Minna Kubin

GLUCOCORTICOID RECEPTORS IN INFLAMMATORY SKIN DISEASES

THE EFFECT OF SYSTEMIC AND TOPICAL GLUCOCORTICOID TREATMENT ON THE EXPRESSION OF GRα AND GRβ
MINNA KUBIN

GLUCOCORTICOID RECEPTORS IN INFLAMMATORY SKIN DISEASES
The effect of systemic and topical glucocorticoid treatment on the expression of GRα and GRβ

Academic Dissertation to be presented with the assent of the Doctoral Training Committee of Health and Biosciences of the University of Oulu for public defence in Auditorium 7 of Oulu University Hospital (Kajaanintie 50), on 9 December 2016, at 12 noon

UNIVERSITY OF OULU, OULU 2016
Kubin, Minna, Glucocorticoid receptors in inflammatory skin diseases. The effect of systemic and topical glucocorticoid treatment on the expression of GRα and GRβ
University of Oulu Graduate School; University of Oulu, Faculty of Medicine; Medical Research Center Oulu; Oulu University Hospital
University of Oulu, P.O. Box 8000, FI-90014 University of Oulu, Finland

Abstract

Glucocorticoids are the most important and widely used treatment modality in dermatology. A large variety of topical as well as systemic preparations is available. Most patients treated with glucocorticoids respond quickly to the treatment, but some are considered insensitive or even resistant to glucocorticoid therapy. Currently, there is no known measurable variable, through which the response can be predicted.

Glucocorticoids mediate their actions through glucocorticoid receptors (GR). Several isoforms of GR exist, but the α (GRα) and β (GRβ) isoforms are clinically the most important. Based on previous studies, it has been proposed that the abundance of GR isoforms or the GRβ: GRα –ratio could affect individual responsiveness to corticosteroid treatment. In particular, up-regulation of GRβ expression has been shown to be linked to resistance to corticosteroid treatment.

This thesis comprises three sub-studies. Firstly, we wanted to determine whether GRα and GRβ are expressed in inflammatory skin diseases. Secondly, we examined if the expression is altered by corticosteroid treatment in eczema atopicum, bullous pemphigoid and psoriasis. Finally, we measured the effects of a topical vitamin D3 analogue (calcipotriol) combined with betamethasone compared with betamethasone monotherapy on inflammatory biomarkers of psoriasis.

Our studies provide detailed novel data about the expression of GRα and GRβ. GRα and GRβ were shown to be expressed in the blood lymphocytes and lesional skin of patients with eczema atopicum, bullous pemphigoid and psoriasis, as well as in the skin of patients with eczema nummulare, lichen simplex chronicus and lichen ruber planus. Systemic corticosteroid treatment was shown to affect the expression of GRα and GRβ in eczema atopicum and bullous pemphigoid, but the inconsistent variation in their expression between patients prevented us from drawing firm conclusions. Neither GRα nor GRβ as a single marker were found to be a suitable predictor of corticosteroid responsiveness. Clinical and laboratory analyses showed that topical treatment of psoriasis with calcipotriol/betamethasone combination ointment is more beneficial measured by both than betamethasone monotherapy.

Keywords: betamethasone, bullous pemphigoid, calcipotriol, dermatology, eczema atopicum, eczema nummulare, glucocorticoid insensitivity, glucocorticoid receptor alpha, glucocorticoid receptor beta, IL-17A, IL-23A, inflammatory skin diseases, lichen ruber planus, lichen simplex chronicus, prednisolone, psoriasis, Th17, TNF-α
Kubin, Minna, Glukokortikoidireseptorit tulehduksellisissa ihosairauksissa. Paikallisen ja systeemisen kortisonhoidon vaikutus GRα:n ja GRβ:n ilmenemiseen
Oulun yliopiston tutkijakoulu; Oulun yliopisto, Lääketieteellinen tiedekunta; Medical Research Center Oulu; Oulun yliopistollinen sairaala
Oulun yliopisto, PL 8000, 90014 Oulun yliopisto

Tiivistelmä
Glukokortikoiditeja ("kortisoni") käytetään tulehduksellisten ihotautien hoidossa paikallisesti tai systeemiseen lääkkeenä. Suurin osa potilaista reagoi hoitoon nopeasti, mutta osalla on hoitovaste hitaasti tai heikompi. Tällä hetkellä ei tunnetta laadukkaita ennustetta, miten kortisonihoidoista vaikuttaa ihossa.

Glukokortikoidit vaikuttavat elimistössä glukokortikoidireseptorin (GR) kautta. GR:ista tunnetaan useita alatyyppejä, joista tärkeimmät ovat α (GRα) ja β (GRβ). Aiemman tiedon pohjalta on pidetty mahdollisena, että GR-alatyyppien suhteella tai määrällä on merkitystä kortisonhoidon vaikutusta. Tässä väitöskirjassa tavoitteen a on selvittää GR-alatyyppien ilmenemiseen tapahtumia tulehduksellisissä ihosairauksissa sairastavilla potilailla sekä tutkia, miten kortisonhoidoissa vaikuttaa GR-tasoihin atooppista ihottumaa, pemfigoidia ja psoriaasian sairastavilla potilailla. Lisäksi olemme verranneet paikallishoitoa, kelotuksesta, kortisonihoidoa ja D-vitamiinijohdosta kalsipotriolin ja kortisonin yhdistelmän painotusta psoriaatikoilla.

Tutkimus on antanut uutta yksityiskohtaisia tietoa GRα:n ja GRβ:n esiintymisestä ihossa ja tulehdussoluissa ihosairauksia sairastavilla potilailla. Tutkimustulosten perusteella voidaan todeta, että GRα ja GRβ esiintyvät atooppista ihottumaa, pemfigoidia ja psoriaasiaan sairastavien potilaiden ihossa ja veren tulehdussoluissa sekä nummulaari-ihottumaa, neurodermatiittia ja punajääkalää sairastavien potilaisten ihossa. Suun kautta annettu kortisonihoito vaikuttaa GRα- ja GRβ-lähetti-RNA:n ilmenemiseen, mutta potilaskohtaiset erot ovat suuret, eikä kumpikin, GRα tai GRβ, soveltu yksinään ennustamaan kortisonhoidon vaikutusta. Paikallisella kortisonhoidolla D-vitamiinijohdos kalsipotriolin ja kortisonin yhdistelmän vaikutus psoriaasin tulehduksellisiin välitädäneisiin ja tulehdussoluuihin on puolestaan vaikutuksen puolesta paikallisella kortisonhoidolla.

Asiasanat: atooppinen ihottuma, betametasoni, glukokortikoidireseptori alfa, glukokortikoidireseptori beta, glukokortikoidiresistenssi, IL-17A, IL-23A, kalsipotrioli, neurodermatiitti, nummulaari-ihottoma, pemfigoidi, prednisoloni, psoriaasi, punajääkäli, Th17, TNF-α, tulehdukselliset ihosairaudet
To the unexpected journeys
Acknowledgements

This study was carried out at the Department of Dermatology and Clinical Research Center Oulu at Oulu University Hospital and Oulu University between 2010 and 2016.

I wish to express my sincerest gratitude to my supervisor, Professor and the Head of the Department of Dermatology, Kaisa Tasanen-Määttä, MD, PhD, for supporting and giving me priceless advice over the years. Her encouragement and guidance have kept me moving steadily towards my PhD degree. I would like to thank my second supervisor, Päivi Hägg, MD, PhD, who together with the former Head of the Department of Dermatology, Professor Emeritus Aarne Oikarinen, MD, PhD, initially suggested the idea of my thesis and gave useful comments on my work. Päivi also helped me to combine the specialist and PhD degrees through practical as well as professional support.

I am grateful to the official pre-examiners of this thesis, Docent Leena Koulu, MD, PhD and Docent Sari Suomela, MD, PhD. Their thought-provoking and valuable comments made me improve the thesis into a more easily understandable story.

I want to acknowledge all the co-authors of the original publications: Docent Riitta Palatsi, MD, PhD; Nina Kokkonen, PhD; Juha Väyrynen MD, PhD; Docent Kirsi-Maria Haapasaari, MD, PhD; Jyri Moilanen, MD, PhD; Docent Matti Kallioinen, MD, PhD; Docent Tiina Hurskainen, PhD; Tatsuya Uchida, PhD; Docent Virpi Glumoff, PhD; Docent Petri Kulmala, MD, PhD, for their help, support and practical comments on my original publications. In particular, Docent Palatsi and Doctor Kokkonen have supported me enormously with their extensive scientific experience, and taught me laboratory methods as well as scientific writing.

I greatly appreciate the help of Ms. Anja Mattila, our skilled laboratory technician, for her assistance and many good laughs during the laboratory work related to this thesis. I also owe a depth of gratitude to technicians Ms. Riitta Vuento and Ms. Birgitta Grekula for their expertise with the laboratory analyses. I would also like to thank Ms. Seija Leskelä for her help with the illustrations related to this thesis.

I am also thankful to Steve Smith, for revising the English language of the thesis and that of the third publication included in this thesis. I thank Anna Vuolteenaho, MA, for revising the English language of two of the publications included in this thesis and the Finnish abstract in this thesis.
I wish to thank my colleagues at the Department of Dermatology of Oulu University Hospital, for support during the years we have worked together. I also thank the nurses and secretaries of the Department for their friendly assistance and quick responses to all my requirements. You make every workday a fun day!

I send warm thoughts to my dear friends Anna-Maria, Toni, Minna, Hannele, Marjo, Sanna, Maija, Noora, Aino and Riku, for believing in my achievements, but placing my feet back on the ground when I was starting to fly too high.

I want to thank Tero’s family: Eija, Paavo, Heli, Ilkka, Aki and Eeva for their support and interest in my research.

I owe my dearest thanks to my mum and dad, for believing in me during my whole life. You never questioned the hours I spent studying. You shared my dreams and supported me with all your heart. I wish all the best in life for my little sister Mari and little brother Matti; may you live your lives to the fullest.

Last, but obviously most importantly, I give my deepest gratitude to my one and only, Tero. Words are not enough to express my love and appreciation of your loyal, loving presence and wisdom when it comes to life itself. You know how much you mean to me, you are my everything. I love you.

This work was financially supported by Research Grants from the Medical Research Center Oulu Doctoral Program, Finnish Dermatological Society, Finnish Medical Foundation, Väinö and Laina Kivi Foundation, The Finnish Medical Society Duodecim, Orion Pharmos and the Finnish Norwegian Medical Foundation.

Oulu, October 2016

Minna Kubin
Abbreviations

Ab  antibody
ACTH  adrenocorticotropic hormone
AD  atopic dermatitis
BMI  body mass index
BP  bullous pemphigoid
BP180  bullous pemphigoid antigen 180
BPDAI  Bullous Pemphigoid Disease Area Index
CCL  CC chemokine ligand
CCR  CC chemokine receptor
CD  cluster of designation
CLA  cutaneous leukocyte antigen
CXCL  CXC chemokine ligand
CXCR  CXC chemokine receptor
DBD  DNA binding domain
DC  dendritic cell
DLQI  Dermatology Life Quality Index
DNA  deoxyribonucleic acid
e.g.  exempli gratia
EASI  Eczema Area and Severity Index
ELISA  enzyme-linked immunosorbent assay
etc.  et cetera
FCM  flow cytometry
FoxP3  forkhead box P3
GAPDH  glyceraldehyde-3-phosphate dehydrogenase
GC  glucocorticoid
GR  glucocorticoid receptor
GRE  glucocorticoid response element
hsp  heat-shock protein
i.e.  id est
IFN  interferon
Ig  immunoglobulin
IL  interleukin
kD  kilo Dalton
LBD  ligand binding domain
LRP  lichen ruber planus
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSC</td>
<td>lichen simplex chronicus</td>
</tr>
<tr>
<td>mAb</td>
<td>monoclonal antibody</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
</tr>
<tr>
<td>PASI</td>
<td>Psoriasis Area and Severity Index</td>
</tr>
<tr>
<td>PBMC</td>
<td>peripheral blood mononuclear cell</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PUVA</td>
<td>combination of a psoralen and ultraviolet A radiation</td>
</tr>
<tr>
<td>RT-qPCR</td>
<td>reverse-transcriptase quantitative PCR</td>
</tr>
<tr>
<td>RXR</td>
<td>retinoid receptor X</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulphate</td>
</tr>
<tr>
<td>T-bet</td>
<td>T-box expressed in T cells</td>
</tr>
<tr>
<td>Th</td>
<td>helper T cell</td>
</tr>
<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
</tr>
<tr>
<td>Treg</td>
<td>regulatory T cell</td>
</tr>
<tr>
<td>UVB</td>
<td>ultraviolet B</td>
</tr>
<tr>
<td>VDR</td>
<td>vitamin D receptor</td>
</tr>
</tbody>
</table>
List of original publications

This thesis is based on the following publications, which are referred to throughout the text by their Roman numerals:


Contents

Abstract ............................. 9
Tiivistelmä .............................. 10
Acknowledgements ............................. 11
Abbreviations ............................. 13
List of original publications ............................. 15
Contents ............................. 15
1 Introduction ............................. 17
2 Review of the literature ............................. 19
  2.1 Inflammatory skin diseases included in the study ............................. 19
     2.1.1 Eczema atopicum ............................. 19
     2.1.2 Eczema nummulare ............................. 24
     2.1.3 Lichen simplex chronicus ............................. 25
     2.1.4 Lichen ruber planus ............................. 26
     2.1.5 Bullous pemphigoid ............................. 27
     2.1.6 Psoriasis ............................. 31
  2.2 Mechanism of action of drugs included in the study ............................. 35
     2.2.1 Glucocorticoids ............................. 35
     2.2.2 Calcipotriol ............................. 42
3 Aims of the present study ............................. 45
4 Materials and methods ............................. 47
  4.1 Patient selection, treatments and samples ............................. 47
     4.1.1 Patients with severe eczema atopicum (I) ............................. 47
     4.1.2 Patients with lichen ruber planus, eczema nummulare and
         lichen simplex chronicus (I) ............................. 48
     4.1.3 Patients with bullous pemphigoid (II) ............................. 49
     4.1.4 Patients with mild or moderate psoriasis (III) ............................. 50
  4.2 Isolation of peripheral blood mononuclear cells (I, II, III) ............................. 51
  4.3 Reverse-transcriptase quantitative polymerase chain reaction (I, II, III) ............................. 52
  4.4 Immunohistochemistry (I, II, III) and image analysis (I, III) ............................. 52
  4.5 Western blot (I, II) ............................. 53
  4.6 Flow cytometry (II, III) ............................. 54
  4.7 Statistical analyses (I, II, III) ............................. 55
5 Results
5.1 The expression of GR$\alpha$ and GR$\beta$ mRNA in studied inflammatory skin diseases (I, II, III) ................................................................. 57
5.2 The expression of GR$\alpha$ and GR$\beta$ protein in studied inflammatory skin diseases (I, II, III) ............................................................................ 57
5.3 Systemic prednisolone therapy in eczema atopicum and bullous pemphigoid (I, II) .................................................................................... 58
5.4 Topical calcipotriol/betamethasone dipropionate treatment in mild and moderate psoriasis (III) ................................................................. 59
5.4.1 Topical treatment in psoriasis and the expression of GR$\alpha$ and GR$\beta$ (III) ............................................................................... 60
5 Discussion
6.1 GR$\alpha$ and GR$\beta$ are expressed in skin and peripheral blood mononuclear cells of patients with inflammatory skin diseases .......... 61
6.2 The expression of GR$\alpha$ and GR$\beta$ does not predict clinical response to prednisolone treatment in patients with eczema atopicum and bullous pemphigoid .............................................. 62
6.3 Topical betamethasone treatment affects GR$\alpha$ and GR$\beta$ expression in patients with psoriasis ............................................................. 63
6.4 Calcipotriol/betamethasone combination therapy is more beneficial in psoriasis than betamethasone monotherapy ...................... 64
6.5 Methodological considerations and limitations of the study .......... 65
6.5.1 Study population ......................................................................... 65
6.5.2 Confounding factors .................................................................... 66
6.6 Future prospects ............................................................................... 66
7 Conclusions
References
Original publications
1 Introduction

Chronic inflammation is at the center of pathogenesis in several skin diseases. Lately, knowledge of the immunopathogenesis of the two most common inflammatory skin diseases has markedly sharpened. Psoriasis has been shown to be linked to the tumor necrosis factor alpha – interleukin-23 – interleukin-17 (TNF-α – IL-23 – IL-17) - inflammatory axis (Furue & Kadono 2016), and the interaction between cytokines and inflammatory cells in atopic eczema has been elucidated (Peng & Novak 2015). In contrast, little is known about the pathogenesis of other inflammatory skin diseases. In psoriasis and atopic eczema, new findings have led to the development of novel, targeted therapies. These biological treatments are used by thousands of psoriatic patients worldwide, and biologicals for atopic eczema are also in development. Inflammatory skin diseases with less well known pathogenesis are, however, still treated with conventional therapies, such as anti-inflammatory drugs, which are often the first-line of treatment.

Glucocorticoids (GC) are the most commonly used anti-inflammatory and immunosuppressive drugs in dermatology. The importance of GCs for a dermatologist cannot be overemphasized: they are the first-line therapy for nearly all inflammatory skin diseases. GCs mediate their actions through glucocorticoid receptors (GR) of which GRα and GRβ are clinically the most important (Oakley & Cidlowski 2013). Based on previous, mainly non-dermatological studies, it has been proposed that the quantity of GR isoforms or the GRβ: GRα –ratio could affect individual responsiveness to corticosteroid treatment. In particular, the up-regulation of GRβ expression has been shown to be linked to resistance to corticosteroid treatment (Lewis-Tuffin & Cidlowski 2006).

Calcipotriol is a vitamin D analogue used topically for the treatment of mild and moderate psoriasis. It mediates its actions through the vitamin D receptor. As our knowledge of psoriasis’ immunopathogenesis continues to extend, the effect of calcipotriol on psoriasis should be re-evaluated with focus on its immunological effects.

This thesis examines GR expression in inflammatory skin diseases. Years of clinical practice have shown the effectiveness of GCs in the treatment of inflammatory dermatoses, but the effects seen in everyday practice have not been extensively analysed using modern laboratory methods. The three studies included in this thesis measured both laboratory and clinical parameters. The effect of topical or systemic GC treatment on GR expression in inflammatory skin diseases was analysed. The literature review at the beginning of the thesis provides an overview
of the skin diseases that were studied. It also illustrates the basis for the use of GCs
and calcipotriol as common anti-inflammatory therapies.
2  Review of the literature

2.1  Inflammatory skin diseases included in the study

2.1.1  Eczema atopicum

Eczema atopicum (atopic dermatitis, atopic eczema, AD) is an inflammatory skin disease characterized by marked pruritus. It is one of the most common chronic skin diseases (Weidinger & Novak 2016). The prevalence of AD in children is 15-20% (Deckers et al. 2012, Lehtonen et al. 2003) and appears to be around 5-10% in adults within the European population (Silverberg & Hanifin 2013, Sinikumpu et al. 2014). AD can affect patients in all age groups and of both genders, but it occurs most frequently in children (Thstrup-Pedersen 2000).

AD is caused by genetic, immunologic and environmental factors (Weidinger & Novak 2016). The strongest known genetic risk factor is the filaggrin null mutation (Irvine et al. 2011). Filaggrin mutations are carried by approximately 10% of people of European ancestry; these mutations increase the risk of developing AD threefold (Irvine et al. 2011). However, a filaggrin mutation alone is not sufficient to cause AD and up to 60% of carriers will not develop atopic skin disease (Irvine et al. 2011). Other known genetic risk factors are linked to immune mechanisms, especially to innate immune signaling, T-cell activation and T-cell subdivision (Weidinger & Novak 2016). Environmental factors that contribute to AD risk include: small family size (Karmaus & Botezan 2002), higher education level (Silverberg & Hanifin 2013), ‘Western diet’ and an urban lifestyle (Flohr & Mann 2014), low exposure to ultra-violet (UV) radiation (Thyssen et al. 2015) as well as exposure to broad-spectrum antibiotics (Tsakok et al. 2013). Different combinations and accumulations of risk factors can initiate the breakdown of the epidermal protective barrier leading to cutaneous inflammation and finally to the commencement of AD (Weidinger & Novak 2016).

Skin barrier impairment, whether based on filaggrin mutations or other mechanisms, is a general feature in almost every AD patient (Peng & Novak 2015). The second hallmark of AD is cutaneous inflammation. There are increased amounts of resident and infiltrated immune cells and proinflammatory cytokines in the skin of AD patients (Peng & Novak 2015, Weidinger & Novak 2016). The functional interplay of these immune cells is essential for the pathogenesis of AD (Peng & Novak 2015). Microbes can also affect the skin barrier and promote helper
T cell type 2 (Th2) -type inflammation (Peng & Novak 2015). In acute AD, the inflammation shows pronounced Th2 cell involvement, alongside moderate numbers of Th22 and Th17 cells, whereas in chronic AD a mixture of Th2/Th1/Th22 cells predominates (Gittler et al. 2012, Peng & Novak 2015). Figure 1 characterizes a schematic presentation of the immunopathogenesis of AD.

Fig. 1. Pathogenesis of eczema atopicum (modified from Peng and Novak 2015).
The manifestation of eczema in AD is quite uniform between patients, but the severity of the disease, frequency of exacerbations and age at the onset of diagnosis leads to apparently heterogeneous phenotypes (Weidinger & Novak 2016). Pruritus is the most common characteristic of AD; other symptoms include erythema, xerosis, excoriations, oozing, crusting and lichenification. AD skin lesions can be located anywhere on the body, but distribution is usually related to the age of the patient; e.g. folds in children and the face in adults (Weidinger & Novak 2016).

Atopic eczema does not have any specific, histopathological features, as histological findings vary with the nature of the lesion biopsied. In acute lesions epidermal edema, spongiosis and lymphohistiocytic infiltrates in the upper dermis are found. Macrophages, neutrophils and eosinophils are rare. In chronic stages, there is less spongiosis, but epidermal acanthosis and fibrosis of the papillary dermis are present (Soter 1989, Uehara 1985).

The diagnosis of AD is based on clinical features (Weidinger & Novak 2016). Anamnesis and clinical signs are considered, and there is normally no need for blood samples or skin biopsies to confirm the diagnosis (Weidinger & Novak 2016). The criteria defined by Hanifin and Rajka (Hanifin & Rajka 1980) are the most commonly used tool in diagnosis (Table 1.) The severity of the disease can be measured using several methods, of which the Eczema Area andSeverity Index (EASI) score (Hanifin et al. 2001) is one of the most preferred (Eichenfield et al. 2014b, Weidinger & Novak 2016). EASI scores above 18 represent severe AD (range 0-72) (Hanifin et al. 2001).
Table 1. The diagnostic criteria for eczema atopicum according to Hanifin and Rajka (Hanifin & Rajka 1980).

<table>
<thead>
<tr>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients must display 3 or more of the following basic features:</td>
</tr>
<tr>
<td>Pruritus</td>
</tr>
<tr>
<td>Typical morphology and distribution:</td>
</tr>
<tr>
<td>Flexural lichenification or linearity in adults</td>
</tr>
<tr>
<td>Facial and extensor involvement in infants and children</td>
</tr>
<tr>
<td>Chronic or chronically-relapsing dermatitis</td>
</tr>
<tr>
<td>Personal or family history of atopy (asthma, allergic rhinitis, eczema atopicum)</td>
</tr>
<tr>
<td>Plus 3 or more of the following minor features:</td>
</tr>
<tr>
<td>Xerosis</td>
</tr>
<tr>
<td>Ichthyosis/palmar hyperlinearity/keratosis pilaris</td>
</tr>
<tr>
<td>Immediate (type I) skin test reactivity</td>
</tr>
<tr>
<td>Elevated serum immunoglobulin E (IgE)</td>
</tr>
<tr>
<td>Early age of onset</td>
</tr>
<tr>
<td>Tendency toward cutaneous infections (especially Staph. aureus and Herpes simplex)/impaired cell-mediated immunity</td>
</tr>
<tr>
<td>Tendency toward non-specific hand or foot dermatitis</td>
</tr>
<tr>
<td>Nipple eczema</td>
</tr>
<tr>
<td>Cheilitis</td>
</tr>
<tr>
<td>Recurrent conjunctivitis</td>
</tr>
<tr>
<td>Dennie-Morgan infraorbital fold</td>
</tr>
<tr>
<td>Keratoconus</td>
</tr>
<tr>
<td>Anterior subcapsular cataracts</td>
</tr>
<tr>
<td>Orbital darkening</td>
</tr>
<tr>
<td>Facial pallor/facial erythema</td>
</tr>
<tr>
<td>Pityriasis alba</td>
</tr>
<tr>
<td>Anterior neck folds</td>
</tr>
<tr>
<td>Itch when sweating</td>
</tr>
<tr>
<td>Intolerance to wool and lipid solvents</td>
</tr>
<tr>
<td>Perifollicular accentuation</td>
</tr>
<tr>
<td>Food intolerance</td>
</tr>
<tr>
<td>Course influenced by environmental/emotional factors</td>
</tr>
<tr>
<td>White dermographismus/delayed blanch</td>
</tr>
</tbody>
</table>

AD follows a chronic course with flares and remissions. Most children diagnosed with AD during infancy have no eczema during later childhood (Weidinger & Novak 2016), although a predisposition for eczema nevertheless persists, i.e. AD is currently incurable. AD is also associated with an elevated prevalence of asthma.

For a differential diagnosis of AD, other eczemas and contact allergies, infectious skin diseases such as scabies, rare congenital immunodeficiencies, keratinization disorders, Netherton syndrome, zinc deficiency and T-cell lymphoma should be excluded (Weidinger & Novak 2016).

Topical corticosteroids are the mainstay and the first-line treatment of AD (Gelmetti & Wollenberg 2014), and are usually the standard with which other topical therapies are compared (Eichenfield et al. 2014a). For acute flares, higher potency GCs are used in the short-term to achieve quick response. For long-term use, the least potent GC that controls symptoms should be selected to minimize the risk of side effects (Eichenfield et al. 2014a). Second-line topical therapy with calcineurin inhibitors (tacrolimus, pimecrolimus) is an option, especially when the adverse events of glucocorticoid treatment are to be minimized (Eichenfield et al. 2014a). Topical calcineurin inhibitors have been used for AD since 2000 and their efficacy is well proven (Eichenfield et al. 2014a). Most of AD patients can achieve clinical improvement with topical therapies only (Sidbury et al. 2014). When topical therapies fail or because of the severity of the disease, phototherapy (primarily with narrow-band ultraviolet B [UVB]) is recommended (Sidbury et al. 2014, Weidinger & Novak 2016). Systemic immunosuppressive therapy is indicated only in severe forms of AD and when topical therapies and/or phototherapy do not sufficiently control the symptoms, or quality of life is substantially impaired (Sidbury et al. 2014, Weidinger & Novak 2016). Systemic corticosteroids, although known to suppress AD and used frequently (Sidbury et al. 2014), are recommended to be used only in the short-term due to their unfavorable risk-benefit profile (Weidinger & Novak 2016). Other immunosuppressive agents that are used to treat AD (such as cyclosporine, azathioprine, methotrexate and mycophenolate mofetil) also have potential side effects and are usually considered only in exceptionally severe cases (Sidbury et al. 2014). If approved, the first biological treatment for AD, the IL-4 receptor-α antibody (Ab), dupilumab, is expected to be launched in early 2017. Dupilumab has proven to be beneficial in moderate and severe AD (Simpson et al. 2016). In addition to topical and/or systemic therapy, nonpharmacological interventions such as emollients, moisturizers and wet wraps, have proven to be highly effective in lessening xerosis and pruritus (Eichenfield et al. 2014a).

Since AD is one of the most common skin diseases, both international (e.g. European Dermatology Forum and American Academy of Dermatology,
Administrative Regulations for Evidence-based Clinical Practice Guidelines) and national (e.g. Finnish Käypä Hoito) guidelines have been established to help clinicians standardize their treatment practices.

2.1.2 Eczema nummulare

Eczema nummulare (nummular eczema, nummular dermatitis) is an inflammatory skin disease characterized by one or multiple pruritic coin-shaped eczematous lesions (Bonamonte et al. 2012, Cowan 1961, Hellgren & Mobacken 1969). Adults aged 20-60 years are most often affected, with predominances also among young female adults and older males (Bonamonte et al. 2012, Cowan 1961). The prevalence of eczema nummulare has been estimated at between 0.1% and 1.9% in Scandinavian studies (Hellgren & Mobacken 1969, Sinikumpu et al. 2014).

Many causative factors have been proposed (dry skin, contact allergies, emotional stress, excessive alcohol intake), but its exact etiopathogenesis remains a mystery (Aoyama et al. 1999, Bonamonte et al. 2012, Jiamton et al. 2012). In comparison to AD, eczema nummulare remains understudied.

Acute lesions show an oozing, crusted surface, while chronic lesions are dry, scaly and lichenified. Eczema nummulare is most commonly found on the limbs and trunk (Bonamonte et al. 2012).

Diagnosis is normally limited to clinical examination (Jiamton et al. 2012). Skin biopsies are rarely needed to confirm the diagnosis. Laboratory tests have not been shown to offer additional diagnostic value.

Histologically, eczema nummulare lacks specific features. Epidermal intercellular edema, spongiotic vesicles of variable size and a lymphohistiocytic infiltrate in the superficial dermis in an acute lesion can indicate to eczema nummulare, but are also common to other forms of acute eczematous condition (Bonamonte et al. 2012). Therefore, when a biopsy is taken, the diagnosis is based on both clinical and histological features.

Once initiated, eczema nummulare can persist chronically or occur in attacks (Jiamton et al. 2012). If eczema nummulare is to clear, it will usually heal within a year of onset; after that it tends to persist or recur (Cowan 1961).

Other types of eczema, contact allergies, psoriasis and tinea are the most probable differential diagnoses and are to be excluded (Jiamton et al. 2012).

Medium or potent topical corticosteroids are the drug of choice, second-line therapy being off-label use of topical calcineurin inhibitors (tacrolimus, pimecrolimus) (Bolognia et al. 2013, Jiamton et al. 2012). The use of topical
therapies is mainly based on clinical expertise; no comparative studies have been done. When eczema nummulare is widespread, phototherapy (narrow-band UVB) may be considered (Larkö 1996). In severe refractory cases, systemic corticosteroid treatment may be used and, with caution, methotrexate can be initiated (Jiamton et al. 2012).

2.1.3 Lichen simplex chronicus

Lichen simplex chronicus (neurodermatitis, LSC) is described as a chronic skin disease with intense pruritus leading to scratching and localized lichenification of the skin (Lotti et al. 2008). The prevalence of LSC in adults is 1.8% in Finland (Sinikumpu et al. 2014), but can be higher depending on the study population, for example 12.1% prevalence has been described in an elderly Thai cohort (Thaipisuttikul 1998). Even though LSC is known to be very common (Ermertcan et al. 2011), there is a need for additional epidemiological data. Typically, patients are adults aged from 30 to 50, and predominantly women (Ermertcan et al. 2011, Lotti et al. 2008).

LSC is generally agreed to be a consequence of continuous rubbing and scratching, often without a clear history of previous skin disease (Lotti et al. 2008). Then again, any pruritic skin lesion, e.g. insect bite, can lead to formation of a LSC lesion (Lotti et al. 2008). The vicious itch-scratch cycle leads to persistence of the lesions (Lotti et al. 2008). The exact molecular pathogenesis remains unresolved (Lotti et al. 2008). Psychogenic factors, especially anxiety disorders, are known to play a relevant role in the formation of LSC lesions (Liao et al. 2014, Lotti et al. 2008).

The neck, ankles, scalp, extensor forearms and genital areas are the predilection sides of LSC (Lotti et al. 2008). One or more lichenified plaques can be seen (Ermertcan et al. 2011). LSC can produce sleep disturbance, sexual dysfunction and affect overall quality of life (An et al. 2013, Ermertcan et al. 2011).

Epidermal hyperplasia, orthokeratosis, hypergranulosis with elongation of the rete ridges and perivascular infiltrate of lymphocytes are characteristic histopathological features of LSC lesions (Lotti et al. 2008). However, the diagnosis of LSC is clinical and neither laboratory testing nor skin biopsy are normally needed.

LSC is not a life-threatening disease, but in the absence of any clear underlying cause, it tends to be resistant to therapy and persistent. The prognosis improves if the psychological aspects are considered and rectified (Lotti et al. 2008).
For differential diagnosis, prurigo nodularis and other causes of pruritus (skin or systemic disease) must be excluded (Lotti et al. 2008).

Treatment of LSC can be frustrating. Potent topical glucocorticoids, possibly under occlusion, are the drug of choice (Lotti et al. 2008). Intralosomal use of GCs is also an option. Topical tacrolimus has also been successfully used (Aschoff & Wozel 2007). For widespread lesions, phototherapy (narrow-band UVB) may be considered (Liao et al. 2014). The critical aspect of treating LSC is to break the itch-scratch cycle by education, support and in severe cases, behavioral therapy (Liao et al. 2014).

### 2.1.4 Lichen ruber planus

Lichen ruber planus (LRP) is a mucocutaneous inflammatory disease. The mean age at diagnosis ranges from 40 to 60 years and gender distribution is balanced (Bhattacharya et al. 2000, Wagner et al. 2013). The prevalence of LRP is estimated to be between 0.5% and 1.0% (Augustin et al. 2011, Wagner et al. 2013); 0.7% in the Finnish adult population (Sinikumpu et al. 2014).

The molecular pathogenesis of LRP is to some degree understood. A cell-mediated destructive autoimmune reaction against basal keratinocytes with a bandlike infiltrate of T cells in the dermo-epidermal junction leads to lesion formation (Sugerman et al. 2002). The autoantigen starting the process is unknown – mechanical trauma, viral and bacterial infections and systemic drugs have been proposed (Sugerman et al. 2002).

The classical form of LRP is characterized by lichenoid, polygonal purpuric papules and plaques with fine white lines (Wichstrom striae) (Wagner et al. 2013). The skin and oral mucosa are the sites most frequently involved, but other mucous membranes, scalp hair and nails can also be affected (Wagner et al. 2013). The predilection sites of skin lesions are extensor surfaces of the limbs and the lower lumbar region (Bhattacharya et al. 2000). Most patients experience pruritus; other symptoms include burning and pain (Bhattacharya et al. 2000).

The histopathological findings of LRP are pathognomic: thickening of the stratum corneum with orthokeratosis, hypergranulosis, sawtooth-like acanthosis and a bandlike inflammatory-cell infiltrate in the upper dermis, consisting primarily of lymphocytes (Le Cleach & Chosidow 2012, Wagner et al. 2013). Direct immunofluorescence investigation of lesional skin yield positive signals, with an intense continuous broad band of staining with antifibrinogen at the dermoeipidermal junction as the best indicator (Kulthanan et al. 2007).
The diagnosis of LRP in its classical cutaneous form is usually done clinically, but sometimes a skin biopsy is needed to exclude other pruritic diseases. Direct immunofluorescence can provide additional evidence when other conditions cannot be excluded (Kulthanan et al. 2007), but it is not routinely carried out. Laboratory tests are not necessary.

Cutaneous LRP usually heals within a year of onset, but is still considered to be a chronic disease (Le Cleach & Chosidow 2012, Sharma et al. 2012, Wagner et al. 2013). Longer, chronically recurrent forms are possible, and the individual prognosis is difficult to predict (Bhattacharya et al. 2000, Wagner et al. 2013).

In its classical form, LRP is easy to diagnose, but variants of LRP sometimes pose a problem with differential diagnosis. Annular LRP can resemble granuloma annulare, linear LRP may look like epidermal nevi or lichen striatus and inverse LRP like intertrigo. Psoriasis, lichen nitidus and lupus erythematosus may also confound diagnosis (Boyd & Neldner 1991).

The treatments of choice, both for cutaneous and mucosal LRP, are potent or very potent topical glucocorticoids, with or without occlusion (Wagner et al. 2013). Second-line treatments include topical tacrolimus and pimecrolimus (off-label), phototherapy (narrow-band UVB) or phototherapy with a light-sensitizing psoralen (PUVA), systemic corticosteroids and retinoids as well as other immunosuppressive agents (cyclosporine, azathioprine, dapsone) (Boyd & Neldner 1991, Wagner et al. 2013). The treatment protocol is chosen by weighting the extent and variant of the disease (Wagner et al. 2013).

2.1.5 Bullous pemphigoid

Bullous pemphigoid (BP) is an autoimmune skin disease characterized by tense bullae and blisters (Feliciani et al. 2015). BP is the most common cutaneous and mucous membrane subepidermal blistering disease globally (Feliciani et al. 2015). The incidence of BP varies from 12.1 to 21.7 new cases per 1 million people per year depending on the study population (Försti et al. 2014, Joly et al. 2012, Marazza et al. 2009). Recent studies show BP to be increasing in prevalence in several countries (Joly et al. 2012, Langan et al. 2008, Schmidt & Zillikens 2013), including Finland (Försti et al. 2014). This increase in incidence is thought to be due to the aging of the general population and the availability of more sensitive and specific diagnostic assays (Schmidt & Zillikens 2013). BP is a disease of the elderly and is extremely rare in children and adolescents (Schmidt & Zillikens 2013).
Genetic predisposition, drug intake and environmental factors (e.g. radiation therapy, viral infections, diet) have been proposed as initiating factors of BP (Lo Schiavo et al. 2013). The main autoantigen in BP is bullous pemphigoid antigen 180 (BP180; BPAG2 or collagen XVII), though BP patients may also have autoantibodies against bullous pemphigoid antigen 230 (BP230 or BPAG1), another hemidesmosomal protein (Hammers & Stanley 2016, Lo Schiavo et al. 2013). The breakdown of self-tolerance is initiated by the capture of self-antigens BP180 and BP230 by antigen-presenting cells, which process and present these antibodies on their cell surface, starting an autoimmune cascade (Lo Schiavo et al. 2013). The pathogenesis then involves the development of BP180–specific autoreactive T cells, the accumulation of other inflammatory cells, complement activation, mast-cell degranulation and protease and chemokine release (Lo Schiavo et al. 2013, Schmidt & Zillikens 2013). The evidence of detailed pathogenesis of BP comes from clinical findings and expenditure of novel animal models (Hurskainen et al. 2015, Nishie 2014). The pathogenesis of BP is illustrated in Figure 2.

Tense blisters, bullae and erosions are seen in the skin and mucous membranes of BP patients (Schmidt & Zillikens 2013, Venning et al. 2012). The inner thighs, groin, axillae, abdomen, neck and flexural aspects of the arms and legs are the predilection sites of the typically symmetrical BP skin lesions (Mutazim 2003). Pruritus, sometimes associated with erythema or urticaria, usually precedes the formation of bullae (Schmidt & Zillikens 2013, Venning et al. 2012). In contrast to the classical form of BP with blisters, up to 20% of patients have no blisters and only pruritus, excoriations and eczematous or urticarial lesions are seen (della Torre et al. 2012, Di Zenzo et al. 2012).
Fig. 2. Pathogenesis of bullous pemphigoid (modified from Lo Schiavo et al. 2013 and Nishie 2014).

A diagnosis of BP is based on a combination of typical clinical presentation, direct immunofluorescence microscopy of perilesional skin samples, and serology. If a formalin-fixed lesional skin biopsy is taken and analysed under a microscope, a subepidermal blister with a moderate to dense inflammatory infiltrate composed of lymphocytes, neutrophils and, characteristically, eosinophils, is typically seen (Hammers & Stanley 2016). In direct immunofluorescence microscopy of perilesional skin biopsies, a linear IgG and/or complement C3 deposit along the basement membrane zone is considered as a specific marker for BP (Hammers & Stanley 2016). Sometimes a weaker linear IgA and/or IgE fluorescence is also seen (Schmidt & Zillikens 2013). Serological analysis by enzyme-linked immunosorbent assay (ELISA) of the autoantibodies against the NC16A domain of
recombinant human BP180 protein is recommended due to both diagnostic value and the importance of monitoring disease severity (Bernard et al. 2009, Sitaru et al. 2007).

The extent of BP can be assessed clinically or by using the Bullous Pemphigoid Disease Area Index (BPDAI) (Murrell et al. 2012). The BPDAI gives a numerical value for the number of erosions and blisters as well as urticaria and erythema, and takes into account the localization of the skin symptoms. The BPDAI has, however, so far been mainly used in clinical studies and still needs further validation (Zhao & Murrell 2015).

The course and prognosis of BP is variable and widely dependent on the comorbidities and the overall health of the patient (Mutasim 2003). BP can be self-limiting and resolve within months to years, but exacerbations and relapses occur, especially if BP is left untreated (Feliciani et al. 2015, Mutasim 2003). BP can also cause severe complications, and affected patients show a significantly increased mortality rate (Försti et al. 2016, Joly et al. 2012, Langan et al. 2008). As well as the disease itself, its treatments, especially high doses of systemic glucocorticoids, are associated with decreased survival rates (Bernard & Charneux 2011, Venning et al. 2012).

Differential diagnosis of BP comprises other autoimmune bullous diseases (pemphigus, linear IgA disease, epidermolysis bullosa acquisita), genetic bullous diseases (epidermolysis bullosa group) and other skin diseases that sometimes form bullae (erythema multiforme, cellulitis, contact dermatitis) (Venning et al. 2012). In the prodromal stage when formation of bullae has not yet begun, all causes of intense pruritus must be considered for differential diagnosis.

Systemic and topical corticosteroids have been shown to be efficacious in the management of BP and are the most commonly used first-line treatments (Feliciani et al. 2015, Venning et al. 2012). Recommendations for dose and duration of GC therapy have been published (Feliciani et al. 2015, Venning et al. 2012). Principally, the severity of the disease is assessed, a topical or systemic GC is selected and during the treatment, the dosage is tapered in proportion to response. With systemic GC therapy, the daily dosage should not exceed a 1 mg dose of prednisolone per kg body weight (Venning et al. 2012). When corticosteroids alone are not sufficient to manage BP symptoms, or a corticosteroid sparing agent is needed, other immunosuppressants (azathioprine, dapsone, methotrexate, mycophenolate mofetil) can be utilized (Feliciani et al. 2015, Schmidt & Zillikens 2013). Tetracyclines could also be considered (Feliciani et al. 2015). In treatment-resistant cases, cyclosporine, intravenous immunoglobulins, omalizumab (a monoclonal antibody
[mAb] that inhibits IgE binding), rituximab (anti- cluster of designation 20 (CD20) antibody) and anti-TNF –antibodies (etanercept, adalimumab) have been used (Feliciani et al. 2015, Venning et al. 2012).

2.1.6 Psoriasis

Psoriasis vulgaris (plaque psoriasis, later: psoriasis) is a chronic, immune-mediated inflammatory systemic disorder (Boehncke & Schön 2015). Psoriasis affects both genders and its prevalence is 1-2% in the general Caucasian population (Augustin et al. 2011, Gudjonsson & Elder 2007, Parisi et al. 2013, Sinikumpu et al. 2014). The symptoms of psoriasis can start at any age (Boehncke & Schön 2015). Positive family history and the presence of certain human leukocyte antigen (HLA) class I antigens are known to be particularly associated with earlier age at onset (Henseler 1997).

Psoriasis is considered a result of the complex interaction of epithelial cells with the innate and adaptive immune systems (Boehncke & Schön 2015). The primary cause, whether keratinocytes or the immune system, however still remains unidentified (Nestle et al. 2009b). Environmental factors, such as mechanical trauma, sunburns, systemic drugs (e.g. β blockers, lithium, antimalarias) and infections (streptococci, Human immunodeficiency virus [HIV]) can provoke psoriasis in genetically predisposed individuals (Boehncke & Schön 2015).

The immunopathogenesis of psoriasis is represented in Figure 3. The predisposing factors mentioned above lead to the release of keratinocyte deoxyribonucleic acid (DNA), which forms a complex with cathelicidin LL-37 (Nestle et al. 2009b). Plasmacytoid dendritic cells (DC) recognise this complex via the intracellular toll-like receptor 9 (TLR 9), and then, by production of interferon-α (IFN-α), and with IL-18, IL-6 and TNF-α produced by keratinocytes, activate dermal DCs (Lowes et al. 2014, Nestle et al. 2009b). Dermal DCs migrate to local lymph nodes and educate specific naïve Th cells (Nestle et al. 2009b). With this signal and several other cytokines, Th cells specialize into Th1, Th17 and regulatory T cells (Tregs) (Nestle et al. 2009b, Sugiyama et al. 2005). Activated T cells recirculate and attach to the dermis and epidermis with their specific surface markers, chemokine receptors (CCR4, CCR6, CCR10, and CXCR3) and cutaneous leukocyte antigen (CLA) (Geginat et al. 2013, Nestle et al. 2009b). T cells are further reactivated in the skin by IFN-γ (Nestle et al. 2009a, Nestle et al. 2009b). DCs also produce IL-12 and IL-23, which leads to Th1 and Th17 proliferation (Furue & Kadono 2016). IL-6 and TNF-α promote differentiation of Th22 cells
Th1 cytokines (IFN-γ and TNF-α) together with IL-17 produced by activated Th17 cells and IL-22 produced by Th22 cells, activate keratinocytes to produce additional mediators such as chemokines (e.g. chemokine CC ligand-20 [CCL-20]), cytokines and antimicrobial peptides (e.g. S100A7 and β defencins) (Furue & Kadono 2016, Harper et al. 2009, Nestle et al. 2009b). Chemokines, e.g. chemokine CXC ligand-1 (CXCL1) and IL-8 (CXCL8) attract neutrophils into the epidermis (Nestle et al. 2009b) and more T cells into the site of inflammation (Harper et al. 2009). Inflammatory cells provoke the proliferation of keratinocytes and they in turn enhance and maintain the chronic inflammation (Nestle et al. 2009b). The inflammatory milieu also leads to activation of several pro-angiogenic factors (Varricchi et al. 2015). This activation of cutaneous cells, recruitment of leucocytes, alteration of keratinocyte differentiation and proliferation as well as vascular proliferation finally leads to formation of skin lesions.
Fig. 3. Pathogenesis of psoriasis (modified from Nestle et al. 2009).

Skin symptoms of psoriasis are clinically characterized by well-defined, raised, scaly, erythematic plaques that are symmetrically distributed (Boehncke & Schön 2015). Elbows, knees, lower back and scalp are most frequently affected (Boehncke & Schön 2015). The Psoriasis Area and Severity Index (PASI) is routinely used to estimate the disease severity (Schmitt & Wozel 2005). The PASI score gives a numerical value for erythema, infiltration, scaling and the extent of the psoriasis lesions (Schmitt & Wozel 2005). It is used to monitor psoriatic patients and to compare disease severity with a PASI score <7 referring to mild, PASI 7-12 to moderate and PASI>12 to severe disease (maximum score 72) (Schmitt & Wozel 2005).
The diagnosis of psoriasis is usually based on clinical findings and no laboratory tests are required (Boehncke & Schön 2015). If a skin biopsy is taken, the histological changes seen in psoriasis lesions are well characterized and pathognomonic (Boehncke & Schön 2015). Epidermal acanthosis (thickening), hyperkeratosis (formation of a thickened cornified layer) and parakeratosis (cell nuclei present in the cornified layer) are seen. Epidermal rete ridges are elongated and dilated blood vessels reach into the tips of the dermal papillae. An inflammatory infiltrate of T cells, macrophages, mast cells and neutrophils accumulates within the epidermis as ‘Kogol pustules’ or subcorneally forming ‘Munro’s microabscesses’ which are pathognomonic for psoriasis (Boehncke & Schön 2015).

Psoriasis is a chronic disease without a known cure. It can have a significant impact on the quality of life of affected individuals (Schmitt & Wozel 2005). The Dermatology Life Quality Index (DLQI) is commonly used to assess the influence of psoriasis on quality of life (Finlay & Khan 1994). A DLQI score over 10 (maximum score 30) signifies very large negative effects on the patient’s life (Mrowietz et al. 2011). However, with the recent development of more targeted therapies, treatment goals have shifted towards more ambitious aims: PASI as well as DLQI scores should be considerably reduced or the treatment should be modified (Mrowietz et al. 2011). The management approach of this disease should include comorbidities, as the systemic inflammation in psoriasis affects organs other than the skin; psoriatic arthritis, Crohn’s disease, depression, non-alcoholic fatty liver, metabolic syndrome and cardiovascular disease are frequently diagnosed in psoriatic patients (Boehncke & Schön 2015).

Differential diagnosis of psoriasis most commonly includes tinea, different eczemas and LRP (Boehncke & Schön 2015).

High-quality evidence-based guidelines (both national and international) have been developed to improve treatment of psoriasis (American Academy of Dermatology Work Group et al. 2011, Mrowietz et al. 2011, Nast et al. 2007, Rantanen et al. 2012). The most frequently used therapies in Finland are listed in Table 2. Topical therapies, such as glucocorticoids and vitamin D3 analogues or combinations of both, are usually sufficient to manage mild and moderate disease. Systemic GC treatment is used only in very exceptional cases: after such treatment there is a known risk of severe rebound worsening of psoriasis (Lowe 1983). Phototherapy is used for more extensive disease. Systemic conventional treatments and biologicals are reserved for patients with the most severe forms of the disease (PASI >10-12) and impaired quality of life (DLQI >10). Biologicals for psoriasis
are therapies developed to target the exact immunopathogenesis of the disease and are therefore highly effective (Nestle et al. 2009b). The therapeutic modalities are also combined and topical therapy is usually continued even if systemic treatment is initiated (Jensen et al. 2010, Rantanen et al. 2012).

Table 2. Treatment of psoriasis.

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Target/mode of action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Topical therapies</strong></td>
<td></td>
</tr>
<tr>
<td>Glucocorticoids</td>
<td>broad spectrum anti-inflammation, immunosuppression</td>
</tr>
<tr>
<td>Vitamin D analogues (calcipotriol, calcitriol)</td>
<td>cellular proliferation, differentiation and anti-inflammation</td>
</tr>
<tr>
<td><strong>Phototherapy</strong></td>
<td></td>
</tr>
<tr>
<td>Narrow-band UVB</td>
<td>immunomodulation (anti-inflammatory)</td>
</tr>
<tr>
<td>PUVA (psoralen and UVA)</td>
<td>immunomodulation (anti-inflammatory)</td>
</tr>
<tr>
<td><strong>Systemic, conventional drugs</strong></td>
<td></td>
</tr>
<tr>
<td>Acitretin</td>
<td>keratinocyte proliferation and differentiation</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>broad spectrum immunosuppression</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>broad spectrum anti-inflammation, immunosuppression</td>
</tr>
<tr>
<td>Apremilast</td>
<td>phosphodiesterase 4 inhibitor</td>
</tr>
<tr>
<td><strong>Biologic treatments</strong></td>
<td></td>
</tr>
<tr>
<td>Adalimumab</td>
<td>TNF-α inhibitor</td>
</tr>
<tr>
<td>Etanercept</td>
<td>TNF-α inhibitor</td>
</tr>
<tr>
<td>Secucinumab</td>
<td>IL-17A inhibitor</td>
</tr>
<tr>
<td>Ixekizumab</td>
<td>IL-17A inhibitor</td>
</tr>
<tr>
<td>Ustekinumab</td>
<td>IL-12 and IL-23 inhibitor</td>
</tr>
</tbody>
</table>

2.2 Mechanism of action of drugs included in the study

2.2.1 Glucocorticoids

Glucocorticoids are the most commonly used anti-inflammatory and immunosuppressive drugs in dermatology, and have been in use since the 1940s. In recognition of the importance of these drugs to modern medicine, Hench, Kendall and Reichstein received a Nobel Prize in 1950 for their discovery (Hench et al. 1950).

To understand the effects of GCs, it is important to understand the normal function of the cortisol hormone. Briefly, neural, endocrine and cytokine signals stimulate and suppress the hypothalamus to control the secretion of corticotropin-releasing hormone into the hypophyseal portal system (Rhen & Cidlowski 2005). With the stimulus from corticotropin-releasing hormone, corticotropin
(adrenocorticotropic hormone, ACTH) is released from the anterior pituitary into the bloodstream (Rhen & Cidlowski 2005). ACTH stimulates the adrenal cortex to produce cortisol, which binds to glucocorticoid receptors in virtually all cells (Rhen & Cidlowski 2005). Cortisol also forms a negative feedback loop with the hypothalamus, regulating the concentration of cortisol in the blood (Webster et al. 2002). GCs and the hypothalamic-adrenal axis are effective in suppressing and resolving inflammatory processes (Webster et al. 2002).

Over the decades, numerous pharmacological analogues of cortisol have been developed. The basic four-ring structure of cholesterol with three hexane rings and one pentane ring has been modified e.g. by hydroxylations, methylations and fluorinations to increase the potency of GC analogues (Fig. 4), but also to reduce side effects (He et al. 2014, Schmit & Rousseau 1979). This has led to a wide range of topical and systemic preparations. Figure 4 represents the structure of cortisol, and as an example, the two GCs investigated in this study: prednisolone and betamethasone.

![Fig. 4. The structure of cortisol, prednisolone and betamethasone (modified from He et al. 2014 and Boland 1962).](image)

The actions of GCs are mediated by the intracellular glucocorticoid receptor first described in detail by Hollenberg and co-workers (Hollenberg et al. 1985). They published the complete amino-acid sequence of two alternative splice variants: glucocorticoid receptor alpha (GRα) and beta (GRβ) (Hollenberg et al. 1985). The GR gene is located on chromosome 5 in the 5q31-q32 region and the genomic structure consists of nine exons (Hollenberg et al. 1985). GRα is 777 amino acid residues long (94 kilo Dalton [kDa]) and identical to GRβ (90 kDa) up to residue 727, after which they diverge due to alternative splicing of exon 9 (Fig. 5). GRβ
comprises 742 residues, so that it differs from GRα only in its shorter carboxy terminus (Hollenberg et al. 1985). The region of the variation is located in the hormone-binding domain, so that GRα binds to cortisol or GC, but GRβ does not (Oakley et al. 1996).

The ligand-free GRα resides predominantly in the cytoplasm of cells, where it is bound to a large multiprotein complex containing two molecules of heat-shock protein 90 (hsp90) (Denis et al. 1988, Oakley et al. 1999). Once the ligand (cortisol) binds to the ligand binding domain (LBD) of GRα, the multiprotein complex dissociates and GRα is translocated to the nucleus (Oakley et al. 1999, Zhou & Cidlowski 2005). GRα binds through its DNA-binding domain (DBD) as a homodimer to specific glucocorticoid response elements (GRE) in DNA, modulating the expression of several glucocorticoid-responsive genes (Leung & Bloom 2003, Xavier et al. 2016). This binding mediates GC signaling. In addition, ligand-activated GRα can act as a monomer, modulating gene expression with other transcription factors, making the response to GCs more complex (Xavier et al. 2016). GRα is the principal mediator of the effects of GCs, and both GRα messenger ribonucleic acid (mRNA) and protein are expressed in most human tissues and cell lines (Pujols et al. 2002). Previous studies have not identified different isoforms, but more data have been gathered recently with GRα-specific antibodies. See Table 3 for a summary of recent studies concerning skin diseases.
Fig. 5. A schematic model of alternative splicing of GR mRNA and the binding of GRα to the glucocorticoid response elements on the nucleus of the cell (modified from Zhou et al. 2005).

The ligand-free GRβ has been localized in both the cytoplasm and nucleus, but mainly in the nucleus (Oakley et al. 1997, Oakley & Cidlowski 2013). It is not
known whether GRβ binds to hsp90 (Oakley et al. 1997). The lack of capacity to bind to the multiprotein complex (as seen with GRα) could explain the principal localization of GRβ in the nucleus. GRβ mRNA has been found in a variety of human tissues, including adult brain, lung, liver, heart, skeletal muscle, kidney, nasal mucosa, eosinophils, peripheral blood mononuclear (PBMC) cells, macrophages and neutrophils (Oakley et al. 1996, Pujols et al. 2002), whereas GRβ protein has been identified in a more limited number of cell types (Lewis-Tuffin & Cidlowski 2006). The studies related to inflammatory skin diseases and conducted with GRβ-specific antibody are listed in Table 3.

GRβ is generally expressed at lower levels than GRα (Oakley & Cidlowski 2013). A high expression level of GRβ has been shown to antagonize the activity of GRα (Oakley & Cidlowski 2013). Competitive binding to GREs and competition for transcriptional co-regulators as well as the formation of inactive GRα/GRβ heterodimers have been proposed to lead to this possible antagonism (Oakley & Cidlowski 2013). Proinflammatory cytokines, e.g. TNF-α (Webster et al. 2001) and Th17-related cytokines (Vazquez-Tello et al. 2013) have been shown to up-regulate GRβ expression leading to glucocorticoid resistance. GRβ can also directly induce and repress several genes independently of GRα (Kino et al. 2009). The up-regulation of GRβ expression has been postulated to be related to glucocorticoid insensitivity in several inflammatory diseases including adult asthma (Leung et al. 1997, Sousa et al. 2000), rheumatoid arthritis (Chikanza & Kozaci 2004), ulcerative colitis (Honda et al. 2000) and AD treated with topical GCs (Hägg et al. 2010). Conversely, down-regulation of GRα expression has been reported to be induced by both long-term GC treatment and short-term boluses of GCs, but up-regulation is also reported, making the analysis of GR isoforms and their in vivo function complex (Chen et al. 2009, Haarman et al. 2004, Hägg et al. 2010).

Furthermore, by alternative splicing, the isoforms GR gamma (GRγ) (Rivers et al. 1999), GR-A and GR-P (Moalli et al. 1993) are generated. GRγ binds glucocorticoids, but GR-A and GR-P do not (Oakley & Cidlowski 2013). The clinical relevance of these isoforms has been studied to some extent, but more studies are needed to evaluate their role in GC responses (Oakley & Cidlowski 2013). Recent studies have also revealed several translational isoforms of GRα, and also post-translational modification occurs, so it is clear that more research is needed to unravel the heterogeneity in GR responses (Duma et al. 2006, Oakley & Cidlowski 2013).
Table 3. Studies related to inflammatory skin diseases conducted with specific GRα and GRβ antibodies that show positive findings.

<table>
<thead>
<tr>
<th>Study group</th>
<th>Antibody</th>
<th>Target cells</th>
<th>Cells obtained from</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hägg et al 2010</td>
<td>GRβ</td>
<td>PBMCs</td>
<td>atopic patients, healthy controls</td>
</tr>
<tr>
<td>Inui et al 2010</td>
<td>GRα</td>
<td>PBMCs</td>
<td>one patient with AD, healthy control</td>
</tr>
<tr>
<td></td>
<td>GRβ</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Lin et al 2014</td>
<td>GRα</td>
<td>oral epithelium</td>
<td>oral lichen planus patients and healthy controls</td>
</tr>
<tr>
<td>Pang et al 2015</td>
<td>GRα</td>
<td>epidermis</td>
<td>psoriasis patients and healthy controls</td>
</tr>
</tbody>
</table>

Through GRs, GCs control the expression of several genes in order to produce anti-inflammatory effects. These interactions lead to diverse cellular mechanisms, including suppression of the production of inflammatory cytokines (e.g. IL-1, IL-6 and TNF-α), inhibition of T cell activation, decrease in the number of eosinophils, mast cells and dendritic cells and changes in the functions of endothelial cells and fibroblasts (Ahluwalia 1998, Leung & Bloom 2003, Umland et al. 2002, Xavier et al. 2016). These mechanisms lead to the well-known responses to GC treatment: anti-inflammation, immunosuppression and antiproliferation. Cutaneous side-effects (such as local cutaneous atrophy, striae, risk of promoting infections, impaired wound healing, hypopigmentation and glaucoma as well as contact sensitization) are also partly due to these effects (Callen et al. 2007, Rhen & Cidlowski 2005). Systemic side effects can occur with exceptionally widespread use of topical GCs or with systemic administration, and include inhibition of adrenal function, increased risk of gastrointestinal ulceration, diabetes and osteoporosis (Callen et al. 2007, Rhen & Cidlowski 2005).

In skin diseases, GCs can be applied topically to the skin, used as intralesional injections, infused intravenously or administered perorally. Regarding potency, topical GCs are classified under a four-point scale by the British National Formulary (in Finland, Pharmaca Fennica): mild, moderate, potent, very potent. Each GC’s classification is based on data from comparative trials as well as animal and human studies, mainly employing vasoconstrictor assays (Barry & Woodford 1974, Marks et al. 1973, Munro et al. 1967, Smith 1957, Woodford & Barry 1982). Topical GCs are used to treat several skin conditions, including eczemas (e.g. AD,
eczema nummulare and allergic contact eczema), LSC and psoriasis to name but a few (Lagos & Maibach 2002). Systemic GCs are reserved for more severe skin diseases, including severe AD (Sidbury et al. 2014) and bullous pemphigoid (Feliciani et al. 2015). They are also used to treat vasculitis, autoimmune connective tissue diseases, such as dermatomyositis and lupus erythematosus, as well as miscellaneous other dermatologic diseases (sarcoidosis, acute angioedema etc.) (Williams & Nesbitt 2001). Comparative investigations of the potency of systemic GCs have also been carried out (Downie et al. 1978, Glyn & Fox 1961, Sparrow & Geelhoed 2006), but there is no classification system for systemic GCs that is used in daily practice.

Systemically administered prednisolone in the treatment of eczema atopicum and bullous pemphigoid

Prednisolone was first synthesized in 1955 and its safety and efficacy profiles are well characterised. It is the delta-1-analogue of hydrocortisone, with enhanced anti-inflammatory potency (Boland 1962). Its efficacy and rapidity in relieving clinical symptoms of skin diseases are widely accepted.

The systemic use of prednisolone in AD has been investigated in only one comparative clinical study (Schmitt et al. 2010). The general opinion is that in AD, prednisolone should only be used short-term and in limited cases, due to the risk of flare after the discontinuation of the treatment and the well-known risk of side effects of long-term use (Ring et al. 2012, Schmitt et al. 2010, Sidbury et al. 2014).

By contrast, prednisolone is the drug of choice for treatment of BP (Feliciani et al. 2015). Several studies concluded in the Cochrane Analysis (by Kirtschig and co-workers) have confirmed the legitimacy of using prednisolone for patients with BP (Kirtschig et al. 2010). However, the risk of side-effects and the heightened risk of mortality must be considered with every patient and prophylactic measures (e.g. osteoporosis prophylaxis) should be noted in the maintenance therapy (Feliciani et al. 2015).

Topical betamethasone in the treatment of psoriasis

Betamethasone is a potent glucocorticoid produced from corticosterone by fluorination and hydroxylation of certain carbon positions (Chambers et al. 1976). It is commercially used in two forms, betamethasone dipropionate and betamethasone valerate. They have both been shown to reduce symptoms of
psoriasis and other corticosteroid-responsive dermatoses (AD, allergic contact eczema, LSC), but betamethasone dipropionate has been shown to be more effective than betamethasone valerate in psoriasis (Chambers et al. 1976). The anti-inflammatory effects of betamethasone dipropionate appear to be somewhat broader than those of betamethasone valerate (Bjerring 1993), and it is usually used at a lower concentration (0.05%) than betamethasone valerate (0.1%) in topical preparations.

When treating psoriasis, betamethasone dipropionate is customarily combined with calcipotriol to form a fixed-ratio combination, which has been proven to be more efficient than either of the ingredients alone (Douglas et al. 2002, Kaufmann et al. 2002, Papp et al. 2003).

2.2.2 Calcipotriol

Calcipotriol (22-ene-26, 27-dehydro-1α, 25(OH)2D₃) is a vitamin D₃ analogue that has been used topically since the early 1990s (Menter & Griffiths 2007). Other topical vitamin D₃ analogues (e.g. calcitriol) are also commercially available, but calcipotriol has been investigated most widely and has been shown to be superior to other analogues in treating mild and moderate psoriasis (Ashcroft et al. 2000). It is rapidly metabolized and safer than the other analogues in higher concentrations (Mortensen et al. 1996).

Vitamin D is a fat-soluble vitamin obtained either from the diet or generated in the skin after UVB-exposure (wavelengths 300-320 nm) (Fraser 1995). Photochemical conversion of 7-dehydrocholesterol forms cholecalciferol, a precursor of active vitamin D metabolites (Fraser 1995). Cholecalciferol requires two hydroxylations for activation. The first takes place mainly in the liver and the second in the kidney, so that calcitriol (1,25-dihydroxyvitamin D₃; 1,25(OH)₂D₃) with three hydroxyl groups is finally formed (Fraser 1995). However, keratinocytes themselves possess the enzymes needed for production of calcitriol, so that the active form of vitamin D can also be produced in the skin (Bikle & Pillai 1988). Calcitriol is a potent agent classified today as a hormone, and is needed for normal calcium homeostasis (Fraser 1995). Calcipotriol (Fig. 6) is a synthetic analogue of calcitriol (Christakos et al. 2016, Leyssens et al. 2014).
The vitamin D receptor (VDR, 60 kDa) belongs to the steroid receptor superfamily along with the glucocorticoid receptors (Christakos et al. 2016). It is capable of moving between the cytoplasm and nucleus and is active as a heterodimer with the retinoid receptor X (RXR) (Christakos et al. 2016). Binding of a vitamin D3 analogue promotes conformational change in the VDR, facilitating contact with the RXR (Christakos et al. 2016). In the nucleus, the VDR/RXR complex binds to vitamin D response elements in DNA regulating numerous genes (Christakos et al. 2016, Saccone et al. 2015). The VDR has been shown to be expressed in most human tissues and cell types (Wang et al. 2012).

The therapeutic effects of vitamin D analogues are a result of antiproliferation effects, the ability to promote differentiation and immunosuppressive activity (Christakos et al. 2016, Gorman et al. 2010). Calcipotriol also increases the expression of the VDR in keratinocytes (Christakos et al. 2016). In psoriasis, calcipotriol favors differentiation but suppresses the proliferation of keratinocytes, leading to decrease in hyperkeratinization and epidermal thickness (Soleymani et al. 2015). Calcipotriol possesses anti-inflammatory properties, and it has been shown to suppress Th17–mediated inflammation (Dyring-Andersen et al. 2015, Lovato et al. 2016) and to regulate immune responses mediated by dendritic cells and T cells (Gorman et al. 2010). It also reduces expression of the antimicrobial peptide S100A7 (Hegyi et al. 2012).
Systemic side-effects of vitamin D3 analogues have been linked to calcium homeostasis. The maximum recommended dose of calcipotriol (50µg/g) ointment is 15 g/day or 100 g/week; higher dosages can lead to hypervitaminosis and hypercalcemia (Georgiou & Tsambaos 1999, Vakirlis et al. 2008). Topical side-effects of calcipotriol are potential irritation and sensitization (Ashcroft et al. 2000). To overcome the risk of topical side-effects, the combination of calcipotriol with a potent GC has proven advantageous and patients treated with the combination experience fewer topical side-effects (Guenther et al. 2002). In addition, calcipotriol counteracts the GC-induced risk of skin atrophy (Norsgaard et al. 2014).

Topical calcipotriol in combination with betamethasone dipropionate is proven to be effective in psoriasis (Douglas et al. 2002, Guenther et al. 2002, Kaufmann et al. 2002, Kragballe et al. 2006, Papp et al. 2003, van der Velden et al. 2010). Calcipotriol has also been used in other skin diseases, e.g. acrodermatitis continua Hallopeau (Sotiriadis et al. 2007), nail psoriasis (Pasch 2016) and, combined with UV light therapy, in vitiligo (Erms et al. 2001, Whitton et al. 2008). There is only limited evidence of the efficacy of calcipotriol in skin diseases other than psoriasis, but there are anecdotal reports of its use in several other dermatoses, including lichen ruber planus and pityriasis rubra pilaris (Bayramgürler et al. 2002, Thiers 1997).
3  Aims of the present study

This work was performed to investigate the expression of glucocorticoid receptors GRα and GRβ in eczema atopicum, eczema nummular, lichen simplex chronicus, lichen ruber planus, bullous pemphigoid and psoriasis. All these inflammatory skin diseases are customarily treated with corticosteroids, topical or systemic, but systemic treatment is used only in severe forms of eczema atopicum and bullous pemphigoid. We investigated whether the expression of GRα and GRβ is associated with the responsiveness to systemic corticosteroid treatment in these two skin diseases. Psoriasis is rarely treated with GC monotherapy, so topical treatment with the common topical combination of calcipotriol and betamethasone was chosen to investigate the effect of GC therapy on GRα and GRβ in psoriatic patients. As psoriasis is considered to be a TNF-α–IL-23–IL-17-axis-mediated disease, the effect of topical treatment on TNF-α–IL-23–IL-17-emphasized inflammation was also studied. The specific aims of the study were to:

1. Investigate, whether GRα and GRβ are expressed in normal, healthy skin and in the skin of patients with inflammatory skin diseases.
2. Explore, how systemic GC therapy affects the expression of GRα and GRβ in patients with severe eczema atopicum and bullous pemphigoid.
3. Determine whether topical treatment with a combination of calcipotriol and betamethasone changes the TNF-α–IL-23–IL-17 mastered inflammation and the expression of GRα and GRβ in psoriatic patients.
4 Materials and methods

4.1 Patient selection, treatments and samples

The Ethical Committee of the Northern Ostrobothnia Hospital District approved the study and it was performed according to the Helsinki Declaration. Written consent for scientific purposes was obtained from all the study patients as well as control patients. The studied skin diseases and samples taken are listed in Table 4.

<table>
<thead>
<tr>
<th>Skin disease</th>
<th>PBMC samples</th>
<th>Skin samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT-qPCR</td>
<td>Western blot</td>
</tr>
<tr>
<td>Eczema atopicum (n=13)</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Eczema nummulare (n=9)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lichen simplex chronicus (n=11)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lichen ruber planus (n=10)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bullous pemphigoid (n=16)</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>Control group (n=17)</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>Psoriasis (n=10)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Control group (n=5)</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

4.1.1 Patients with severe eczema atopicum (I)

For the first study, 13 patients referred to Oulu University Hospital for severe atopic dermatitis (EASI score > 18, Fig. 7) were selected. All patients were noted to have a long disease history; all had had AD since early childhood. Patients receiving current topical therapies were included, and no washout period was required before entering the study. Patients receiving systemic therapies and/or phototherapy were excluded. Per oral prednisolone (Prednisolon®; Leiras Finland, Helsinki, Finland) was administered daily, at a starting dose of 0.5 mg/kg, which was gradually tapered over the two-week treatment period. Topical therapy was allowed during the study and patients used mild, moderate or potent GCs depending on the treated skin area.

Blood samples were taken three times: before entering the study and after 3 and 14 days of prednisolone treatment. Skin biopsies were taken from eczematous
skin from three patients. Patients’ EASI index was recorded at all three time points. Serum IgE, eosinophil count and body mass index (BMI) were measured at baseline. No control group was formed due to ethical reasons: prednisolone has well known side-effects and it would have been unethical to give a systemic GC to healthy volunteers.

![Image of severe eczema](image.jpg)

**Fig. 7. Forearm of a patient with severe eczema atopicum.**

**4.1.2 Patients with lichen ruber planus, eczema nummulare and lichen simplex chronicus (1)**

The National Supervisory Authority for Welfare and Health (Finland) approved the use of archival routine patient samples for research purposes.

We selected skin specimens from patients with lichen ruber planus (n=10), eczema nummulare (n=9) and lichen simplex chronicus (n=11) diagnosed and treated in the Department of Dermatology, Oulu University Hospital. The samples
were originally taken for clinical purposes. Histologic findings were analysed by a dermatopathologist. The clinical picture was compared to the histologic finding, and only cases where the diagnosis was confirmed both clinically and histologically, were included.

### 4.1.3 Patients with bullous pemphigoid (II)

Sixteen patients with severe bullous pemphigoid (recently started symptoms, generalized itch, widespread blistering, Fig. 8) were included in the second study. The study was initiated before the BPDAI was published, and therefore BPDAI score was not measured. The diagnosis of BP was based on a composite of typical clinical presentation of BP, with positive findings from direct immunofluorescence microscopy and serology investigations. For the direct immunofluorescence assay, a perilesional 3-4 mm skin punch biopsy was taken from uninvolved skin. Linear IgG and/or complement C3 deposit along the basement membrane zone was considered as a specific marker for BP. Diagnosis was further strengthened by the presence of circulating anti-BP180 antibodies detected by ELISA (HUSLAB, Helsinki, Finland). Lesional skin punch biopsies were taken from 11 patients.

Prednisolone treatment was initiated at a mean dosage of 0.49 mg/kg/day depending on the extent of the disease and the presence of comorbidities and continued for 60 days. Prednisolone dosage was gradually diminished if the response for treatment was considered to be good. Topical corticosteroid treatment with a moderate (hydrocortisone butyrate, n=2) or potent (betamethasone valerate, n=14) corticosteroid was applied to lesions only. All patients underwent a peripheral blood test before the treatment was started, and thereafter on days 5, 14 and 60 of the treatment period. Azathioprine (Azamun®; Takeda Oy, Helsinki, Finland) was started for three patients as an adjuvant therapy.

A control group of 17 elderly patients was formed. Controls underwent a peripheral blood test during their visit in the hospital. No skin biopsies or follow-up blood samples were taken from the controls.
4.1.4 Patients with mild or moderate psoriasis (III)

The third study was conducted on 10 psoriatic patients (Fig. 9). Patients were included if they had chronic mild or moderate plaque psoriasis (the mean PASI score before treatment was 9.1) and had not received any systemic or UV-treatment in the two months prior to entering the study. Five of the patients received therapy with a two-compound ointment (calcipotriol 50 µg/g and betamethasone dipropionate 0.5 mg/g [0.05%] Daivobet®; Leo Pharma, Vantaa, Finland) and five with betamethasone valerate 1 mg/g (0.1%) ointment monotherapy (Bemetson®; Orion Pharma, Espoo, Finland). The patients were advised to use topical treatment once a day after a shower. The tubes were weighed before and after the treatment period. Two 6 mm punch biopsies were taken before entering the study; one from lesional skin and one from uninvolved, healthy skin. Another 6 mm punch biopsy was taken after one week treatment period from a symmetrical contralateral plaque. Skin biopsies were cut in halves, one half was fixed in formalin for
immunohistochemical analyses, and the other was snap frozen in liquid nitrogen for RNA extraction. Blood samples were taken twice; before treatment and after one week of treatment. Each patients’ PASI score was recorded before and after the treatment. BMI was measured at baseline.

Fig. 9. Symmetrical distribution of psoriasis lesions on the lower extremities of a patient with moderate psoriasis.

Control subjects were healthy controls or patients who had benign skin tumors (verrucae, nevi); five control subjects were recruited. A 6 mm skin punch biopsy was obtained from controls’ healthy truncal skin. Blood samples were taken twice, a week apart. BMI was measured. Control patients received no therapy.

4.2 Isolation of peripheral blood mononuclear cells (I, II, III)

Lymphocytes (PBMCs) were isolated from heparinized blood samples (20 mL) using the Ficoll-Paque Density Gradient method (GE Healthcare Biosciences, Uppsala, Sweden) at room temperature. Heparinized blood was diluted in 20 mL phosphate buffered saline (PBS), divided into ten 4 mL samples and transferred into 10 mL tubes containing 3 mL of Ficoll-Paque Plus -reagent. The tubes were then centrifuged at 400 x g for 40 minutes, and the mononuclear cell layer was collected with a pipette. The collected cells were centrifuged twice with 30 mL PBS for 100 x g for 20 minutes to remove any platelets, Ficoll-Paque Plus and plasma.
After the isolation, the cells were deep-frozen (-70°C) until further use for quantitative polymerase chain reaction (PCR) and flow cytometric (FCM) analyses.

4.3 Reverse-transcriptase quantitative polymerase chain reaction (I, II, III)

Total mRNA was isolated from AD (I) and BP (II) PBMC samples with the Oligotex Direct mRNA Mini Kit (Qiagen, Crawley, UK) and reverse transcription was performed using M-MuLV reverse transcriptase (Fermentas, Helsinki, Finland).

The QIAamp RNA Blood Mini Kit (Qiagen) was used for total RNA isolation from psoriatic patients’ PBMC samples and the RNeasy Plus Universal Mini Kit (Qiagen) for skin samples (III). Reverse transcription was performed by using RevertAid reverse transcriptase (Thermo Fisher Scientific, Waltham, MA, USA).

Reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) was performed with the IQ5 Real-Time PCR Detection System and iQ™ SYBR® Green Supermix (both from Bio-Rad, Hercules, CA, USA) was used to quantify the transcripts. Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH; I-III) and human β-actin (II, III) served as reference genes. The samples were run as duplicates (II) or triplicates (I, III). Melt curve analysis was performed to ensure the amplification of a single product. Expression levels were assessed by the normalized expression method (ΔΔCt) according to the manufacturer’s instructions (Bio-Rad).

4.4 Immunohistochemistry (I, II, III) and image analysis (I, III)

Skin biopsies were fixed in formalin, embedded in paraffin and sectioned at 3.5 μm. Sections were deparaffinised in xylene and rehydrated in a graded series of alcohol baths. The Invitrogen Histostain® Plus Bulk kit (Invitrogen, Camarillo, CA, USA) was used for staining for GRα and GRβ, and antigen retrieval was performed using a microwave oven and a Citrate buffer (pH 6.0), 17 min for GRα and 12 min for GRβ. For CD3, CD4, CD8 and forkhead box P3 (FoxP3) staining, the Envision kit (K5007, Dako, Glostrup, Denmark) was used with antigen retrieval in a Tris-ethylenediaminetetraacetic acid (EDTA) buffer (pH 9.0) in a microwave oven for 17 min. For IL-17A staining, the Goat HRP-polymer kit (Biocare GHP 516L; Biocare Medical, Concord, CA, USA) was used with antigen retrieval in a Citrate buffer (pH 6.0) for 12 min in a microwave oven. Endogenous peroxidase activity was blocked with peroxidase blocking solution (S2023, Dako).
3,3’-diaminobenzidine (K5007, DAB; Dako) was used as the chromogen and Hematoxylin as the counterstain. PBS was used as a negative control replacing the primary antibodies. The primary antibodies used in immunostainings are listed in Table 5.

For analyses of keratinocyte staining with GRα and GRβ, the method described by Zlobec and co-workers was used (Zlobec et al. 2007). The estimation was done semi quantitatively in steps of 5%, and the average proportion of keratinocyte nuclei with positive immunoreaction was counted. To analyse the staining for CD3, CD4, CD8, FoxP3 and IL-17A, ImageJ (NIH, Bethesda, MA, USA), the Java-based open source image processing software was used. This cell counting method counts the average number of positively stained cells per 20× field image (0.14 mm²).

Table 5. Primary antibodies used for immunohistochemical staining of skin biopsies (I-III) (III, published by permission of Medicaljournals.se).

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Source/Target</th>
<th>Catalogue number and producer</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRα</td>
<td>polyclonal/rabbit, antihuman</td>
<td>sc-1002 (p20), Santa Cruz Biotechnology, Santa Cruz, CA, USA</td>
<td>1:500</td>
</tr>
<tr>
<td>GRβ</td>
<td>polyclonal/rabbit, antihuman</td>
<td>ab3581, Abcam Ltd., Cambridge, UK</td>
<td>1:1000</td>
</tr>
<tr>
<td>CD3</td>
<td>monoclonal/mouse, antihuman</td>
<td>NCL-CD3-PS1, Novocastra, Newcastle, UK</td>
<td>1:50</td>
</tr>
<tr>
<td>CD4</td>
<td>monoclonal/mouse, antihuman</td>
<td>NCL-CD4-368, Novocastra, Newcastle, UK</td>
<td>1:40</td>
</tr>
<tr>
<td>CD8</td>
<td>monoclonal/mouse, antihuman</td>
<td>NCL-CD8-4B11, Novocastra, Newcastle, UK</td>
<td>1:200</td>
</tr>
<tr>
<td>FoxP3</td>
<td>monoclonal/mouse, antihuman</td>
<td>ab20034, Abcam Ltd., Cambridge, UK</td>
<td>1:100</td>
</tr>
<tr>
<td>IL17A</td>
<td>polyclonal/goat, antihuman</td>
<td>AF-317-NA, R&amp;Dsystems, Minneapolis, MN, USA</td>
<td>1:100</td>
</tr>
</tbody>
</table>

4.5 Western blot (I, II)

A novel polyclonal GRβ-specific IgG antibody to detect GRβ was designed for Western blotting. The antibody was raised against amino acids 728-742 at the carboxyterminus of the human GRβ protein, therefore allowing detection of GRβ but not GRα. The Eurogentec antibody production service (Eurogentec, Seraing, Belgium) produced the antibody.
The PBMC extracts were lysed in Blue Sample Buffer (8 M urea, 1 M Tris pH 6.8, 2% sodium dodecyl sulphate [SDS], 5% glycerol, bromphenolblue), separated in 4-15 % gradient SDS-polyacrylamide gel electrophoresis (PAGE) (Bio-Rad) and transferred to the nitrocellulose membrane. Unspecific binding was blocked by 60 min incubation with 5% milk in Tween-Tris -buffered saline (TBS). The primary antibody for detecting both GRα and GRβ isoforms (sc-1003, Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used at 1:200 dilution and the novel GRβ specific antibody at 1:500 dilution. Peroxidase conjugated anti-rabbit IgG (A0545, Sigma- Aldrich, St. Louis, MO, USA) was used as a secondary antibody at 1:20 000 dilution. Immunoreactivity was visualized using an enhanced chemiluminescence technique (ECL Prime, GE Healthcare, Buckinghamshire, UK) as specified by the manufacturer. Equal protein levels were normalized using an antibody against GAPDH at 1:500 dilution (sc-24778, Santa Cruz). HaCaT cells (a spontaneously transformed immortal keratinocyte cell line from adult human skin) served as a positive control sample.

4.6 Flow cytometry (II, III)

After isolation, PBMCs were resuspended in Dulbecco’s Modified Eagle cell culture medium (DMEM) containing 10% dimethyl sulfoxide (DMSO) and the suspensions were transferred into cryotubes (300-1000 μl/tube). The tubes were placed in a -70°C freezer inside a freezing container to allow slow-freezing and higher cell viability.

BP patients’ samples were analysed using a 4-color flow cytometry panel with a FACSCalibur cytometer (Becton Dickinson, Mountain View, CA, USA) and calculated with FlowJo software version 7.6.5 (Treestar, Ashland, USA). Cells were counted and 0.5 x 10^7 PBMCs were fixed in 4% paraformaldehyde and permeabilized with 0.5% saponin. Then, in PBS buffer containing 0.5% bovine serum albumin (BSA) and 0.5% saponin, PBMCs were stained with anti-GRβ rabbit polyclonal Ab (Eurogentec) followed by phycoerythrin (PE)-conjugated anti-rabbit Ig goat polyclonal secondary Ab (ab97070, Abcam). Anti-CD3-peridinin chlorophyll protein complex (PerCP) (345766, BD), anti-CD4-allophycocyanin (APC) (555349, BD) and anti-GR mouse monoclonal fluorescein isothiocyanate (FITC) detecting GRα and GRβ isoforms (G3030-01M, USBiological, Salem, MA, USA) were used for antigen staining of the cells. 10 000 cells were acquired from the cytometer for further analyses. Corresponding isotype control antibodies were used as negative controls in all assays. Expression of
GR\(\alpha\)+\(\beta\) and GR\(\beta\) proteins was analysed in subgroups of CD4+/CD3+; CD4-/CD3+; CD4low/CD3- and CD4-/CD3- cells.

The memory CD4+ Th17/Th1 cells from psoriatic patients’ PBMCs were analysed using a 6-color flow cytometry panel with a LSRFortessa flow cytometer (BD) and FlowJo software (TreeStar). mAbs from BioLegend (San Diego, CA, USA) against the surface antigens CD4-PerCP (344624), CD45RA-FITC (304106), CXCR3-APC (353708), CCR6-brilliant violet 421 (BV421) (353408), CCR10-PE (341504), CLA-phycoerythrin-cyanin 7 (PECy7) (321316); and CD8-FITC (345772) from Becton Dickinson, were used. The protocol described by Ma and co-workers (Ma et al. 2015) was followed. To analyse the amount of Tregs, anti-CD4-PerCP (345770), anti-CD25-APC (555434) and anti-CD127-PE (557938) mAbs from Becton Dickinson were used for surface staining and the anti-FoxP3-Alexa Fluor 488 (AF488) (320112) mAb from BioLegend was used for intracellular staining. The FoxP3 / Transcription Factor Staining Buffer protocol was followed (eBioscience, San Diego, CA, USA).

4.7 Statistical analyses (I, II, III)

Statistical analyses were all performed using IBM SPSS Statistics 22.0 program (IBM, Armonk, NY, USA). Spearman’s rho was used to analyse correlations between variables to determine any covariance. In the first study, the Kruskal – Wallis test was used to analyse the association between GR isoform expression in keratinocytes and the biopsied lesion type. The Wilcoxon signed-rank test was used to compare the expression of GR isoforms before, during and after prednisolone treatment, and in the first study, the EASI scores and GR isoform levels. In the third study, the Wilcoxon signed-rank and Mann-Whitney tests were used to analyse the results of immunohistochemical staining and the paired sample t-test was used to compare RT-qPCR and FCM results before and after the treatment period. A two-tailed p value < 0.05 was considered statistically significant.
5 Results

5.1 The expression of GRα and GRβ mRNA in studied inflammatory skin diseases (I, II, III)

Glucocorticoid receptor isoform mRNA expression has been previously studied only in a few cutaneous diseases, and in vivo data is particularly sparse. We aimed to study whether GR isoforms are expressed in inflammatory dermatoses.

Using RT-qPCR we showed that both isoforms, GRα and GRβ, were indeed expressed in all PBMC samples from patients with AD and psoriasis. All psoriatic patients’ controls also expressed both GR isoforms. In BP, GRα was expressed in all 16 PBMC samples from BP patients, whereas GRβ was detected only in 13 samples. All 17 controls for BP patients expressed GRα, but only 12 expressed GRβ in their PBMCs.

In addition to PBMC samples, we analysed GRα and GRβ mRNA expression in skin samples from psoriatic patients and their controls. GRα was expressed in all samples: both normal and lesional samples from psoriatic patients as well as all control samples. GRβ was found in all psoriasis lesional samples, whereas one of the 10 psoriatic patients did not express GRβ in their normal skin. All controls expressed GRβ.

5.2 The expression of GRα and GRβ protein in studied inflammatory skin diseases (I, II, III)

To investigate the expression of glucocorticoid receptor isoforms at the protein level, we analysed skin and PBMC samples using immunohistochemical staining, Western blotting and flow cytometry. The immunohistochemical stainings showed that GRα and GRβ were detectable in all the skin samples from patients with in AD, eczema nummulare, LSC, LRP, BP and psoriasis. The nuclear staining varied between samples, so that nuclear positivity of keratinocytes for GRα was between 40% and 80% and for GRβ between 37.5% and 70%. The most prominent nuclear positivity for both GRα and GRβ was detected in LRP, whereas AD samples showed the least nuclear positivity. We did not calculate the nuclear positivity in keratinocytes of BP samples, since mostly only cytoplasmic staining for both isoforms was seen. As an interesting new finding, we demonstrated that granulocytes (mainly neutrophils and eosinophils) showed strong positive GRβ.
nuclear staining in AD, eczema nummulare, LSC, LRP and in some BP samples (Fig. 10). To the best of our knowledge, this is the first time that cutaneous granulocytes have been shown to be positive for GRβ.

**Fig. 10.** In skin biopsies stained with GRα (A) neutrophils (asterisk) were negative, whereas showing strong positive staining (asterisk) for GRβ antibody (B).

Western blot analysis was done for all 13 PBMC samples from AD patients and six samples from BP patients as well as five of their controls. We detected GRα in all AD and BP samples and nearly all control samples. In contrast, GRβ was found in the majority of AD patients (10 out of 13) and all control patients, but in only four out of six BP patients.

By FCM in five BP patients and their controls, we investigated whether GRα and GRβ were differently distributed into different subclasses of PBMCs. CD4+, CD4- and CD4+ non-T cells were analysed. GRα+β and GRβ protein were detected in all the samples and the results did not differ between patients and controls. The staining was similar between CD4+ and CD4- T cells, but CD4+ non-T cells (CD4low, CD3- cells; monocytes, macrophages, dendritic cells, NK cells) stained more for GRβ than T cells.

### 5.3 Systemic prednisolone therapy in eczema atopicum and bullous pemphigoid (I, II)

To investigate whether the level of GRβ could act as a potential indicator of glucocorticoid sensitivity during prednisolone treatment, the expression of GRα and GRβ mRNA was analysed by RT-qPCR from PBMC samples of patients with AD and BP.
In AD, the mean level GRα mRNA was down-regulated during 14 days of prednisolone treatment and the mean level of GRβ mRNA was up-regulated. Three of the 13 AD patients were considered to be GC insensitive since their EASI scores decreased by only <7%. In five patients, the EASI score decreased by between 17.4 and 37.4% and their clinical response was considered moderate. In the remaining patients the decrease in EASI score was over 46.8% and these patients were considered to have responded well to prednisolone therapy. However, due to inter-individual variation we could not detect a statistically significant connection between GR isoform levels and the response to prednisolone treatment.

In BP, the mRNA levels of both isoforms were markedly changed after only five days of prednisolone treatment, and during the 60 days treatment, the levels varied greatly without any clear pattern. Azathioprine had to be initiated as an adjuvant therapy to GCs in three patients with BP because these patients’ response to GCs was considered insufficient. However, since these three patients responded to a certain degree to GC therapy, they were not considered to be totally GC insensitive. Our aim was to find a correlation between the expression level of GRα and GRβ and the response to prednisolone treatment, but statistically significant correlations were not found.

To summarize, the GR isoform mRNA levels were altered by treatment with prednisolone both in AD and BP, but the level of GRβ mRNA was not connected to treatment response.

5.4 Topical calcipotriol/betamethasone dipropionate treatment in mild and moderate psoriasis (III)

Since the in vivo effects of topical use of calcipotriol/ betamethasone dipropionate ointment on Th17 cytokine expression in psoriasis have until now been published only by Hino and co-workers (Hino et al. 2011), we wanted to elucidate more this interesting and fashionable topic.

We compared the effects of topical calcipotriol/betamethasone combination and betamethasone monotherapy on the numbers of T cells and molecular markers by RT-qPCR, immunohistochemistry and FCM. The one week combination therapy down-regulated the mRNA expression of TNF-α, IL-23A, IL-17A, S100A7, CCL-20 and IFN-γ in lesional skin samples and the expression of IL-6, IL-23A, TNF-α, T-box expressed in T cells (T-bet) and IFN-γ in PBMCs. In contrast, the effect of one week’s betamethasone monotherapy on these inflammatory markers was lesser. The expression of FoxP3 as a Treg marker was down-regulated by the combination,
but not by betamethasone monotherapy, in both the skin and PBMC samples. Immunohistochemical analyses showed that the combination therapy reduced the number of CD4+, CD8+ T cells and Tregs in psoriatic lesions more than betamethasone monotherapy. The FCM analysis demonstrated that the combination decreased the numbers of circulating CD8+ T cells, Tregs, skin homing Th17 memory cells and Th22 memory cells, but betamethasone alone had little or no effect.

5.4.1 Topical treatment in psoriasis and the expression of GRα and GRβ (III)

Both treatments, calcipotriol/betamethasone ointment and betamethasone monotherapy, up-regulated the expression of GRα mRNA in the skin samples, monotherapy significantly so. In contrast, the expression of GRα in PBMCs decreased during both treatments. GRβ expression varied greatly and no constant changes or statistical correlations to Th17 related cytokines were observed. Both treatments had a tendency to decrease the levels of nuclear staining of keratinocytes for GRα and GRβ. Both treatments also resulted in clinical improvement of psoriatic lesions, but combination therapy was more efficient.
6 Discussion

6.1 GRα and GRβ are expressed in skin and peripheral blood mononuclear cells of patients with inflammatory skin diseases

The glucocorticoid receptor was discovered in healthy skin already before GRα and GRβ were separately described in 1985 (Epstein & Bonifas 1982, Leiferman et al. 1983). One can nowadays speculate that these first findings most probably represented GRα, which is generally expressed at higher levels than other isoforms (Oakley & Cidlowski 2013). The expression of GRα and GRβ has since been studied in only a few skin diseases (Hägg et al. 2010, Inui et al. 2010, Lin et al. 2014, Pang et al. 2015). In AD, poor response to topical GC therapy has been associated with increased expression of GRβ (Hägg et al. 2010), but in the small study by Inui and co-workers (Inui et al. 2010), no correlation was found between the expression of GRβ and response to GC treatment. Lin and co-workers showed that GRα is expressed in oral lichen planus lesions (Lin et al. 2014). In psoriasis, administration of topical GCs did not affect GRα expression in the study by Pang and co-workers (Pang et al. 2015). GRα mRNA as well as protein has been detected in healthy human skin (Lin et al. 2014, Pang et al. 2015), but no previously published study has examined the expression of GRβ in healthy skin. We aimed to broaden the findings of previous study groups and to investigate, whether GRα and GRβ are expressed in the skin and PBMCs of patients with AD, eczema nummulare, LSC, LRP, BP and psoriasis.

We showed that patients with AD and BP express GRα and GRβ mRNA and protein in their PBMCs. Patients with psoriasis were shown to express GRα and GRβ mRNA in their PBMCs. GRα and GRβ proteins were also found in the skin samples of AD, BP and psoriasis patients as well as those of patients with eczema nummulare, LSC and LRP. The results of the skin biopsies were confirmed with two laboratory methods (immunohistochemistry and RT-qPCR) in psoriatic patients only, but based on these and the findings of previously published studies, we are confident that these two isoforms are found in the skin of patients with all the studied inflammatory skin diseases. It remains interesting to note that the studied skin diseases differed in terms of the nuclear positivity of keratinocytes.

As a conclusion, these results confirm that GCs have a receptor located in the PBMCs and in the skin, supporting the use of glucocorticoid-based therapies in
inflammatory skin diseases. This result was not unexpected, given the common clinical response to GC treatment in inflammatory dermatoses.

6.2 The expression of GRα and GRβ does not predict clinical response to prednisolone treatment in patients with eczema atopicum and bullous pemphigoid

Previously, glucocorticoid treatment has been shown to affect the expression of GR isoforms in other inflammatory diseases, mainly non-cutaneous conditions. The up-regulation of GRβ has been linked to GC resistance in asthma (Christodouloupolous et al. 2000, Hamid et al. 1999, Leung et al. 1997, Sousa et al. 2000), nasal polyposis (Hamilos et al. 2001), rheumatoid arthritis (Kozaci et al. 2007) and colitis ulcerosa (Fujishima et al. 2009, Honda et al. 2000) in particular. Our hypothesis was that the expression of GRβ could also be used as a marker of glucocorticoid responsiveness in AD and BP patients treated with prednisolone. This hypothesis arose from our previously published work, in which elevated expression of GRβ was found in AD patients unresponsive to a topical GC (Hägg et al. 2010).

The patients in the first two studies of this thesis received prednisolone and their response to treatment was monitored clinically. All patients responded to a certain degree: EASI scores decreased in all AD patients and blistering was reduced or eliminated in all BP patients, although there was a clear variation between patients in response. Thus the treatment response of our study patients resembles the known variation in corticosteroid responsiveness described in other inflammatory diseases (Leung & Bloom 2003); e.g. in rheumatoid arthritis, up to 30% of patients are clinically GC resistant (Chikanza & Kozaci 2004). Unfortunately, we did not find a connection between GR isoform mRNA levels measured by RT-qPCR and the clinical response to GC treatment. The mRNA levels were obviously altered during GC treatment, but no connection was found to any of the studied parameters.

The role of GRβ as a potential inhibitor of corticosteroid responsiveness may be significant in some inflammatory diseases, but our current data do not support the idea of the high expression level of GRβ explaining corticosteroid resistance in dermatological diseases. The differences between our findings and those of studies in other diseases could be explained by the limited number of patients in our study or confounding factors, such as additional topical GC treatment. In the previous non-dermatological studies the number of patients varied between 10 and 38, which is at the similar level as 13 AD patients and 16 BP patients in our studies. However,
our first and second studies are not the only ones which do not support the significance of GRβ in corticosteroid resistance: Hausmann and co-workers demonstrated in study of 86 patients using only RT-qPCR, that GR isoforms levels do not predict corticosteroid responsiveness in inflammatory bowel disease (Hausmann et al. 2007).

The differences between the findings of our previous study on AD patients (Hägg et al. 2010) and those of the first study included in this thesis might be due to confounding factors in the latter study (e.g. topical GC therapy). Also, given the very low expression level of GRβ compared with GRα, the sensitivity of the laboratory methods could have affected the results.

In healthy individuals, responsiveness to GCs has been shown to be tissue-specific (Chriguer et al. 2005, Ebrecht et al. 2000). In our study, the results were based on analyses of PBMCs. The studies of other inflammatory diseases, representing GRβ as a marker of GC responsiveness, were also performed using only PBMC or biopsy samples. Only Hamid and co-workers used PBMCs as well as bronchoalveolar lavage samples (BAL) in their study (Hamid et al. 1999). This raises the question as to whether the results of studies that analyse only one tissue type (such as ours) can be generalized to represent the responsiveness in the whole individual.

6.3 Topical betamethasone treatment affects GRα and GRβ expression in patients with psoriasis

Until now, the only study to have analysed the expression of GR in psoriasis in vivo was that of Man and colleagues (Man et al. 2013). However, that study did not analyse the expression of GRα and GRβ separately. The third study included in this thesis had, in that aspect, an interesting setting. Our study revealed that GRα and GRβ are both expressed in lesional psoriatic skin. The two topical treatments, both containing betamethasone, increased the expression of GRα mRNA in the skin, whereas the number of GRα immunopositive keratinocytes and the GRα mRNA level in PBMCs were decreased. The expression of GRβ varied between individuals. Possibly due to this variation, no statistical correlations between the expression level of GRβ mRNA and Th17 related cytokines were observed. This observation differs from the results of the study by Vazquez-Tello and co-workers (Vazquez-Tello et al. 2013).

The nuclear immunopositivity of keratinocytes was decreased by both topical treatments. That GRα mRNA expression increased in the skin, but
immunopositivity of keratinocytes and mRNA expression in PBMCs decreased, appears to be a conflicting result. This may be explained by differences between the sample types in term of cell types present. The expression of GRα mRNA in the skin was analysed from the whole skin biopsy with all the cells present in the skin, whereas we measured immunopositivity only in keratinocytes. In addition PBMCs are purely lymphocytes.

Unfortunately these results did not increase our current understanding of the potential role of GRα or GRβ as a marker of glucocorticoid sensitivity in psoriasis. All patients responded to topical treatment, but the combination of calcipotriol and betamethasone was clinically more efficient. Since we had no treatment resistant patients, the effect of GRβ expression on the treatment response in psoriasis cannot be analysed. Nonetheless, we can conclude that betamethasone has an effect on the expression of GRα and GRβ in psoriatic skin.

6.4 Calcipotriol/betamethasone combination therapy is more beneficial in psoriasis than betamethasone monotherapy

Calcipotriol/betamethasone combination ointment has been shown to be more efficient than monotherapy with each of its components evaluated using markers of the skin immune system and epidermal proliferation (van der Velden et al. 2010). Since data of its effects on inflammatory pathways in psoriasis mainly come from in vitro studies (Hegyi et al. 2012, Lovato et al. 2016), we decided to compare the mechanism of action and clinical efficiency of this combination with that of betamethasone monotherapy in vivo in a real-life clinical setting.

Psoriasis is considered to be linked to the TNF-α–IL-23–IL-17-axis. Blocking of TNF-α, IL-23 or IL-17A has proven to be an excellent therapeutic option in psoriasis (Boehncke & Schön 2015, Nestle et al. 2009b). In our third study, both topical therapies decreased the expression of TNF-α in skin and PBMC samples. Our study was also the first to describe the effect of calcipotriol/betamethasone ointment on the expression of IL-23A in vivo, and the combination was more effective in decreasing its expression than monotherapy. Both topical therapies, more so the combination therapy, decreased the expression of IL-17A mRNA and the numbers of circulating skin-homing CLA+ Th17 cells. The other cytokines and cellular markers studied also supported the more advantageous effects of calcipotriol/betamethasone ointment, so we conclude that, after only one week therapy, the combination therapy has a more beneficial effect than betamethasone monotherapy on the TNF-α–IL-23–IL-17–immunological axis in psoriasis.
The results presented here should be confirmed with a longer study period and, of course, a larger and more stringently selected study population would be beneficial.

6.5 Methodological considerations and limitations of the study

6.5.1 Study population

An important difference between \textit{in vitro} and \textit{in vivo} studies is that \textit{in vitro} studies are usually easier to perform and analyses can be repeated several times, if needed. The recruitment of patients for \textit{in vivo} studies can be challenging and sometimes takes years. Furthermore, the results of \textit{in vivo} studies are usually unique and cannot always be repeated.

The first study continued for a long period of time, between 14\textsuperscript{th} of January 2008 and 15\textsuperscript{th} of May 2013, since it was challenging to find enough AD patients suitable for prednisolone treatment who matched our inclusion criteria. Samples for the analyses of eczema nummulare, LSC and LRP were chosen from archival specimens and as described earlier in the literature review, skin samples are not needed for the diagnosis of these dermatoses and are only occasionally taken. Thus the number of suitable biopsy samples was limited.

Every patient referred to Oulu University Hospital between 15\textsuperscript{th} of September 2010 and 17\textsuperscript{th} of April 2013 for suspected severe BP was invited to take part in the second study. The long study period reflects the rarity of BP in the area of Northern Ostrobothnia and the low incidence of new, severe cases during the study period.

In the third study, the patient recruitment and sampling was done between 18\textsuperscript{th} of March and 3\textsuperscript{rd} of July 2013. We planned this as a preliminary study and our intention is to broaden the study later.

All these reasons limited our available material. Firstly, the low number of samples limited the statistical analyses. Had a larger cohort of AD and BP samples been available, more GC treatment resistant patients may have been found and maybe even two subgroups of responders and non-responders could have been formed. With a larger psoriatic cohort, statistically significant differences between the two topical therapies would perhaps have been found in contrast to the marked, but not significant differences that we detected. However, even with the small numbers of patients we were able to show that GR\(\alpha\) and GR\(\beta\) are expressed in inflammatory dermatoses.
6.5.2 Confounding factors

In the first study, the patients were allowed to continue topical GC treatment during the therapy with prednisolone. This might have had a noteworthy impact on the GR isoforms’ expression, since topical GCs are systemically absorbed, especially when extensive areas of skin are treated (Aalto-Korte & Turpeinen 1995). The same confounding use of topical therapies might also have affected the results of the second study. We were, however, on a quest to find a marker of glucocorticoid responsiveness that could be used in everyday clinical practice, so these confounding factors were accepted.

In the second study, the patients had other systemic diseases and were treated with other medications, any of which might have changed their response to glucocorticoid treatment. This is a problem related to most clinical studies in elderly patients, but due to the nature of BP, a younger cohort cannot be formed.

Because of the confounding factors, the results in the first two studies may not be repeatable.

6.6 Future prospects

In the three studies included in this thesis, we aimed to elucidate the complex interaction of glucocorticoids and their cellular receptors GRα and GRβ, but were only able to scratch the surface of the subject. The responsiveness to GC treatment may not be predictable using a single measurable marker. This observation is supported by the fact that individual responsiveness varies within healthy patients as well as patients with inflammatory diseases, but also within different cell types (Quax et al. 2013).

Since the exact targets and mechanisms of action of GC therapy still remain unresolved, more studies on this subject are needed. In the era of biological therapies, most patients are still treated with conventional therapies and bear the potentially life-threatening side-effects. Glucocorticoids, as the mainstays of therapy for most dermatological conditions, should, by any means possible, be kept in the spotlight of modern dermatological research.
7 Conclusions

Topical and systemic glucocorticoid based therapies are the mainstay treatment of most skin diseases. However, potential side effects limit their use. If a poor response could be predicted, the patient could be treated with something else as first-line therapy, thus sparing them the risk of GC side-effects for little potential benefit. The effects of GCs are mediated through GR isoforms, and some previous, mostly non-dermatological studies, support the idea that GRβ could be a clinical marker of corticosteroid insensitivity. We aimed to test this hypothesis in skin diseases in a real-life setting. As knowledge of the immunopathogenesis of psoriasis expands, we investigated whether the commonly used topical therapies, betamethasone or calcipotriol/betamethasone, affected the general immunological markers in psoriasis.

Based on our results the following conclusion can be made:

1. GRα and GRβ are expressed in the skin of patients with eczema atopicum, eczema nummulare, lichen simplex chronicus, lichen ruber planus, bullous pemphigoid and psoriasis. This affirms the use of topical GC therapy in these diseases.

2. Both GRα and GRβ mRNA transcripts are produced in PBMCs of patients with severe AD and BP; GRα to a greater extent. This supports the use of per oral GC treatment, since a systemic response is needed.

3. Systemic GC therapy affects the expression levels of GR isoforms in AD and BP, but the degree of the effect varies widely between patients. This means that measuring the amounts of GRα or GRβ mRNA cannot be used to predict the response to corticosteroid treatment.

4. In psoriatic patients, calcipotriol together with betamethasone dipropionate is more efficient in suppressing the inflammatory TNF-α–IL-23–IL-17–axis than betamethasone valerate monotherapy. These in vivo data advocate the use of combination treatment as the first line therapy in mild and moderate psoriasis.
References


Bjerring P (1993) Comparison of the bioactivity of mometasone furoate 0.1% fatty cream, betamethasone dipropionate 0.05% cream and betamethasone valerate 0.1% cream in humans. Inhibition of UV-B-induced inflammation monitored by laser Doppler blood flowmetry. Skin Pharmacol 6(3): 187-192.


Original publications


Reprinted with permission from John Libbey Eurotext (I) and Medicaljournals.se (II, III).

Original publications are not included in the electronic version of the dissertation.
1376. Koivukangas, Jenni (2016) Brain white matter structure, body mass index and physical activity in individuals at risk for psychosis : The Northern Finland Birth Cohort 1986 Study

1377. Väyrynen, Sara (2016) Histological and molecular features of serrated colorectal adenocarcinoma and its precursor lesions


1380. Pinola, Pekka (2016) Hyperandrogenism, menstrual irregularities and polycystic ovary syndrome : impact on female reproductive and metabolic health from early adulthood until menopause


1383. Raatiniemi, Lasse (2016) Major trauma in Northern Finland


1386. Lappalainen, Olli-Pekka (2016) Healing of cranial critical sized defects with grafts, stem cells, growth factors and bio-materials

1387. Ronkainen, Justiina (2016) Role of Fto in the gene and microRNA expression of mouse adipose tissues in response to high-fat diet

1388. Ronkainen, Eveliina (2016) Early risk factors influencing lung function in schoolchildren born preterm in the era of new bronchopulmonary dysplasia

1389. Casula, Victor (2016) Quantitative magnetic resonance imaging methods for evaluation of articular cartilage in knee osteoarthritis : free-precession and rotating-frame relaxation studies at 3 Tesla

1390. Alahuhta, Ilkka (2016) The microenvironment is essential for OTSCC progression

1391. Hagnäs, Maria (2016) Health behavior of young adult men and the association with body composition and physical fitness during military service

Book orders:
Granum: Virtual book store
http://granum.uta.fi/granum/
Minna Kubin

GLUCOCORTICOID RECEPTORS IN INFLAMMATORY SKIN DISEASES

THE EFFECT OF SYSTEMIC AND TOPICAL GLUCOCORTICOID TREATMENT ON THE EXPRESSION OF GRα AND GRβ