Ingrid Asp Psoriasis Research Center

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A generous donation to Linköping University made possible the establishment of the Ingrid Asp Foundation for Psoriasis Research to strengthen, broaden and deepen research into psoriasis. As a result, the Ingrid Asp Psoriasis Research Center was inaugurated in spring 2012. Since then, we have built a strong research platform devoted to psoriasis research. The research group includes Cecilia Bivik Eding and Deepti Verma (PhD, principal research engineers) and Gunna Sigurdardóttir and Ines Köhler (MD, dermatologists, PhD students). At the Division of Dermatology, we have a well-equipped laboratory for cell culture, biomedical analyses and molecular biology. The Medical Faculty at Linköping University provides core facilities, including advanced equipment and technical support for flow cytometry, molecular biology and microscopy. Animal studies are performed at the Center for Biomedical Research core facility.

Specific aims

Psoriasis is an immunologically-mediated inflammatory disease with strong genetic susceptibility. It is a painful, disabling condition that affects 2–3% of the population of the Western world. The disease is characterized by distinctive skin eruptions showing intense hyperproliferation and disturbed maturation of the epidermal cells and an inflammatory dermal infiltrate, a combination of changes that is reminiscent of those seen in cancer. However, one important distinction is that the epithelial proliferation in psoriasis remains under very strict control. While much progress has been made in our understanding of the pathogenesis of psoriasis, many of the fundamental cellular activities that are crucial for the development of psoriasis are poorly understood. In fact, the role of keratinocytes (KCs) in the formation of the psoriatic lesion has received very limited attention. Furthermore, the genetic predisposition in psoriasis is well described, but there is an urgent need to clarify the biological consequences of the disease-associated genetic variants in order to better understand the pathophysiological mechanisms that drive the disease.

Our specific aims are as follows:
Dermal lesion: To explore the equilibrium between proliferation, differentiation and apoptosis in the formation of psoriatic plaque and to evaluate experimentally whether increased apoptosis by MTH1 inhibitors may hamper this process.

The genetic and epigenetic predisposition: To investigate the mechanism by which genetic variants in susceptibility genes lead to psoriasis and to investigate whether epigenetic alterations contribute to the heredity and disease susceptibility in psoriasis.

Research areas

Studies of the dermal lesion in psoriasis

We have previously focused our interest on psoriasin (S100A7), a calcium-binding protein of the S100 family, which was originally identified as being highly expressed in psoriatic KCs (1). Our previous results suggest possible mechanisms for psoriasin to contribute to the development of the psoriatic lesion. Psoriasin was shown to be induced by reactive oxygen species (ROS) and its expression correlates with increased survival and NF-κB signalling in epithelial cells (2–4).

Moreover, we observed that the expression of psoriasin leads to an increase in the expression of reactive oxygen species (ROS)-induced angiogenic factors and neovascularization *in vitro* and in mice. Psoriasin was further shown to induce the proliferation, migration and tube formation of dermal-derived endothelial cells (HMVEC-d) and to promote the release of pro-angiogenic mediators (3, 4).

A role for psoriasin in KC differentiation was supported by the inhibited KC differentiation following psoriasin downregulation. Psoriasin was shown to correlate with the degree of KC differentiation *in vivo* and, using tetracycline (TET)-driven conditional psoriasin expression, we recently found a change in the regulation of differentiation genes and an expression pattern reminiscent of that in psoriatic epidermis (5).

Cellular proliferation and apoptosis maintain tissue homeostasis, whereas dysregulated apoptosis contributes to numerous pathological conditions. In normal skin, KCs in the superficial layer of the epidermis undergo apoptosis in equilibrium with the proliferation of cells in the basal layer. The disturbed termi-

nal differentiation in the psoriatic epidermis, which results in the retention of granulated and cornified cells, may be a consequence of massive proliferation that does not allow sufficient time for the proper differentiation of the cells. We hypothesized that dysregulated cell death contributes to the thickened epidermal plaques in psoriasis. Using cultured KCs obtained from skin punch biopsies, we have demonstrated higher viability and resistance to ultraviolet (UV)-B apoptosis in the KCs derived from both involved and uninvolved skin, compared with KCs from healthy controls. We positioned the dysregulation upstream of cytochrome c release in the mitochondrial pathway and observed a change in the expression of apoptosis-related genes using microarray transcriptome analysis (6).

In addition, the proliferating KC sub-population, which is twice as large compared with normal skin, is still poorly defined. In one of our current projects, we aim to define the level of cellular differentiation in proliferating psoriatic KCs. We apply multicolour flow cytometry on psoriatic epidermal KCs and control KCs. In recent preliminary data we describe an overall more immature phenotype of psoriasis KCs compared with normal KCs, with higher expression levels of the stem cell-associated markers p63, CD44 and CD29. We are currently addressing the mechanism and consequences of this decreased cellular differentiation.

In collaboration with Professor Helleday's team at the Karolinska Institute, we evaluated the potential use of MTH1 inhibitors in the treatment of psoriasis. To counteract the damaging effects of increased ROS production, cells upregulate defence mechanisms including MTH1, which detoxifies oxidized nucleotides. MTH1 was identified as a potential cancer therapeutic target (7) and several potent small molecule inhibitors have been developed. Interestingly, we found an increase in MTH1 expression in involved psoriatic skin compared with normal skin, suggesting a potential beneficial effect of MTH inhibition.

Studies of the genetic and epigenetic predisposition in psoriasis

Genome-wide association studies (GWAS) and more targeted candidate gene approaches have identified more than 80 single nucleotide polymorphisms (SNPs) associated with psoriasis. Many of them are situated near genes that are involved in adaptive and innate immune pathways and, more specifically, the IL-23/Th17 axis. The work conducted by our collaborator, Professor J. T. Elder at the University of Michigan, whom I joined for a sabbatical period in 2013–2014, has directed many of these genetic studies. This collaboration has led to several publications, such as the recent international ExomeChip genotyping experiment of more than 6,000 cases and controls (8).

Our present project plan includes studies of selected, predicted causal variants in the coding sequence that will help us to understand the biological and mechanistic connections between each locus and disease pathogenesis, disease onset and severity. This could also lead to the identification of new drug targets. We previously collected a large psoriasis patient sample, including 1,988 individuals from 491 families, in collaboration with the Swedish Psoriasis Association. Using this sample, we analysed how components of the inflammasomes (NLRP3, NLRP1 and CARD8) contribute to psoriasis susceptibility (9,10).

We have ongoing studies on tyrosine kinase 2 (*TYK2*) that transmits signals from activated cytokine receptors. In an in-depth analysis of the *TYK2* genomic region using GWAS and resequencing data, we found a strong genetic association between three non-synonymous variants in the exonic regions of the *TYK2* gene. We found that individuals who carried the protective I684S variant had significantly reduced p-STAT4 levels in CD4+CD25+CD45RO+ and CD8+CD25+CD45RO+ cells compared with controls homozygous for the ancestral haplotype (Fig. 2). Similar reductions in p-STAT4 were also observed in skin-homing, cutaneous lymphocyte-associated antigen (CLA)-positive CD4 and CD8 cells from I684S carriers.

These data establish the functional significance for the TYK2 I684S variant in psoriasis susceptibility (11) DNA methylation is the most widely studied epigenetic mechanisms regulating gene transcription. We have used reduced representation bisulphite sequencing (RRBS) to determine the methylome and the associated gene expression pattern in involved (PP) and uninvolved (PN) psoriatic epidermis. Through the RRBS assay, we were able to compare the methylation status of ~2–3

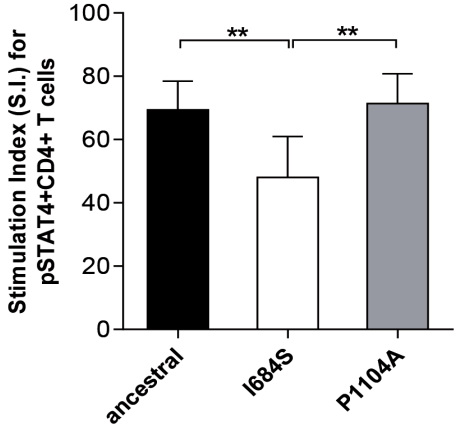

Fig. 1. Flow cytometry data describing IL-12-induced pSTAT4 signaling in CD4+CD25+CD45RO+ cells in different TYK2 haplotypes. $n = 5–9$ in each group.

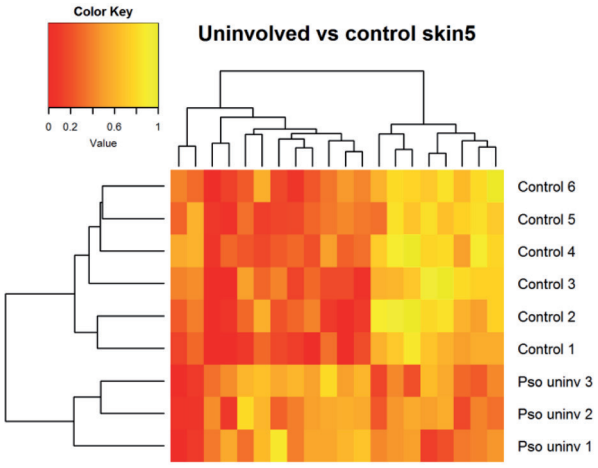

Fig. 2. Unsupervised hierarchical clustering of methylation ratios shows a distinct separation of the uninvolved and healthy epidermis. yellow: hypermethylation, red: hypo methylation.

million CpG sites in the epidermal DNA. Our findings suggest the overall hypermethylation of the involved and uninvolved psoriatic epidermis compared with the healthy epidermis and reveal a large number of sites whose methylation pattern differs between the separate conditions. In particular, we have observed the differential methylation pattern in several psoriasis risk-associated loci, IL23R, TRAF3IP2 and TNFAIP3. Interestingly, we detect a specific methylation pattern in uninvolved skin compared with normal skin (12).

Studies of the cardiovascular comorbidites in psoriasis

These studies are part of Gunna Sigurdardottir's thesis. She will defend her thesis in 2018.

We have screened a panel of cardiovascular and inflammatory markers for their utility as biomarkers. Using Luminex technology, we have analysed a large number of markers related to the metabolic syndrome and endothelial dysfunction in psoriasis patients and body mass index (BMI)-matched controls. Among them, thrombomodulin and *plasminogen activator inhibitor-1* (PAI-1) were found to be significantly altered, which we analyse further in independent samples. The levels of some of these markers were effectively diminished by treatment with a tumour necrosis factor alpha (TNF-α) inhibitor, but they were not reduced by the commonly used UV therapy. Patients with psoriasis and cardiovascular risk may therefore benefit from systemic treatment rather than UV therapy (13, 14).

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