Functional genomics of psoriasis

Alicia Lledo Lara
Hertford College
University of Oxford

A thesis submitted in partial
fulfilment of the requirements for the degree of
Doctor of Philosophy
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Abstract

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This is my abstract...

Acknowledgements

Thank you, thank you, thank you.

Declarations

I declare that unless otherwise stated, all work presented in this thesis is my own. Several aspects of each project relied upon collaboration where part of the work was conducted by others.

Submitted Abstracts

Title **Year**
Authors

Associated Publications

Title
Journal
Authors

Other Publications

Title
Journal
Authors

Contents

List of Figures

List of Tables

Abbreviations

Abbreviation	Definition
Ab	Antibody
ATAC-seq	
ChIPm	
DMARDs	disease-modifying antirheumatic drugs
Fast-ATAC	
NSAID	nonsteroidal antiinflammatory drug
Omni-ATAC	
PI	Protein inhibitor
PsA	
PSO	
QC	
qPCR	quantitative polymerase chain reaction
RA	Rheumatoid arthritis
SDS	Sodium dodecyl sulfate
SF	Synovial fluid

Chapter 1

Introduction

1.1 Psoriasis and psoriatic arthritis

Psoriasis (PSO) and psoriatic arthritis (PsA) are considered different common complex disease entities. PSO is a chronic inflammatory skin disease with episodes of relapse and remitance included in the group of inflammatory dermatose diseases (Nestle et al. 2009). On the other hand, PsA is a seronegative, chronic, inflammatory disease within the family of spondyloarthritis (Moll et al. 1973; Coates et al. 2016), usually developed after the manifestation of PSO (**Villanova2016**). PS and PsA have both, similar and different, clinical features, which are likely a reflection of shared and individual genetic loci contributing to the disease development (Variants in RUNX3 contribute to susceptibility to PsA, exhibiting further common ground with ankylosing spondylitis, PsA Immunochip). It is important to understand the commonalities and differences between PSO and PsA at the physiological and genetic level in order to better understand the relevance of the genetic variability in the risk to develop these conditions.

1.1.1 Epidemiology and global impact

PSO represents a serious global problem that currently affects about 100 million people worldwide, including children and adults regardless sex (Organization 2016). Although PSO prevalence presents a very weak correlation

with geographic latitude (Jacobson et al. 2011), it has been reported to vary upon ethnicity. For example, PSO prevalence in adults is comparatively lower among African, African American and Asian (0.4-0.7%) compared to American and Canadian (4.6 and 4.7%, respectively) populations. In the UK, PSO prevalence's estimate ranges between 2-3% and it affects approximately 1.8 million people (Perera et al. 2012).

PsA in the general population ranges between 0.04-1.2% (Perera et al. 2012) but prevalence dramatically increases to 10-30% within PSO cases (**Reich2008**; Gelfand et al. 2005), showing evidence of association between the two diseases. Particularly, in the UK, 14% of the PSO patients develop chronic inflammatory arthritis in the form of PsA at some point of the disease course (Ibrahim et al. 2009). Overall, data suggests an steady increase in both, PSO and PsA, prevalence over time (**Springate2007**; Organization 2016).

Although PSO can be developed at any age, onset of disease seems to have a bimodal distribution strongly influenced by the Human Leukocyte Antigen (HLA) Cw*06 (HLA-Cw6), which encodes an allele for one of the genes in the Major Histocompatibility Complex (MHC), involved in antigen presentation and immune cells regulation (Henseler and Christophers 1985). It is also the strongest genetic association with PSO and PsA risk identified in GWAS studies (()Ellinghaus2010, Strange2010, Stuart2010; Sun2010). The'early-onset' or Type I is characterised by development of disease around 16-22 and 30-39 years and a greater prevalence for HLA-C*06:02 (85.4% of the cases). In contrasts, the late-onset or Type II group manifests disease between 50-60 years old and presents positive HLA-C*06:02 only in 14.6% of the cases. This classification based on the age of onset has also distinctive features from a clinical point of view, as they have different severity, relapse frequency and family history.

PSO and PsA also represent a burden for the economy of the countries due to treatment and associated morbidity. For example, in the UK treatment and

management of PSO in 2015 ranged between 4,000 to 14,000, before and after requirements of biological therapy, respectively (Burgos-Pol and Dermo 2016). Regarding PsA, yearly National Health Service (NHS) cost ranges from 11 to 20,782 with a mean of 1,446 per person and significant variation depending on the disease severity (Poole et al. 2010).

1.1.2 PSO and inflammatory dermatoses

The International Classification of Disease 10 (IDC-10) includes more than 1,000 skin or skin-related diseases with heterogeneous prevalence. As a group, it is one of the most prevalent condition that can affect up to 70% of the population, regardless age and geographic location (**ICD-10**). An study in 2010 concluded that skin disease causes a huge burden in the global context of health, being the 4th leading cause of nonfatal burden (**Roderick2014**). The skin is the biggest organ in the human body and it constitutes an effective barrier between the environment and the internal organs. The most external layer, the epidermis, plays a relevant role in the innate and adaptive immunity (Proksch et al. 2008). Therefore, alterations of this barrier due to exogenous (e.g fungus, bacteria) or endogenous (e.g genetic) factors can lead to development of inflammatory dermatose conditions such as PSO, atopic dermatitis (AD), seborrheic dermatitis and cutaneous lupus erythematosus (CLE) (**Johnson-Huang**; **2009**).

The characteristics of the epidermal lesion allows a further classification of the inflammatory dermatoses into non-pustular, pustular and vesiculo-bullous. Due to the great diversity in the type, location and severity the psoriatic lesions can belong to the non-pustular and pustular groups, which demonstrates the heterogeneity of the disease (Perera et al. 2012). As a result, several clinical phenotypes of PSO including vulgaris, guttate, pustular, erythroderma and nail pitting have been defined and it is under debate whether some of those should be considered a different disease entity (**Marrakchi2011**).

1.1.3 PsA and spondyloarthropaties

PsA belong to the family known as spondylarthropaties (SpA) which also includes other subtypes such as ankylosing spondylitis (AS), reactive arthritis (ReA), idiopathic inflammatory bowel disease (IBD) and undifferentiated SpA (). All SpA subtypes are characterised by structural damage (bone formation and erosion) as well as inflammation of joints and extraarticular sites, such as eyes, gut and skin. Additional SpA criteria have led to a reduced classification of SpA into axial and peripheral SpA based on the affected joint (spine/sacroilicac or peripheral) and the presence of extraarticular features (**Runwaleit2001**; **Runwaleit2001**). Familial studies with HLA-B27 positive status have shown manifestation of different SpA forms, such as PSO and IBD, within a single family (**Said-Nahal2000**). Consistently, a transgenic rat model overexpressing the human HLAB27 allele also presented several features of SpA simultaneously (**Hammer1990**). All these observations partially support the hypothesis that SpA subtypes may be a single multifaceted condition with share genetic, immunophatological and structural features, with dynamic phenotypes determined by ubiquitous genetic or environmental factors (**Baeten2013**). Nevertheless, validation of this theory remains challenging due to lack of complete understanding of the cellular and molecular processes that explain SpA pathophysiology. For example, some studies suggest that multiple genetic factors may be involved in the determination of the axial and peripheral arthritis (**Porcher2005**) and partially explain the immunopathological differences (**Appel2011**; **Noordenbos2012**).

As a phenotype, PsA can be further subdivided in five clinical groups based on Moll and Wright criteria, : distal, destructive, symmetric, asymmetric and spinal (Moll et al. 1973). These subclasses mainly differed upon the location, number and distribution of the affected joints. Later studies have questioned this method of classification due overlapping of the different subsets and lack of

inclusion of dactylitis (diffuse swelling of a digit), a typical feature of PsA absent in RA (Reich et al. 2009). This phenotypic heterogeneity increases the difficulty in the design and achievement of meaningful outcomes from clinical studies.

1.2 Pathophysiology of psoriasis and psoriatic arthritis

1.2.1 Clinical presentation and diagnosis

The most common form of PSO (approximately 90% of all cases) is plaque PSO vulgaris that manifests with raising well demarcated plaques with, erythema and scaling. The epidermis undergoes an increase in thickness (acanthosis) and vascularisation that causes pinpoint bleeding when the scales are broken (Perera et al. 2012). The plaques vary in thickness and size and tend to be symmetrically distributed, mostly on the elbows, knees, scalp, lumbosacral region, palms and soles (Griffiths and Lancet 2007). A particularly relevant variant of PSO vulgaris is the inverse or flexural, in which the plaques are thinner,non-scaly and shiny and they are located armpits and genitals (Sampogna et al. 2012). The second most common type (10%), PSO guttate, is characterised by acute onset of small droplike papules usually distributed in the trunk and the proximal extremities (Vence et al. 2015). Type I PSO commonly appears in the form of guttate lesions after bacterial infection whilst type II involves spontaneous chronic plaques (Perera et al. 2012). In terms of severity, pustular PSO is the main one potentially life threatening (**Moura2015**).

In PsA the most common manifestation is the symmetric/polyarticular (more than 50%) followed by the asymmetric/oligoarticular (around 30%) PsA, that affects single or few distal interphalangeal (DIP),proximal interphalangeal (PIP) and metatarsal phalangeal (MP) joints, predominantly (**McGonagle2011**; Reich et al. 2009). Clinical or sub-clinical inflammation of the connective tissue

between tendon or ligament and bone (enthesitis) is also found in 35% of the PsA patients (**McGonagle2011**; **Polachek2017**). Axial involvement, mainly spinal, is also present in a quarter of the PsA patients and it increases the risk to develop extrarticular features (**Jadon2016**).

As previously mentioned, PsA prevalence is greater in patients with diagnosed PSO, particularly type II (**Gu"dh *j"'–o˝nsson2002**). The psoriatic lesions precede joint inflammation in approximately 60-70% of the cases(**Gladman2005**; **McGonagle**; **2011**). Particularly, prevalence of nail lesions is 2.6-fold greater in PSO patients that later develop PsA than in those with uncomplicated PSO (**Moll1976**; Griffiths and Lancet 2007). Similar observations have been made for scalp and intergluteal regions lesions, which together with nail affection constitute a predictive biomarker for development of joint inflammation (**McGonagle**; **2011**). This reinforces the need of appropriate coordination between dermatologists and rheumatologists for an early diagnostic and treatment that could prevent functional joint disability.

Several comorbidities have been associated with PSO and PsA, with comparatively greater prevalence in PsA. For example, intraocular inflammation known as uveitis affects 8% of PsA patients compared to 2% of the PSO ones (**Husted2011**; **Oliveira2015**). Other classic and emerging comorbidities include Inflammatory Bowel Disease (IBD), cardiovascular disease , myocardial infarction (Gelfand et al. 2006),angina and hypertension (Gladman et al. 2009), type II diabetes (T2D) (**Saphiro2007**), metabolic syndrome (**Cohrn20017**) and cancer (Gelfand et al. 2006). PSO and PsA have also important implication in the mental health of the patients and they are associated with an increased prevalence of depression and suicidal ideation (Sampogna et al. 2012).

The diagnosis of PSO and PsA is mainly based in clinical assessment since there is a lack of appropriate biomarkers at early stages of disease (Villanova et al. 2013). PSO skin lesions can be evaluated using the the Psoriasis Area and Severity

Index (PASI). This test quantifies lesional burden weighted by body part based on the amount of affected body surface area and the degree of severity of erythema, induration and scale (**Fredriksson1978**) (Table 1.1). To evaluate PsA, analysis of performance of the previously mentioned Moll and Wright criteria together with additional ones such as the Vasey and Espinoza, Gladman et al. and McGonagle led to the confirguration of the Classification Criteria for Psoriatic Arthritis (CASPAR), the most widely used. It requires the patient displaying inflammatory arthritis, enthesitis, and/or spondylitis and 3 points from a list of associated elements (Table **??**) (**Taylor2006**). Another composite measure commonly used to evaluate treatment efficacy for PsA is the PsA Response Criteria (PSARC) based on the number of tender joints (TJC) and swollen joints (SJC) over 68 and 66, respectively, as well as a physician global assessment based on a short questionnaire (**Philipp2011**; **Clegg1996**)

Feature	Scoring scale
Body location	Head and neck, upper limbs, trunk and lower limbs
Intensity	Redness, thickness and scaling
Severity	Absent, mild, moderate, severe or very severe
Affected area	Nil (0), 1-9%, 10-29%, 30-49%, 50-69%, 70-89% or 90-100%

Table 1.1:

A patient must have inflammatory articular disease (joint, spine, or enthesial) with 3 points from 5 categories	
PSO	a. Current skin or scalp disease b. History of PSO c. Family history of PSO
Psoriatic nail involvement	Typical psoriatic nail distrophy
A negative test for RF	Using preferrably by enzyme-linked immunosorbent assay (EMSA)
Dactylitis	a. Swelling of an entire finger b. History of dactylitis
Radiologic evidence of juxtaarticular new bone formation	Ossification near joint margins

Table 1.2: Name and full sequence of the PCR primers used for amplification, indexing and pooling of the ATAC-seq and ChIPm samples in this thesis. These primers were designed by Buenrostro *et al.*, 2013 and they are an modified version of the Nextera Illumina primers optimised for larger molecular weight DNA fragments from low input samples. All samples were indexed with the universal primer Ad1.noMx and one of the additional 18 primers. The indexing sequence of each of the primers is in blue text.

1.2.2 Aetiology of PSO and PsA

PSO and PsA are both complex chronic inflammatory diseases where genetically predisposed individuals after exposure to a particular environmental trigger undergo a dysregulated immune response that initiates disease (Figure1.1). One of the greater controversies has been characterising the origin of the pathologies as well as the connection between skin and joint inflammation. Particularly, for PSO it remains unclear whether disruption of the skin triggers activation of the immune response or viceversa.

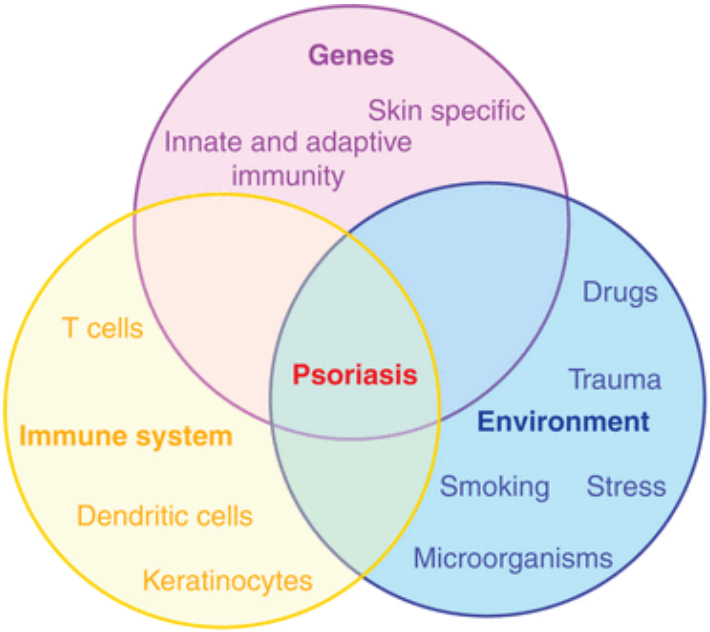

Figure 1.1: Figure adapted from (Meglio2014)

Dysregulation of the innate and adaptive immune response

The dysregulation of the immune response in PSO and PSA is the result of the interaction between cells of the innate and immune system (Section xx) as well as a complex cytokine milieu involving several feedback loops that perpetuate the response.

KC play a very important role in the disease initiation through production of different proinflammatory cytokines (section xx) that mediate activation of the innate immune response. These activated KC induce the release of IFN-α and IFN-γ by circulating plasmacytoid DC (pDC) and myeloid DC (mDC), respectively. They represent two of the most relevant cytokines in PSO and PsA pathogenesis and they contribute to lymphocyte recruitment and maintenance of DC activation (ref) and appear to be particularly upregulated at the mRNA level in the lesional skin (**Schmid1994**). Another cytokine produced by mDC is interleukin-12 (IL-12). The increase of downstream targets including Th1 infiltration and IFN-γ in the skin lesion and inflamed synovium also suggest a role of this mDC cytokine in the innate component of PSO and PsA pathogenesis.

Another key cytokine in this dysregulated inflammatory response is TNF-α, very relevant in bone turnover and bone remodeling in PsA (**Mensah2008**). It is produced by activated KC, mast cells but also by adaptive immune cells types, including Th1 and Th17 cells infiltrated in the psoriatic lesion and PsA inflamed joints (red) and it induces activation of Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-B) signaling pathways (ref). It also activates several kinase signaling pathways as well as cell death programs (ref). In the context of inflammation, NF-B represents a master transcriptional regulator of both, the innate and adaptive immune system that induces expression of proinflammatory cytokines, antiapoptotic genes and genes involved in chronic inflammation maintenance (ref). The importance of this transcription factor (TF) in PSO and PsA pathogenesis is reflected by the association with disease of

several genetic variants in some of the negative regulators of its proinflammatory activity, including Nuclear Factor Of Kappa Light Polypeptide Gene Enhancer In B-Cells Inhibitor Alpha *NFKBIA* and TNF Receptor-Associated Factor 3 Interacting protein 2 *TRAF3IP2* (ref).

Interleukin-23 (IL-23) and Th17 axis represents a key loop for the maintenance of PSO and PsA inflammatory response and a very important link between innate and adaptive immunity. IL-23 is an innate regulatory cytokine, mainly produced by mDC and macrophages homing the inflamed skin and it binds to the IL-23 receptor (IL-23R), which expression is upregulated in the DC and T cells of the lesion and in circulating Th cells (ref). In PSO, IL-23 is the mediator for the pathogenic loop between activated KC and T cells (ref). Both IL-23 and IL-23R present protective and pathogenic genetic variants associated with PSO and PsA risk (ref). The activation of the IL-23 pathway leads importantly to increased IL-17 production through NF-activation by *TRAF3IP2* (ref). IL-17 favors maintenance of the adaptive immune mediated Th17 response through recruitment and activation of neutrophils, induction of proinflammatory cytokines including IL-1β and $IL-6$ and $also$ $perpetuation$ of KC $activation$ (ref) $(https$: $//www.ncbi.nlm.nih.gov/pmc/articles/PMC3580541/).$ $More$ $recently,$ $interleukin-22 (IL-22)$ has $arisen$ as $another$ of the key $cytokines$ in $mediating$ the $dysregulated$ $crosstalk$ $between$ 22 $levels$ are $increased$ in the $psoriatic$ $lesions$ and $serum$ of $patients$ and it is $mainly$ $produced$ by a sub cells known as Th22 (ref). It mediates some of the histological changes in skin as well as AMP production by KC (ref).

Histopathological alterations in skin and joints

As previously mentioned, the epidermis is the most external structure of the skin and it is formed approximately by 90% by keratinocytes (KC) organised in a layer structure that self-renews in a time dependent manner from the

bottom to the surface (Wikramanayake et al. 2014). As the KC differentiate they undergo changes in their shape, replication ability and keratin composition of their intracellular matrix. The KC of the most superficial layer of the epidermis (stratum corneum) are the terminally differentiated form of these cells and they are surrounded by a protein and lipid rich layer known as cornified envelop Proskch2008, Wikramanayake2014. In the context of PSO, dysregulation of the epidermis cell renewal leads to alterations of its normal histological features and development of the psoriatic lesions. KC undergo upregulation in the proliferation rate (hyperplasia) causes aberrant cell differentiation, phenomena known as parakeratosis (ref). It also leads to thickening of the epidermis (acantosis) and the subsequent scale formation (ref). Concomitantly, inflammation causes immune cell infiltration and hypervascularisation of the lesion driven by upregulation in the expression of angiogenic factors such Vascular Endothelial Growth Factor (*VEGF*) and activation of the endothelium (Perera et al. 2012).

In PsA joint affection usually follows skin lesions and it involves a wide range of histological changes in the joints driven the connection between the immune and skeletal systems, particularly bone remodeling (ref). One of the most common structural changes is caused by the swelling and inflammation of the joints which define the arthritic component (ref). As result of this inflammation, alterations in bone remodeling leads to osteolysis with subsequent bone resorption and erosion at the affected joints (**Mensah2017**). This phenomenon is particularly relevant in arthritis mutilans or chronic absortive arthritis, one of the most severe forms of PsA (**Haddad2013**). Bone erosion is also the main histopathological process driving dactylatis, where bone lysis resolves in shortening of the digits (**Gladman2005**). On the other hand, 35% of the PsA patients undergo enthesitis, where the inflammation occurs at the insertion of tendons, ligaments or joint capsule fibers (**Polachek2017**). Overtime,

it causes structural changes due to formation of bony spurs along the insertion sites(**Schett2011**).

Environmental factors and disease

There are several environmental triggers known to be associated with increased risk and worsening of PSO and PsA development. A wide range of drugs including antidepressant, antihypertensive and anticytokine therapies have been clinically associated with initiation, exacerbation and worsening of PSO. As examples, therapies such as IFN-$\alpha for the treatment of hepatitis C virus and anti-TNF antibodies for the treatment of several chronic inflammatory diseases, including PSO$(**Kim2

Preceding streptococcal throat infection has likewise been associated with development of type I PSO lesions only (**Gudjonsson2003**). This is partially explained by the molecular mimicry found for several streptococcal antigens, such as the M protein (highly similar to the structure of certain human keratins) and bacterial peptidoglycans (**Valdimarsson2009**). Also, there is evidence of homologous T cell clones homing tonsils and psoriatic lesions (**Diluvio2006**). Recent studies looking at the microbiome and disease development have also suggested perturbation in the composition of the gut and skin microbiota of PSO and PsA patients compared to the controls (**Yan2017**). Consistently with other chronic inflammatory disease such as IBD and AS, these differences in the microbiome could lead to dysregulation of inflammatory pathways in skin and joints through immunomodulation (**Eppinga2014**).

Physical trauma, including tattoos and surgical incisions, has also been associated with the appearance of psoriatic lesions in uninvolved skin as well as joint inflammation in digits (Nestle et al. 2009) due to initiation of the Koebner phenomenon (**Weiss2002**). Lastly, there are several behavioral factors such as smoking, alcohol and stress with suggested with PSO and PsA. However, there are not consistent conclusions of their involvement in triggering disease (**Meglio2014**).

1.2.3 Cell types involved in PSO and PsA pathogenesis

There is great debate about the most relevant cell types contributing to PSO and PsA pathogenesis. Progressively, both are understood as dynamic and continuous processes where different cell types became predominantly important at different stages of the pathology. Regarding levels of circulating immune cells, severe PSO patients (PASI¿12) showed significantly decreased numbers of PBMC compared to moderate (PASI¡12) and healthy individuals (**Langewouters2008**). ; KC are one of the most relevant cell type at early stages of PSO pathogenesis. Several studies have shown the role of KC as immune sentinels through antigen presentation and production of antimicrobial peptides (AMP), cytokines and chemokines. There is evidence of complex formation between the cationic AMP cathelicidin or LL-37 and self-DNA/RNA released by the damaged KC in the psoriatic skin upon trigger by environmental factors (Lande et al. 2007). It leads to initiation of the skin inflammation through activation of skin-resident DC (**Nestle2005**) and secretion of pro-inflammatory cytokines, importantly IL-1, IL-6 and TNF-α, that reinforce activation of DC and Th lymphocytes (**Feldmeyer2007**; **Arend2008**; Nestle et al. 2009). Moreover, expression of the MHC-II allows KC to act as APC and activate memory $CD4^+$ and $CD8^+$ T cells inducing a recall immune response (**Black2007**). Studies in mouse models have shown development of psoriatic lesions in immunodeficient mice upon human xenotransplant of PSO skin only (Boyman et al. 2004). The importance of KC in PSO development is also reinforced by the association with PSO risk of genes of the late cornified envelope (LCE) family (Tsoi et al. 2012). Overall, these findings would support the hypothesis attributing the initiation of the chronic inflammatory response in PSO as the consequence of the epidermis dysfunction Proskch2008.

mDC and pDC have also been considered important innate immune cells in disease initiation (**Mahil20016**). They are professional APC that induce T

cell activation and the subsequent adaptive immune response. The relevance of antigen presentation in disease has been highlighted at the cellular (**Rusell1972**; **Tiilikainen1980**) and also at the genetic level with the PSO and PsA GWAS association of HLA-Cw*06:02 and ERAP1 (Strange et al. 2010), which encodes for an aminopeptidase involved in the trimming of peptide antigens. Although pDC are circulating cells absent in healthy skin, they infiltrate into the lesional and uninvolved dermis of PSO lesions (**Nestle2005**) and get activated by the aforementionedd self-DNA and LL-37 complex through Toll-like Receptor (TLR)-9 (Lande et al. 2007). In contrast, quiescent mDC are epidermal resident and upon secretion of IFN-α by pDC a 30-fold increase of mature mDC is observed in lesional skin but not in uninvolved or healthy tissue (ref). Different mDC subpopulation mediate the Th1 and Th17 response as well perpetuation of KC activation through IL-23 production (ref). Studies in immunodeficient PSO mice models have shown that blockage of downstream IFN-α signaling or its production by pDC failed to induce T cell activation and onset of PSO (**Nestle2005**).

Neutrophils are also though to be closely involved in disease initiation through their ability to form neutrophil extracellular traps (NET)that contain host DNA and AMP, particularly LL-37 (**Hu2016**). There is evidence of increased NET formation in peripheral blood and lesional skin of PSO patients and they seem to be contributing to pDC and $CD4^+$ T activation (**Hu2016**). Neutrophils have also been identified in recent studies as one of the main sources of IL-17 production in the skin lesions (**Lin2011**) and they also release a wide range of proteases which some induce KC proliferation (**Mahil2006**).

In the context of the innate immunity, the involvement of monocytes and macrophages in PSO and PsA has not been extensively studied. Resident macrophages in the healthy dermis undergo a 3-fold increase in lesional skin and they are involved in disease development through TNFα production

(**Mahil2016**; Perera et al. 2012). Different mice models for chronic psoriasiform skin inflammation have shown a key role of macrophage migration into the affected skin and TNF-α production for maintenance of the lesions (**Stratis2006**; **Wang2006**). Some studies using PSO and PsA patients derived monocytes have also highlighted the systemic aspects of both pathologies. PSO PBMC isolated monocytes have shown greater phagocytic and bactericidal activity compare to those from healthy individuals (**Bar-Eli1979**). Later studies have also shown increased circulating intermediate monocytes ($CD14^+$ high $CD16^+$ high) and monocyte aggregation in PSO patients with subsequent enhanced platelet activation and angiogenesis (**Golden2015**).

Historically, T lymphocytes have been considered one of the most relevant cell types in initiation and maintenance of PSO and PsA, and GWAS association with MHC-I also supports the role of T cells in disease. Report cases in humans have demonstrated that bone marrow transplantation can initiate or terminate PSO and, therefore, the role of bone marrow-derived T cells in disease pathophysiology (**Gardembas1990**; Eedy et al. 1990). The percentage of circulating T cells in PSO has been reported to be dependent of severity. Different studies have shown reduced number of T cells in moderate-to-severe and severe PSO patients when compared to milder phenotypes and healthy controls. Despite this reduction, increased percentage of the memory populations $CD4^+CD45RO^+$ and $CD8^+CD45RO^+$ have been demonstrated in the same individuals(**Lecewicz-Toru2001**; **Langewouters2008**). There is still controversy regarding the total $CD4^+$ and $CD8^+$ abundance and $CD4^+/CD8^+$ ratios in PBMC, which may be due to the phenotype heterogeneity of PSO patients in the different studies (**Lecewicz-Toru2001**; **Cameron2003**; **Langewouters2008**). In PsA, no differences abundance of circulatings T cells have been identified compared to healthy individuals (**Costello1999**).

In healthy skin, $CD4^+$ and $CD8^+$ are found in the dermis and epidermis, respectively (Clark et al. 2006; Perera et al. 2012) and upon lesion development an increase in activated memory $CD4^+CD45RO^+$and $CD8^+CD45RO^+$ in the respective compartments can be detected as soon as 3 days after its appearance (Clark et al. 2006), highlighting the importance of the memory population. *In vivo* studies conducted in mice by Boyman and colleagues showed that development of PSO following engrafted human pre-lesional skin was dependent of local T cell proliferation and it did not required injection of additional factors (**Boyle2013**). This supports the theory where recruitment of circulating T cells is restricted to the priming event and it is minimal afterward (Perera et al. 2012). The relative importance of $CD4^+$versus $CD8^+$ in PSO initiation has been tested immunodeficient mice with pre-lesional skin xenografts followed with injection of purified activated T cell populations (Nickoloff and Wrone-Smith 1999). These observations suggested a model where $CD4^+$ but not $CD8^+$ T cells where required for the progression of uninvolved to lesional skin in mice. Interestingly, injection of $CD4^+$ activated cells was followed by an increase in activated resident $CD8^+$ T cells expressing the acute activation marker CD69. It suggest a hypothesis where in the skin $CD4^+$ drive signaling for activation of resident T cells and the activated $CD8^+$ resident population are the main effector cells. In PsA, $CD4^+$ are significantly more abundant than $CD8^+$ cells in synovial tissues (**Diani2015**). In contrast, $CD8^+$ expressing CD45RO are prevalent in the SF and they are also significantly increased when compared to controls (**Costello1999**).

In addition to memory T cells, the contribution of regulatory T (Treg) cells have also been investigated to some extent due their role in immunosurveillance and self-tolerance in the context of autoimmune disease. Nevertheless, controversial results have been found regarding relative abundance and impaired function (Perera et al. 2012).

Based on the cytokine profile, PSO and PsA has been demonstrated to be a type 1 Th/Tc disease, where naive $CD4^+$ and $CD8^+$ cells get activated and proliferate in the presence of IL-12 and IFN-γ (**Austin1999**; Perera et al. 2012). Later studies also identified additional subsets including Th-17/Tc-17 and Th-22/Tc-22, which are mainly dependent on IL-23 and IL-6 for their activation, respectively (**Mahil2016**). The importance Th17 cells and their IL-17 production has been assessed in skin, joints and blood, where increased IL-17 and also IL-23 mRNA and protein levels have been found in PSO and PsA patients compared to controls (**Cai2012**; **reference for joints**). It has been shown that the predominant $CD8^+$ cells in the SF are also IL-17 producers and their abundance correlates with markers of inflammation and structural changes in the joint (**Menon2014**). This finding distinguishes PsA from other forms of arthritis such as RA and is in line with findings on skin that suggest a prominent role of $CD8^+$ IL-17 producing cells in the different stages of both pathologies. There is also evidence of the synergistic interaction between Th1 and Th17 cells which overall enhances the production of AMPs by KC (**Kryczek2008**). Understanding of the importance of IL-17 has also led to the discovery of other immune cells producing this pivotal cytokine including innate immune lymphoid (ILC) cells and $\gamma\delta$ T cells which have also started to been investigated in the context of PSO and PsA pathophysiology and treatment (**Meglio2014**; **Leijten2015**). IL-17 producing cell have also been hypothesised to be responsible for the link between skin and joint lesions. Although the precise mechanisms for transition between PSO and PsA is unknown, studies using PSO and RA mice models have shown that skin lesions facilitate arthritis and joint inflammation It has been hypothesised that the presence of IL-17 producer cells in inflamed skin located nearby the enthesis of joint under physical stress could trigger the development of PsA.

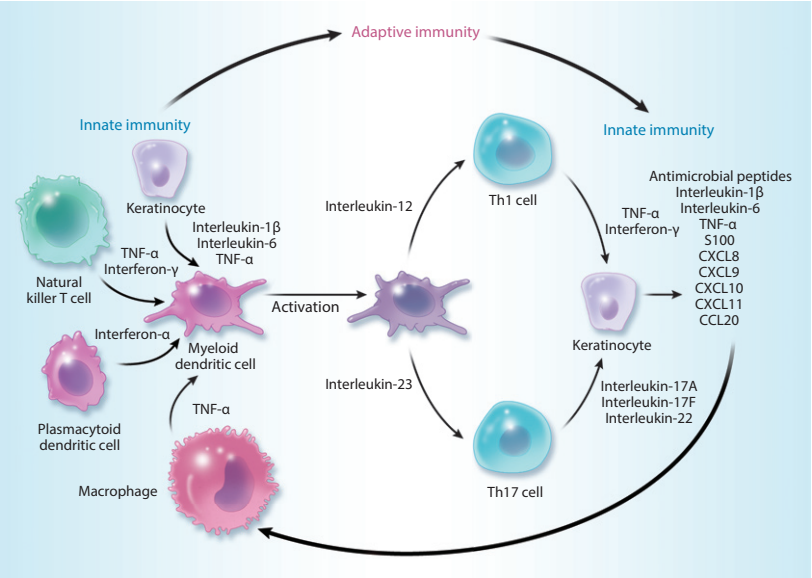

Figure 1.2: Figure adapted from (Nestle et al. 2009)

1.2.4 Therapeutic intervention and prognosis

Currently, there is no cure for either PSO or PsA and the different treatments available are focused in managing the disease manifestations and symptoms. The approach to treat them are usually dependent on the disease severity. Cases of mild-to-moderate PSO are usually managed with topical and systemic therapies (**Menter2009**). Among the topical agents, corticosteroids and emollients are the most commonly used and affordable ones. Emollients are non-medicated agents that contribute to keep the skin soft and moist minimising the symptoms of itching and tenderness and they have been accepted as an adjunctive for the treatment of PSO. On the other hand, cortocosteroid in creams, shampoo and spray remain as the most widely spread medical topical treatment due to their antiinflammatory, antiproliferative, and immunosuppressive actions. Corticosteroids are classified based on the potency and they all exert their effect through binding to intracellular corticosteroid receptors and regulation of gene transcription (**Tadicherla2009**). Although they are the most common way to treat mild forms of disease, some of them are approved only for short term treatments as side effects have been reported at a local and systemic level (**Menter2009**). In the case of PsA presenting swelling of two or less joints intraarticular injection of glucocorticosteroids together with joint aspiration have shown to reduce pain and inflammation as a short-time measure (Coates et al. 2016).

For PSO, other topical treatments are used in combination with topical corticosteroids including ultra violet (UV) light therapy and vitamin D analogues, which inhibit KC proliferation, stimulate KC differentiation and inhibits T cell proliferation and other inflammatory mediators (**Rizova2001**). Topical retinoids and calcineurin inhibitors are also used less frequently for the treatment of PSO and they have shown reduced systemic absorption and better suitability for long term treatments in some studies (**Weinstein2003**; **Menter2009**).

Treatment of most forms of PsA and more moderate-to-severe PSO require the use of a broad range of systemic therapies. For mild cases of PsA with involvement of less than four joints and non radiological evidence of structural changes, nonsteroidal antiinflammatory drug (NSAID) are the most commonly used to help controlling the mild inflammatory symptoms and also helping to alleviate the pain and stiffness (Coates et al. 2016). However, the use of NSAID is not recommended in patients presenting CVD comorbidities due to the increased risk associated with the use of these drugs (**Bhala2013**). For those NSAID-resistant or more severe forms of PsA, disease-modifying antirheumatic drugs (DMARDs) including the an antagonist of folic acid methotrexate (MTX) and the phosphodiesterase 4 inhibitor apremilast have shown to immunosupressive effects on activated T cells and reduction of cytokine production, respectively (**Schmitt2014**; **Gossec2016**; **Keating2017**; **Polachek2017**). However, increased risk of hepatotoxicity for the MTX Menter2009 and gastrointestinal side effect of apremilast Menter2009 require requires ensuring appropriate dosing and surveillance.

The use of biologic systemic agents tend to be the most specific but also expensive treatment for severe cases of PSO and PsA and they are not used as the first-line of treatment. These are molecular species generated in cell-based that modulate the immune response in a physiological based manner (Perera et al. 2012). Among the biologic agents targeting cytokines, TNFα inhibitors (TNFi) have been broadly used for the past five decades to treat both, PSO and PsA since this is a pivotal cytokine in initiation and perpetuation of the inflammatory response. There are three TNFi approved for the treatment of PSO etanercept, infliximab and adalimumab (**Ahil2016**) and another two, certolizumab pegol and golimumab, also used in the management of PsA and other rehumatoid diseases (**Coates2016b**). All the TNFi are antibody-based agents but etanercep, that is a soluble receptor, and they also show differences in the frequency and via of

administration as well as the efficacy, particularly in the specific improvement of skin or joint lesions (**Mease2000**). Although TNF-α blockade is one of the most effective treatments some patients experience common side effects such as increased risk of infection, reactivation of latent infections, demyelinating disease and induced pustular PSO have been identified (Nickoloff and Nestle 2004). Between 20 to 50% of the patients fail to respond to the first TFNi and require switching to a second or third one (**Abramson2016**). Lately, new biologic therapies have been developed to target other key cytokines in the pathogenesis of PsA and PSO, such as IL-12, IL-23 (ustekinumab) or IL-17 (secukinumab and ixekizumab) (**Mahil2016**). These new biologics represent a substantial benefit for treating patients and they are routenely administered to individuals failing to respond after a switch to a second TNFi (**Coates2016b**).

Chapter 2

Material and Methods

2.1 Ethical approval and recruitment of study participants

Sample recruitment for the two different phenotypes and the healthy volunteers were conducted under different ethics.

2.1.1 PSO patient recruitment

Patient blood samples and normal or psoriatic skin biopsies were collected in collaboration with the Dermatology Department research nurses at the Churchill Hospital, Oxford University Hospitals NHS Trust and Professor Graham Ogg at the Weatherall Institute of Molecular Medicine, University of Oxford under approval from the Oxfordshire Research Ethics Committee (REC 09/H0606/71 and 08/H0604/129). After written informed consent, up to 60 mL of blood from eligible PSO patients were collected into 10 mL anticoagulant EDTA-containing blood tubes (Vacutainer System, Becton Dickson).

PSO patients were eligible for recruitment when meeting the following criteria:

- over 18 years old
- previously or newly diagnose, in a flare and going into biologic therapy for the first time

- fulfillment of the clinically accepted Psoriasis Area and Severity Index (PASI) classification for PSO diagnosis (**Fredriksson1978**)
- moderate to severe disease (PASI¿5)
- less than 2 weeks without antibiotics unless used for prophylaxis
- available clinical information and written consent

Detailed clinical information of the PSO cohort is included in (Chapter **??**)(Table **??**).

2.1.2 PsA patient recruitment

Sample recruitment was performed as part of the Immune Function in Inflammatory Arthritis (IFIA) study established in 2013 (REC/06/Q1606/139)in collaboration with research nurses at the Nuffield Orthopaedic Centre, Oxford University Hospitals NHS Trust and Dr Hussein Al-Mossawi at the Botnar Research Centre. Following informed written consent, blood (30 mL) and synovial fluid aspirate (variable upon disease severity) were recruited into 10 mL anticoagulant sodium heparin coated tubes (Vacutainer System, Becton Dickson).

Eligibility of the PsA patients was upon fulfillment of the following criteria:

- over 18 years old
- previously or newly diagnose, with concomitant PSO, in a flare and going into biologic therapy for the first time
- fulfillment of the clinically accepted PsA Response Criteria (PSARC) including a physician global assessment questionnaire (**Philipp2011**; **Clegg1996**)
- oligoarticular phenotype and naïve for any treatment
- less than 2 weeks without antibiotics unless used for prophylaxis

- available clinical information and written consent

Further details about the cohort and clinical information can be found in (Chapter **??**)(Table **??**).

2.1.3 Healthy volunteer recruitment

Recruitment of healthy volunteers was conducted as part of the study Genetic Diversity and Gene Expression in White Blood Cells with approval from the Oxford Research Ethics Committee (REC 06/Q1605/55). Up to 80 mL of blood were collected into 10 mL anticoagulant EDTA-containing blood tubes, similarly to the PSO sample recruitment.

The criteria for healthy individuals to participate in the study was:

- over 18 years old and preferably British or European
- no family history of PSO, PsA, RA or SpA
- matched sex and age with the PSO cohort
- less than 2 weeks since last infectious process
- available clinical information and written consent

2.2 Sample processing

Blood, synovial fluid and skin biopsies were processed straight after recruitment following the appropriate protocols.

2.2.1 PBMC and synovial fluid cells isolation

PBMC were isolated from blood samples through density gradient separation using Ficoll-Paque. Total synovial fluid (SF) cells (SFC) were isolated by centrifugation at 500g for 5 min. Both were washed twice in Hanks balanced

salt solution without calcium or magnesium (Thermo Fisher Scientific) and resuspended in phosphate saline buffer (PBS, Gibco) supplemented with 0.5% fetal calf serum (FCS, Invitrogen) and 2mM ethylenediaminetetraacetic acid (EDTA, Sigma)prior to cell types separation. Cell numbers and viability were determined by manual count using a haemocytometer and trypan blue (Sigma).

2.2.2 Primary cell isolation using magnetic-activated cell sorting

For the work related to PSO and healthy volunteers, primary cell subpopulations were separated using magnetic-activated cell sorting (MACS, Miltenyi). Positive selection was performed for consecutive isolation of $CD19^+$ B cells, $CD8^+$ T cells, $CD14^+$ monocytes and $CD4^+$ T cells with AutoMACS Pro (Miltenyi) and cells were manually counted as previously described. MACS separation was chosen over Fluorescence-associated cell sorting (FACS) due to time and logistic constrains in the sample processing and therefore cell numbers in down stream application may not be as exact.

2.2.3 Primary cell isolation using fluorescence-activated cell sorting

Primary cell subpopulations from controls to study the effect of cryopreservation in chromatin states (Chapter 3) or PsA blood and SF samples were isolated by FACS. PBMC and SFC were resuspended in PBS 1mM EDTA (FACS buffer) at $10x10^6$ cells/mL, stained with the appropriate antibody cocktail (Table 2.1) for 30 min at 4°C, washed with FACS buffer and centrifuged at 500g for 5 min at 4°C. For the samples used in Chapter 3, a modified FACS buffer supplemented with 3 mM EDTA , 2% FCS and 25mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Invitrogen) was used

to avoid cell clumping after cryopreservation and short recovery.After removing the supernatant, cells were resuspended in FACS buffer prior to separation.

In the controls samples of Chapter 3 only $CD14^+$ monocytes and $CD3^+$ $CD14^-$ $CD4^+$ T cells were isolated in the SONY SH800 cell sorter. For the PsA samples, separation of $CD19^+$ B cells, memory T cells ($CD3^+$ $CD14^-$ $CD4^-$ $CD45RA^-$ and $CD3^+$ $CD14^-$ $CD8^+$ $CD45RA^-$) ,$CD14^-$ monocytes and $CD56^-$ NK was performed using FACS Aria (BD) cell sorter from both PBMC and SFC. Bulk sorted cells were collected in 1.5mL tubes in PBS 1% FCS, whilst single cell and small bulk sorting was performed in 96-well plates in the appropriate buffer (See RNA-seq section). Different nozzle sizes were chosen for bulk and single-cell sorting and OneComp eBeads (eBioscience) were used for compensation of fluorescence spill over.

Surface marker	Fluorochrome PsA/CTL	Manufacturer PsA/CTL	Clone PsA/CTL	Dilution PsA/CTL
Viability	eFluor780	-	eBioscience	1:250
CD3	FITC/AF700	SK7/UCHT1	BioLegend	xxx/1:50
CD4	APC	RPA-T4/RPA-T4	BioLegend	1:50/1:50
CD8a	PE	RPA-T8	BioLegend	xxx
CD45RA	BV421	HI100	BioLegend	xxx
CD19	PerCP-Cy5.5	SJ25C1	BioLegend	xxx
CD14	Pe-Cy7/FITC	M5E2/TUK4	BioLegend/Miltenyi	xxx/1:100
CD56	BV510	NCAM16.2	BD	xxx

Table 2.1: Details regarding target molecule, fluorochrome, clone, supplier and dilution used for PBMC and SFC staining are provided for each of the antibodies in the panel. In controls only CD3, CD4 and CD4 markers were used.

2.2.4 Skin biopsies processing and adherent assay

KC enrichment from skin biopsies was performed as described in Gutowska-Owsiak and colleagues (GutowskaOwsiak and Schaupp 2012). Skin biopsies (approximately 4mm) were washed with PBS, cut in 1mm width strips and incubated in 2U/mL of dispase II (Sigma) overnight at 4°C. The epidermis

was separated from the dermis and either snap-frozen in liquid nitrogen (for RNA extraction) or further digested in trypsin (Invitrogen) at 37°C for 5 min, when used for chromatin accessibility assay. After digestion the resulting cell suspension was filtered through a 70µm nylon strainer (BD) and washed with PBS. In some instances cells were manually counted and aliquoted for ATAC-seq processing. In others, cell from each of the biopsies were resuspended in KGM-2 BulletKit (Lonza) supplemented with 0.06mM Ca^2+ and cultured in a collagen IV coated 96-well plate at 4°C for 10 min or 3 hours, upon experimental requirements (see Chapter X). After culturing, cells were washed twice with 200µL of PBS and kept at 37°C for downstream processing.

2.3 Experimental protocols

2.3.1 Cryopreservation and cell culture

For the controls samples in Chapter 3, 40-50x10^6 of PBMC were freeze-thawing using a modified version of the (Kent 2009) protocol, where cells were pre-conditioned in RPMI 1640 (brand) complete medium supplemented with2 mM L-glutamine, 100U penicillin and strep 100µg/mL and 50% FCS for 30 minutes and afterwards diluted 1 in 2 in complete RPMI 1640 (supplemented as previously described) with 20% dimethyl sulfoxide (DMSO, Sigma). PBMC underwent slow cryopreservation at -80°CC in isopropanol at -1°C per minute and stored for a minimum of two weeks in liquid nitrogen. PBMC were thaw, resuspended in supplemented complete RPMI 1640 with 10% FCS at a density of 10^6 cells/mL and rested for 30 min at 37C, 5% CO2 in 25mL non-adherent polypropylene cell culture flasks followed by filtering through a 40µm to obtain an homogenous cell suspension undergoing FACS separation. Frozen Normal human epidermal keratinocytes (NHEK) in passage 3 were recovered and cultured at a cell density of 5x10^6 cells/mL in a 75 mL adherent cell

culture flask (brand) in EpiLife basal medium (Gibco) following manufacturer's instructions. After recovery NHEK were trypsinised at room temperature for 8 minutes followed by trypsin inactivation with EpiLife 10% FCS, centrifugation at 180g for 10 min at room temperature and manual counting with trypan blue. NHEK were seeded in a 96-well plate in 100uL of medium at a cell density of 160 cells/µL. NHEK were cultured for 2 days to a 90-100% confluence before being used downstream.

2.3.2 ATAC-seq, Fast-ATAC and Omni-ATAC

Improved versions of the ATAC-seq protocol were progressively used in the thesis for assessment of chromatin accessibility in different primary cells, including $CD14^+$ monocytes, $CD4^+$ and $CD8^+$ T cells, $CD19^+$ B cells and $CD56^+$ NK cells. The subsequent version aimed to reduce the amount of mitochondrial DNA and improve the ratio of signal to noise for this technique.

After MACS separation, primary cells were manually counted as above specified and they were resuspended in PBS with 1% FCS. As previously stated, due to reduced accuracy of manual cell counting compared to FACS sorting, in my experiments ATAC-seq was performed using an estimated number of cells between 50,000 to 100,000. ATAC-seq was performed as described in Buenrostro *et al.*, 2013 with minor modifications. Cells were centrifuged at 500g for 5 min at 4°C. After removing the supernatant cells were lysed for 10 min, the nuclei were transposed using the Nextera Tn5 transposase (Illumina) for 40 min at 37°C and DNA was purified using the PCR MinElute kit (Qigen). Additional modifications and performance in 96-well plates were implemented for KC and they will be described in

After appropriate determination of the amount of DNA amplification using qPCR, samples were amplified and singled indexed for 11 PCR cycles using modified Nextera primers from Buenrostro *et al.*,2013 (Table2.2). The

resulting DNA libraries were purified using the MinElute kit and additional Agencourt AMPure XP Magentic Beads (Beckman Coulter), according to the manual specifications, to remove the remaining adaptors and primer dimers.

Primer name	Full sequence
Ad1.noMX	AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGCGTCAGATGTG
Ad2.1	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGT
Ad2.2	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGT
Ad2.3	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGT
Ad2.4	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGGAGATGT
Ad2.5	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGGAGATGT
Ad2.6	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGGAGATGT
Ad2.7	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGGAGATGT
Ad2.8	CAAGCAGAAGACGGCATACGAGATCCTCTCTGGTCTCGTGGGCTCGGAGATGT
Ad2.9	CAAGCAGAAGACGGCATACGAGATAGCGTAGCGTCTCGTGGGCTCGGAGATGT
Ad2.10	CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTCTCGTGGGCTCGGAGATGT
Ad2.11	CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTCTCGTGGGCTCGGAGATGT
Ad2.12	CAAGCAGAAGACGGCATACGAGATTCCTCTACGTCTCGTGGGCTCGGAGATGT
Ad2.13	CAAGCAGAAGACGGCATACGAGATATCACGACGTCTCGTGGGCTCGGAGATGT
Ad2.14	CAAGCAGAAGACGGCATACGAGATACAGTGGTGTCTCGTGGGCTCGGAGATGT
Ad2.15	CAAGCAGAAGACGGCATACGAGATCAGATCCAGTCTCGTGGGCTCGGAGATGT
Ad2.16	CAAGCAGAAGACGGCATACGAGATACAAACGGGTCTCGTGGGCTCGGAGATGT
Ad2.22	CAAGCAGAAGACGGCATACGAGATTGTGACCAGTCTCGTGGGCTCGGAGATGT
Ad2.23	CAAGCAGAAGACGGCATACGAGATAGGGTCAAGTCTCGTGGGCTCGGAGATGT

Table 2.2: Name and full sequence of the PCR primers used for amplification, indexing and pooling of the ATAC-seq and ChIPm samples in this thesis. These primers were designed by Buenrostro *et al.*, 2013 and they are an modified version of the Nextera Illumina primers optimised for larger molecular weight DNA fragments from low input samples. All samples were indexed with the universal primer Ad1.noMx and one of the additional 18 primers. The indexing sequence of each of the primers is in blue text.

Following the Nature Methods publication of Corces et al., 2016, the initial ATAC-seq protocol was replaced by a modified version named Fast-ATAC. It was specifically optimised for hematopoietic cells and combined cell lysis and transposition in a single step. Fast-ATAC was performed as described by Corces et al., 2016 with minor modifications. Since 5,000 cells was considered the lower limit to generate good quality data in Fast-ATAC, in my experiments I used 20,000 MACS or FACS sorted cells, to account for inaccurate manual cell counting as well as possible cell loss over centrifugation steps. The Fast-ATAC reaction was performed for 30 min at 37°C and agitation at 400rpm. DNA was purified as in ATAC-seq and libraries were generated following 13 cycles of PCR amplification, following appropriate cell cycle determination. Purification following PCR were performed using Agencourt AMPure XP Magentic Beads only.

Omni-ATAC, a third generation of ATAC-seq was published by Corces et al., 2017. It consisted in an universal protocol with individual cell lysis and transposition reactions intercalated with a washing step, to remove mitochondrial DNA and other cell debri Omni-ATAC was performed as described by Corces and colleagues (**Corces2017**) using 50,000 cells.

Following either of the three protocols, DNA tagmentation profiles were assessed with the D1000 high sensitivity DNA tape (Agilent) as part of the quality control and quantified using the Kapa kit (Roche), following the manufacturer's instructions. Pools of 12 to 16 libraries were sequenced in one to 3 lanes of the HiSeq4000 Illumina platform by the Oxford Genomics Centre at the Wellcome Trust Centre for Human Genetics.

2.3.3 Chromatin Immunoprecipitation with sequencing library preparation by Tn5 transposase

Assessment of histone marks modification in the chromatin of PSO patients from Cohort 1B and four age matched healthy volunteers was performed

using a low cell input Chromatin Immunoprecipitation (ChIP) method know as ChIPmentation (ChIPm). For each individual and cell type three histone marks, including H3K27ac, H3K4me1 and X were tested in 100,000 cells and compared to an input control processed in parallel. Samples were processed following the protocol published by Schmidl and colleagues (**Schmidl2015**) with some modifications. Aliquots of 600,000 cells of MACS sorted cell types, as described in 2.2, were fixed with 1% formaldehyde (Sigma) and snap frozen in dry ice and ethanol prior to storage at -80°C. Chromatin sonications of the different individuals and cell types were performed in one batch using Covaris M220(Covaris). Each of the aliquots was resuspended in 130µL of SDS lysis buffer (Table2.3), sonicated for 8 min using a duty factor of 5% and aliquoted for single ChIPm reactions prior to long term storage at -80°C.

Sonicated chromatin aliquots were thawed and resuspended in appropriate volume of ChIP equilibration buffer (Table2.3), in order to achieve the appropriate concentration of NaCl and Triton-X100. For the immunoprecipitation step, samples were incubated with the appropriate amount of antibody (Table 2.4) overnight in rotation at 4°C. Protein-A Dynabeads (Invitrogen) were also washed three times with Beads wash buffer (Table2.3) and blocked with yeast tRNA (supplier) overnight in rotation at 4°C. Beads were washed twice with the aforementioned washing buffer and resuspended in SDS lysis buffer before being added to the sample-antibody mix. One of the advantages of this protocol is the tagmentation of the chromatin when still bound to the beads prior to protein decross-linking, which allows preventing overtagmentation of the DNA.

qPCR was performed in each of the purified ChIPm DNA samples to identify the number of full cycles required to reach one-third of the final fluorescence. Libraries were amplified for the number of cycles minus one determined with this strategy, allowing to minimise the total number of PCR replicates. The primers used for amplification and indexing are the ones

optimised by Buensotro and colleagues (Table2.2). The number of amplification cycles for each of the samples is recorded in

Reagent	Final concentration	Supplier
SDS lysis buffer		
SDS	0.25%	Sigma
EDTA	1mM	X
Tris-HCl pH 8	10mM	Sigma
PI	1X	Roche
Water	-	Ambion
ChIP equilibration buffer		
Triton-X100	1.66%	Sigma
EDTA	1mM	X
NaCl	233mM	X
Tris-HCl pH 8	10mM	Sigma
PI	1X	Roche
Water	-	Ambion
Beads washing buffer		
SDS	0.1%	Sigma
EDTA	50mM	X
NaCl	150mM	X
NP-40	1%	Sigma
Tris-HCl pH 8	10mM	Sigma
PI	1X	Roche
Water	-	Ambion

Table 2.3: Composition of the three modified buffers in house for the ChIPm protocol: SDS lysis buffer, ChIP equilibration buffer and beads washing buffer. For each of the buffers the reagents, composition and supplier are indicated.The final volume prepared for each buffer was adjusted depending on the number of samples processed at the time. Sodium dodecyl sulfate (SDS), PI (proteinase inhibitor).

Histone mark	Feature	µL per sample	Manufacturer and catalo
H3K427ac	Promoter	X	
H3K4me1	Active enhancer	X	
X	X	X	

Table 2.4: Details regarding the histone marks, the the most likely chromatin state delineated, the amount of antibody required per reaction and the supplier and catalogue num of the antibodies.

Bibliography

Boyman, O., Hefti, H. P., Conrad, C., Nickoloff, B. J., Suter, M., and Nestle, F. O. (2004). "Spontaneous development of psoriasis in a new animal model shows an essential role for resident T cells and tumor necrosis factor-alpha". *J Exp Med* 199: pp. 731–6. ISSN: 0022-1007 (Print)0022-1007. DOI: 10.1084/jem.20031482. URL: http://dx.doi.org/10.1084/jem.20031482.

Burgos-Pol, R. and Dermo, Martnez-Sesmero-J. M. (2016). "The cost of psoriasis and psoriatic arthritis in 5 European countries: a systematic review". *Actas Dermo*. URL: http://www.sciencedirect.com/science/article/pii/S1578219016301524.

Clark, R. A., Chong, B., Mirchandani, N., Brinster, N. K., Yamanaka, K., Dowgiert, R. K., and Kupper, T. S. (2006). "The vast majority of CLA+ T cells are resident in normal skin". *J Immunol* 176: pp. 4431–9. ISSN: 0022-1767 (Print)0022-1767. URL: http://dx.doi.org/.

Coates, L. C., FitzGerald, O., Helliwell, P. S., and and, Paul-C. in arthritis (2016). "Psoriasis, psoriatic arthritis, and rheumatoid arthritis: Is all inflammation the same?" *Seminars in arthritis and*. URL: http://www.sciencedirect.com/science/article/pii/S0049017216300646.

Eedy, D. J., Burrows, D., Bridges, J. M., and Jones, F. G. (1990). "Clearance of severe psoriasis after allogenic bone marrow transplantation". *BMJ* 300: p. 908. ISSN: 0959-8138 (Print)1468-5833 (Electronic). URL: http://dx.doi.org/.

Gelfand, J. M., Gladman, D. D., Mease, P. J., and Academy, Smith-N. (2005). "Epidemiology of psoriatic arthritis in the population of the United States". *the American Academy*. URL: http://www.sciencedirect.com/science/article/pii/S0190962205010108.

Gelfand, J. M., Neimann, A. L., Shin, D. B., and Jama, Wang-X. (2006). "Risk of myocardial infarction in patients with psoriasis". *Jama*. URL: http://jamanetwork.com/journals/jama/fullarticle/203598.

Gladman, D. D., Ang, M., Su, L., and the, B. D. M. of (2009). "Cardiovascular morbidity in psoriatic arthritis". *Annals of the*. DOI: 10.1136/ard.2008.094839. URL: http://dx.doi.org/10.1136/ard.2008.094839.

Griffiths, C. E. M. and Lancet, Jnwn (2007). "Pathogenesis and clinical features of psoriasis". *The Lancet*. URL: http://www.sciencedirect.com/science/article/pii/S0140673607611283.

GutowskaOwsiak, D. and Schaupp, A. L. (2012). "IL17 downregulates filaggrin and affects keratinocyte expression of genes associated with cellular adhesion". *Experimental dermatology* 21: pp. 104–110. DOI: 10.1111/j.1600-0625.2011.01412.x. URL: http://dx.doi.org/10.1111/j.1600-0625.2011.01412.x.

Henseler, T. and Christophers, E. (1985). "Psoriasis of early and late onset: characterization of two types of psoriasis vulgaris". *J Am Acad Dermatol* 13: pp. 450–6. ISSN: 0190-9622 (Print) 0190-9622.

Ibrahim, G., Waxman, R., and Helliwell, P. S. (2009). "The prevalence of psoriatic arthritis in people with psoriasis". *Arthritis Rheum* 61: pp. 1373–8. ISSN: 0004-3591 (Print) 0004-3591. DOI: 10.1002/art.24608.

Jacobson, Christine C., Kumar, Sandeep, and Kimball, Alexa B. (2011). "Latitude and psoriasis prevalence". *Journal of the American Academy of Dermatology* 65: pp. 870–873. ISSN: 0190-9622. DOI: 10.1016/j.jaad.2009.05.047. URL: http://dx.doi.org/10.1016/j.jaad.2009.05.047.

Kent, L. (2009). "Freezing and thawing human embryonic stem cells". *Journal of visualized experiments: JoVE*. URL: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3152242/.

Lande, R., Gregorio, J., Facchinetti, V., Chatterjee, B., Wang, Y. H., Homey, B., Cao, W., Su, B., Nestle, F. O., Zal, T., et al. (2007). "Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide". *Nature* 449: pp. 564–9. ISSN: 0028-0836. DOI: 10.1038/nature06116. URL: http://dx.doi.org/10.1038/nature06116.

Moll, J. M. H., in, arthritis, and rheumatism, Wright-V. (1973). "Psoriatic arthritis". *Seminars in arthritis and rheumatism*. URL: http://www.sciencedirect.com/science/article/pii/0049017273900358.

Nestle, Frank O., Kaplan, Daniel H., and Barker, Jonathan (2009). "Psoriasis". *New England Journal of Medicine* 361: pp. 496–509. DOI: doi:10.1056/NEJMra0804595. URL: http://www.nejm.org/doi/full/10.1056/NEJMra0804595.

Nickoloff, B. J. and Nestle, F. O. (2004). "Recent insights into the immunopathogenesis of psoriasis provide new therapeutic opportunities". *J Clin Invest*. Vol. 113: pp. 1664–75. ISBN: 0021-9738 (Print). DOI: 10.1172/jci200422147. URL: http://dx.doi.org/10.1172/jci200422147.

Nickoloff, B. J. and Wrone-Smith, T. (1999). "Injection of pre-psoriatic skin with CD4+ T cells induces psoriasis". *Am J Pathol* 155: pp. 145–58. ISSN: 0002-9440 (Print)0002-9440. DOI: 10.1016/s0002-9440(10)65109-7. URL: http://dx.doi.org/10.1016/s0002-9440(10)65109-7.

Organization, World Health (2016). *Global report on psoriasis*. Report.

Perera, G. K., Di Meglio, P., and Nestle, F. O. (2012). "Psoriasis". *Annu Rev Pathol* 7: pp. 385–422. ISSN: 1553-4006. DOI: 10.1146/annurev-pathol-011811-132448.

Poole, C. D., Lebmeier, M., Ara, R., and Rheumatology, Rafia-R. (2010). "Estimation of health care costs as a function of disease severity in people with psoriatic arthritis in the UK". *Rheumatology*. DOI: 10.1093/rheumatology/keq182. URL: http://dx.doi.org/10.1093/rheumatology/keq182.

Proksch, E., Brandner, J. M., and Jensen, J. M. (2008). "The skin: an indispensable barrier". *Exp Dermatol* 17: pp. 1063–72. ISSN: 0906-6705.

Reich, K., Krger, K., and Dermatology, Mssner-R. of (2009). "Epidemiology and clinical pattern of psoriatic arthritis in Germany: a prospective interdisciplinary epidemiological study of 1511 patients with plaquetype psoriasis". *Journal of Dermatology*. DOI: 10.1111/j.1365-2133.2008.09023.x. URL: http://dx.doi.org/10.1111/j.1365-2133.2008.09023.x.

Sampogna, Francesca, Tabolli, Stefano, and Abeni, Damiano (2012). "Living with psoriasis: prevalence of shame, anger, worry, and problems in daily activities and social life". *Acta dermato-venereologica* 92: pp. 299–303. DOI: 10.2340/00015555-1273. URL: http://dx.doi.org/10.2340/00015555-1273.

Strange, A., Capon, F., Spencer, C. C., Knight, J., Weale, M. E., Allen, M. H., Barton, A., Band, G., Bellenguez, C., Bergboer, J. G., et al. (2010). "A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1". *Nat Genet* 42: pp. 985–90. ISSN: 1061-4036. DOI: 10.1038/ng.69410.1038/ng.694.Epub20100ct17.. URL: http://dx.doi.org/10.1038/ng.694.

Tsoi, L. C., Spain, S. L., Knight, J., Ellinghaus, E., Stuart, P. E., Capon, F., Ding, J., Li, Y., Tejasvi, T., Gudjonsson, J. E., et al. (2012). "Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity". *Nat Genet* 44: pp. 1341–8. ISSN: 1061-4036. DOI: 10.1038/ng.2467.

Vence, L., Schmitt, A., and Medical, Meadows-C. E. (2015). "Recognizing guttate psoriasis and initiating appropriate treatment". *West Virginia Medical*. URL: http://go.galegroup.com/ps/i.do?id=GALE%7CA422778063&sid=googleScholar&v=2.1&it=r&linkaccess=fulltext&issn=00433284&p=AONE&sw=w.

Villanova, Federica, Meglio, Paola, and Nestle, Frank O. (2013). "Biomarkers in psoriasis and psoriatic arthritis". *Annals of the rheumatic diseases* 72. DOI: 10.1136/annrheumdis-2012-203037. URL: http://dx.doi.org/10.1136/annrheumdis-2012-203037.

Wikramanayake, T. C., Stojadinovic, O., and Tomic-Canic, M. (2014). "Epidermal Differentiation in Barrier Maintenance and Wound Healing". *Adv Wound Care (New Rochelle)*. Vol. 3: pp. 272–280. ISBN: 2162-1918 (Print) 2162-1918 (Linking). DOI: 10.1089/wound.2013.0503.