most prevalent sites of itch reported were lower back and legs. Stress, cold weather and skin dryness were the aggravating factors. Relieving factors for itch were sunbathing and application of emollients with or without corticosteroids. Quality of life was negatively affected in some patients.

Using the comprehensive questionnaire the different aspects of itch in psoriasis were studied. The frequency and intensity of pruritus were higher in women. Characteristics of pruritus were similar as those reported in other studies. The lower leg and scalp were reported as the most commonly affected sites. Major aggravating factors for pruritus were stress and dryness of skin. Sun, sleep and vacation could relieve to pruritus. The most common anti-pruritic treatments used by patients were topical glucocorticoids, vitamin D and emollients, while antihistamines were used by a small number of patients. Mood, concentration, and sleep were negatively affected by pruritus.

Members of the tachykinin family were expressed on nerves and inflammatory cells on the biopsy sections of patients of psoriasis with pruritus. Substance P, NKA, NKB and NK-2R, immunoreactive nerves and substance P, NKA, NKB and their respective receptors NK-1, NK-2 NK-3 reactive inflammatory cells were higher in number in psoriasis as compared to healthy controls. The pruritus intensity and number of NK-2R positive cells in lesional psoriatic skin were significantly correlated. The cortisol ratio was inversely correlated with the number of NK-1R positive inflammatory cells in lesional skin. There was also a positive correlation between the BDI score and the number of substance P positive cells in non-

lesional skin. These results suggest a role of members of the tachykinin family in psoriasis *per se*, and also a role of NK-2R in pruritus.

Intradermally injected substance P induced pruritus in psoriasis patients. Substance P ( $10^{-5}$  mol/L) induced a tendency to a larger intensity of pruritus in lesional than in non-lesional psoriatic skin. The responses of pruritus as well as wheal and flare reactions from intradermally injected substances did not differ significantly from those of the healthy controls.

Thus this study indicates that pruritus is a common and very important symptom in psoriasis. Members of the tachykinin family might play a role as mediators of pruritus in psoriasis.

## 7 FUTURE ASPECTS

The qualitative and epidemiological studies indicate that pruritus is a very important symptom for patients with plaque psoriasis. In the future, it would be interesting to further study on the aggravating and relieving factors of pruritus especially focusing on food and clothing and also to investigate the gender differences with respect to characteristics of pruritus using qualitative research tools such as in-depth interviews.

Expression of tachykinin genes (TAC 1 and TAC 3) in blood and skin biopsies of pruritic psoriasis patients will be studied using RT-PCR, and their mRNA expression will be correlated with the intensity of pruritus.

Immunohistochemical staining of biopsy sections of psoriasis patients strongly suggest a role of members of the tachykinin family in psoriasis as well as pruritus in this disease. Like substance P, a new member of the tachykinin family hemokinin-1 (HK-1) has an affinity to bind to NK-1R. HK-1 was determined to be as equally potent as substance P in inducing plasma extravasation and mast cell degranulation in a rat model (Zhang et al., 2000). Expression of HK-1 will be studied on biopsy sections of pruritic psoriasis skin. The role of HK-1 in pruritic psoriasis skin will be further studied through intradermal injections into pruritic psoriasis skin.

Finally, antagonists of the tachykinin receptors, particularly of NK-1 and NK-2R, antagonists in pruritic psoriasis lesions in order to investigate if itching is reduced. Tachykinin antagonists might be one of the effective anti-pruritic treatments for psoriasis patients in the future.

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