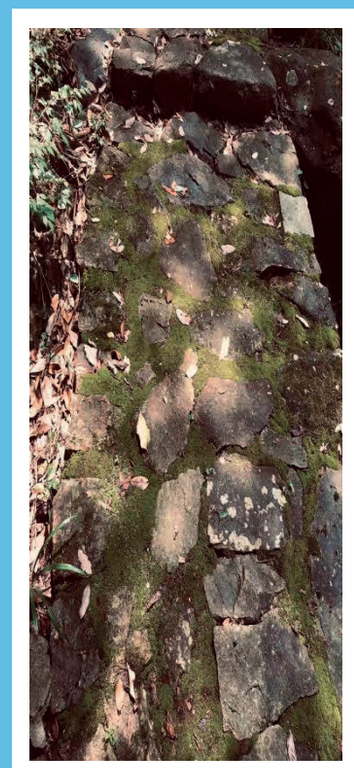
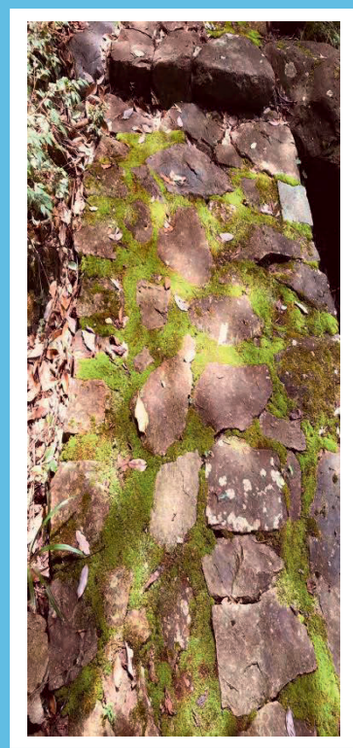
# The IL-17A/IL-17R Pathway in Immune-Mediated Inflammatory Diseases

Xiaofei XU



The IL-17A/IL-17R Pathway in Immune-Mediated Inflammatory Diseases

Xiaofei XU



The IL-17A/IL-17R Pathway in Immune-Mediated Inflammatory Diseases

Xiaofei XU

# The IL-17A/IL-17R Pathway in Immune-Mediated Inflammatory Diseases

Xiaofei XU

The work presented in this thesis was performed at the department of Rheumatology, Dermatology and Immunology, Erasmus MC, Rotterdam, The Netherlands.
Publication of this thesis was financially supported by Erasmus Postgraduate School Molecular Medicine and the Erasmus University Rotterdam.
X.Xu were financially supported by the China Scholarship Council for funding PhD fellowships (No. 201406100056).

The IL-17A/IL-17R Pathway in Immune-Mediated Inflammatory Diseases.

Layout: Xiaofei Xu
Printing: Ridderprint BV - www.ridderprint.nl
Cover: Xiaofei Xu, photograph by Xiaofei Xu

ISBN: 978-94-6458-482-0

# The IL-17A/IL-17R Pathway in Immune-Mediated Inflammatory Diseases

# De IL-17A/IL-17R route in immuun gemedieerde ontstekingsziekten

Thesis

to obtain the degree of Doctor from the
Erasmus University Rotterdam
by command of the
rector magnificus

Prof.dr. A.L. Bredenoord

and in accordance with the decision of the Doctorate Board.
The public defense shall be held on
Thursday 13th October, 2022
at 13:00 hrs
by

Xiaofei XU
born in Shandong, P.R. China.

Erasmus University Rotterdam

**DOCTORAL COMMITTEE**

| | |
|---|---|
| **Promotors:** | Prof. dr. E.P. Prens |
| | Dr. E. Lubberts |
| **Other members:** | Prof. dr. A.W. Langerak |
| | Prof. dr. E.M.G. de Jong |
| | Prof. dr. P.M. van Hagen |

# TABLE OF CONTENTS

# 1

# Molecular and cellular signatures in immune-mediated inflammatory diseases related to joint and skin disorders: similarities and differences

Xiaofei Xu, Koen Dudink, Errol Prens, Erik Lubberts

Manuscript in preparation

# I. Introduction

### The immune system

In nature, defense against other intrusions proposes surviving advantages in the selection arena of “survival of the fittest” during evolution. Prokaryotic microorganisms such as bacteria have already developed mechanisms to defend themselves from foreign invasions (1). Restriction enzymes, for example, protects bacteria from foreign DNA (2). Fungi and plants produce a wide range of antibacterial products including the most well-known penicillin (3,4). Among animals, a sophisticated set of molecules and cells dedicated to self-defense emerges in all metazoans, which can be grouped into the innate immune system (5). Later in vertebrates with jaws, the remarkable adaptive immunity with a distinct feature of immune memory transforms immunity into the higher level of specificity (5).

So, what is exactly immunity? In biology, immunity is the ability of multicellular organisms to recognize “self” and “non-self” components, eliminate pathogen infections and establish a self-sustaining balance (6). Therefore, three major tasks are involved in immune functions: (1) immune defense- to resist harmful invasions, (2) immune surveillance- to recognize “self” and “mutations”, and (3) immune homeostasis- to maintain a balance between tolerance and immunogenicity (6).

To accomplish the mission of immunity, two sets of arsenals are deployed, specific and non-specific. The innate immunity, which is non-specific and developed early in evolution, reacts fast during immune responses (7). It includes physical (e.g. skin), chemical (e.g. anti-microbial peptides), and microbiological barriers (e.g. microbiota), and importantly, the innate immune system including cytokines, chemokines, complements and innate immune cells such as neutrophils and macrophages (7). The adaptive immunity, on the other hand, is specific with prolonged immune memory, and responds at a later

stage compared to the innate immunity during immune responses. It consists of antigen-specific reactions by T and B lymphocytes (8).

**Innate immunity**

The first line of defense in an organism is provided by the surface directly exposed to external environments. In humans, such areas include skin, lung and intestinal mucosa. Skin holds the largest area in direct contact with outside, and thereby can be deemed as the largest immune organ in the body (9). Cornification with expression of keratins and tight junctions between epithelial cells in the epidermis insulate the body from direct harms (10). Besides the physical barrier, keratinocytes and skin microbiota produce antimicrobial peptides such as defensins as chemical barriers, and reduce local pH levels to facilitate barrier functions (10,11). Upon infection, keratinocytes recognize invading pathogens through pathogen recognition receptors (PRR) such as Toll-like receptors (TLR), and release inflammatory cytokines and chemokines to recruit immune cells and amply immune reactions (12). During viral infection, after PRR recognition, skin resident cells such as plasmacytoid dendritic cells can be a major source of type I interferons, which protect uninfected cells by interfering viral RNA and DNA replication (13).

As a typical innate immune cell, neutrophil infiltration usually occurs in sites of inflammation in response to released chemokines (14). Exodus of neutrophils involves coordinated expression of adhesion molecules (addresins) and gradient chemokine concentrations (15). Once present in the local infection, neutrophils phagocytose invading pathogens and digest the engulfed pathogens within the phagocytic vacuoles (16). However, the capacity of neutrophils to ingest bacteria is limited with a total number of 5-25 kills, and thereafter, together with killed microorganisms, neutrophils undergo apoptosis and can form yellowish liquid called pus (17). On the contrary, the other phagocyte, macrophages can digest more than 100 invading pathogens.

Dendritic cells (DC) act as a bridge between innate and adaptive immunity.

In skin, for instance, Langerhans cells (DC) in the epidermis capture antigens from pathogens, and migrate into draining lymph nodes to relay the antigen to T lymphocytes (12,14). There they induce a vast proliferation of naïve T lymphocytes, and in turn, activated T cells home to the inflammation sites to combat the pathogens (18).

## Adaptive immunity

### T cells

Antigen-presenting cells (APC) are cells that display antigen bound with major histocompatibility complex (MHC) molecules on their surface. This process is called antigen presentation. Almost all cell types express MHC class I molecules and thereby can present peptides with 8-10 amino acids to CD8+ cytotoxic T cells (19). Together with other innate immune cells such as natural killer cells (NK), CD8+ T cells are a strong component of the cell-mediated immunity.

Professional APCs are a group of immune cells including dendritic cells, macrophages and B cells that can activate naïve CD4+ T cells. They capture antigens from invading pathogens, digest them into short peptides with usually 13-25 amino acids in length, and present the peptides in the form of MHC class II- peptide (MHCII-p) to CD4+ T cells (20). Upon recognition of the MHCII-p complex by T cell receptor (TCR) and co-receptor molecule CD4, a secondary signal provided by molecules like CD80/86 on APC determines whether naïve T cells will be fully activated or undergo anergy (21). This constitutes the check points of immune reactions. When these check points are in imbalance, either uninhibited as in autoimmune diseases or suppressed as in carcinogenesis, diseases occur (22,23).

Fully activated CD4+ T cells are instructed by cytokines present in the local milieu to differentiate into different subsets of CD4+ T helper cells (Th) (**Figure 1**, 24). Presence of IL-12 induces the differentiation of Th1 cells which express

the typical transcription factor T-bet and produce the IFN-γ cytokine, while IL-4 induces Th2 cells to express GATA3 and cytokines such as IL-4, IL-5 and IL-13 (24). Combination of IL-1, IL-6 and TGF-β induces the differentiation of Th17 cells with specific expression of the transcription factor RORγt (RORc) and cytokine IL-17. In contrast, TGF-β alone induces T regulatory cells (Treg) to express the transcription factor Foxp3 and the cytokine IL-10 (24,25). Different functions have been attributed to these different CD4+ Th cell subsets (25). Th1 cells are believed to be important in combating intracellular bacterial and viral infections. Th2 cells are beneficial to immunoglobulin production and protection from extracellular parasites. Th17 cells contribute to control fungi infections, while Treg are pivotal in regulating immune tolerance. However, post-differentiation plasticity has been observed in CD4+ Th differentiation, especially regarding the Th17 lineage (26,27). And dysregulation of Th subsets is involved in various human diseases (28). For instance, the Th17 cells and related cytokines are pathogenic in diverse autoimmune or autoinflammatory diseases, which are the main topics of this thesis (**Chapter 2-6**).

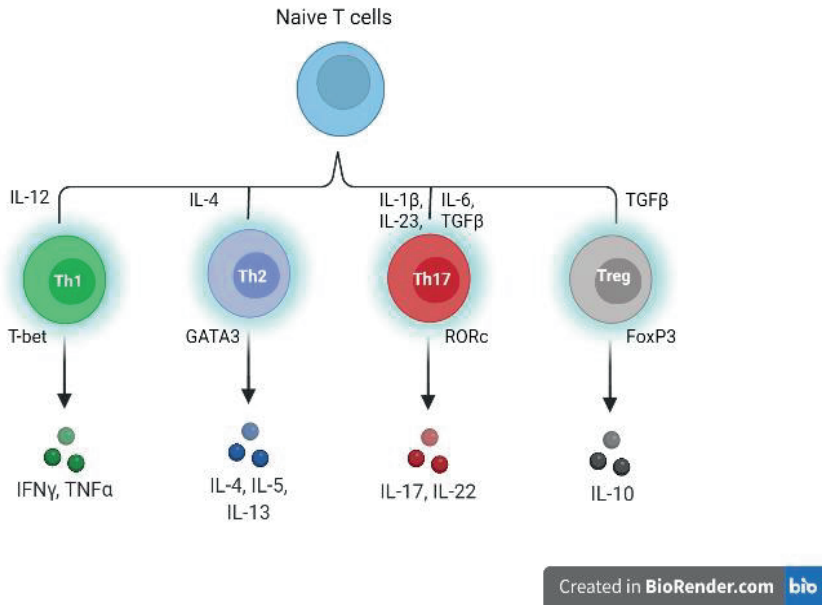



**Figure 1. Differentiation of CD4+ T cells into diverse Th cell subsets.** After activation

of naïve CD4+ T cells with TCR and costimulatory signals, T cells exposed to different cytokines induce various transcription factors and express various corresponding cytokines.

**B cells**

B cells are antibody-producing cells. Each B cell expresses a specific B cell receptor (BCR), which, in the secreted form, constitutes the immunoglobulin (Ig) pool. The BCR is composed of two light chains (IgL) and two heavy chains (IgH), both of which undergo V(D)J recombination (29). This V(D)J recombination process determines the structure and therefore the BCR specificity of the antigen-binding fragments (Fab). The specific antigen-binding motifs are termed complementarity-determining regions (CDRs) (30). The IgH chain is different from the IgL chain with the presence of the fragment crystallizable region (Fc). Due to different lengths and structures of the Fc part, Ig can be grouped into IgM (pentamer), IgG, IgD, IgE, and IgA (dimer) (30). All these antibodies contribute to humoral immunity with IgM serving as the default choice.

Upon antigen stimulation and T cell costimulation (i.e. Th2, T follicular helper cells (Tfh)), B cells in the germinal center of draining lymph nodes undergo class switch recombination to produce high affinity antibodies such as IgG instead of low affinity IgM (31). Simultaneously, somatic hypermutation will occur in the CDRs and improve the reservoir of binding antibodies. Due to the somatic hypermutation process, the diversity of antibodies is estimated to increase to around $10^{10}$ compared to a repertoire of over $10^{8}$ TCRs (32).

During immune responses, antibodies neutralize pathogens by binding to specific antigens. Antibody-bound pathogens substantially increase the efficacy of phagocytosis by immune cells such as macrophages and neutrophils (33). This process is called opsonization. For example, antibody opsonization is 100-fold more effective compared to pathogen alone (34). This phenomenon indicates that innate and adaptive immunity influence each other and immunity

is not a one-way path, but a closed circle enhancing each other's reactions. Similarly, cytokines produced by Th1, Th2, and Th17 cells can modulate the responses of innate immunity including the function of innate immune cells and the stimulation of tissue resident cells such as fibroblasts (34-36). Therefore, immunity is not just a singular reaction to external distress to prevent infection, but also an orchestrated cross-talk between diverse cell types to achieve new immune homeostasis.

**Autoinflammatory/autoimmune disease**

The tasks of immunity are best illustrated when it goes awry (34). For instance, under-performance of immune systems during immune defense against pathogens will result in uncontrolled infections. Frail immune surveillance, specially at old ages, will miss malignant "mutations", and will evade immune responses against tumors. Last but not least, loss of immune homeostasis will shift the paradigm of immune tolerance into immunogenicity, and cause immune reactions to unharmful "self" antigens. It is in this realm of loss of immune tolerance that we will continue our discussion.

Breach on immune tolerance can be caused by aberrant innate and adaptive immunity. By a recent definition, overactivity of innate immune responses is termed autoinflammatory diseases, while overactivity of the adaptive systems is called autoimmune diseases (37). However, as discussed above, innate and adaptive immunity are intertwined and influence each other. It is difficult to restrict diseases into one category, especially during the late stage of full-blown immune reactions. Therefore, immune-mediated inflammatory diseases (IMID) may serve as a better definition that covers everything under the same umbrella (38). In addition, each IMID can vary in a wide spectrum from predominantly autoinflammatory (i.e. innate immunity dominant) to predominantly autoimmune (i.e. adaptive immunity dominant) diseases (39,40).

## II. Immune-mediated inflammatory diseases (IMID)

Immune-mediated inflammatory diseases (IMID) is an umbrella term used to describe a group of diseases or conditions that are characterized by common inflammatory pathways (41). The estimated incidence of IMID is around 5-7% in Western population, and multiple organ involvements including the joint, skin and intestines may be observed in patients (42). Genetic predisposition and familial heredity are crucial determinants of disease susceptibility, especially relating to gene polymorphism of human leukocyte antigen (HLA) molecules and various cytokines (42,43). Besides, environmental factors such as trauma, infection and smoking may serve as external triggers for IMID disease developments (42,43).

Though a definitive etiology is still elusive, IMID share similar features of molecular and cellular immune dysregulation. Recent advances with biologics targeting specific immune cytokines or pathways have provided new insights regarding the shared pathogenesis and the management of IMID (41,43). Of particular interests are the wide efficacy of certain biologics observed across diverse IMID diseases, such as antibodies targeting TNF and the IL-23/IL-17 pathway (41,43). However, it is worth noting that, despite of the clinical successes with biologics in IMID, divergency has also occurred in various treatment results (41). Particularly, these differential effects emphasized the facts of mixed autoimmune and autoinflammation nature of IMID contributing to disease pathogenesis (44). Therefore, we set out to discuss similarities and differences of molecular and cellular mechanisms in IMID with focus on skin and joint conditions, and to invite a reflection on what can be learnt from current biological treatments in IMID. Diseases pertaining to the scope of discussion will be limited to psoriasis, hidradenitis suppurativa as templates of skin conditions, and rheumatoid arthritis, psoriatic arthritis as examples of joint diseases.

**Psoriasis**

Psoriasis is a chronic autoimmune disease that mainly affects the skin. Estimated prevalence of psoriasis is around 2-3% world-wide (45). Five different types of psoriasis are described including plaque, guttate, inverse, pustular, and erythrodermic (45). Plaque psoriasis, also known as psoriasis vulgaris, accounts for around 90% of psoriasis cases (46). Red, dry, and itchy scales of inflamed skin are the most common symptoms. The scaling plaques are results of rapid growth of epidermal keratinocytes. In psoriasis, keratinocytes are regenerated every 3-5 days instead of the usual 28-30 days (47). Excessive proliferation together with premature differentiation causes epidermal hyperplasia and parakeratosis in epidermis (46,47).

Genetic factors together with environmental triggers are thought to cause the disease onset. The psoriasis susceptibility 1 locus (PSORS1), which is linked to functions of antigen presentation and recognition, is a major genetic determinant locus located in the HLA region (45-48). HLA-Cw6 is reportedly the strongest gene variant associated with psoriasis, and is particularly common in early-onset and more-severe diseases (45,48). Besides HLA genes, mutations involving the epidermal differentiation complex (PSORS4), tyrosine kinase 2 (TYK2, PSORS6) and IL-23 receptor (PSORS7) all have been confirmed to contribute to psoriasis susceptibility (45,47).

The pathogenesis of psoriasis can be postulated in two phases: (1) an initiation phase that can be triggered by trauma (for instance the Koebner phenomenon), pathogens, or drugs; (2) a maintenance phase with activation of T cell subsets and involvement of the adaptive immunity (48). The exact cause of psoriasis is not clear, but it is believed to be a combination of external triggers and internal hyperactive immune reactions. As shown in **Figure 2**, in response to external triggers such as trauma or infection, antimicrobial peptides (AMPs) are released by keratinocytes (48,49). Genetic predisposition makes susceptible subjects to produce more of these AMPs such as cathelicidin LL37,

β-defensins, and S100 proteins (48,49). The AMPs bind with self RNA/DNA released during external stress stimulated plasmacytoid dendritic cells (pDC) via toll-like receptor (TLR) 9 to produce type I interferons (IFN-α and IFN-β) (50). After exposure to the type I interferons, myeloid dendritic cells (mDC) mature into inflammatory phenotypes and secret cytokines such as IL-12 and IL-23, which promote the differentiation of Th1 and Th17 cells, respectively (46,51). The activation of Th cell subsets, particularly the Th17 cells, marks the start of maintenance phase and immune perpetuation in disease development (48). Activated Th17 cells release typical Th17 cytokines including IL-17A, IL-17F and IL-22 and induce inflammatory cytokines such as TNF, IL-6 (52). These cytokines further stimulate skin resident cells such as keratinocytes and fibroblasts, recruit immune cells, and establish a feed-forward loop to perpetuate inflammation (46,53,54).

The IL-23/IL-17 axis plays a fundamental role in the pathogenesis of psoriasis (48,53). Genetic variations of the IL-23 receptor and its downstream signaling cascade Tyk2 have been implicated in psoriasis susceptibility (45). Congruently, aberrant expression of IL-23 cytokine in psoriasis lesions is significantly higher compared to normal skin (55,56). IL-23 is a heterodimer cytokine formed by the IL-23p19 and the shared IL-12/IL-23 p40 subunits. In combination with cytokines such as IL-6 and TGFβ, IL-23 promotes the differentiation of Th17 cells (57-59). Concomitantly, marked increases of IL-17 family cytokines such as IL-17A, IL-17C and IL-17F are observed in psoriasis (60). Potential sources of IL-17A (and IL-17F) are predominantly immune cells such as CD4+ Th17 cells, CD8+ T cells, γδT cells and innate lymphoid cells 3 (ILC3) (52,61). Skin resident cells such as keratinocytes can be major sources of IL-17C, which contribute significantly to keratinocyte hyperproliferation via autocrine manners (62). IL-17A is a more potent inducer of inflammatory stimulation than IL-17F, as observed in our own and others' studies (54,63). Both cytokines function through the shared IL-17 receptor composed of subunits IL-17RA and IL-17RC,

though preferential binding of different subunits is reported for each cytokine during signaling (64). Recently, IL-17RD is also reported to be a functional receptor subunit for these cytokines (65,66). IL-17A and IL-17F, alone or in combination with other cytokines such as IL-1β and TNF, can directly or indirectly exacerbate keratinocyte hyperproliferation (53,61,67). IL-22, another Th17 cytokine, is a prominent inducer of keratinocyte hyperplasia, and IL-20 subfamily cytokines such as IL-19 and IL-24, which are downstream targets of IL-17 cytokines, can further sustain epidermal acanthosis (68,69). The IL-20 subfamily cytokines are conducive to keratinocyte hyperproliferation especially during the chronic phase as has been found in the imiquimod-induced psoriasis-like mouse model according to our observation (54). Though keratinocytes may play a fundamental role in psoriasis initiation, our research results suggested that fibroblasts are probably the primary stromal skin cells that sustain inflammation during the maintenance stage (54). Firstly, fibroblasts are located in the dermis, where most of the recruited immune cells aggregate, and therefore are better positioned to interact with immune infiltrates. Secondly, *in vitro* culture results suggest that fibroblasts, compared to keratinocytes, produce more inflammatory cytokines when stimulated or cocultured with T cells. However, we do acknowledge that, *in vitro* culture system is not representative for the *in vivo* situations. The observed stronger production of inflammatory cytokines by fibroblasts *in vitro* may be confounded by the different culture conditions compared to keratinocytes.

Psoriasis is not a unanimous disease but comprises of different types. The underlying cellular and molecular mechanisms in disease pathogenesis may also vary (70). Take pustular psoriasis for example. In the pustular form, increased expression of IL-1 family cytokines including IL-1β and IL-36 family cytokines have been reported in comparison to plaque psoriasis (70,71). Neutrophil infiltration in the epidermis such as Munro's microabscesses is one of the hallmark histological features of psoriasis (45). However, the number of

neutrophils is relatively low in plaque psoriasis compared to the massive recruitment of neutrophils in pustular psoriasis (72). Therefore, it can be stated that, while the chronic plaque psoriasis is predominantly an autoimmune response maintained by adaptive immune reactions, the pustular psoriasis shows more features of autoinflammation caused by the innate immunity (70). In different types of psoriasis, the contribution of autoimmune and autoinflammatory responses may tilt one way or the other. How each type of psoriasis is immunologically distinct, and how the diverse combinations of innate and adaptive immunity contribute to psoriasis pathogenesis still need greater appreciation.

**Hidradenitis suppurativa**

Hidradenitis suppurativa (HS), also known as acne inverse, is a chronic inflammatory skin disease that primarily affects the axillary, inguinal, gluteal and under the breast areas (73). Painful inflamed nodules, abscesses and tunnel formation such as sinus tracts and fistulas are typical features of HS (73). Acute and chronic forms are observed in HS. Acute HS shows symptoms of a few red deep-seated nodules that may coalesce into cord-like structures (74). Chronic HS shows characteristics of multiple abscesses, interwoven sinus tracts deep in the dermis, and foul exudate (74). As the disease progresses, fibrosis, scarring and dermal contractures may incur. The prevalence of HS is estimated to be about 1% in population, and females appear to be more commonly affected by HS than males (75). HS severity can be divided into three stages according to the Hurley's staging system (76). Stage I HS indicates isolated nodules with abscess formation, stage II involves separated lesions with recurrent abscesses and sinus tract formation, and stage III includes diffuse regions of lesional areas with interconnected sinus tracts (73,76).

The exact cause of HS is unclear, but a combination of genetic, environmental factors, lifestyle and microbiota are believed to contribute to HS pathogenesis (73,77). Recently, gene polymorphism related to the subunit of γ-

secretase among others was suggested to contribute to HS pathogenesis (77,78). Besides, uncontrolled inflammation, especially massive accumulation of neutrophils, is detrimental in HS (79). Various inflammatory cytokines are overexpressed in HS lesions including IL-1β, IL-36 family, and IL-17 cytokines (73,77,78). Changes of the microbiome around HS lesional skin have been implicated in disease pathogenesis (73,77). However, whether these changes play an eliciting role or just by-products of immune responses of HS pathology remains to be further investigated. For simplicity, a theorized arbitrary division into “initiation phase” and “progression phase” is discussed to facilitate understanding (73). A historical view of the disease suggests that occlusion of the hair follicles may be the initial triggering factor (80,81). Hyperplasia of the follicular epithelium leads to follicular occlusion and resident bacteria propagation (73,80,81). Bacteria and damaged cells release pattern molecules that activate skin resident macrophages, which produce inflammatory cytokines including IL-1β and TNF (**Figure 2**, 82). These inflammatory cytokines further induce the secretion of chemokine (such as CXCL1, CCL2 and CCL20) and cytokines (such as IL-6 and IL-8), leading to recruitment of more immune cells and exacerbation of inflammatory reactions (73,83, unpublished data). During the “progression phase”, diverse infiltration of immune cells are typical features (73). Of particular interests are the involvement of T cell subsets such as Th1 and Th17 cells (84,85). Enhanced expression of interferon-γ (IFN-γ) and IL-17 are observed in HS lesions (85). Our data suggests increased local accumulation of IL-17+ CD4+ and CD8+ T cells in HS lesions (unpublished data). Interestingly, our results also show that expression of IL-17 receptor, particularly IL-17RC, is upregulated in lymphoid cells in HS (unpublished data). Stimulated by inflammatory cytokines such as IL-1β, TNF, and IL-17, HS skin fibroblasts produce large quantity of degrading enzymes including matrix metalloproteinases (MMP-1 and MMP-3), which contribute to disease progression and sinus tract formation (unpublished data). Scarring and dermal

contractures are common end-stage results of severe HS due to prolonged inflammation and wound healing (74).

Current treatment options for HS is rather limited due to the elusive nature of the disease (86). Lifestyle changes such as weight loss and quitting smoking are recommended, and warm baths may help in mild cases (86-88). Systemic or locally administered antibiotics can be prescribed as first-line treatments (73,87-89). However, these antibiotics are used more for their anti-inflammatory effects rather than to treat infections (86). Biologics, for instance, TNF blocking agents, are among choices for moderate to severe HS after failure of antibiotic options (90). Biologics targeting other pathways such as the IL-23/IL-17 axis

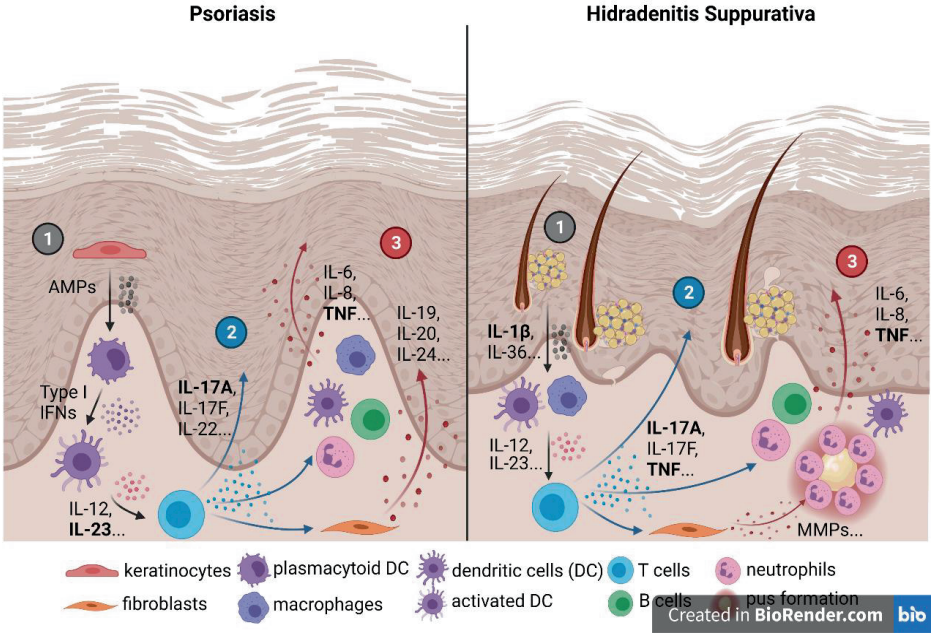



**Figure 2. Immune pathogenesis of psoriasis and hidradenitis suppurativa (HS).** Diverse immune cells and cytokines contribute to the pathogenesis of psoriasis and HS. Generally, three stages can be proposed during the disease developments: ① genetic predisposition and environmental factors trigger inflammation, ② innate and adaptive immune responses exacerbate autoinflammation and autoimmunity, ③ stromal cells amplify immune responses and perpetuate the feed-forward loop of autoinflammation and autoimmunity.

are being evaluated in several HS clinical trials (91,92). Some case report data indicate that these options are promising in HS therapy even after failure to above treatments (93-95). For patients with excessive disease, surgical removal can be a good choice to improve the patients' quality of life (73,86).

**Psoriasis versus HS**

As in psoriasis, HS shares the characteristics of many other immune-mediated inflammatory diseases (IMID). Inflammatory cytokines, in particular the Th1 (IFN-γ) and Th17 cytokines (IL-17), are similarly increased in HS as in psoriasis (84). This strongly suggests the involvement of adaptive immunity in both diseases (**Figure 2**). TNF expression is also suggested similar between progressed HS and psoriatic lesions (83,84). Nevertheless, massive accumulation of neutrophils and excessive expression of IL-1β and IL-1 family members including the IL-36 family cytokines suggest a strong signature of innate immunity activation in HS (83,96). Increased IL-1β may contribute to enhanced MMP induction and tissue degradation in HS (97). Though keratinocyte hyperplasia is present in HS, it is apparently less prominent compared to psoriasis. This is probably due to lower expression of IL-22 in HS lesions compared to psoriasis (84,98). Contrarily, higher expression of IL-10 is observed in HS than in psoriasis (84). As observed in our study, this may lead to downregulation of other cytokines such as IL-17 and IL-20 family cytokines IL-19 and IL-24, which are also conducive to keratinocyte hyperproliferation. Whether lower expression of IL-19 and IL-24 is present in HS compared to psoriasis still needs future verification. Taken together, HS, perhaps more like pustular than plaque psoriasis, poses a mixed presence of innate and adaptive immunity in the spectrum of autoimmune and autoinflammatory diseases (78).

**Rheumatoid arthritis**

Rheumatoid arthritis (RA) is a typical chronic autoimmune disease that primarily affects the joints. The prevalence rate is around 0.5-1% varying among regions, and more frequent occurrence in females than males is observed (99). Common clinical symptoms include arthralgia, morning stiffness, and symmetrical swollen joints (99). Despite of intensive research, the exact cause of rheumatoid arthritis is still not clear, but it is believed that a combination of genetic and environmental factors is involved (99). Inflammation, thickening of the synovial membrane known as pannus, and erosion of bone and cartilage are typical features present in RA (100). “Swan neck” deformation of fingers can develop in advanced stages of RA due to long-term damages to the joint (101). Rheumatoid factors (autoantibodies against the Fc portion of IgG) and antibodies to citrullinated proteins (ACPA) are observed in around 80% cases of RA (102).

Primary manifestations of RA involve the inflammation of synovial membrane and concomitant joint destruction (99). Three phases of RA progression are currently suggested: (1) an initiation phase due to non-specific inflammation; (2) an amplification phase with adaptive immunity activation; (3) the chronic phase with prolonged inflammation, tissue restructuring and joint damage. In the initiation stage, genetic variations such as HLA polymorphism predispose individuals to be more susceptible, and together with environmental factors such as cigarette smoking, immune tolerance is disturbed and replaced with immune activation (103). In the amplification stage, autoantibodies such as rheumatoid factors and ACPA augment the activation of innate immune cells, which consequently amply immune reactions by recruiting more T cells (104, **Figure 3**). Activated T cells secrete cytokines such as TNF, IFN-γ and IL-17, and interact with innate immune cells and local resident cells, which form a positive feed-forward loop of immune responses in joint inflammation (105). In the chronic phase, prolonged inflammation causes the formation of granulation

tissue by fibroblast-like synoviocytes (FLS), and activated FLS further contribute to disease progression by production of pro-inflammatory cytokines (such as IL-6, IL-8) and proteases involved in joint erosion (such as matrix metalloproteinases, MMPs) (106).

As an autoimmune disorder, primary immune cells involved in RA pathogenesis are B cells, T cells and macrophages. The mechanism of autoantibody production by autoreactive B cells is still not clear in RA. Detection of these autoantibodies usually precedes clinical disease onset and continues to expand during disease progression (99). Two groups of the most studied antibodies are anti-citrullinated protein antibodies (ACPA) and rheumatoid factors (RF). ACPA positive RA is strongly associated with genetic variants HLA-DR1 and HLA-DR4 (107). As an indication of breaching immune tolerance, the presence of ACPA can last years before joint symptoms, and initial autoantibody titer holds predictive values regarding disease development (107). Autoreactive B cells coupled with HLA-DR susceptible polymorphism promote CD4+ Th cell proliferation and differentiation. During the early stage, Th2 cells secrete cytokines such as IL-4, which in turn activate B cell proliferation and immunoglobulin class switching to IgE (108). However, as RA deteriorates, highly inflammatory and pathogenic Th1 and Th17 cells are induced and accumulate in local joints (109). Th1 cells produce pro-inflammatory cytokines such as IFN-γ and TNF, while Th17 secrete IL-17 among other inflammatory cytokines. Both Th cell subsets contribute to RA progression via production of inflammatory cytokines and cellular interactions with other immune or resident cells. For instance, Th1 cells promote the antigen presentation function of macrophages to CD4+ Th cells (110). In our studies, cellular contacts between *ex vivo* cultured RA CD4+ T cells and synoviocytes are conducive to enhanced cytokine production compared to contact deprivation (unpublished observations).

In the synovium, lining fibroblast-like synoviocytes (FLS) are important

amplifiers of immune reactions. In response to inflammatory cytokines such as IL-17 and TNF, FLS cells produce large amounts of inflammatory cytokines including IL-6, IL-8 and MMPs (111). This formed a feed-forward loop between FLS and immune cells, in particular CCR6+Th17 cells to perpetuate local inflammation (111). Similarly, macrophages are primed into inflammatory M1 phenotype, and together with activated FLS, contribute heavily to local tissue destruction (110). Due to stimulation of inflammatory cytokines, FLS in RA joint exhibits increased resistance to apoptosis and impaired contact inhibition (106). This collectively contributes to synovial hyperplasia and eventual pannus formation. Moreover, RA FLS provide a niche for the survival of local immune cells. For instance, they provide cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and type I interferons to support the survival of neutrophils and T cells, respectively (106,112). Another pathology that can be largely attributed to FLS is the destruction of cartilage. MMPs, primarily produced by FLS and macrophages in RA, are mainly responsible for the degradation of type II collagen network and the disruption of cartilage biomechanical functions (113). Besides, cytokines such as IL-1 and IL-17 promote chondrocytes to undergo apoptosis, and thereby deprive the cartilage of possible regeneration (114). Another hallmark feature of RA is bone erosion. One possible mechanism of bone damage in RA is that inflammatory cytokines such as IL-1β, IL-6, IL-17 and TNF induce higher expression of receptor activator of nuclear factor kappa-B ligand (RANKL) and macrophage colony-stimulating factor (M-CSF), which subsequently promote osteoclastogenesis and reduce osteoblast proliferation (115). The combined result is more bone destruction, less bone formation, and therefore bone erosion in inflicted joints.

It should be noted that RA is a heterogenous disease (116). Subgroups of RA with more prominent features of systemic inflammation respond poorly to conventional disease-modifying anti-rheumatic drugs (DMARDs), but can be successfully treated with IL-1 pathway blockade (117). This indicates that

diverse contributions of mixed autoimmune and autoinflammatory phenotypes exist in RA pathogenesis.

**Psoriatic arthritis**

Psoriatic arthritis (PsA) is a form of inflammatory arthritis that occurs predominantly in patients with psoriasis or a history of psoriasis including family history. Around 15% of PsA patients show symptoms of arthritis before onset of psoriatic skin lesions (118). Typical features of PsA include sausage-like swelling fingers and toes (dactylitis), pitting and ridging nails and nail detachment (onycholysis), and inflammation around the tendon insertion (enthesitis). PsA is a heterogeneous disease, and five clinical subtypes have been suggested including distal, oligoarticular, polyarticular, arthritis mutilans and spondylitis subtypes (118). Prevalence of PsA in both sexes is around equal, though in some subtypes of PsA, predominance in females is observed (118).

The exact cause of PsA is unknown. However, strong heritable influences have been observed in genome-wide association studies. In contrast to rheumatoid arthritis, PsA is more associated with class I rather than class II major histocompatibility complex (MHC) alleles. For instance, HLA-B alleles has been associated with about 40-50% of patients with PsA (118). Interestingly, HLA-C*06 is a risk factor for psoriasis but not for PsA (118). Besides, other risk alleles associated with psoriasis and psoriatic arthritis include IL-12A, IL-12B, IL-23R and genes involving the NF-κB signaling pathway (118,119). Environmental factors like obesity and some forms of psoriasis also contribute to increased risks of PsA.

PsA is a multiple-phased disease that can involve diverse organs including the joint, the skin, the gut and bone marrow. As in psoriasis, the IL-23/IL-17 pathway plays a detrimental role in the pathogenesis of PsA (120). Common features of disease initiation at different sites are believed to involve the production of IL-23 and the activation of IL-17+ cells (118, **Figure 3**). Pathogenic events of psoriatic skin lesions have been stated in above sections

of psoriasis. In the gut, microbial dysbiosis may induce IL-23 release and potentiate Th17 differentiation (118). During enthesitis, it was proposed that stress or trauma induces the local release of IL-23, which activates IL-17 producing cells at the tendon-insertion sites (121).

Th17 cells, CD8+ T cells, γδ T cells and type III innate lymphoid cells (ILC3) are all suggested cellular sources of IL-17. Especially, the percentages of IL-17+ CD8+ cells have been associated with the disease severity of PsA, but not that of RA (122). However, our *ex vivo* data showed that, with T cell receptor activation, CD4+, but not CD8+ T cells produce measurable IL-17A in PsA (123). The induction of IL-17A in CD8+ T cells seems to be an effect resulting from strong stimulation of phorbol myristate acetate (PMA) and ionomycin during flow cytometry (123). Similarly, ILC3 reportedly fail to express IL-17A upon *in vitro* stimulation in spondyloarthritis (124). Other cell types including mast cells and neutrophils are also found IL-17+ in PsA joints (125). Sequestering of IL-17 by these cells has been suggested instead of self-production (126-128). Activated IL-17+ cells in turn produce inflammatory cytokines including IL-17, IL-22 and TNF, which exacerbate inflammation and cause bone damage. Of particular importance is the fibroblast-like synovial cells (FLS) in amplifying inflammation. Data from our own and other groups suggest that FLS in PsA contribute importantly to local production of inflammatory cytokines (IL-6, IL-8) and damaging enzymes (MMPs) (123,129). For immune cells, inhibition mechanisms such as T regulatory cells and the production of IL-10 cytokine act as brakes to control inflammation. However, for stromal cells such as FLS, activation by inflammatory cytokines seem less well regulated and can contribute significantly to disease progression.

It should be noted that pathogenic bone formation including joint ankylosis also occur at sites of inflammation around the enthesis (129). The receptor activator of NF-kB (RANK) and corresponding ligand (RANKL) have been implicated in the metabolic regulation of bone destruction via osteoclast

proliferation, while IL-17A, a potential bone morphogenetic protein, and molecules in the Wnt signaling pathway are conducive to pathogenic bone formation in PsA (130). Psoriatic arthritis is a heterogeneous disease both among patients and within the same individuals (118). Axial or spinal involvements of PsA make it similar to ankylosing spondylitis (130). In contrast, bone erosion and destruction in PsA resemble more of rheumatoid arthritis (131). This puts PsA in between the characteristics of both ankylosing spondylitis and rheumatoid arthritis.

**RA versus PsA**

PsA is usually asymmetrical and involves distal joints, while RA is commonly symmetrical and involves wrist and metacarpophalangeal joints (131). Autoantibodies such as RF and ACPA are typical features of RA, and not found in PsA (131). In contrast, axial involvement is typical in PsA, but not in RA (131). As mentioned above, bone damage is present in both RA and PsA. Nevertheless, new bone formation can happen in late stage PsA, but not in RA (131). Hyperplasia of the synovium, known as pannus, can be seen in both RA and PsA. Neoangiogenesis is observed in both PsA and RA synovia with tortuous characteristics in PsA and straight and branching features in RA (132). Among immune cells infiltrating the synovium, more mast cells, CD15+ neutrophils and CD163+ macrophages are found in PsA compared to RA (133). In contrast, more infiltrating T lymphocytes and plasma cells are suggested in RA than in PsA (133). In line with this observation, biologics targeting B cells (anti-CD20) and T cells (CTLA4-Ig) have been approved for treatments of RA, while biologics targeting the IL-23/IL-17 pathway have been successful in treating PsA (131). Details regarding the differential effects of biologics in IMID will be discussed in **Chapter 7**.

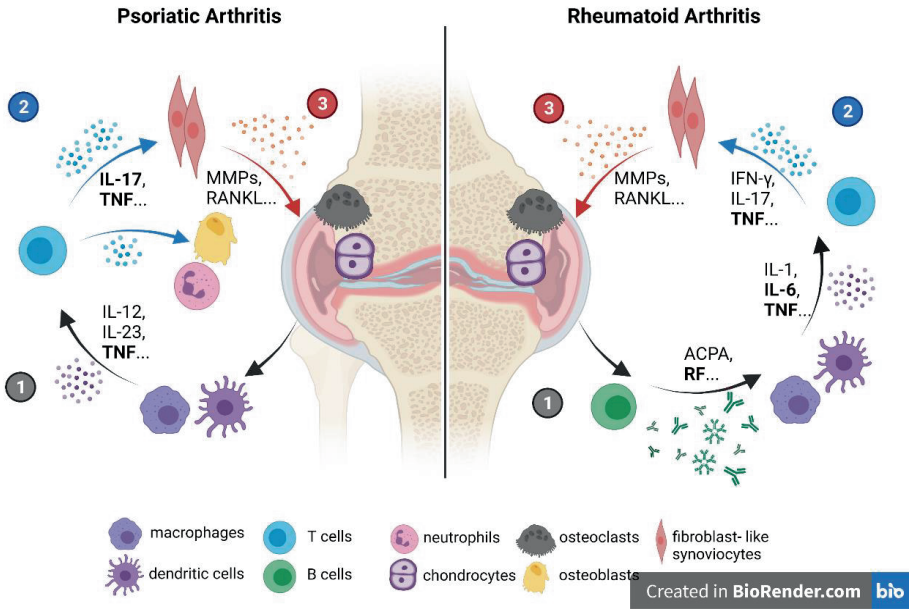



**Figure 3. Immune pathogenesis of psoriastic arthritis (PsA) and rheumatoid arthritis (RA).** Diverse immune cells and cytokines contribute to the pathogenesis of PsA and RA. Generally, three stages can be proposed during the disease developments: ① genetic predisposition and environmental factors trigger inflammation, ② innate and adaptive immune responses exacerbate autoinflammation and autoimmunity, ③ stromal cells amplify immune responses and perpetuate the feed-forward loop of autoinflammation and autoimmunity.

## Summary

Genetic predisposition and environmental triggers are commonly shared etiological factors for most IMID. Disease developments of IMID generally can be divided into three stages: an initiation stage with little or none clinical manifestations, a maintenance phase with explosive inflammation involving both innate and adaptive responses, and a chronic phase with stromal cells heavily contributing to disease symptoms (48, 99).

Diverse cell types and cytokines are conducive to IMID pathogenesis, and

differential contributions of innate and adaptive immunity are observed in IMID such as psoriasis, HS, RA and PsA. Even within the same disease, different subtypes may be caused by divergent immune dysregulation. The surging usage of biologics targeting specific pathway provides the unique opportunity of observing their individual contributions to the disease pathology. Up till now, anti-TNF agents are the most widely applicable drugs in treatments of IMID such as psoriasis, HS, RA and PsA (43). This highlights the pivotal role of TNF in the pathogenesis of IMID. However, anti-TNF biologics can induce paradoxical psoriasis in some RA and IBD patients, and this casts another layer of immune complexity in psoriasis (135).

Biologics targeting the IL-23/IL-17 pathway prove invaluable in treating psoriasis and PsA with especially remarkable improvements of skin symptoms (43). Reportedly, IL-12/IL-23p40 antibody can still benefit paradoxical psoriasis induced by anti-TNF agents (136). This suggests that TNF and IL-23/IL-17 pathway may contribute to different aspects of psoriasis developments. Considering the dynamics of soluble and membrane TNF and the bifurcating functions of receptors TNFR1 and TNFR2, the concept of selective suppressing deleterious effects of TNF has been introduced (137). The observance that IL-23 and IL-17 inhibitors appear more effective in relieving skin symptoms than joint manifestations in PsA patients indicates that the autoimmune signatures contributing to skin and joint disease developments might differ (43). Anti-IL-17 biologics fall short of achieving the expected efficacy in RA. This further reiterates the fact that during the maintenance phase of IMID joint diseases such as RA, the contribution of IL-23/IL-17 pathway may be limited, though this pathway may be detrimental during disease initiation (105). Whether the IL-23/IL-17 pathway inhibitors will achieve desirable and sustained efficiency in HS treatments is currently under intensive investigation (73).

Both IL-1 and IL-6 inhibitors are clinically available for treatments of RA patients. Anti-IL-1 biologics are reportedly less effective compared to TNF

inhibitors in treating RA (138). In psoriasis and PsA, anti-IL-6 agents show no efficacy in alleviating skin symptoms, and incidental occurrence of paradoxical psoriasis was reported in some RA cases (139,140). Pustular psoriasis, a subtype of psoriasis, shows prominent expression of IL-1 family cytokines and massive infiltration of neutrophils. Both IL-1 and IL-6 inhibitors demonstrate clinical benefits of treating pustular psoriasis in case studies (141,142). Potential beneficial effects of these biologics are also expected in HS patients as increased IL-1 family cytokines and neutrophil recruitment are common features of HS lesions (73). However, more detailed random clinical trials will be necessary to prove these ideas.

Together, it can be concluded that diverse contributions of innate versus adaptive immunity may play parts in the pathogenesis of IMID, and even in different subgroups of the same disease, diverse molecular and cellular mechanisms are involved. Immune stratification will help to shed light on the pathophysiology of IMID and improve targeted therapeutic effects in the personalized medicine era.

## III. Aims of this thesis

In this thesis we aim to identify critical IL-17A-producing cells and to explore the expression and regulation of the IL-17 receptor, consisting of IL-17 receptor A (IL-17RA) and IL-17RC on IL-17 target cells in different immune-mediated inflammatory diseases (IMID) with focus on joint inflammation and skin disorders. In **chapter 1**, a general introduction, including similarities and differences in molecular and cellular signatures, is given for these four IMID that includes rheumatoid arthritis (RA), psoriatic arthritis (PsA), psoriasis (PsO), and hidradenitis suppurativa (HS).

Different cellular sources of IL-17 in PsA have been described in literature such as CD4+ and CD8+ T cells, γδ T cells. Particularly, IL-17A+ CD8+ T cells has been implicated in the pathogenesis of PsA and correlate with disease severity. In **chapter 2**, we investigated the production of IL-17A by CD4+ and CD8+ T cells in synovial fluid from PsA patients, and sorted them out to culture under anti-CD3/anti-CD28 stimulation with or without allogeneic fibroblasts or autologous CD14+ monocytes. Moreover, the differential roles of IL-17A and TNF were compared using the *ex vivo* CD4+ T cell- synovial fibroblast coculture system.

IL-23/IL-17 pathway is involved in the pathogenesis of psoriasis. And the imiquimod (IMQ)-induced psoriasis-like skin inflammation is a well-established mouse model to study psoriasis. In **chapter 3**, we utilized the IMQ-induced psoriasis model to study the chronic skin inflammation due to aberrant IL-17 expression. Early and late stages of disease progression were studied to understand the time-course changes of inflammatory cytokines and skin symptoms when IL-23/IL-17 pathway was heightened with IL-10 neutralization. *In vivo* mouse model findings were confirmed with *ex vivo* culture systems, and were further validated with open source clinical transcription analysis results.

Targeting IL-17A in HS treatments has been reported in sporadic cases and currently being evaluated in wider clinical trials. In **chapter 4**, we first screened the dysregulated cytokines and immune molecules in HS skin compared to normal control skin. Then we evaluated the IL-17A- and IL-17RA/RC-expressing cells in HS skin lesions compared to peripheral blood. Finally, a HS fibroblast and T cell coculture system was set up to evaluate the differential therapeutic effects of TNF and IL-17A neutralization in HS.

Tyrosine kinase 2 (Tyk2) belongs to the signal-transducing Janus kinase (JAK) family which also includes JAK1, JAK2, and JAK3. Tyk2 is reportedly involved in the down-stream signaling of IFN-α, IL-6, IL-10 and IL-12/IL-23. Tyk2 inhibitors showed positive results in clinical trials, and Deucravacitinib, a selective Tyk2 inhibitor, is being assessed for drug approvals of psoriasis treatments. Using the imiquimod-induced psoriasis-like mouse model, we evaluated the therapeutic benefits of a Tyk2 inhibitor, compared to the standard treatment dexamethasone and an inhibitor of lymphocyte-specific protein tyrosine kinase (Lck) in **chapter 5**. We found that, the Tyk2 inhibitor alleviates inflammation in lesional psoriasiform skin but shows negligible effects in reducing skin thickness such as acanthosis in this model.

Biologics targeting the IL-17 pathway are successful in treating PsA patients. Contrarily, limited success is observed in targeting IL-17 for RA patients. The discrepancy in clinical responses between RA and PsA leads to our investigation described in **chapter 6**. Analyzing immune infiltrates of synovial fluid, we found higher IL-17A+ CD8+ T cells in PsA compared to RA, though percentages of IL-17A+ CD4+ T cells are similar. Interestingly, higher expression of IL-17 receptor, especially the IL-17RC subunit, is observed in T and B lymphocytes in RA compared to PsA. To understand the potential

regulating mechanism of IL-17R expression, sorted CD4+ T cells were stimulated with anti-CD3/CD28 antibodies, with/without cytokines or cocultured with synovial fibroblasts.

In **chapter 7** we discussed the biological treatment options for different IMID such as psoriasis, HS, RA and PsA, with focus particularly on the pathologic role of cytokines including the IL-23-IL-17 pathway, TNF, IL-1, and IL-6 in IMID.

A general discussion, including future perspectives, regarding the IL-17A/IL-17R pathway studies in IMID as described in this thesis is given in **chapter 8**.

## References


1. Brenner S. (1989) The Evolution of the Immune System: An Introduction. In: Melchers F. et al. (eds) Progress in Immunology. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-83755-5_164.
2. Oliveira PH, Touchon M, Rocha EP. The interplay of restriction-modification systems with mobile genetic elements and their prokaryotic hosts. Nucleic Acids Res. 2014;42(16):10618-31. doi: 10.1093/nar/gku734.
3. Deshmukh SK, Verekar SA, Bhave SV. Endophytic fungi: a reservoir of antibacterials. Front Microbiol. 2015 Jan 8;5:715. doi: 10.3389/fmicb.2014.00715.
4. Khameneh B, Iranshahy M, Soheili V, Fazly Bazzaz BS. Review on plant antimicrobials: a mechanistic viewpoint. Antimicrob Resist Infect Control. 2019 Jul 16;8:118. doi: 10.1186/s13756-019-0559-6.
5. Cooper MD, Alder MN. The evolution of adaptive immune systems. Cell. 2006 Feb 24;124(4):815-22. doi: 10.1016/j.cell.2006.02.001.
6. Abbas, A.K., Lichtman, A.H. and Pillai, S. (2016) Basic Immunology: Functions and Disorders of the Immune System, Elsevier, Amsterdam.
7. Iwasaki A, Medzhitov R. Control of adaptive immunity by the innate immune system. Nat Immunol. 2015 Apr;16(4):343-53. doi: 10.1038/ni.3123.
8. Nicholson LB. The immune system. Essays Biochem. 2016 Oct 31;60(3):275-301. doi: 10.1042/EBC20160017.
9. Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. Cell Res. 2020 Jun;30(6):492-506. doi: 10.1038/s41422-020-0332-7.
10. Nguyen AV, Soulika AM. The Dynamics of the Skin's Immune System. Int J Mol Sci. 2019 Apr 12;20(8):1811. doi: 10.3390/ijms20081811.
11. Schmid-Wendtner MH, Korting HC. The pH of the skin surface and its impact on the barrier function. Skin Pharmacol Physiol. 2006;19(6):296-302. doi: 10.1159/000094670.
12. Matejuk A. Skin Immunity. Arch Immunol Ther Exp (Warsz). 2018 Feb;66(1):45-54. doi: 10.1007/s00005-017-0477-3.
13. McNab F, Mayer-Barber K, Sher A, Wack A, O'Garra A. Type I interferons in infectious disease. Nat Rev Immunol. 2015 Feb;15(2):87-103. doi: 10.1038/nri3787.
14. Kabashima K, Honda T, Ginhoux F, Egawa G. The immunological anatomy of the skin. Nat Rev Immunol. 2019 Jan;19(1):19-30. doi: 10.1038/s41577-018-0084-5.
15. Williams MR, Azcutia V, Newton G, Alcaide P, Luscinskas FW. Emerging mechanisms of neutrophil recruitment across endothelium. Trends Immunol. 2011 Oct;32(10):461-9. doi: 10.1016/j.it.2011.06.009.
16. Segal AW. How neutrophils kill microbes. Annu Rev Immunol. 2005;23:197-223. doi:

10.1146/annurev.immunol.23.021704.115653.

17. Sabin CA, Lundgren JD. The natural history of HIV infection. Curr Opin HIV AIDS. 2013 Jul;8(4):311-7. doi: 10.1097/COH.0b013e328361fa66.

18. Clayton K, Vallejo AF, Davies J, Sirvent S, Polak ME. Langerhans Cells-Programmed by the Epidermis. Front Immunol. 2017 Nov 29;8:1676. doi: 10.3389/fimmu.2017.01676.

19. Rock KL, Reits E, Neefjes J. Present Yourself! By MHC Class I and MHC Class II Molecules. Trends Immunol. 2016 Nov;37(11):724-737. doi: 10.1016/j.it.2016.08.010.

20. ten Broeke T, Wubbolts R, Stoorvogel W. MHC class II antigen presentation by dendritic cells regulated through endosomal sorting. Cold Spring Harb Perspect Biol. 2013 Dec 1;5(12):a016873. doi: 10.1101/cshperspect.a016873.

21. Schwartz RH. T cell anergy. Annu Rev Immunol. 2003;21:305-34. doi: 10.1146/annurev.immunol.21.120601.141110.

22. Postow MA, Callahan MK, Wolchok JD. Immune Checkpoint Blockade in Cancer Therapy. J Clin Oncol. 2015 Jun 10;33(17):1974-82. doi: 10.1200/JCO.2014.59.4358.

23. Paluch C, Santos AM, Anzilotti C, Cornall RJ, Davis SJ. Immune Checkpoints as Therapeutic Targets in Autoimmunity. Front Immunol. 2018 Oct 8;9:2306. doi: 10.3389/fimmu.2018.02306.

24. Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 T cell populations (*). Annu Rev Immunol. 2010;28:445-89. doi: 10.1146/annurev-immunol-030409-101212.

25. Luckheeram RV, Zhou R, Verma AD, Xia B. $CD4^+$T cells: differentiation and functions. Clin Dev Immunol. 2012;2012:925135. doi: 10.1155/2012/925135.

26. Geginat J, Paroni M, Maglie S, Alfen JS, Kastirr I, Gruarin P, De Simone M, Pagani M, Abrignani S. Plasticity of human CD4 T cell subsets. Front Immunol. 2014 Dec 16;5:630. doi: 10.3389/fimmu.2014.00630.

27. Diller ML, Kudchadkar RR, Delman KA, Lawson DH, Ford ML. Balancing Inflammation: The Link between Th17 and Regulatory T Cells. Mediators Inflamm. 2016;2016:6309219. doi: 10.1155/2016/6309219.

28. Raphael I, Nalawade S, Eagar TN, Forsthuber TG. T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. Cytokine. 2015 Jul;74(1):5-17. doi: 10.1016/j.cyto.2014.09.011.

29. Jung D, Alt FW. Unraveling V(D)J recombination; insights into gene regulation. Cell. 2004 Jan 23;116(2):299-311. doi: 10.1016/s0092-8674(04)00039-x.

30. Schroeder HW Jr, Cavacini L. Structure and function of immunoglobulins. J Allergy Clin Immunol. 2010 Feb;125(2 Suppl 2):S41-52. doi: 10.1016/j.jaci.2009.09.046.

31. Stavnezer J, Guikema JE, Schrader CE. Mechanism and regulation of class switch recombination. Annu Rev Immunol. 2008;26:261-92. doi:

10.1146/annurev.immunol.26.021607.090248.

32. Arstila TP, Casrouge A, Baron V, Even J, Kanellopoulos J, Kourilsky P. A direct estimate of the human alphabeta T cell receptor diversity. Science. 1999 Oct 29;286(5441):958-61. doi: 10.1126/science.286.5441.958.

33. Walters MN, Papadimitriou JM. Phagocytosis: a review. CRC Crit Rev Toxicol. 1978 Sep;5(4):377-421. doi: 10.3109/10408447809081012.

34. Parkin J, Cohen B. An overview of the immune system. Lancet. 2001 Jun 2;357(9270):1777-89. doi: 10.1016/S0140-6736(00)04904-7.

35. Khader SA, Gaffen SL, Kolls JK. Th17 cells at the crossroads of innate and adaptive immunity against infectious diseases at the mucosa. Mucosal Immunol. 2009 Sep;2(5):403-11. doi: 10.1038/mi.2009.100.

36. Holley MM, Kielian T. Th1 and Th17 cells regulate innate immune responses and bacterial clearance during central nervous system infection. J Immunol. 2012 Feb 1;188(3):1360-70. doi: 10.4049/jimmunol.1101660.

37. Doria A, Zen M, Bettio S, Gatto M, Bassi N, Nalotto L, Ghirardello A, Iaccarino L, Punzi L. Autoinflammation and autoimmunity: bridging the divide. Autoimmun Rev. 2012 Nov;12(1):22-30. doi: 10.1016/j.autrev.2012.07.018.

38. Rahman P, Inman RD, El-Gabalawy H, Krause DO. Pathophysiology and pathogenesis of immune-mediated inflammatory diseases: commonalities and differences. J Rheumatol Suppl. 2010 May;85:11-26. doi: 10.3899/jrheum.091462.

39. Möller B, Villiger PM. Inhibition of IL-1, IL-6, and TNF-alpha in immune-mediated inflammatory diseases. Springer Semin Immunopathol. 2006 Jun;27(4):391-408. doi: 10.1007/s00281-006-0012-9.

40. Teng MW, Bowman EP, McElwee JJ, Smyth MJ, Casanova JL, Cooper AM, Cua DJ. IL-12 and IL-23 cytokines: from discovery to targeted therapies for immune-mediated inflammatory diseases. Nat Med. 2015 Jul;21(7):719-29. doi: 10.1038/nm.3895.

41. McInnes IB, Gravallese EM. Immune-mediated inflammatory disease therapeutics: past, present and future. Nat Rev Immunol. 2021 Oct;21(10):680-686. doi: 10.1038/s41577-021-00603-1.

42. Kuek A, Hazleman BL, Ostör AJ. Immune-mediated inflammatory diseases (IMID) and biologic therapy: a medical revolution. Postgrad Med J. 2007 Apr;83(978):251-60. doi: 10.1136/pgmj.2006.052688.

43. Schett G, McInnes IB, Neurath MF. Reframing Immune-Mediated Inflammatory Diseases through Signature Cytokine Hubs. N Engl J Med. 2021 Aug 12;385(7):628-639. doi: 10.1056/NEJMra1909094.

44. Szekanecz Z, McInnes IB, Schett G, Szamosi S, Benkő S, Szűcs G. Autoinflammation and autoimmunity across rheumatic and musculoskeletal diseases. Nat Rev Rheumatol. 2021 Oct;17(10):585-595. doi: 10.1038/s41584-021-00652-9.

45. Greb JE, Goldminz AM, Elder JT, Lebwohl MG, Gladman DD, Wu JJ, Mehta NN, Finlay AY, Gottlieb AB. Psoriasis. Nat Rev Dis Primers. 2016 Nov 24;2:16082. doi: 10.1038/nrdp.2016.82.

46. Nestle FO, Kaplan DH, Barker J. Psoriasis. N Engl J Med. 2009 Jul 30;361(5):496-509. doi: 10.1056/NEJMra0804595.

47. Schön MP, Boehncke WH. Psoriasis. N Engl J Med. 2005 May 5;352(18):1899-912. doi: 10.1056/NEJMra041320.

48. Rendon A, Schäkel K. Psoriasis Pathogenesis and Treatment. Int J Mol Sci. 2019 Mar 23;20(6):1475. doi: 10.3390/ijms20061475.

49. Morizane S, Gallo RL. Antimicrobial peptides in the pathogenesis of psoriasis. J Dermatol. 2012 Mar;39(3):225-30. doi: 10.1111/j.1346-8138.2011.01483.x.

50. Lande R, Gregorio J, Facchinetti V, Chatterjee B, Wang YH, Homey B, Cao W, Wang YH, Su B, Nestle FO, Zal T, Mellman I, Schröder JM, Liu YJ, Gilliet M. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. Nature. 2007 Oct 4;449(7162):564-9. doi: 10.1038/nature06116.

51. Hänsel A, Günther C, Ingwersen J, Starke J, Schmitz M, Bachmann M, Meurer M, Rieber EP, Schäkel K. Human slan (6-sulfo LacNAc) dendritic cells are inflammatory dermal dendritic cells in psoriasis and drive strong TH17/TH1 T-cell responses. J Allergy Clin Immunol. 2011 Mar;127(3):787-94.e1-9. doi: 10.1016/j.jaci.2010.12.009.

52. Miossec P, Kolls JK. Targeting IL-17 and TH17 cells in chronic inflammation. Nat Rev Drug Discov. 2012 Oct;11(10):763-76. doi: 10.1038/nrd3794.

53. Furue M, Furue K, Tsuji G, Nakahara T. Interleukin-17A and Keratinocytes in Psoriasis. Int J Mol Sci. 2020 Feb 13;21(4):1275. doi: 10.3390/ijms21041275.

54. Xu X, Prens E, Florencia E, Leenen P, Boon L, Asmawidjaja P, Mus AM, Lubberts E. Interleukin-17A Drives IL-19 and IL-24 Expression in Skin Stromal Cells Regulating Keratinocyte Proliferation. Front Immunol. 2021 Sep 20;12:719562. doi: 10.3389/fimmu.2021.719562.

55. Lee E, Trepicchio WL, Oestreicher JL, Pittman D, Wang F, Chamian F, Dhodapkar M, Krueger JG. Increased expression of interleukin 23 p19 and p40 in lesional skin of patients with psoriasis vulgaris. J Exp Med. 2004 Jan 5;199(1):125-30. doi: 10.1084/jem.20030451.

56. Piskin G, Sylva-Steenland RM, Bos JD, Teunissen MB. In vitro and in situ expression of IL-23 by keratinocytes in healthy skin and psoriasis lesions: enhanced expression in psoriatic skin. J Immunol. 2006 Feb 1;176(3):1908-15. doi: 10.4049/jimmunol.176.3.1908.

57. Dong C. Diversification of T-helper-cell lineages: finding the family root of IL-17-producing cells. Nat Rev Immunol. 2006 Apr;6(4):329-33. doi: 10.1038/nri1807.

58. Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton

RD, Wahl SM, Schoeb TR, Weaver CT. Transforming growth factor-beta induces development of the T(H)17 lineage. Nature. 2006 May 11;441(7090):231-4. doi: 10.1038/nature04754.

59. McGeachy MJ, Bak-Jensen KS, Chen Y, Tato CM, Blumenschein W, McClanahan T, Cua DJ. TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. Nat Immunol. 2007 Dec;8(12):1390-7. doi: 10.1038/ni1539.

60. Johansen C, Usher PA, Kjellerup RB, Lundsgaard D, Iversen L, Kragballe K. Characterization of the interleukin-17 isoforms and receptors in lesional psoriatic skin. Br J Dermatol. 2009 Feb;160(2):319-24. doi: 10.1111/j.1365-2133.2008.08902.x.

61. Brembilla NC, Senra L, Boehncke WH. The IL-17 Family of Cytokines in Psoriasis: IL-17A and Beyond. Front Immunol. 2018 Aug 2;9:1682. doi: 10.3389/fimmu.2018.01682.

62. Lauffer F, Jargosch M, Baghin V, Krause L, Kempf W, Absmaier-Kijak M, Morelli M, Madonna S, Marsais F, Lepescheux L, Albanesi C, Müller NS, Theis FJ, Schmidt-Weber C, Eyerich S, Biedermann T, Vandeghinste N, Steidl S, Eyerich K. IL-17C amplifies epithelial inflammation in human psoriasis and atopic eczema. J Eur Acad Dermatol Venereol. 2020 Apr;34(4):800-809. doi: 10.1111/jdv.16126.

63. Iwakura Y, Ishigame H, Saijo S, Nakae S. Functional specialization of interleukin-17 family members. Immunity. 2011 Feb 25;34(2):149-62. doi: 10.1016/j.immuni.2011.02.012.

64. Wright JF, Bennett F, Li B, Brooks J, Luxenberg DP, Whitters MJ, Tomkinson KN, Fitz LJ, Wolfman NM, Collins M, Dunussi-Joannopoulos K, Chatterjee-Kishore M, Carreno BM. The human IL-17F/IL-17A heterodimeric cytokine signals through the IL-17RA/IL-17RC receptor complex. J Immunol. 2008 Aug 15;181(4):2799-805. doi: 10.4049/jimmunol.181.4.2799.

65. Mellett M, Atzei P, Horgan A, Hams E, Floss T, Wurst W, Fallon PG, Moynagh PN. Orphan receptor IL-17RD tunes IL-17A signalling and is required for neutrophilia. Nat Commun. 2012;3:1119. doi: 10.1038/ncomms2127.

66. Su Y, Huang J, Zhao X, Lu H, Wang W, Yang XO, Shi Y, Wang X, Lai Y, Dong C. Interleukin-17 receptor D constitutes an alternative receptor for interleukin-17A important in psoriasis-like skin inflammation. Sci Immunol. 2019 Jun 7;4(36):eaau9657. doi: 10.1126/sciimmunol.aau9657.

67. Chiricozzi A, Guttman-Yassky E, Suárez-Fariñas M, Nograles KE, Tian S, Cardinale I, Chimenti S, Krueger JG. Integrative responses to IL-17 and TNF-α in human keratinocytes account for key inflammatory pathogenic circuits in psoriasis. J Invest Dermatol. 2011 Mar;131(3):677-87. doi: 10.1038/jid.2010.340.

68. Sa SM, Valdez PA, Wu J, Jung K, Zhong F, Hall L, Kasman I, Winer J, Modrusan Z, Danilenko DM, Ouyang W. The effects of IL-20 subfamily cytokines on reconstituted human epidermis suggest potential roles in cutaneous innate defense and pathogenic

adaptive immunity in psoriasis. J Immunol. 2007 Feb 15;178(4):2229-40. doi: 10.4049/jimmunol.178.4.2229. Erratum in: J Immunol. 2007 Jun 1;178(11):7487.

69. Wolk K, Witte E, Warszawska K, Schulze-Tanzil G, Witte K, Philipp S, Kunz S, Döcke WD, Asadullah K, Volk HD, Sterry W, Sabat R. The Th17 cytokine IL-22 induces IL-20 production in keratinocytes: a novel immunological cascade with potential relevance in psoriasis. Eur J Immunol. 2009 Dec;39(12):3570-81. doi: 10.1002/eji.200939687.

70. Liang Y, Sarkar MK, Tsoi LC, Gudjonsson JE. Psoriasis: a mixed autoimmune and autoinflammatory disease. Curr Opin Immunol. 2017 Dec;49:1-8. doi: 10.1016/j.coi.2017.07.007.

71. Johnston A, Xing X, Wolterink L, Barnes DH, Yin Z, Reingold L, Kahlenberg JM, Harms PW, Gudjonsson JE. IL-1 and IL-36 are dominant cytokines in generalized pustular psoriasis. J Allergy Clin Immunol. 2017 Jul;140(1):109-120. doi: 10.1016/j.jaci.2016.08.056.

72. Ikeda S, Takahashi H, Suga Y, Eto H, Etoh T, Okuma K, Takahashi K, Kanbara T, Seishima M, Morita A, Imai Y, Kanekura T. Therapeutic depletion of myeloid lineage leukocytes in patients with generalized pustular psoriasis indicates a major role for neutrophils in the immunopathogenesis of psoriasis. J Am Acad Dermatol. 2013 Apr;68(4):609-617. doi: 10.1016/j.jaad.2012.09.037.

73. Sabat R, Jemec GBE, Matusiak Ł, Kimball AB, Prens E, Wolk K. Hidradenitis suppurativa. Nat Rev Dis Primers. 2020 Mar 12;6(1):18. doi: 10.1038/s41572-020-0149-1.

74. Alikhan A, Lynch PJ, Eisen DB. Hidradenitis suppurativa: a comprehensive review. J Am Acad Dermatol. 2009 Apr;60(4):539-61; quiz 562-3. doi: 10.1016/j.jaad.2008.11.911.

75. Jemec GB. Clinical practice. Hidradenitis suppurativa. N Engl J Med. 2012 Jan 12;366(2):158-64. doi: 10.1056/NEJMcp1014163.

76. Hurley HJ. Axillary hyperhidrosis, apocrine bromhidrosis, hidradenitis suppurativa, and familial benign pemphigus: surgical approach. In: Roenigk RK, Roenigk HH, editors. Dermatologic surgery. Marcel Dekker, New York, 1989, pp. 729–739.

77. Vossen ARJV, van der Zee HH, Prens EP. Hidradenitis Suppurativa: A Systematic Review Integrating Inflammatory Pathways Into a Cohesive Pathogenic Model. Front Immunol. 2018 Dec 14;9:2965. doi: 10.3389/fimmu.2018.02965.

78. Nomura T. Hidradenitis Suppurativa as a Potential Subtype of Autoinflammatory Keratinization Disease. Front Immunol. 2020 May 20;11:847. doi: 10.3389/fimmu.2020.00847.

79. Lima AL, Karl I, Giner T, Poppe H, Schmidt M, Presser D, Goebeler M, Bauer B. Keratinocytes and neutrophils are important sources of proinflammatory molecules in hidradenitis suppurativa. Br J Dermatol. 2016 Mar;174(3):514-21. doi: 10.1111/bjd.14214.

80. von Laffert M, Helmbold P, Wohlrab J, Fiedler E, Stadie V, Marsch WC. Hidradenitis suppurativa (acne inversa): early inflammatory events at terminal follicles and at interfollicular epidermis. Exp Dermatol. 2010 Jun;19(6):533-7. doi: 10.1111/j.1600-0625.2009.00915.x.

81. von Laffert M, Stadie V, Wohlrab J, Marsch WC. Hidradenitis suppurativa/acne inversa: bilocated epithelial hyperplasia with very different sequelae. Br J Dermatol. 2011 Feb;164(2):367-71. doi: 10.1111/j.1365-2133.2010.10034.x.

82. van der Zee HH, de Ruiter L, van den Broecke DG, Dik WA, Laman JD, Prens EP. Elevated levels of tumour necrosis factor (TNF)-α, interleukin (IL)-1β and IL-10 in hidradenitis suppurativa skin: a rationale for targeting TNF-α and IL-1β. Br J Dermatol. 2011 Jun;164(6):1292-8. doi: 10.1111/j.1365-2133.2011.10254.x.

83. Witte-Händel E, Wolk K, Tsaousi A, Irmer ML, Mößner R, Shomroni O, Lingner T, Witte K, Kunkel D, Salinas G, Jodl S, Schmidt N, Sterry W, Volk HD, Giamarellos-Bourboulis EJ, Pokrywka A, Döcke WD, Schneider-Burrus S, Sabat R. The IL-1 Pathway Is Hyperactive in Hidradenitis Suppurativa and Contributes to Skin Infiltration and Destruction. J Invest Dermatol. 2019 Jun;139(6):1294-1305. doi: 10.1016/j.jid.2018.11.018.

84. Wolk K, Warszawska K, Hoeflich C, Witte E, Schneider-Burrus S, Witte K, Kunz S, Buss A, Roewert HJ, Krause M, Lukowsky A, Volk HD, Sterry W, Sabat R. Deficiency of IL-22 contributes to a chronic inflammatory disease: pathogenetic mechanisms in acne inversa. J Immunol. 2011 Jan 15;186(2):1228-39. doi: 10.4049/jimmunol.0903907.

85. Moran B, Sweeney CM, Hughes R, Malara A, Kirthi S, Tobin AM, Kirby B, Fletcher JM. Hidradenitis Suppurativa Is Characterized by Dysregulation of the Th17:Treg Cell Axis, Which Is Corrected by Anti-TNF Therapy. J Invest Dermatol. 2017 Nov;137(11):2389-2395. doi: 10.1016/j.jid.2017.05.033.

86. Vinkel C, Thomsen SF. Hidradenitis Suppurativa: Causes, Features, and Current Treatments. J Clin Aesthet Dermatol. 2018 Oct;11(10):17-23.

87. Shah N. Hidradenitis suppurativa: a treatment challenge. Am Fam Physician. 2005 Oct 15;72(8):1547-52.

88. Jemec GB. Clinical practice. Hidradenitis suppurativa. N Engl J Med. 2012 Jan 12;366(2):158-64. doi: 10.1056/NEJMcp1014163.

89. Bettoli V, Join-Lambert O, Nassif A. Antibiotic Treatment of Hidradenitis Suppurativa. Dermatol Clin. 2016 Jan;34(1):81-9. doi: 10.1016/j.det.2015.08.013.

90. Kimball AB, Okun MM, Williams DA, Gottlieb AB, Papp KA, Zouboulis CC, Armstrong AW, Kerdel F, Gold MH, Forman SB, Korman NJ, Giamarellos-Bourboulis EJ, Crowley JJ, Lynde C, Reguiai Z, Prens EP, Alwawi E, Mostafa NM, Pinsky B, Sundaram M, Gu Y, Carlson DM, Jemec GB. Two Phase 3 Trials of Adalimumab for Hidradenitis Suppurativa. N Engl J Med. 2016 Aug 4;375(5):422-34. doi: 10.1056/NEJMoa1504370.

91. Lee RA, Eisen DB. Treatment of hidradenitis suppurativa with biologic medications. J Am Acad Dermatol. 2015 Nov;73(5 Suppl 1):S82-8. doi: 10.1016/j.jaad.2015.07.053.

92. Casseres RG, Prussick L, Zancanaro P, Rothstein B, Joshipura D, Saraiya A, Turkowski Y, Au SC, Alomran A, Abdat R, Abudu M, Kachuk C, Dumont N, Gottlieb AB, Rosmarin D. Secukinumab in the treatment of moderate to severe hidradenitis suppurativa: Results of an open-label trial. J Am Acad Dermatol. 2020 Jun;82(6):1524-1526. doi: 10.1016/j.jaad.2020.02.005.

93. Thorlacius L, Theut Riis P, Jemec GBE. Severe hidradenitis suppurativa responding to treatment with secukinumab: a case report. Br J Dermatol. 2018 Jul;179(1):182-185. doi: 10.1111/bjd.15769.

94. Jørgensen AR, Yao Y, Thomsen SF. Therapeutic Response to Secukinumab in a 36-Year-Old Woman with Hidradenitis Suppurativa. Case Rep Dermatol Med. 2018 Apr 16;2018:8685136. doi: 10.1155/2018/8685136.

95. Villegas-Romero I, Collantes-Rodríguez C, Valenzuela-Ubiña S, Jiménez-Gallo D. Moderate to Severe Hidradenitis Suppurativa Successfully Treated With Secukinumab. Actas Dermosifiliogr (Engl Ed). 2020 Oct;111(8):696-698. English, Spanish. doi: 10.1016/j.ad.2019.07.007.

96. Hessam S, Sand M, Gambichler T, Skrygan M, Rüddel I, Bechara FG. Interleukin-36 in hidradenitis suppurativa: evidence for a distinctive proinflammatory role and a key factor in the development of an inflammatory loop. Br J Dermatol. 2018 Mar;178(3):761-767. doi: 10.1111/bjd.16019.

97. Rosi E, Fastame MT, Scandagli I, Di Cesare A, Ricceri F, Pimpinelli N, Prignano F. Insights into the Pathogenesis of HS and Therapeutical Approaches. Biomedicines. 2021 Sep 6;9(9):1168. doi: 10.3390/biomedicines9091168.

98. Hotz C, Boniotto M, Guguin A, Surenaud M, Jean-Louis F, Tisserand P, Ortonne N, Hersant B, Bosc R, Poli F, Bonnabau H, Thiébaut R, Godot V, Wolkenstein P, Hocini H, Lévy Y, Hüe S. Intrinsic Defect in Keratinocyte Function Leads to Inflammation in Hidradenitis Suppurativa. J Invest Dermatol. 2016 Sep;136(9):1768-1780. doi: 10.1016/j.jid.2016.04.036.

99. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. Lancet. 2016 Oct 22;388(10055):2023-2038. doi: 10.1016/S0140-6736(16)30173-8. Epub 2016 May 3. Erratum in: Lancet. 2016 Oct 22;388(10055):1984.

100. Schett G, Gravallese E. Bone erosion in rheumatoid arthritis: mechanisms, diagnosis and treatment. Nat Rev Rheumatol. 2012 Nov;8(11):656-64. doi: 10.1038/nrrheum.2012.153.

101. Shapiro JS. Wrist involvement in rheumatoid swan-neck deformity. J Hand Surg Am. 1982 Sep;7(5):484-91. doi: 10.1016/s0363-5023(82)80045-2.

102. Nielsen SF, Bojesen SE, Schnohr P, Nordestgaard BG. Elevated rheumatoid factor and long term risk of rheumatoid arthritis: a prospective cohort study. BMJ. 2012 Sep

6;345:e5244. doi: 10.1136/bmj.e5244.

103. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med. 2011 Dec 8;365(23):2205-19. doi: 10.1056/NEJMra1004965.

104. Edilova MI, Akram A, Abdul-Sater AA. Innate immunity drives pathogenesis of rheumatoid arthritis. Biomed J. 2021 Apr;44(2):172-182. doi: 10.1016/j.bj.2020.06.010.

105. Lubberts E. The IL-23-IL-17 axis in inflammatory arthritis. Nat Rev Rheumatol. 2015 Jul;11(7):415-29. doi: 10.1038/nrrheum.2015.53. Epub 2015 Apr 28. Erratum in: Nat Rev Rheumatol. 2015 Oct;11(10):562.

106. Bartok B, Firestein GS. Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. Immunol Rev. 2010 Jan;233(1):233-55. doi: 10.1111/j.0105-2896.2009.00859.x.

107. van Drongelen V, Holoshitz J. Human Leukocyte Antigen-Disease Associations in Rheumatoid Arthritis. Rheum Dis Clin North Am. 2017 Aug;43(3):363-376. doi: 10.1016/j.rdc.2017.04.003.

108. Gerli R, Bistoni O, Russano A, Fiorucci S, Borgato L, Cesarotti ME, Lunardi C. In vivo activated T cells in rheumatoid synovitis. Analysis of Th1- and Th2-type cytokine production at clonal level in different stages of disease. Clin Exp Immunol. 2002 Sep;129(3):549-55. doi: 10.1046/j.1365-2249.2002.01913.x.

109. Chen J, Li J, Gao H, Wang C, Luo J, Lv Z, Li X. Comprehensive evaluation of different T-helper cell subsets differentiation and function in rheumatoid arthritis. J Biomed Biotechnol. 2012;2012:535361. doi: 10.1155/2012/535361.

110. Roberts CA, Dickinson AK, Taams LS. The Interplay Between Monocytes/Macrophages and CD4(+) T Cell Subsets in Rheumatoid Arthritis. Front Immunol. 2015 Nov 19;6:571. doi: 10.3389/fimmu.2015.00571.

111. Paulissen SM, van Hamburg JP, Davelaar N, Asmawidjaja PS, Hazes JM, Lubberts E. Synovial fibroblasts directly induce Th17 pathogenicity via the cyclooxygenase/prostaglandin E2 pathway, independent of IL-23. J Immunol. 2013 Aug 1;191(3):1364-72. doi: 10.4049/jimmunol.1300274.

112. Katano M, Okamoto K, Arito M, Kawakami Y, Kurokawa MS, Suematsu N, Shimada S, Nakamura H, Xiang Y, Masuko K, Nishioka K, Yudoh K, Kato T. Implication of granulocyte-macrophage colony-stimulating factor induced neutrophil gelatinase-associated lipocalin in pathogenesis of rheumatoid arthritis revealed by proteome analysis. Arthritis Res Ther. 2009;11(1):R3. doi: 10.1186/ar2587.

113. Orr C, Vieira-Sousa E, Boyle DL, Buch MH, Buckley CD, Cañete JD, Catrina AI, Choy EHS, Emery P, Fearon U, Filer A, Gerlag D, Humby F, Isaacs JD, Just SA, Lauwerys BR, Le Goff B, Manzo A, McGarry T, McInnes IB, Najm A, Pitzalis C, Pratt A, Smith M, Tak PP, Thurlings R, Fonseca JE, Veale DJ, Tas SW. Synovial tissue research: a state-of-the-art review. Nat Rev Rheumatol. 2017 Aug;13(8):463-475. doi: 10.1038/nrrheum.2017.115. Epub 2017 Jul 13. Erratum in: Nat Rev Rheumatol. 2017

Sep 22;13(10 ):630. Erratum in: Nat Rev Rheumatol. 2017 Dec 19;14 (1):60.

114.Na HS, Park JS, Cho KH, Kwon JY, Choi J, Jhun J, Kim SJ, Park SH, Cho ML. Interleukin-1-Interleukin-17 Signaling Axis Induces Cartilage Destruction and Promotes Experimental Osteoarthritis. Front Immunol. 2020 May 5;11:730. doi: 10.3389/fimmu.2020.00730. Erratum in: Front Immunol. 2020 Aug 19;11:1862.

115.Tanaka Y. Clinical immunity in bone and joints. J Bone Miner Metab. 2019 Jan;37(1):2-8. doi: 10.1007/s00774-018-0965-5.

116.van der Pouw Kraan TC, van Gaalen FA, Kasperkovitz PV, Verbeet NL, Smeets TJ, Kraan MC, Fero M, Tak PP, Huizinga TW, Pieterman E, Breedveld FC, Alizadeh AA, Verweij CL. Rheumatoid arthritis is a heterogeneous disease: evidence for differences in the activation of the STAT-1 pathway between rheumatoid tissues. Arthritis Rheum. 2003 Aug;48(8):2132-45. doi: 10.1002/art.11096.

117.Bresnihan B, Alvaro-Gracia JM, Cobby M, Doherty M, Domljan Z, Emery P, Nuki G, Pavelka K, Rau R, Rozman B, Watt I, Williams B, Aitchison R, McCabe D, Musikic P. Treatment of rheumatoid arthritis with recombinant human interleukin-1 receptor antagonist. Arthritis Rheum. 1998 Dec;41(12):2196-204. doi: 10.1002/1529-0131(199812)41:12<2196::AID-ART15>3.0.CO;2-2.

118.Ritchlin CT, Colbert RA, Gladman DD. Psoriatic Arthritis. N Engl J Med. 2017 Mar 9;376(10):957-970. doi: 10.1056/NEJMra1505557. Erratum in: N Engl J Med. 2017 May 25;376(21):2097.

119.Stuart PE, Nair RP, Tsoi LC, Tejasvi T, Das S, Kang HM, Ellinghaus E, Chandran V, Callis-Duffin K, Ike R, Li Y, Wen X, Enerbäck C, Gudjonsson JE, Kõks S, Kingo K, Esko T, Mrowietz U, Reis A, Wichmann HE, Gieger C, Hoffmann P, Nöthen MM, Winkelmann J, Kunz M, Moreta EG, Mease PJ, Ritchlin CT, Bowcock AM, Krueger GG, Lim HW, Weidinger S, Weichenthal M, Voorhees JJ, Rahman P, Gregersen PK, Franke A, Gladman DD, Abecasis GR, Elder JT. Genome-wide Association Analysis of Psoriatic Arthritis and Cutaneous Psoriasis Reveals Differences in Their Genetic Architecture. Am J Hum Genet. 2015 Dec 3;97(6):816-36. doi: 10.1016/j.ajhg.2015.10.019.

120.Suzuki E, Mellins ED, Gershwin ME, Nestle FO, Adamopoulos IE. The IL-23/IL-17 axis in psoriatic arthritis. Autoimmun Rev. 2014 Apr-May;13(4-5):496-502. doi: 10.1016/j.autrev.2014.01.050.

121.Sherlock JP, Joyce-Shaikh B, Turner SP, Chao CC, Sathe M, Grein J, Gorman DM, Bowman EP, McClanahan TK, Yearley JH, Eberl G, Buckley CD, Kastelein RA, Pierce RH, Laface DM, Cua DJ. IL-23 induces spondyloarthropathy by acting on ROR-γt+ CD3+CD4-CD8- entheseal resident T cells. Nat Med. 2012 Jul 1;18(7):1069-76. doi: 10.1038/nm.2817.

122.Menon B, Gullick NJ, Walter GJ, Rajasekhar M, Garrood T, Evans HG, Taams LS, Kirkham BW. Interleukin-17+CD8+ T cells are enriched in the joints of patients with psoriatic arthritis and correlate with disease activity and joint damage progression. Arthritis Rheumatol. 2014 May;66(5):1272-81. doi: 10.1002/art.38376.

123. Xu X, Davelaar N, Mus AM, Asmawidjaja PS, Hazes JMW, Baeten DLP, Vis M, Bisoendial RJ, Prens EP, Lubberts E. Interleukin-17A Is Produced by CD4+ but Not CD8+ T Cells in Synovial Fluid Following T Cell Receptor Activation and Regulates Different Inflammatory Mediators Compared to Tumor Necrosis Factor in a Model of Psoriatic Arthritis Synovitis. Arthritis Rheumatol. 2020 Aug;72(8):1303-1313. doi: 10.1002/art.41271.

124. Blijdorp ICJ, Menegatti S, van Mens LJJ, van de Sande MGH, Chen S, Hreggvidsdottir HS, Noordenbos T, Latuhihin TE, Bernink JH, Spits H, Rogge L, Baeten DLP, Yeremenko NG. Expansion of Interleukin-22- and Granulocyte-Macrophage Colony-Stimulating Factor-Expressing, but Not Interleukin-17A-Expressing, Group 3 Innate Lymphoid Cells in the Inflamed Joints of Patients With Spondyloarthritis. Arthritis Rheumatol. 2019 Mar;71(3):392-402. doi: 10.1002/art.40736.

125. Lin AM, Rubin CJ, Khandpur R, Wang JY, Riblett M, Yalavarthi S, Villanueva EC, Shah P, Kaplan MJ, Bruce AT. Mast cells and neutrophils release IL-17 through extracellular trap formation in psoriasis. J Immunol. 2011 Jul 1;187(1):490-500. doi: 10.4049/jimmunol.1100123.

126. Noordenbos T, Blijdorp I, Chen S, Stap J, Mul E, Cañete JD, Lubberts E, Yeremenko N, Baeten D. Human mast cells capture, store, and release bioactive, exogenous IL-17A. J Leukoc Biol. 2016 Sep;100(3):453-62. doi: 10.1189/jlb.3HI1215-542R.

127. Tamassia N, Arruda-Silva F, Calzetti F, Lonardi S, Gasperini S, Gardiman E, Bianchetto-Aguilera F, Gatta LB, Girolomoni G, Mantovani A, Vermi W, Cassatella MA. A Reappraisal on the Potential Ability of Human Neutrophils to Express and Produce IL-17 Family Members *In Vitro*: Failure to Reproducibly Detect It. Front Immunol. 2018 Apr 17;9:795. doi: 10.3389/fimmu.2018.00795.

128. Monin L, Gaffen SL. Interleukin 17 Family Cytokines: Signaling Mechanisms, Biological Activities, and Therapeutic Implications. Cold Spring Harb Perspect Biol. 2018 Apr 2;10(4):a028522. doi: 10.1101/cshperspect.a028522.

129. Veale DJ, Fearon U. The pathogenesis of psoriatic arthritis. Lancet. 2018 Jun 2;391(10136):2273-2284. doi: 10.1016/S0140-6736(18)30830-4.

130. McGonagle DG, McInnes IB, Kirkham BW, Sherlock J, Moots R. The role of IL-17A in axial spondyloarthritis and psoriatic arthritis: recent advances and controversies. Ann Rheum Dis. 2019 Sep;78(9):1167-1178. doi: 10.1136/annrheumdis-2019-215356. Epub 2019 Jul 5. Erratum in: Ann Rheum Dis. 2020 Jan;79(1):e12.

131. Merola JF, Espinoza LR, Fleischmann R. Distinguishing rheumatoid arthritis from psoriatic arthritis. RMD Open. 2018 Aug 13;4(2):e000656. doi: 10.1136/rmdopen-2018-000656.

132. Reece RJ, Canete JD, Parsons WJ, Emery P, Veale DJ. Distinct vascular patterns of early synovitis in psoriatic, reactive, and rheumatoid arthritis. Arthritis Rheum. 1999 Jul;42(7):1481-4. doi: 10.1002/1529-0131(199907)42:7<1481::AID-ANR23>3.0.CO;2-E.

133. Kruithof E, Baeten D, De Rycke L, Vandooren B, Foell D, Roth J, Cañete JD, Boots AM, Veys EM, De Keyser F. Synovial histopathology of psoriatic arthritis, both oligo- and polyarticular, resembles spondyloarthropathy more than it does rheumatoid arthritis. Arthritis Res Ther. 2005;7(3):R569-80. doi: 10.1186/ar1698.

134. Celis R, Cuervo A, Ramírez J, Cañete JD. Psoriatic Synovitis: Singularity and Potential Clinical Implications. Front Med (Lausanne). 2019 Feb 11;6:14. doi: 10.3389/fmed.2019.00014.

135. Li SJ, Perez-Chada LM, Merola JF. TNF Inhibitor-Induced Psoriasis: Proposed Algorithm for Treatment and Management. J Psoriasis Psoriatic Arthritis. 2019 Apr;4(2):70-80. doi: 10.1177/2475530318810851.

136. Tillack C, Ehmann LM, Friedrich M, Laubender RP, Papay P, Vogelsang H, Stallhofer J, Beigel F, Bedynek A, Wetzke M, Maier H, Koburger M, Wagner J, Glas J, Diegelmann J, Koglin S, Dombrowski Y, Schauber J, Wollenberg A, Brand S. Anti-TNF antibody-induced psoriasiform skin lesions in patients with inflammatory bowel disease are characterised by interferon-γ-expressing Th1 cells and IL-17A/IL-22-expressing Th17 cells and respond to anti-IL-12/IL-23 antibody treatment. Gut. 2014 Apr;63(4):567-77. doi: 10.1136/gutjnl-2012-302853.

137. Kalliolias GD, Ivashkiv LB. TNF biology, pathogenic mechanisms and emerging therapeutic strategies. Nat Rev Rheumatol. 2016 Jan;12(1):49-62. doi: 10.1038/nrrheum.2015.169.

138. Sims JE, Smith DE. The IL-1 family: regulators of immunity. Nat Rev Immunol. 2010 Feb;10(2):89-102. doi: 10.1038/nri2691.

139. Choy EH, De Benedetti F, Takeuchi T, Hashizume M, John MR, Kishimoto T. Translating IL-6 biology into effective treatments. Nat Rev Rheumatol. 2020 Jun;16(6):335-345. doi: 10.1038/s41584-020-0419-z.

140. Wendling D, Letho-Gyselinck H, Guillot X, Prati C. Psoriasis onset with tocilizumab treatment for rheumatoid arthritis. J Rheumatol. 2012 Mar;39(3):657. doi: 10.3899/jrheum.111166.

141. Viguier M, Guigue P, Pagès C, Smahi A, Bachelez H. Successful treatment of generalized pustular psoriasis with the interleukin-1-receptor antagonist Anakinra: lack of correlation with IL1RN mutations. Ann Intern Med. 2010 Jul 6;153(1):66-7. doi: 10.7326/0003-4819-153-1-201007060-00030.

142. Blauvelt A. IL-6 Differs from TNF-α: Unpredicted Clinical Effects Caused by IL-6 Blockade in Psoriasis. J Invest Dermatol. 2017 Mar;137(3):541-542. doi: 10.1016/j.jid.2016.11.022.

# 2

## IL-17A is produced by synovial fluid CD4+ but not CD8+ T cells after TCR activation and regulates different inflammatory mediators compared to TNF in a synovitis model of psoriatic arthritis

Xiaofei Xu, Nadine Davelaar, Anne-Marie Mus, Patrick S. Asmawidjaja, Johanna M.W. Hazes, Dominique L.P. Baeten, Marijn Vis, Radjesh J. Bisoendial, Errol P. Prens, Erik Lubberts

Arthritis Rheumatol. 2020 Aug;72(8):1303-1313.

## ABSTRACT

***Objective:*** Interleukin-17A (IL-17A) and TNFα contribute to the pathogenesis of psoriatic arthritis (PsA). However, their functional relationship in PsA synovitis has not been fully elucidated. In addition, although CD8+ T-cells in PsA have been recognized as a source of IL-17A using flow cytometry, it is not clear whether CD8+ T-cells secrete IL-17A under more physiological conditions. Clarifying these issues are the objectives of this study.

***Methods:*** IL-17A+ T-cells were identified in PsA synovial fluid (SF, n=20), treatment-naïve PsA blood and healthy blood (n=22 each) by flow cytometry. IL-17A+ T-cells were sorted from PsA SF (n=12) and stimulated using anti-CD3/anti-CD28 or phorbol myristate acetate and ionomycin (PMA/ion) *ex vivo* alone (n=3), with autologous monocytes (n=3) or with allogeneic PsA fibroblast-like synoviocytes (PsA-FLS, n=5-6). To evaluate the differential effects of neutralizing IL-17A and TNFα, SF CD4+ T-cell and PsA-FLS co-cultures were used (n=5-6).

***Results:*** PsA-SF CD4+ (0.71 (0.35-1.50)%, median (IQR)) and CD8+ T-cells (0.44 (0.17-1.86)%) are IL-17A+ using flow cytometry. However, only CD4+ T-cells secreted IL-17A after anti-CD3/anti-CD28 activation ($p<0.05$). Similar results were observed in co-cultures with PsA monocytes or PsA-FLS ($p<0.05$). Remarkably, CD8+ T-cells only secreted IL-17A after 4- or 72-hours stimulation with PMA/ion. Anti-IL-17A and anti-TNF treatments both inhibited PsA synovitis *ex vivo*. Neutralizing IL-17A strongly inhibited IL-6 ($p<0.05$) and IL-1β ($p<0.01$), while anti-TNF was more potent in reducing MMP-3 ($p<0.05$) and MMP-13.

***Conclusion:*** PsA-SF CD8+ T-cells, in contrast to CD4+ T-cells, did not secrete IL-17A after TCR activation. Overlapping but also distinct effects at the level of inflammatory cytokines and MMPs were found after neutralizing IL-17A or TNFα in a human *ex vivo* PsA synovitis model.

## INTRODUCTION

Psoriatic arthritis (PsA) is a chronic inflammatory arthritis that develops in up to 30% of patients with a history of or active psoriasis (1). Activated T cells have long been reported to contribute to arthropathies including PsA pathogenesis (2), and therapies depleting lymphocytes were tested in PsA patients with limited clinical response (3). The lack of efficacy during depletion therapy was attributed to a modest synovial lymphopenia despite of significant reduction in peripheral blood (3). This pinpoints to the pathogenic role of local T cells in PsA joints. Moreover, enhanced local clonal expansions of CD4+ and CD8+ T cells were identified in PsA synovial fluid compared to PsA peripheral blood (4), further suggesting that intra-articular T cell activation drives PsA joint inflammation.

Activated T cells excrete a wide range of proinflammatory cytokines including IL-17A and TNFα, both of which have been shown to be elevated in PsA synovial fluid or synovium (5-7). Evidence from studies of PsA patients and other arthropathies points to the involvement of IL-17A in arthritis pathogenesis (8,9). It has been suggested that CD4+ (10,11), CD8+ T cells (12-14) and group 3 innate lymphoid cells (ILC3) (15) can be potential sources of IL-17A in PsA synovial fluid or synovium. However, which of the above cell types is the main producer of IL-17A in local PsA joints is still not clear. Recently, ILC3 reportedly fail to express IL-17A upon *in vitro* stimulation in spondyloarthritis joints (16). Nevertheless, direct *ex vivo* comparison of IL-17A production upon T cell receptor (TCR) activation by CD4+ and CD8+ T cells remains unknown in PsA synovial fluid.

TNFα is a proinflammatory cytokine present at high levels in PsA (5,6). Neutralization of TNFα proved to be effective in reducing local inflammation while slowing or halting joint destruction in patients with PsA (17). However, with success rates of around 60-70% in active PsA patients, standard anti-TNF therapies fail to achieve satisfactory results in remaining non-responding patients (18,19). Even among responders, initial efficacy with TNFα inhibition fails to sustain in subgroups of patients (18). Interestingly, in psoriasis patients not responsive to the TNF blocker, etanercept, persistent levels of serum IL-17A were observed (20). Recent research showed that antibodies targeting IL-

17A, including secukinumab and ixekizumab, were effective in treating PsA patients (21,22) and both were approved for treatment in active PsA. Similar to anti-TNF biologics, IL-17A blockades also successfully suppress joint inflammation and prevent radiographic progression (21,22). Patients intolerant or irresponsive to TNFα inhibition still showed disease improvement with anti-IL-17A therapy (23,24). Further understanding of potential overlapping and distinct roles between anti-IL-17A and anti-TNF treatments is still relevant. It will help to guide future clinical practice and potentially achieve more sustainable therapeutic effects for patients with PsA.

## MATERIAL & METHODS

### Study design

IL-17A+ CD4+ and CD8+ T cells were first identified using flow cytometry in: 1) synovial fluid (SF) of active PsA patients; 2) peripheral blood from treatment naïve early PsA patients; and 3) peripheral blood from age and sex matched healthy volunteers as controls. SF CD4+ and CD8+ T cells were then sorted to compare ex vivo IL-17A secretion with or without stimulation (anti-CD3/anti-CD28 or PMA/ion). Sorted CD4+ and CD8+ T cells were further co-cultured with autologous PsA monocytes or allogeneic PsA fibroblasts to check ex vivo IL-17A secretion. With TCR activation, sorted SF CD4+ T cells were co-cultured with PsA fibroblasts to evaluate the effects of IL-17A neutralization in comparison to TNF blockade.

### Patients

Synovial fluid (SF) of active PsA patients (n=20) were collected. Peripheral blood of treatment naïve early PsA patients and age-/sex-matched healthy volunteers (n=22 each) were also included. All PsA diagnoses were performed by rheumatologists according to classification for psoriatic arthritis (CASPAR) criteria. This study is part of the Dutch South-West Psoriatic Arthritis Register (DEPAR) study and is approved by medical ethics review board of Erasmus Medical Center Rotterdam. Details of PsA patients and healthy volunteers are summarized in supplementary table S1.

### Flow cytometry and cell sorting

Cell pellets from PsA-SF were stained for surface markers following standard practice with or without density gradient centrifugation. Peripheral blood of early PsA patients and healthy volunteers were additionally lysed for red blood cells and stained for surface markers as stated above. For intracellular staining, parts of the cell pellets were stimulated for 4 hours (hrs) with 50 ng/ml phorbol myristate acetate (PMA) (Sigma-Aldrich), 500 ng/ml ionomycin and Golgistop (BD Biosciences). Afterwards cells were stained for surface markers and Fixable Viability Dye eF506 (eBioscience 65-0866-14) following manufacturer's instructions. Cells were then fixed with 2% paraformaldehyde in phosphate buffered saline (PBS) and permeabilized with 0.5% saponin buffer (0.5% BSA, 0.05% NaN3 in PBS).

Sorted CD4+ and CD8+ T cells were *ex vivo* cultured or co-cultured for 72 hours and restimulated to examine intracellular staining as described above. LSR II flow cytometer (BD Biosciences) was used to analyze samples and FlowJo software (TreeStar) was used to process results. Cells were sorted with FACSAria cell sorter (BD Biosciences) and purity of obtained cell populations was ≥ 98%.

**Flow cytometry antibodies**

Following antibodies were used during staining. From BD Biosciences: CD8-PE-CF594 (clone RPA-T8), TCRγδ-PE Cy7 (clone 11F2), CD19-PE Cy7 (clone SJ25C1), CD45-PE-CF594 (clone HI30), CD14-APC H7 (clone MϕP9), CD45RO-PerCP Cy5.5 (clone UCHL1). From BioLegend: CD56-AF700 (clone 5.1H11), CD15-AF700 (clone W6D3), CD16-BV785 (clone 3G8), IFN-γ-AF488 (clone 4S.B3), TNFα-BV421 (clone MAb11), CD25-PE Cy7 (clone BC96). From eBioscience: IL-17A-PE (clone eBio64DEC17), IL-22-eF660 (clone 22URTI). From Sony Biotechnology: CD45-PerCP Cy5.5 (clone HI30), CD3-BV785 (clone OKT3), CD4-BV711 (clone OKT4), CD4-FITC (clone RPA-T4).

**Cell culture**

Sorted T cells were seeded $2.5\times10^4$ per well in 96-well round bottom culture plates and stimulated with 0.3 µg/ml soluble anti-CD3 and 0.4 µg/ml soluble anti-CD28 (both from Sanquin, Amsterdam, The Netherlands) for 4 or 72 hrs in IMDM (Lonza) supplemented with 10% fetal calf serum (Invitrogen), 100 U/ml penicillin/streptomycin, 2 mM L-Glutamine (both from Lonza) and 50 µM β-

mercapto-ethanol (Merck). PsA-FLS was isolated and cultured as described previously (25). After reaching 90% confluence, allogeneic PsA-FLS was seeded $1.0\times10^4$ per well in 96-well flat bottom culture plates and after overnight incubation, $2.5\times10^4$ T cells per well were added with soluble anti-CD3/anti-CD28 as above for 72 hrs. In co-cultures with autologous monocytes, $1.0\times10^4$ per well CD14+ monocytes were seeded with $2.5\times10^4$ per well T cells in 96-well round bottom culture plates and incubated with soluble anti-CD3/anti-CD28 for 72 hrs.

During neutralization experiments, 100 µg/ml anti-IL-17 (secukinumab, Novartis) and 1µg/ml anti-TNF (adalimumab, AbbVie) were used. An isotype IgG1κ (Sigma-Aldrich) was included as control and soluble anti-CD3/anti-CD28 activation was used as stimulation.

**Enzyme-linked immunosorbent assay (ELISA)**

IFN-γ, IL-6 and IL-8 in culture supernatants were measured with ELISA (Invitrogen) following manufacturer's instructions. IL-17A, TNFα, MMP-1 and MMP-3 were measured with ELISA Duoset (R&D systems) following manufacturer's instructions.

**Reverse transcription and real-time polymerase chain reaction (RT-PCR)**

RNA was isolated with Total RNA Miniprep Kit (Sigma Aldrich). cDNA was synthesized with 10U/µl Superscript II after treatment with 0.1 U/µl DNAse (both from Invitrogen). RT-PCR was performed with ViiA7 sequence detection system (Life Technologies). Probes were chosen from the universal probe library (Roche Applied Science) and primers were designed using ProbeFinder software. Gene expression data was normalized to house-keeping gene hypoxanthine-guanine phosphoribosyltransferase (HPRT). Primer sequences are available upon request.

**Statistical analysis**

Flow cytometry results were shown as median (interquartile range (IQR)) and *ex vivo* stimulation data are mean ± standard error (SEM). Statistical differences were determined with paired or unpaired student's *t* test. All data analyses were performed with GraphPad Prism V5 and P-values $<0.05$ were considered as significant.

## RESULTS

### Subsets of lymphoid and myeloid cells, except B cells and NK cells, are comparable in PsA synovial fluid and peripheral blood

Peripheral blood from early PsA and matched healthy volunteers (n=22 each) as well as synovial fluid (SF) from active PsA patients (n=20), were stained for surface markers to identify immune cells. Gating strategies for cell populations were shown in supplementary figure S1. As shown in figure 1A, percentages of CD3+ T cells were 44.8 (8.6-55.4)% (median (IQR)) in PsA-SF, 43.3 (25.6-58.2)% in PsA blood and 47.5 (26.7-60.3)% in blood of healthy volunteers. For myeloid cells, percentages of CD14+

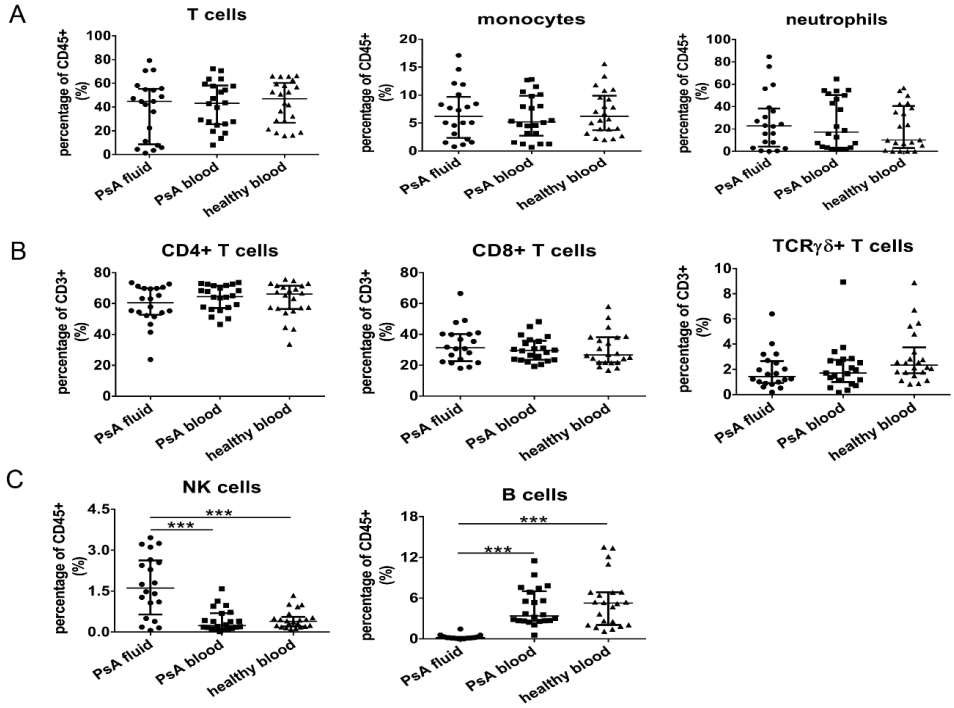



**Figure 1**. **Differences in percentages of B cells and NK cells in PsA synovial fluid compared to PsA and healthy blood.** SF (n=20) of active PsA patients, peripheral blood of early PsA patients (n=22) and age/sex- matched healthy volunteers (n=22) were stained for surface markers. Gating as shown in supplementary figure S1. **A**. Frequencies of T cells (CD3+), myeloid cells (CD14+), and neutrophils (CD15+CD16+) among CD45+ immune cells. **B.** Among T cells, percentages of CD4+, CD8+ and γδT subsets were shown. **C.** Percentages of B cells (CD3-CD19+) and NK cells (CD3-CD56bright) among CD45+ immune cells. Data are median values and interquartile ranges, and asterisks indicate significance: ***p<0.001.

monocytes in PsA-SF, PsA blood and healthy blood, were 6.2 (2.4-9.7)%, 5.2 (2.7- 9.9)%, and 6.2 (3.7-9.9)%, respectively, while percentages of CD15+CD16+ neutrophils were 22.8 (4.3-38.3)%, 17.3 (3.3-50.4)%, and 10.2 (3.1-40.6)% (figure 1A).

Among CD3+ T cell subsets, CD4+ T cells on average accounted for over 60% of the T cell population with 60.5 (52.7-69.9)% in PsA-SF, 64.6 (57.1-71.7)% in PsA blood, and 66.2 (56.5-71.6)% in healthy blood (figure 1B). About 30% of total T cells were CD8+ with 31.3 (22.6-40.3)% in PsA-SF, 29.7 (23.5-35.8)% in PsA blood, and 26.7 (21.9-38.2)% in healthy blood (figure 1B). TCRγδ+ T cell percentages were around 1.5-2.5% among groups with 1.4 (0.92-2.7)% in PsA-SF, 1.7 (0.99-2.8)% in PsA blood, and 2.3 (1.7-3.7)% in healthy blood (figure 1B).

As shown in figure 1C, significant enhanced accumulation of CD56+(bright) NK cells in PsA-SF was observed comparing to blood of either treatment naïve early PsA patients or healthy volunteers (1.62 (0.64-2.62)% PsA-SF versus 0.24 (0.13-0.69)% PsA blood and 0.39 (0.20-0.56)% healthy blood). On the contrary, CD19+ B cell percentage in PsA-SF was significantly lower in comparison to either blood samples with 0.17 (0.08-0.30)% in PsA-SF, 3.38 (2.65-7.71)% in PsA blood, and 5.26 (2.05-6.87)% in healthy blood, respectively (figure 1C).

**Enriched percentage of IL-17A+ CD8+ T cells in PsA synovial fluid compared to peripheral blood**

Cell pellets from active PsA-SF (n=20), blood from treatment naïve early PsA and healthy volunteers (n=22 each) were intracellularly stained for IL-17A, IFN-γ, TNFα and IL-22. Surface marker CD4 but not CD8 was down-regulated by PMA and ionomycin (PMA/iono) stimulation during intracellular staining (supplementary figure S2). Therefore, the CD45+CD3+CD8- population was considered CD4+ T cells. Figure 2A showed representative staining results of IL-17A, IFN-γ, TNFα and IL-22 among CD4+ and CD8+ T cells in PsA-SF, PsA blood and blood of healthy volunteers.

In PsA-SF, 0.71 (0.35-1.50)% (median (IQR)) of CD4+ T cells was IL-17A+ and not significantly different comparable to 0.44 (0.17-1.86)% IL-17A+ CD8+ T cells (figure 2B). In PsA blood, however, the percentage of IL-17A+ CD8+ T

cells (0.13 (0.09-0.22)%) was significantly lower than IL-17A+ CD4+ T cells (0.95 (0.77-1.36)%) (figure 2B). Similar result was found in healthy blood (1.15 (0.73-1.78)% CD4 vs 0.13 (0.08-0.21)% CD8) (figure 2B). For IFN-γ staining, 44.4 (24.7-64.8)% CD8+ T cells were positive in PsA-SF, which is significantly higher than 28.8 (13.5-41.2)% of IFN-γ+ CD4+ T cells.

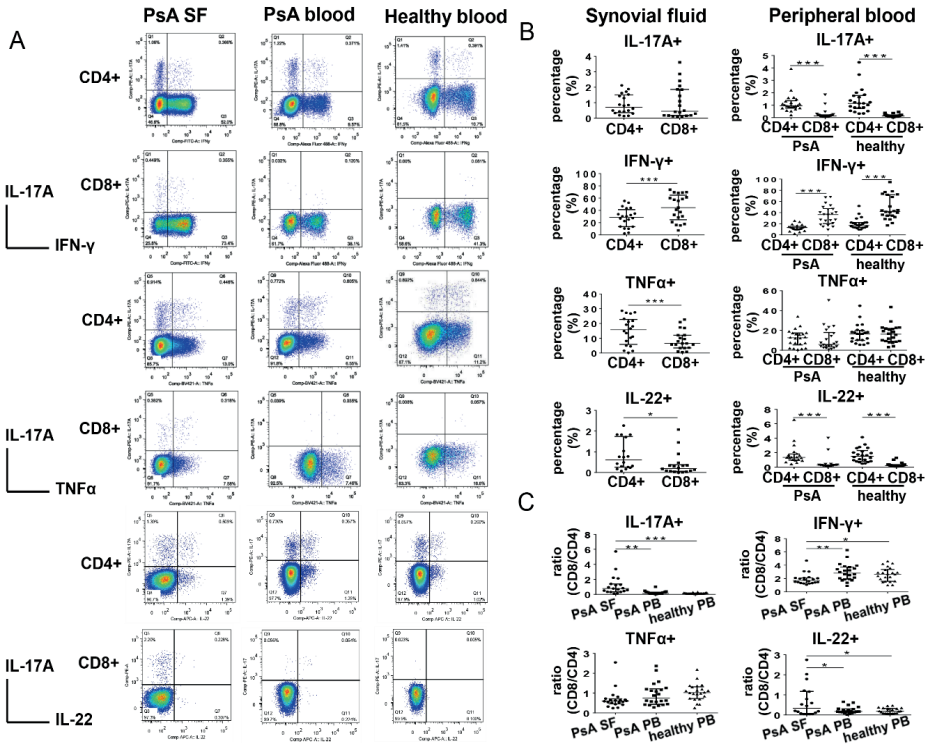



**Figure 2. Enriched percentage of IL-17A+ CD8+ T cells in PsA-SF compared to peripheral blood. A.** Representative intracellular staining results of IL-17A, IFN-γ TNFα and IL-22 among PsA-SF (n=20), PsA blood (n=22) and healthy blood (n=22) after 4hrs' PMA/ion stimulation. **B.** Summary of IL-17A+, IFN-γ TNFα and IL-22 staining results in CD4+ and CD8+ T cells in PsA-SF (left) and peripheral blood (right). **C.** Percentage ratio between CD8+ and CD4+ T cells of each individual sample was calculated and summarized for each cytokine staining. Data are median values and interquartile ranges, and asterisks indicate significance: *p<0.05 **p<0.01 ***p<0.001.

Similar differences were observed in PsA blood (13.1 (10.0-15.2)% CD4 vs 36.4 (20.3-47.2)% CD8) and healthy blood (17.6 (13.3-22.2)% CD4 vs 42.1 (34.0-72.0)%) (figure 2B). The percentage of TNFα+ CD4+ T cells was significantly higher than TNFα+ CD8+ T cells in PsA-SF (15.7 (5.8-22.7)% CD4 vs 6.5 (3.4-

12.1)% CD8) (figure 2B). However, similar results were observed between CD4+ and CD8+ T cells in PsA blood (12.5 (5.3-17.4)% CD4 vs 5.9 (3.5-17.9)% CD8) and healthy blood (16.5 (9.9-20.1)% CD4 vs 16.2 (9.1-22.0)% CD8) (figure 2B). IL-22+ cells were higher in CD4+ T cells than in CD8+ T cells in PsA-SF (0.61 (0.26-1.74)% CD4 vs 0.20 (0.06-0.38)% CD8) (figure 2B). Similar results were observed in PsA blood (1.35 (0.96-1.82)% CD4 vs 0.27 (0.16-0.32)% CD8) and healthy blood (1.51 (0.81-2.30)% CD4 vs 0.20 (0.15-0.34)% CD8).

To compare directly percentage differences between CD4+ and CD8+ T cells between PsA-SF, PsA blood and healthy blood, percentage ratios (PR) were calculated. The percentage of IL-17A+ CD8+ T cells was divided by the percentage of IL-17A+ CD4+ T cells. Similar calculations were performed for IFN-γ, TNFα and IL-22. With PR close to 1 in PsA-SF (0.77 (0.33-1.39), median (IQR)), it indicated that nearly equal percentages of CD8+ and CD4+ T cells were IL-17A+ (figure 2C). In contrast, PR in PsA and healthy blood were 0.12 (0.09-0.30) and 0.11 (0.08-0.18), both significantly lower than PsA-SF (figure 2C). This suggested that a specific enrichment of IL-17A+ CD8+ T cells over CD4+ T cells in PsA-SF compared to peripheral blood. For IFN-γ+ cells, PR equals to 1.72 (1.34-2.10) in PsA-SF, 2.81 (1.90-3.75) in PsA blood, and 2.67 (1.59-3.36) in healthy blood (figure 2C), indicating 2-3 times more IFN-γ+ CD8+ T cells versus IFN-γ+ CD4+ T cells in all groups. TNFα+ PRs were comparable in PsA-SF (0.60 (0.46-0.71)), PsA blood (0.75 (0.44-1.21)), and healthy blood (1.02 (0.72-1.31)) (figure 2C). This suggested that almost equal percentages of CD8+ and CD4+ T cells were TNFα+ in PsA-SF and peripheral blood. Similar to IL-17A, IL-22+ PR were significantly higher in PsA-SF (0.33 (0.07-1.17)) compared to in PsA blood (0.17 (0.14-0.31)) and healthy blood (0.19 (0.09-0.32)), indicating enhanced accumulation of IL-22+ CD8+ T cells over CD4+ T cells in PsA-SF compared to peripheral blood.

**CD4+ but not CD8+ T cells in PsA synovial fluid secrete IL-17A upon TCR activation**

To compare IL-17A production and secretion between CD4+ and CD8+ T cells, PsA-SF CD4+ and CD8+ T cells were sorted from synovial fluid mononuclear cells (SFMC). These sorted cells were anti-CD3/anti-CD28 stimulated and *ex vivo* cultured for 72 hours. Sorted CD4+ T cells did not include CD4+CD14+

monocytes and CD4+CD25high T regulatory cells. After 72 hours, the concentration of IL-17A was measured in culture supernatants using ELISA. In addition, cells were used for intracellular staining of IL-17A. For this, cells were stimulated with PMA/ion for another 4 hours. As shown in figure 3A, intracellular staining of IL-17A was positive for both CD4+ and CD8+ T cells. However, in contrast to the intracellular IL-17A staining, IL-17A was only secreted and measured in supernatants of CD4+ but not CD8+ T cells. Similar results were observed for IL-17F as shown in supplementary figure S3.

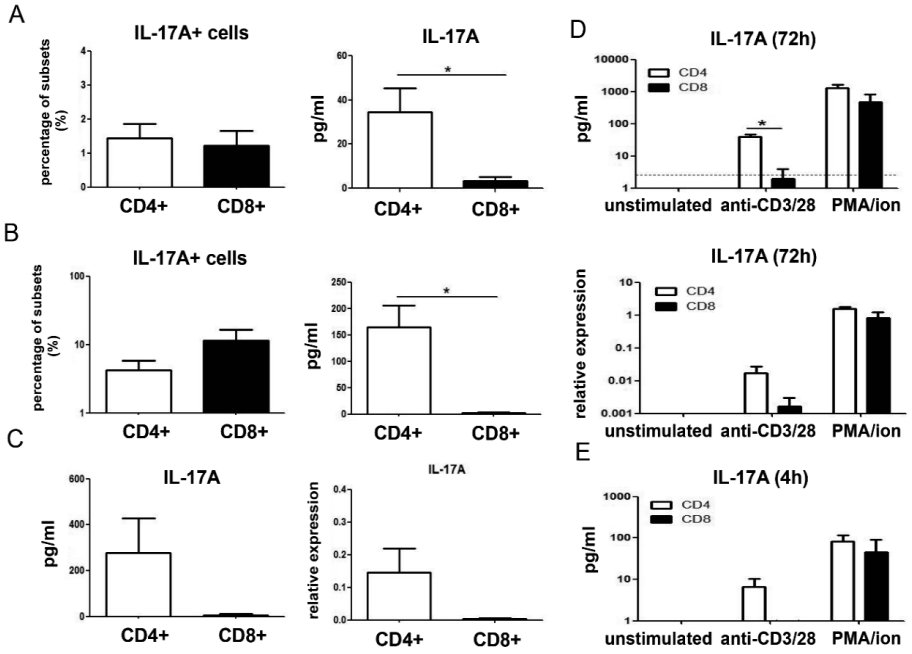



**Figure 3. PsA-SF CD4+ but not CD8+ T cells produce IL-17A upon anti-CD3/anti-CD28 activation. A.** IL-17A staining in cells and protein level in culture supernatants for sorted SF CD4+ and CD8+ T cells after 72 hrs' *ex vivo* anti-CD3/anti-CD28 activation (n=5 or 6 from two independent experiments). **B.** IL-17A staining in cells and protein level in culture supernatants for SF CD4+ and CD8+ T cells co-cultured with allogeneic PsA-FLS and activated with anti-CD3/anti-CD28 after 72 hrs (n=5 or 6 from two independent experiments). **C.** IL-17A in culture supernatants and mRNA expression in cells for SF CD4+ and CD8+ T cells after 72 hrs' *ex vivo* co-culture with autologous CD14+ monocytes and anti-CD3/anti-CD28 activation (different patients from A/B, n=3). **D.** IL-17A in culture supernatants and mRNA expression in cells for sorted SF CD4+ and CD8+ T cells after *ex vivo* stimulation with anti-CD3/anti-CD28, PMA/ion, or without stimulation for 72 hrs (different patients from A/B, n=3) (dash line indicates IL-17A

detection limit for IL-17A) and (**E**) IL-17A in culture supernatants after similar stimulation for 4 hrs (n=3). Data are mean ± SEM, and asterisks indicate significance: *p<0.05.

To further confirm that anti-CD3/anti-CD28 activation didn't result in secretion of IL-17A from CD8+ T cells, CD4+ and CD8+ T cells were sorted from PsA SFMC and co-cultured with allogeneic PsA fibroblast-like synoviocytes (FLS) or autologous CD14+ PsA monocytes. In line with the findings described above, flow cytometry revealed that CD8+ T cells stained positive for IL-17A after 3 days of co-culture with PsA-FLS (figure 3B). However, excreted IL-17A levels were not detectable in supernatants of anti-CD3/anti-CD28 activated CD8+ T cells co-cultures with PSA-FLS (figure 3B). Similarly, in co-cultures of 72 hours with autologous monocytes, anti-CD3/anti-CD28 activation resulted IL-17A secretion in co-culture supernatants and mRNA IL-17A expression only in CD4+ but not CD8+ T cells (figure 3C). To further explain this discrepancy, PsA-SF CD4+ and CD8+ T cells were cultured with anti-CD3/anti-CD28, PMA/ion, or without stimulation for 4 and 72 hrs. As shown in figure 3D, at 72 hrs, only CD4+ T cells produced IL-17A after anti-CD3/anti-CD28 activation at both protein and mRNA levels, while PMA/ion stimulated both CD4+ and CD8+ T cells to produce IL-17A. Similar results were confirmed with 72 hrs co-cultures of T cells and autologous monocytes (supplementary figure S4). More importantly, as shown in figure 3E, even after 4 hrs of PMA/ion stimulation, CD8+ T cells produced as much IL-17A as CD4+ T cells, but no IL-17A secretion was detectable by CD8+ T cells after 4 hrs of anti-CD3/anti-CD28 activation. Time-course analysis including 24, 48 and 72 hrs with another three PsA patients confirmed above findings (data not shown).

**IL-17A induces overlapping and differential effects in PsA fibroblast-like synoviocytes compared to TNFα**

As established above, PsA-SF CD4+ T cells are the main IL-17A producers with anti-CD3/anti-CD28 activation. To evaluate overlapping and differential therapeutic effects of TNFα and IL-17A blockers, CD4+ T cells were isolated from PsA SFMCs, stimulated with anti-CD3/anti-CD28 and co-cultured with allogeneic PsA fibroblast-like synoviocytes (FLS). Expression of IL-17RA and IL-17RC was confirmed on both PsA-FLS and CD14+ PsA monocytes

(supplementary figure S5). Pre-titrations of antibodies were performed as shown in supplementary figure S6, and concentrations were chosen to comparably achieve 60-70% of inhibition for each cytokine.

Anti-IL-17A and anti-TNF antibody treatment specifically and significantly reduced the production of either IL-17A or TNFα, respectively in the co-culture system (figure 4A). IFN-γ was not affected compared to an isotype antibody control (figure 4A). Both anti-IL-17A and anti-TNF treatments significantly reduced IL-8 levels compared with the isotype antibody group (figure 4B). Combining two antibodies showed a significant additive effect compared to anti-IL-17A alone (figure 4B). Interestingly, anti-TNF did not significantly influence IL-6 production in these co-cultures compared to isotype control, while anti-IL-17A significantly suppressed IL-6 level compared to both isotype control and anti-TNF (figure 4B). Similar results were observed during antibody titrations (supplementary figure S6). Although anti-TNF significantly reduced mRNA expression of IL-1β, it was significantly less effective compared to anti-IL-17A (figure 4B). Levels of MMP-1 were significantly down-regulated by the combination treatment of anti-IL-17A and anti-TNF compared to isotype control or anti-IL-17A alone (figure 4C). MMP-3 levels were significantly suppressed after neutralizing TNFα compared to both anti-IL-17A and isotype control (figure 4C). Similar to MMP-1, expression of MMP-9 was significantly reduced only by the combination of anti-IL-17A and anti-TNF compared to isotype control (figure 4C). Similar to MMP-3, anti-TNF significantly down-regulated the expression of MMP-13 compared to isotype control, and combination with anti-IL-17A achieved a significant further reduction of MMP-13 compared to either antibody alone (figure 4C).

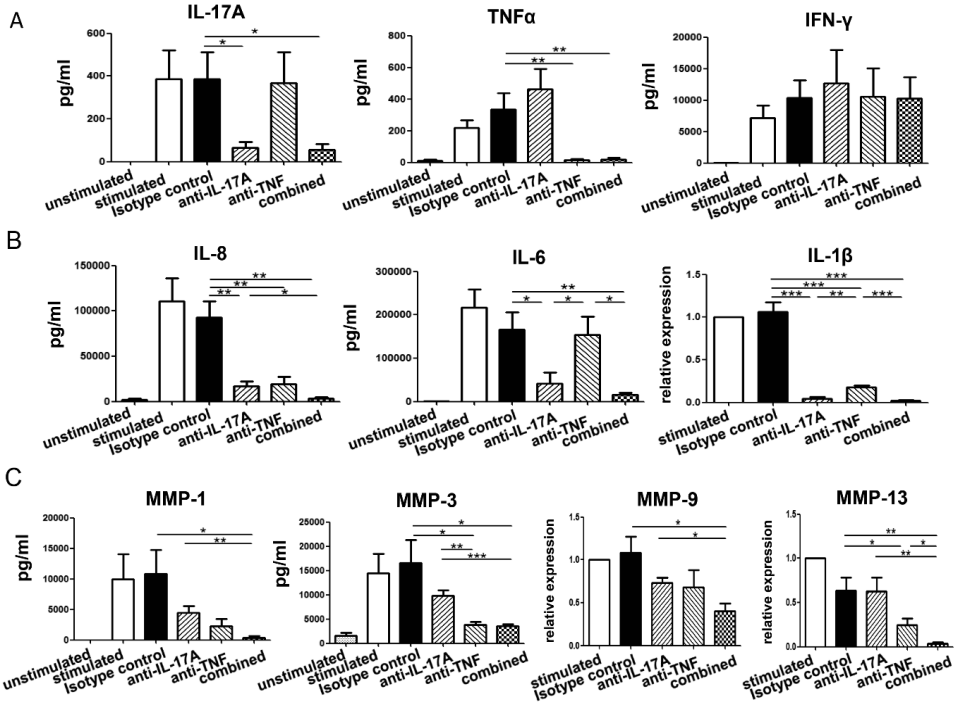



**Figure 4. IL-17A induces differential effects compared to TNFα in PsA FLS.** CD4+ T cells were sorted from PsA-SF and co-cultured with allogeneic PsA-FLS for 72 hrs with or without anti-CD3/anti-CD28 activation. **A.** Anti-IL-17A and anti-TNF specifically reduced IL-17A and TNFα, respectively, in culture supernatants compared to isotype control antibody, but not IFN-γ, in co-culture system. **B.** IL-6 and IL-8 levels in culture supernatants and IL-1β expression level in co-cultured cells after 72 hrs' co-culture. **C.** MMP-1 and MMP-3 levels in culture supernatants and MMP-9 and MMP-13 expression levels in co-culture cells after 72 hrs. Data (n=5 or 6) are pooled from two independent experiments and plotted as mean ± SEM. Asterisks indicate significance: *p<0.05 **p<0.01 ***p<0.001.

## DISCUSSION

The role of IL-17A in PsA pathogenesis is currently widely accepted following the clinical success of biologicals targeting the IL-17 pathway (21,22). Various cell types have been suggested to be a potential source of IL-17A in PsA. However, a detailed *ex vivo* comparison of IL-17A producing cells with various techniques is still relevant. Here we present evidence that CD4+ but not CD8+ T cells from synovial fluid of PsA patients excrete IL-17A upon *ex vivo* activation with anti-CD3/anti-CD28. This indicates that upon TCR activation, CD4+ T cells will be the main producer of IL-17A in local PsA joints despite that both are IL-17A-producing with PMA and ionomycin stimulation. To better reflect in-tissue situations, synovial fluid T cells were co-cultured with fibroblast-like synoviocytes. With this *ex vivo* PsA co-culture assay, we showed that anti-IL-17A and anti-TNF both have therapeutic effects in inhibiting the proinflammatory activation loop between T cells and stromal cells. Interestingly, differential effects between anti-IL-17A and anti-TNF were also observed. Anti-IL-17A exhibits stronger inhibition of inflammatory cytokines such as IL-6 and IL-1β, while anti-TNF is more potent in reducing the production of MMPs.

Earlier reports suggested that CD8+ T cells are predominant in PsA (26). However, our findings show that CD4+ T cells are the major T cell subset in PsA-SF similar to PsA blood. Possible explanations for the discrepancy may lie in the methods of handling samples. Firstly, during our experiments, surface staining was performed directly after cell pelleting without density gradient separation to keep all subsets as genuine as *in vivo.* Secondly, percentages of cell subsets were analyzed without *ex vivo* stimulation and separated from intracellular staining. PMA and ionomycin stimulation conventionally used during intracellular staining can strongly downregulate CD4 but not CD8 surface expression as shown in supplementary figure S1. Therefore, research conditions should represent the infiltrated immune cell characteristics in PsA synovial fluid of patients with PsA as closely as possible.

IL-17A+ CD8+ T cells have been reported in both psoriasis and PsA (12-14,27,28), and particularly in PsA, it was found that percentages of IL-17A+ CD8+ T cells correlate with disease activity and progression of joint damage (12). Our results confirmed the enrichment of IL-17A+ CD8+ T cells in PsA

synovial fluid. However, most of the previous published IL-17A+ results were obtained via intracellular staining with PMA and ionomycin stimulation (11-14,27,28). Such strong stimuli don't resemble the physiological state of T cell activation. On the contrary, anti-CD3/anti-CD28 simulation used in our study activates T cells *in vitro* by providing both primary TCR signal and secondary costimulatory signal. Our findings proved that CD8+ T cells *ex vivo* activated with anti-CD3/anti-CD28 did not produce measurable amounts of IL-17A in contrast to CD4+ T cells. Of note, both CD4+ and CD8+ T cells were IL-17A positive during intracellular staining. Furthermore, in a separate study by Raychaudhuri SP, et al (10), anti-CD3/anti-CD28 activation for three days was used during intracellular staining instead of PMA and ionomycin, and they found that percentages of IL-17A+ CD8+ T cells were negligible in PsA synovial fluid compared to distinct IL-17A+ CD4+ staining (10). However, in their study, mixed T cell subsets were activated simultaneously and chances of IL-17A endocytosis can't be ruled out. We avoided this caveat by sorting CD4+ and CD8+ T cells from PsA synovial fluid and clearly showed that CD4+ but not CD8+ T cells secrete IL-17A upon *ex vivo* anti-CD3/anti-CD28 activation. This indicated that in PsA joints, TCR activation promotes CD4+ T cells but not CD8+ T cells to produce IL-17A. Similar results were observed for IL-17F (supplementary figure S3), and it implies that CD4+ T cells may be the dominant source of both IL-17A and IL-17F in PsA synovial fluid upon TCR activation. Recently, a dual specific antibody targeting both IL-17A and IL-17F was tested in PsA and supported that neutralizing both could achieve rapid and sustained therapeutic effects (29). Group 3 ILCs were another cell type that has been reported as a potential source of IL-17A in PsA synovial fluid (15). However, a recent study reported that though these ILC3s are expanded in inflamed arthritis joints, they failed to express IL-17A upon *in vitro* stimulation (16). These data resemble what we have found for CD8+ T cells. Therefore, the contribution of CD8+ T cells and ILC3 to IL-17 production in PsA joints may be limited under physiological conditions in contrast to CD4+ T cells.

Biologics targeting IL-17A or TNFα prove effective in treating PsA patients, but clinical response rates vary considerably. Failure with one biologic will need to switch to an alternative, which can be a different biological from the same class or a biological with a different mode of action. We used an *ex vivo* model

of human PsA synovitis to explain differential effects and showed that although both biologics efficiently inhibited the proinflammatory loop in the co-culture system, distinct strengths existed between anti-IL-17A and anti-TNF. We provided evidence that neutralizing IL-17A reduced more strongly inflammatory cytokines such as IL-6 and IL-1β, while anti-TNF was more potent in reducing MMPs like MMP-3 and MMP-13. Therefore, to achieve better and sustainable treatment results, it is rational to target both IL-17A and TNFα. Recently, a bispecific antibody, ABT-122, has been developed, and first phase II study in PsA showed that dual inhibition had efficacy and safety similar to adalimumab, a TNF blocker (30, 31). Synergistic effects of anti-IL-17A and anti-TNF were also observed in our experiments and by others (32). Combinations or sequential therapy using both neutralizing agents may be another choice. However, adverse effects such as infection should be closely monitored during all dual neutralizations.

The percentage of IFNγ positive CD4+ and CD8+ T cells are relatively high in blood of patients with PsA compared to IL-17A. In addition, the levels of IFNγ were much higher compared to IL-17A and TNF in the human ex vivo PsA synovitis model. However, neutralization of IL-17A or TNF did not influence the level of IFNγ in these co culture experiments but significantly decreased the inflammation in our synovitis model of PsA, indicating that IFNγ is not a major player in the T cell – PsA synovial fibroblast activation.

We found lower percentages of CD19+ B cells but higher percentages of CD56+(bright) NK cells in PsA synovial fluid which is in line with data from other groups (33-35). B cells tend to function in a protective way in psoriasis or PsA, and can be a source anti-inflammatory cytokines, such as IL-10 (34, 36). Interestingly, increased percentage of CD56+(bright) NK subset was reported to trigger differentiation of monocytes into dendritic cells in PsA synovial fluid (37). Monocytes and dendritic cells are major sources of IL-23 and the IL-23-IL-17 axis plays a pivotal role in inflammatory arthritis (38, 39). Biologicals targeting IL-23 subunits, p40 and p19, were successfully tested in PsA clinical trials (40,41). Whether synovial fluid CD56+(bright) NK cells modulate IL-23 expression by monocytes/dendritic cells and thereby influence IL-23-IL-17 axis is still not known and warranted further research.

In summary, our study showed that the contribution of CD8+ T cells to IL-17A local production in PsA synovial fluid needs further examination as TCR activation mimicked by anti-CD3/anti-CD28 didn't induce IL-17A release from CD8+ in contrast to CD4+ T cells. IL-17A and TNF blockades showed differential effects in our human *ex vivo* PsA synovial inflammation model with the former more potent in dampening inflammatory cytokines while the latter more potent in lowering MMPs. We acknowledged the limitations of our *ex vivo* study systems, and further validation of a combination or sequential therapy in patients with IL-17A and TNFα neutralizing agents is needed. This study indicates that targeting both IL-17A and TNFα may complement each other and may possess additive benefits in coping with destructive synovitis in patients with PsA.

## REFERENCES


1. Ritchlin CT, Colbert RA, Gladman DD. Psoriatic arthritis. N Engl J Med. 2017;376(10):957-70.
2. Poulter LW, Duke O, Panayi GS, Hobbs S, Raftery MJ, Janossy G. Activated T lymphocytes of the synovial membrane in rheumatoid arthritis and other arthropathies. Scand J Immunol. 1985;22(6):683-90.
3. Takada K, Danning CL, Kuroiwa T, Schlimgen R, Tassiulas IO, Davis JC Jr, et al. Lymphocyte depletion with fludarabine in patients with psoriatic arthritis: clinical and immunological effects. Ann Rheum Dis. 2003;62(11):1112-5.
4. Costello PJ, Winchester RJ, Curran SA, Peterson KS, Kane DJ, Bresnihan B, et al. Psoriatic arthritis joint fluids are characterized by CD8 and CD4 T cell clonal expansions appear antigen driven. J Immunol. 2001;166(4):2878-86.
5. Partsch G, Steiner G, Leeb BF, Dunky A, Bröll H, Smolen JS. Highly increased levels of tumor necrosis factor-alpha and other proinflammatory cytokines in psoriatic arthritis synovial fluid. J Rheumatol. 1997;24(3):518-23.
6. Ritchlin C, Haas-Smith SA, Hicks D, Cappuccio J, Osterland CK, Looney RJ. Patterns of cytokine production in psoriatic synovium. J Rheumatol. 1998;25(8):1544-52.
7. van Baarsen LG, Lebre MC, van der Coelen D, Aarrass S, Tang MW, Ramwadhdoebe TH, et al. Heterogeneous expression pattern of interleukin 17A (IL-17A), IL-17F and their receptors in synovium of rheumatoid arthritis, psoriatic arthritis and osteoarthritis: possible explanation for nonresponse to anti-IL-17 therapy? Arthritis Res Ther. 2014;16(4):426.

8. Lubberts E. Th17 cytokines and arthritis. Semin Immunopathol. 2010;32(1):43-53.
9. Wang EA, Suzuki E, Maverakis E, Adamopoulos IE. Targeting IL-17 in psoriatic arthritis. Eur J Rheumatol. 2017;4(4):272-277.
10. Raychaudhuri SP, Raychaudhuri SK, Genovese MC. IL-17 receptor and its functional significance in psoriatic arthritis. Mol Cell Biochem. 2012;359(1-2):419-29.
11. Wade SM, Canavan M, McGarry T, Low C, Wade SC, Mullan RH, et al. Association of synovial tissue polyfunctional T-
cells with DAPSA in psoriatic arthritis. Ann Rheum Dis. 2019;78(3):350-354.
12. Menon B, Gullick NJ, Walter GJ, Rajasekhar M, Garrood T, Evans HG, et al. Interleukin-17+CD8+ T cells are enriched in the joints of patients with psoriatic arthritis and correlate with disease activity and joint damage progression. Arthritis Rheumatol. 2014;66(5):1272-81.
13. Diani M, Casciano F, Marongiu L, Longhi M, Altomare A, Pigatto PD, et al. Increased frequencey of activated CD8+ T cell effectors in patients with psoriatic arthritis. Sci Rep. 2019;9(1):10870.
14. Steel KJA, Srenathan U, Ridley M, Durham LE, Wu SY, Ryan SE, et al. Synovial IL-17A+ CD8+ T cells display a polyfunctional, pro-inflammatory and tissue-resident memory phenotype and function in psoriatic arthritis. Arthritis Rheumatol. 2019. doi: 10.1002/art.41156.
15. Leijten EF, van Kempen TS, Boes M, Michels-van Amelsfort JM, Hijnen D, Hartgring SA, et al. Brief report: enrichment of activated group 3 innate lymphoid cells in psoriatic arthritis synovial fluid. Arthritis Rheumatol. 2015;67(10):2673-8.
16. Blijdorp ICJ, Menegatti S, Van Mens LJJ, van de Sand MGH, Chen S, Hreggvidsdottir HS, et al. IL-22- and GM-CSF-expressing but not IL-17A-expressing group 3 innate lymphoid cells are expanded in the inflamed spondyloarthritis joint. Arthritis Rheumatol. 2018. doi: 10.1002/art.40736.
17. Mease PJ. Tumour necrosis factor (TNF) in psoriatic arthritis: pathophysiology and treatment with TNF inhibitors. Ann Rheum Dis. 2002;61(4):298-304.
18. Aaltonen K, Heinonen A, Joensuu J, Parmanne P, Karjalainen A, Varjolahti-Lehtinen T, et al. Effectiveness and drug survival of TNF-inhibitors in the treatment of psoriatic arthritis: A prospective cohort study. Semin Arthritis Rheum. 2017;46(6):732-739.
19. Taurog JD, Chhabra A, Colbert RA. Ankylosing Spondylitis and Axial Spondyloarthritis. N Engl J Med. 2016;374(26):2563-74.
20. Fitz L, Zhang W, Soderstrom C, Fraser S, Lee J, Quazi A, et al. Association between serum interleukin-17A and clinical response to tofacitinib and etanercept in moderate to severe psoriasis. Clin Exp Dermatol. 2018;43(7):790-797.

21. van der Heijde D, Landewé RB, Mease PJ, McInnes IB, Conaghan PG, Pricop L, et al. Brief report: secukinumab provides significant and sustained inhibition of joint structural damage in a phase III study of active psoriatic arthritis. Arthritis Rheumatol. 2016;68(8):1914-21.
22. Mease PJ, van der Heijde D, Ritchlin CT, Okada M, Cuchacovich RS, Shuler CL, et al. Ixekizumab, an interleukin-17A specific monoclonal antibody, for the treatment of biologic-naive patients with active psoriatic arthritis: results from the 24-week randomised, double-blind, placebo-controlled and active (adalimumab)-controlled period of the phase III trial SPIRIT-P1. Ann Rheum Dis. 2017;76(1):79-87.
23. Pelechas E, Memi T, Voulgari PV, Drosos AA. A case of recalcitrant psoriatic arthritis to TNF inhibitors improved after administration of secukinumab, an IL-17A inhibitor. Rheumatol Ther. 2017;4(2):509-513.
24. Nash P, Kirkham B, Okada M, Rahman P, Combe B, Burmester GR, et al. Ixekizumab for the treatment of patients with active psoriatic arthritis and an inadequate response to tumour necrosis factor inhibitors: results from the 24-week randomised, double-blind, placebo-controlled period of the SPIRIT-P2 phase 3 trial. Lancet. 2017;389(10086):2317-2327.
25. van Hamburg JP, Asmawidjaja PS, Davelaar N, Mus AM, Colin EM, Hazes JM, et al. Th17 cells, but not Th1 cells, from patients with early rheumatoid arthritis are potent inducers of matrix metalloproteinases and proinflammatory cytokines upon synovial fibroblast interaction, including autocrine interleukin-17A production. Arthritis Rheum. 2011;63(1):73-83.
26. Costello P, Bresnihan B, O'Farrelly C, FitzGerald O. Predominance of CD8+ T lymphocytes in psoriatic arthritis. J Rheumatol. 1999;26(5):1117-24.
27. Ortega C, Fernández-A S, Carrillo JM, Romero P, Molina IJ, Moreno JC, et al. IL-17-producing CD8+ T lymphocytes from psoriasis skin plaques are cytotoxic effector cells that secrete Th17-related cytokines. J Leukoc Biol. 2009;86(2):435-43.
28. Res PC, Piskin G, de Boer OJ, van der Loos CM, Teeling P, Bos JD, et al. Overrepresentation of IL-17A and IL-22 producing CD8 T cells in lesional skin suggests their involvement in the pathogenesis of psoriasis. PLoS One. 2010;5(11):e14108.
29. Glatt S, Baeten D, Baker T, Griffiths M, Ionescu L, Lawson ADG, et al. Dual IL-17A and IL-17F neutralisation by bimekizumab in psoriatic arthritis: evidence from preclinical experiments and a randomised placebo-controlled clinical trial that IL-17F contributes to human chronic tissue inflammation. Ann Rheum Dis. 2018;77(4):523-532.

30. Genovese MC, Weinblatt ME, Mease PJ, Aelion JA, Peloso PM, Chen K, et al. Dual inhibition of tumour necrosis factor and interleukin-17A with ABT-122: open-label long-term extension studies in rheumatoid arthritis or psoriatic arthritis. Rheumatology (Oxford). 2018;57(11):1972-1981.
31. Mease PJ, Genovese MC, Weinblatt ME, Peloso PM, Chen K, Othman AA, et al. Phase II study of ABT-122, a tumor necrosis factor- and interleukin-17A-targeted dual variable domain immunoglobulin, in patients with psoriatic arthritis with an inadequate response to methotrexate. Arthritis Rheumatol. 2018;70(11):1778-1789.
32. Fischer JA, Hueber AJ, Wilson S, Galm M, Baum W, Kitson C, et al. Combined inhibition of tumor necrosis factor α and interleukin-17 as a therapeutic opportunity in rheumatoid arthritis: development and characterization of a novel bispecific antibody. Arthritis Rheumatol. 2015;67(1):51-62.
33. Spadaro A, Scrivo R, Moretti T, Bernardini G, Riccieri V, Taccari E, et al. Natural killer cells and gamma/delta T cells in synovial fluid and in peripheral blood of patients with psoriatic arthritis. Clin Exp Rheumatol. 2004;22(4):389-94.
34. Mavropoulos A, Varna A, Zafiriou E, Liaskos C, Alexiou I, Roussaki-Schulze A, et al. IL-10 producing Bregs are impaired in psoriatic arthritis and psoriasis and inversely correlate with IL-17- and IFNγ-producing T cells. Clin Immunol. 2017;184:33-41.
35. Dalbeth N, Callan MF. A subset of natural killer cells is greatly expanded within inflamed joints. Arthritis Rheum. 2002;46(7):1763-72.
36. Dass S, Vital EM, Emery P. Development of psoriasis after B cell depletion with rituximab. Arthritis Rheum. 2007;56(8):2715-8.
37. Zhang AL, Colmenero P, Purath U, Teixeira de Matos C, Hueber W, Klareskog L, et al. Natural killer cells trigger differentiation of monocytes into dendritic cells. Blood. 2007;110(7):2484-93.35.
38. Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B, et al. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity. 2000;13(5):715-25.
39. Lubberts E. The IL-23-IL-17 axis in inflammatory arthritis. Nat Rev Rheumatol. 2015;11(7):415-29.
40. Kavanaugh A, Ritchlin C, Rahman P, Puig L, Gottlieb AB, Li S, et al. Ustekinumab, an anti-IL-12/23 p40 monoclonal antibody, inhibits radiographic progression in patients with active psoriatic arthritis: results of an integrated analysis of radiographic data from the phase 3, multicentre, randomised, double-blind, placebo-controlled PSUMMIT-1 and PSUMMIT-2 trials. Ann Rheum Dis. 2014;73(6):1000-6.

41. Deodhar A, Gottlieb AB, Boehncke WH, Dong B, Wang Y, Zhuang Y, et al. Efficacy and safety of guselkumab in patients with active psoriatic arthritis: a randomised, double-blind, placebo-controlled, phase 2 study. Lancet. 2018;391(10136):2213-2224.

## Supplementary Figures

| Parameters | PsA fluid | PsA blood | healthy blood |
|---|---|---|---|
| Age (mean ± SD, years) | 47.7 ± 13.7 | 45.4 ± 12.4 | 45.3 ± 12.3 |
| Sex (female/male) | 7/13 | 16/6 | 16/6 |
| DAS28 (mean ± SD) | 3.16 ± 1.39 | n.a. | 0 |
| DMARD therapy, n(%) | 18(90) | 0(0) | 0(0) |
| oral predisone therapy, n(%) | 0(0) | 0(0) | 0(0) |
| Biologic therapy, n(%) | 4(20) | 0(0) | 0(0) |

**Table S1. Clinical characteristics of PsA patients and healthy volunteers**
Synovial fluid (SF) of established PsA patients (n=20), peripheral blood of treatment naïve early PsA patients and age-/sex-matched healthy volunteers (n=22 each) were collected. (n.a. indicates not available.)

Supplementary
Figure S1

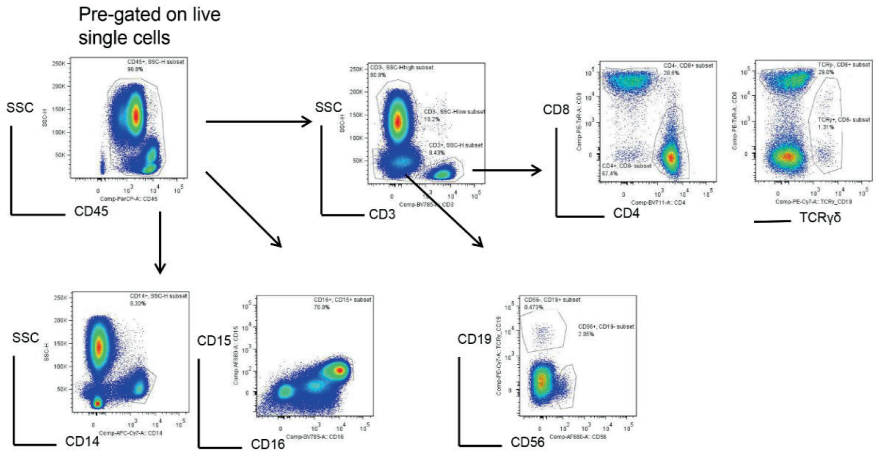



**Figure S1. Gating strategy for cell subsets in surface staining results.** Cell pellets directly from PsA-SF, PsA blood and healthy blood were stained and analyzed for frequencies in samples. T cells (CD3+), B cells (CD19+), NK cells (CD56+), monocytes (CD14+), and neutrophils (CD15+CD16+) were identified among CD45+ immune cells. Among T cells, CD4+, CD8+, and TCRγδ T cells were further identified.

Supplementary Figure S2

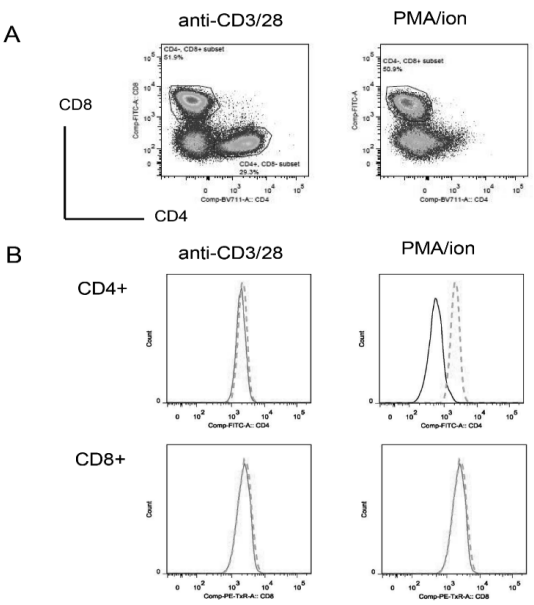



**Figure S2. PMA and ionomycin stimulation down-regulated CD4 surface marker. A.** Healthy peripheral blood mononuclear cells (PBMCs) were stimulated with anti-CD3/anti-CD28 or PMA/ion for 4 hrs and percentages of CD4+ and CD8+ T cells were examined after stimulation. **B.** CD4+ and CD8+ T cells were sorted from healthy PBMCs, and *ex vivo* stimulated for 4 hrs. Histogram of CD4 and CD8 surface expression were plotted after stimulation with anti-CD3/anti-CD28 (solid red) or PMA/ion (solid dark) and compared with unstimulated (dash blue) conditions (n=3).

Supplementary Figure S3

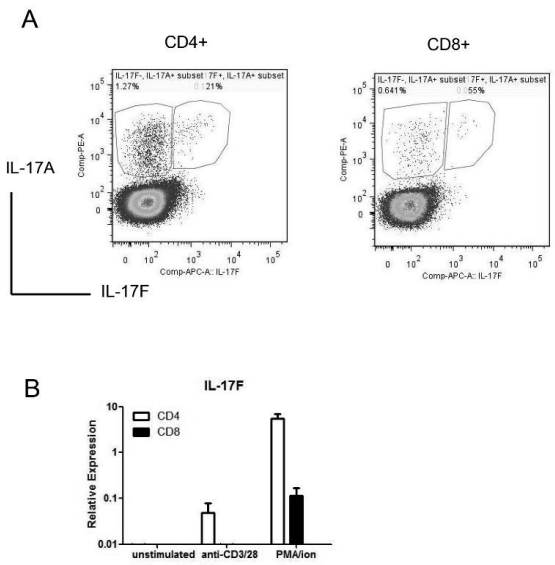



**Figure S3. Expression and induction of IL-17F in CD4+ and CD8+ T cells from PsA-SF. A.** Representative flow cytometry staining of IL-17A and IL-17F in CD4+ and CD8+ T cells of PsA-SF. **B.** mRNA expression of IL-17F in CD4+ and CD8+ T cells sorted from PsA-SF and stimulated with anti-CD3/anti-CD28, PMA/ion, or without stimulation for 72 hrs (n=3).

Supplementary Figure S4

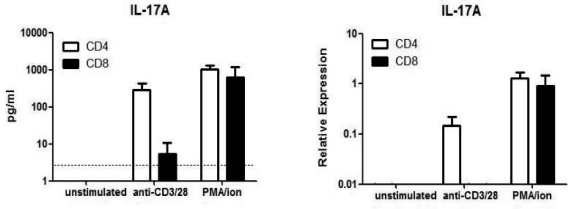



**Figure S4. PMA/ion, but not anti-CD3/anti-CD28, induced IL-17A from PsA-SF CD8+ T cells and monocytes co-cultures.** IL-17A levels in co-culture supernatants and mRNA expression in cells after SF CD4+ and CD8+ T cells were *ex vivo* co-cultured with autologous CD14+ monocytes and activated with anti-CD3/anti-CD28, PMA/ion, or without stimulation for 72 hrs (n=3) (dash line indicates ELISA detection limit for IL-17A).

Supplementary Figure S5

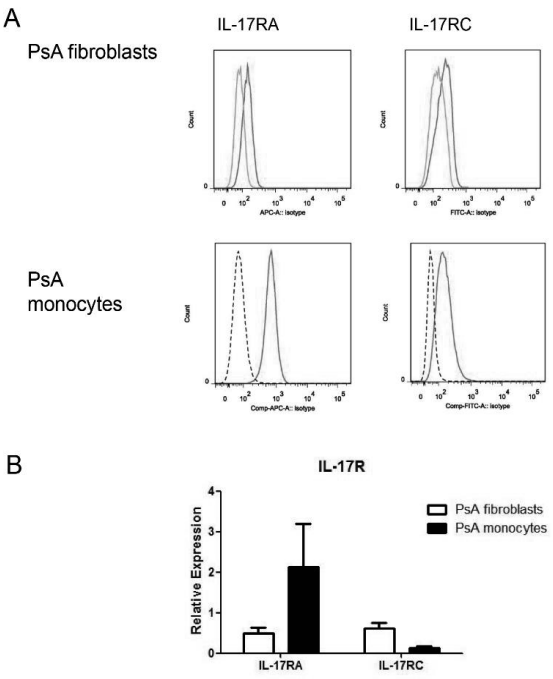
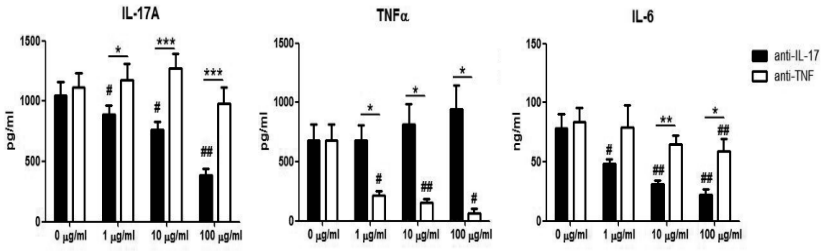



**Figure S5. PsA-FLS and monocytes expressed IL-17 receptor. A.** Representative flow cytometry staining of IL-17RA and IL-17RC on PsA-FLS and monocytes (solid red) compared to isotype antibody (solid blue or dash dark) controls (n=2). **B.** mRNA expression of IL-17RA and IL-17RC in PsA-FLS and monocytes (n=2).

Supplementary
Figure S6

A




B

| IL-17A | | |
|---|---|---|
| anti-IL-17A | Average concentration (pg/ml) | Percentage reduction (%) |
| 0 μg/ml | 1045 | 0 |
| 1 μg/ml | 889 | 15 |
| 10 μg/ml | 765 | 27 |
| 100 μg/ml | 387 | 63 |

| TNF | | |
|---|---|---|
| anti-TNF | Average concentration (pg/ml) | Percentage reduction (%) |
| 0 μg/ml | 683 | 0 |
| 1 μg/ml | 214 | 69 |
| 10 μg/ml | 154 | 77 |
| 100 μg/ml | 67 | 90 |

**Figure S6. Pre-titration of anti-IL-17A and anti-TNF in co-cultures of PsA-FLS and CD4+ T cells from PBMCs of healthy donors. A.** IL-17A, TNFα, and IL-6 ELISA results in co-culture supernatants after 72 hrs' activation with anti-CD3/anti-CD28 with 0, 1, 10, 100 μg/ml of either antibody added. Data (n=6) are pooled from two independent experiments. (* indicates significant differences between two antibody groups and # shows significant differences compared to respective null antibody group.) **B.** Average concentrations of IL-17A and TNFα in culture supernatants of each group were summarized and percentage of reduction was calculated compared to null antibody group.

# 3

# Interleukin-17A drives IL-19 and IL-24 expression in skin stromal cells regulating keratinocyte proliferation

Xiaofei Xu, Errol Prens, Edwin Florencia, Pieter Leenen, Luis Boon, Patrick Asmawidjaja, Anne-Marie Mus, Erik Lubberts

Front Immunol. 2021 Sep 20;12:719562.

## Abstract

IL-17A has been shown to be up-regulated in psoriasis lesions and is central to psoriasis pathogenesis. IL-19, along with other IL-20 subfamily cytokines such as IL-20 and IL-24, is induced by IL-17A and contributes especially to epidermal hyperplasia in psoriasis. However, the regulation, cellular sources of IL-19 and whether targeting of IL-17A by biologics influence IL-19 expression is not completely understood. To investigate the regulation of IL-19 by IL-17A in psoriasis, the imiquimod-induced psoriasis mouse (IMQ) model was used. Enhanced expression of IL-17A in the IMQ model was achieved by anti-IL-10 antibody treatment. Assessments of skin inflammation macroscopically, by histology and flow cytometry, all confirmed increased psoriatic symptoms. Interestingly, depletion of IL-10 markedly upregulated IL-23/IL-17 pathway related cytokines followed by a significant increase in IL-19 and IL-24. The up-regulation of IL-19 and IL-24, but not IL-17A, coincided with increased keratinocyte proliferation. To investigate the cellular source and effects of biologics on IL-19, human skin fibroblasts from healthy controls and psoriasis patients were cultured alone or co-cultured with activated memory CD4+ T cells. Besides IL-1β, IL-17A induced direct expression of IL-19 and IL-24 in skin fibroblasts and keratinocytes. Importantly, intrinsic higher expression of IL-19 in psoriatic skin fibroblasts was observed in comparison to healthy skin fibroblasts. Neutralization of IL-17A in the human skin fibroblast-T cell co-culture system significantly suppressed IL-19 and IL-24 expression. Together, our data show that IL-17A-induced IL-19 and IL-24 expression in skin stromal cells contribute to keratinocyte proliferation.

## INTRODUCTION

Psoriasis is a chronic autoimmune skin disease affecting around 2 to 3% of the Western population (1,2). Psoriasis vulgaris, the plaque-forming phenotype, is the most common type and accounts for 85-90% of all patients with psoriasis (1,3). Aberrant regulation of pro-inflammatory and anti-inflammatory cytokines is considered important in the pathogenesis of psoriasis (1-4). Increased levels of IL-17A and elevated percentages of IL-17A-producing lymphocytes are found in psoriatic plaques (5-9). IL-17A alone, and in combination with other inflammatory cytokines such as TNFα, stimulate keratinocyte activation, proliferation, amplifies the immune response and perpetuates cell infiltration (10,11). Biologics targeting IL-17A alone or in combination with IL-17F or the IL-17 receptor A (IL-17RA) are efficacious in the treatment of plaque psoriasis, highlighting the central role of the IL-17A pathway in psoriasis pathogenesis (12). Interleukin-19, a member of the IL-20 subfamily together with IL-20 and IL-24, is up-regulated in psoriatic lesions and contributes to keratinocytes hyperplasia in psoriasis (13). IL-19 is reportedly down-stream of the IL-23/IL-17 cascade and autocrine production of IL-19 by keratinocytes is shown to elicit keratinocyte proliferation (14). Recent data show that serum IL-19 levels reflect clinical improvement induced by anti-IL-17 biologic treatment (15). However, whether other resident cells, such as skin fibroblasts, are a source of IL-19 and whether in situ IL-19 expression is normalized in patients with psoriasis treated with anti-IL-17 therapy is not clear.

Previously, our group established a psoriasis-like skin inflammation model in mice using topical application of imiquimod (IMQ) (16). This model successfully re-captures most critical features of acute plaque formation in psoriasis such as keratinocyte hyper proliferation, acanthosis and parakeratosis (16). Like in human psoriasis, enhanced activity of the IL-23/IL-17 pathway was also involved in the IMQ-induced psoriasis mouse model (16). However, in contrast to the chronic natural course in human psoriasis, this mouse model does not develop into a chronic state of psoriasis, because of stabilization and even improvement of skin inflammation after 5 to 6 days. Interestingly, a clinical study in psoriasis patients showed that, non-lesional skin treated with IMQ initially developed typical features of psoriasis such as acanthosis and parakeratosis

(17). Nevertheless, both clinical and histological features subsided thereafter and in this human model of IMQ-induced psoriasis, the induced lesions showed spontaneous improvement after 5 to 6 days. This improvement was accompanied by significantly lower expression of IL-17A and with a higher expression of IL-10 (17). This suggests that upregulation of IL-10 is involved in the spontaneous improvement of psoriasis symptoms after 5 to 6 days in murine IMQ model and probably explains the spontaneous improvement observed in the IMQ mouse model. Therefore, we used an anti-IL-10 antibody to investigate whether we could achieve enhanced expression of IL-17 in the IMQ-induced psoriasis mouse model and the accompanying visible psoriatic symptoms beyond day 5. *In vitro* assays with human skin fibroblasts from patients with psoriasis and healthy skin were performed to evaluate the direct induction of IL-19 by IL-17. In addition, an ex vivo human psoriasis skin co-culture system was used to examine the effects of biologics targeting IL-17A on IL-19 expression.

## MATERIAL AND METHODS

### IMQ-induced psoriasis mouse model

BALB/C mice (8-11 week-old) received daily topical application of 62.5mg 5% Aldara (3M Pharmaceuticals) on their shaved back skin. Control mice (n=6, pooled from two independent experiments) were treated with a thin layer of petrolatum (Fagron). Daily evaluation of the local psoriasis area and severity index (PASI) has been described previously (16). Every other day, 20 mg/kg body weight of anti-IL-10 or isotype control antibody (n=10 each, pooled from two independent experiments) was intraperitoneally (i.p.) injected, or 5 mg/kg body weight of dexamethasone (n=7, pooled from two independent experiments) was subcutaneously (s.c.) injected as an anti-inflammatory gold standard. Five and ten days after IMQ induction, mice were sacrificed for analysis. Food and water were provided ad libitum, and mice were kept under specific pathogen-free conditions. All experiments were approved by the Erasmus MC Dutch Animal Ethics Committee (DEC).

**Histology and Immunohistochemistry**

After sacrifice, skin biopsies were taken and snap-frozen in TissueTek (Bayer). Sections were cut with a Leica cryostat. Gr-1 antibody (clone RB6-8C5) and Ki-67 antibody (Dako, A0047) were used for IHC staining. Subsequent steps were performed as described earlier (15).

Images were analyzed with LAX V4.12 program (Leica microsystems) or NDP view2 (Hamamatsu photonics). To measure epidermal thickness, the average of four measurements was used as the representative thickness per sample. To reduce variance between different experiments, thickness ratios were calculated. Specifically, each skin thickness was divided by the mean skin thickness of the isotype group from that experiment, and thereby mean values of thickness for isotype groups were always set at one.



**Flow cytometry**

Back skin (ca. 1 cm2) was digested in 50 µg/mL Liberase (Roche) at $4^0$C overnight and then at $37^0$C for 1 hour to create a single cell suspension and cells were stained with the following antibodies: CD45-BV785 (Biolegend, clone 104), CD11b-eF450 (eBioscience, clone M1/70), Ly6C-APC-Cy7 (BD pharmingen, clone AL-21) and Ly6G-PE-CF594 (BD horizon, clone 1A8). Samples were analyzed with LSR II flow cytometer (BD Biosciences) and results processed with FlowJo software (TreeStar).

Healthy peripheral blood mononuclear cells (PBMC) were obtained from buffycoats (Sanquin, Amsterdam, the Netherlands) and harvested with Ficoll density gradient centrifugation. For the co-culture experiments, memory T cells (CD4+CD45RO+CD14-CD25low/int) were sorted using the FACSAria cell sorter (BD Biosciences) and co-cultured with skin fibroblasts. Cells were stained with the following antibodies: CD14-APC-H7 (clone MϕP9), CD45RO-PerCP-Cy5.5 (clone UCHL1), both from BD Biosciences, and CD4-FITC (clone RPA-T4), CD25-PE Cy7 (clone BC96), both from Sony Biotechnology. The obtained cell purity was $\geqslant$ 98%.

**Reverse transcription and real-time polymerase chain reaction (RT-PCR)**

RNA was isolated with TRIzol (Thermo Fisher) or Total RNA Miniprep Kit (Sigma-Aldrich). cDNA was synthesized with Superscript II after DNase treatment (both from Invitrogen). RT-PCR was performed with ViiA7 sequence

detection system (Life Technologies). Gene expression of IL-17A, IL-17F, IL-19, IL-22, IL-23p19, IL-24 were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in mouse samples and hypoxanthine-guanine phosphoribosyltransferase (HPRT) in human samples. Primer sequences were summarized in supplementary Table S1.

**Human primary skin fibroblast cultures and co-cultures with T cells**

Fibroblasts (n=6) from lesional skin of psoriasis patients were obtained from our biobank collection. Healthy skin samples (n=6) were obtained from healthy individuals who underwent cosmetic surgeries in the Sint Franciscus Hospital (Rotterdam, The Netherlands). Signed consents were provided by all healthy participants. Human primary skin fibroblasts were cultured from above skin samples as described previously (18). Passages 3-8 fibroblasts were seeded $1.0\times10^4$ per well in 96-well culture plates and stimulated with IL-1β (0.01 ng/mL, 201-LB), TNFα (5 ng/mL, 210-TA), IL-17A (50 ng/mL, 317-IL), and IL-17F (500 ng/mL, 1335-IL) (all from R&D systems) for 24 hours (hrs).

In co-culture experiments, skin fibroblasts ($1.0x10^4$) were co-cultured with CD4+CD45RO+CD14-CD25low/int T memory cells ($2.5\times10^4$) sorted from buffy coats (n=6). Soluble anti-CD3 and anti-CD28 (both from Sanquin, Amsterdam, The Netherlands) were added for 72 hrs. In addition, 100 µg/ml anti-IL-17Aantibody (secukinumab, Novartis), 1µg/ml anti-TNF antibody (adalimumab, AbbVie), and an isotype IgG1κ antibody (Sigma-Aldrich) were used.

**Enzyme-linked immunosorbent assay (ELISA)**

Human IL-8 and IL-19 in culture supernatants was measured with ELISA (Invitrogen) and ELISA Duoset (R&D systems) following manufacturers' instructions.

**Data set analysis**

Public microarray data (GSE13355) were analyzed to compare mRNA expression of psoriatic lesional (n=64), non-lesional skin (n=58) and skin from healthy controls (n=58). Based on GSE13355, values of 216876_s_at (IL-17A), 220745_at (IL-19), 206569_at (IL-24) and 212021_s_at (MKI67) from gene expression profile GDS4602 were plotted. Dataset GSE53552 was analyzed for mRNA expression of psoriatic skin lesions following treatment with

brodalumab (AstraZeneca). Psoriatic non-lesional (n=23), lesional (n=25), and day 8 (n=4), day 15 (n=19), day 43 (n=16) after treatments were included. 220745_at (IL-19), 206569_at (IL-24), 212022_s_at (MKI67) values from GDS5420 were plotted based on GSE53552.

**Statistics**

Statistical differences were determined with paired or unpaired student's *t* test. All data analyses were performed with GraphPad Prism V5 and P-values <0.05 were considered as significant.

# RESULTS

## IL-10 neutralization enhances skin thickness and scaling in the IMQ-induced psoriasis mouse model

The design of the IL-10 neutralization experiments is summarized in **Supplementary Figure S1A,B**. Local psoriasis area and severity index (PASI) score was used to evaluate psoriasis symptoms including skin scaling, thickness and redness. As shown in **Figure 1A**, ten days after IMQ treatment, macroscopic scores of skin scaling and thickness were significantly higher in the anti-IL-10 treated group (anti-IL-10) compared to the isotype antibody control group (isotype). This resulted in a significant higher PASI score after neutralizing IL-10 compared to the isotype control (**Figure 1A**). Dexamethasone treatment significantly improved both symptoms compared to either the anti-IL-10 or isotype group. No significant difference for skin redness was observed among groups. Details of kinetic data of the macroscopic scores were summarized in **Supplementary Figure S2A**. These data indicate that IL-10 neutralization enhances skin thickness and scaling in the IMQ-induced psoriasis mouse model beyond day 5.

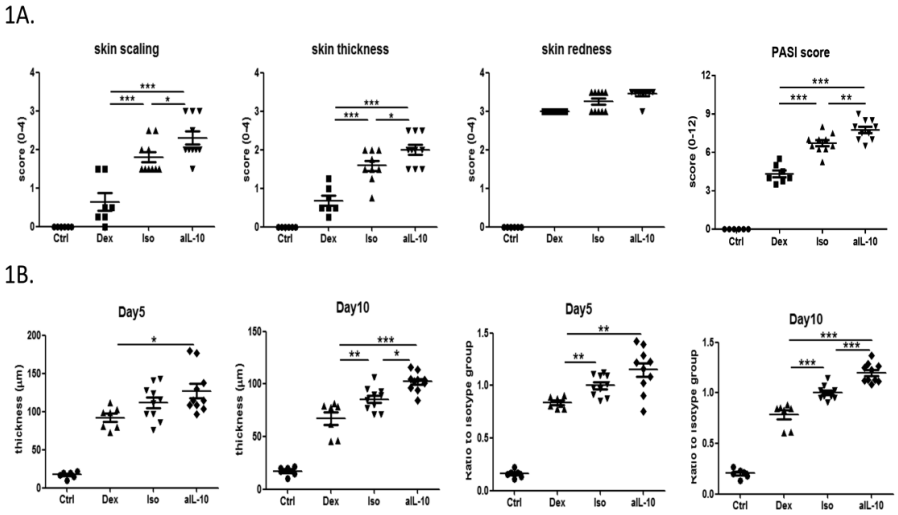



**Figure 1. IL-10 neutralization worsens psoriatic symptoms and epidermal thickness in the IMQ-induced psoriasis mouse model. (A)** At day 10, scores for skin scaling, thickness, redness and PASI in various groups following IMQ treatment. **(B)** Measured average epidermal

thickness in psoriasis-like skin at days 5 and 10, and thickness ratios compared to isotype antibody controls at days 5 and 10. Data are shown as means ± SEMs. * P<0.05, ** P<0.01, and *** P<0.001.

**IL-10 neutralization increases epidermal thickness and keratinocyte proliferation in the IMQ-induced psoriasis mouse model**

To confirm the increased skin thickness observed macroscopically after anti-IL-10 treatment, H&E staining was performed and epidermal thickness was measured microscopically (**Figure 2A**). At day 5, epidermal thickness was significantly higher in the anti-IL10 compared to dexamethasone treatment (**Figure 1B**). At day 10, anti-IL-10 significantly increased epidermal thickness compared to the isotype group, whereas dexamethasone significantly reduced this compared to both groups (**Figure 1B**). This is in line with our macroscopic findings of increased skin thickness with the PASI score in the anti-IL-10 treated group (**Figure 1A**).



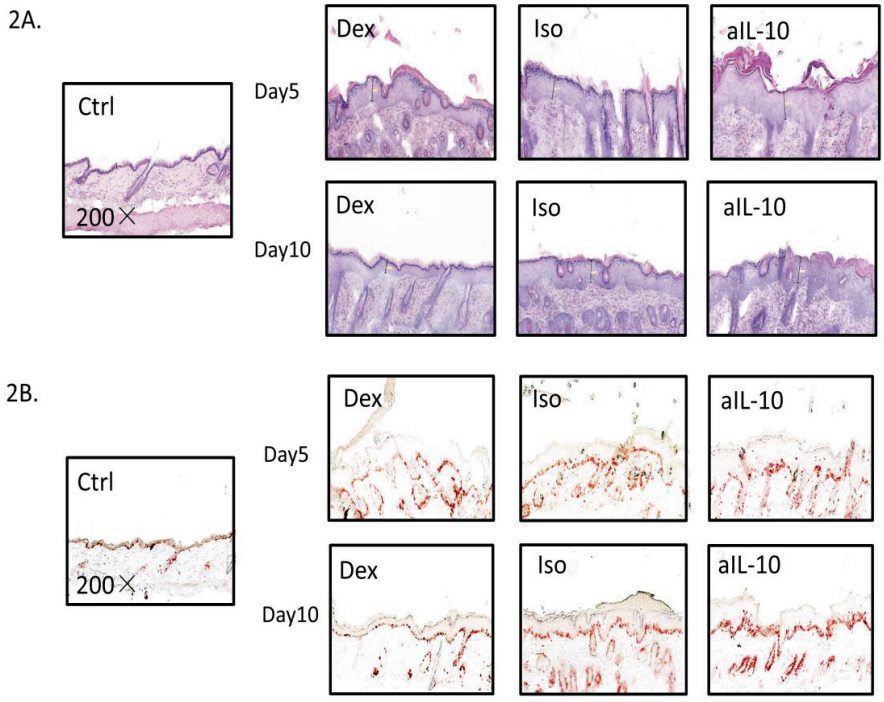



**Figure 2. IL-10 neutralization increases keratinocyte proliferation and neutrophil accumulation in the IMQ-induced psoriasis mouse model.**

**(A)** Representative H&E staining results of skin sections in IMQ-induced psoriasis mouse model at days 5 and 10. **(B)** Representative IHC staining of Ki-67+ proliferating keratinocytes in skin sections of IMQ-induced psoriasis mouse model at days 5 and10. All images were taken with 200× magnification.

As indicated before, compared to isotype group, ratios of epidermal thickness in the anti-IL-10 treated group were slightly increased at day 5, but significantly increased at day 10 (**Figure 1B**). Both epidermal thickness and thickness ratios in the isotype group were comparable to saline-treated IMQ groups (**Supplementary Figure S2B**). Ki-67 staining was performed to further identify proliferating cells. As shown in **Figure 2B**, 1-2 layers of keratinocytes in the epidermal stratum basale were Ki-67+ in dexamethasone group, 2-3 layers were Ki-67+ in isotype group, while 4-5 layers were Ki-67+ in anti-IL-10 group (at days 5 and 10). This is in line with the increased thickness of the epidermis in the anti-IL-10 treated group at day 10 (**Figure 1B**). Taken together, our data indicate that in the IMQ-induced psoriasis mouse model, IL-10 neutralization enhances skin thickness through facilitating keratinocyte proliferation.

**IL-10 neutralization increases the recruitment of neutrophils and monocytes into the skin**

Flow cytometry showed representative staining results of infiltrating neutrophils (CD11b+Ly6CintLy6G+) in lesional skin at day 10 (**Figure 3A**). Both neutrophils numbers and percentages were increased after neutralization of IL-10 compared to the isotype group (**Figure 3B**). Treatment with dexamethasone significantly reduced both parameters compared to the other groups (**Figure 3B**). At days 5 and 10, IHC staining confirmed the enhanced recruitment of Gr-1+ neutrophils in IMQ lesional skin in the anti-IL-10 treated group compared to the isotype group (**Figure 3C**). Neutrophil chemokine CXCL2, but not CXCL1, was upregulated after neutralizing IL-10 and correlated with enhanced neutrophil recruitment (**Figure 3B**). Similar results were found for CD11c+Ly6Cint monocytes-derived dendritic cells (mono/DCs) (**Figure 3D,E**). The isotype group showed similar cell numbers and percentages of neutrophils and mono/DCs compared to the saline-treated IMQ group (**Supplementary Figure S2C,D**), indicating that no specific immune effects were induced by the isotype antibody injections. These data indicate that IL-10 neutralization in the

IMQ-induced psoriasis mouse model results in enhanced persistent inflammation, as evidenced by enhanced influx of neutrophils and mono/DCs into lesional skin.

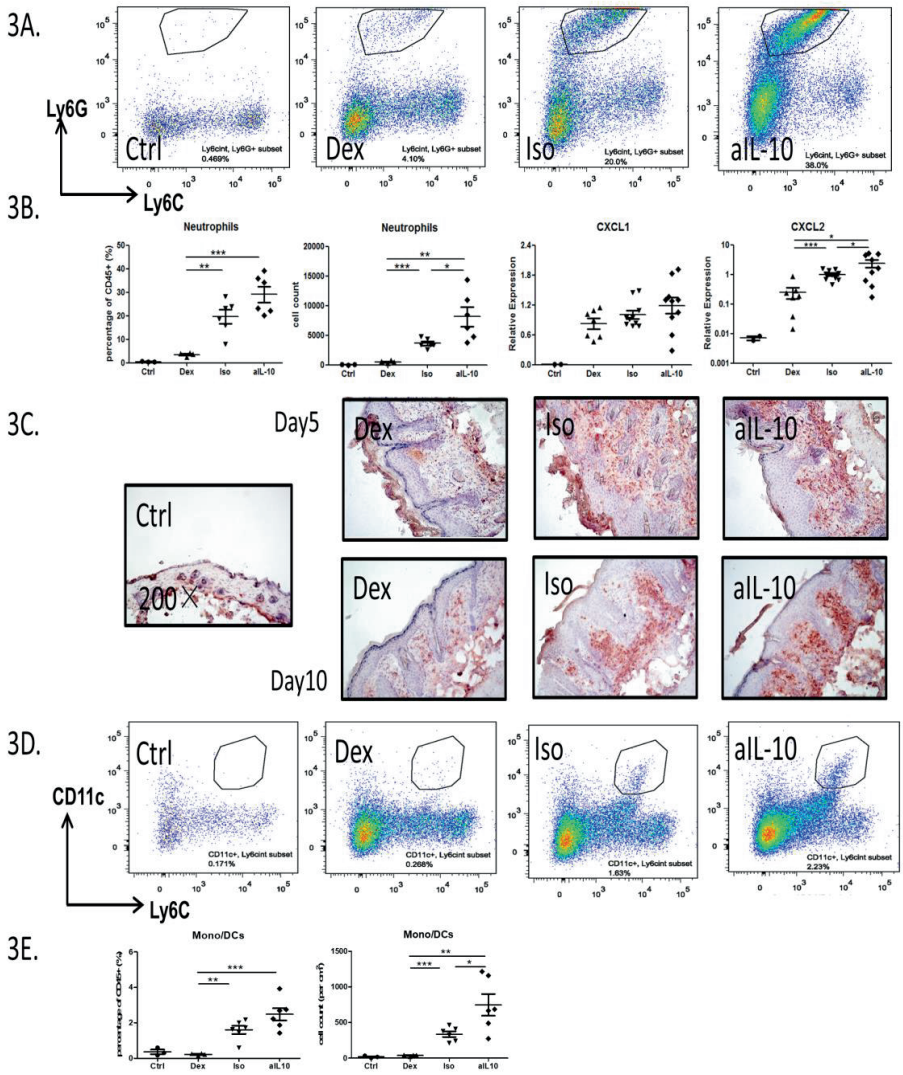



**Figure 3. IL-10 neutralization increases neutrophil and monocyte/DC infiltration in the IMQ-induced psoriasis mouse model.**

**(A)** Flow cytometry staining of Ly6G+Ly6Cint neutrophils among pregated CD45+CD11b+ cells in lesional psoriasis-like skin at day10. **(B)** Percentages and cell numbers of neutrophils among

total CD45+ immune cells at day10 in flow cytometry analysis and levels of the neutrophil chemokines, CXCL1 and CXCL2, in lesional psoriasis-like skin. **(C)** Representative IHC staining of Gr-1+ neutrophils in skin sections of the IMQ-induced psoriasis mouse model at days 5 and 10. **(D)** Flow cytometry staining of CD11c+Ly6Cint monocyte-derived dendritic cells in lesional psoriasis-like skin at day10. **(E)** Percentages and cell numbers of monocyte-derived dendritic cells (mono/DCs) among total CD45+ immune cells at day10 using flow cytometry. Data are shown as means ± SEMs. * $P$<0.05, ** $P$<0.01, and *** $P$<0.001.

**IL-10 neutralization in the IMQ-induced psoriasis mouse model results in an early upregulation of the IL-23/IL-17 immune pathway related cytokines followed by a subsequent later increase of IL-19 and IL-24 in the skin**

In psoriasis, IL-17-producing T helper cells (Th17) are central in the pathogenesis and Th17-related cytokines such as IL-17A, IL-17F and IL-22, together with TNFα, drive epidermal hyperplasia (9,16,19). Therefore, we investigated the effects of IL-10 neutralization on T cells and T cell cytokine expression in the IL-23/IL-17-dependent IMQ-induced psoriasis mouse model (16). Neutralizing IL-10 did not change the number of CD3+ T cells, including CD4+ and γδ T cells at day 10 (**Supplementary Figure S3A-C**). However, at day 5, IL-23p19, IL-22, IL-17A and IL-17F were significantly increased in the anti-IL-10 treated group compared to the isotype control group (**Figure 4A**). Interestingly, no difference in expression of these cytokines was found between these two groups at day 10 (**Figure 4A**). In contrast, the IL-20 subfamily cytokines, IL-19 and IL-24, were significantly upregulated only at day 10 but not at day 5 (**Figure 4B**) which correlated with the significant increase of epidermal thickness at day 10. This indicates that IL-19 and IL-24, rather than IL-22, IL-17A or IL-17F, were responsible for the late stage (day 10) keratinocyte hyper-proliferation and acanthosis in the IMQ-induced psoriasis mouse model during anti-IL-10 treatment.

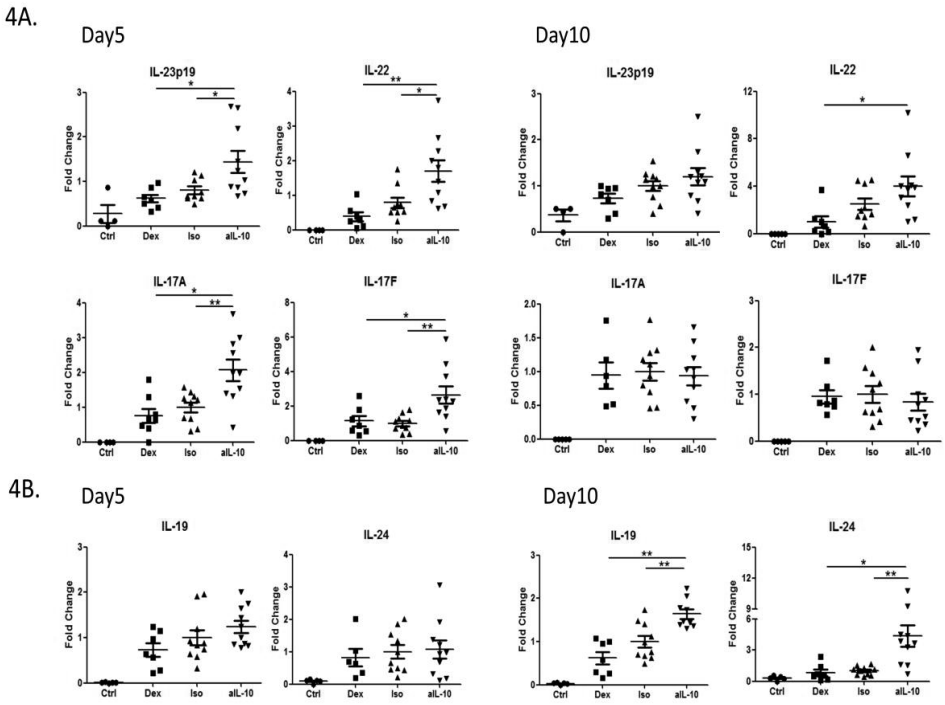



**Figure 4. Early and late up-regulation of IL-23/IL-17 cytokines and IL-20 subfamily cytokines in the skin during anti-IL-10 treatment in IMQ-induced psoriasis mouse model.** **(A)** At days 5 and 10, mRNA expression of IL-23, IL-22, IL-17A and IL-17F in various groups following IMQ treatment. **(B)** At days 5 and 10, mRNA expression of IL-19 and IL-24 in various groups following IMQ treatment. Data are shown as means ± SEMs. * $P$<0.05, ** $P$<0.01.

### IL-17 induces human skin fibroblasts to produce IL-19 and IL-24

As the increase in IL-17 family cytokines preceded the upregulation of IL-19 and IL-24, we assumed that the IL-17 family cytokines induced IL-19 and IL-24. Therefore, the expression of IL-19 and IL-24 in skin fibroblasts was examined after stimulation with IL-1β, TNF, IL-17A, or IL-17F for 24 hours. The expression of the IL-17 receptor, IL-17RA and IL-17RC, was confirmed on skin fibroblasts from psoriasis patients or healthy volunteers (**Supplementary Figure S4A**). As shown in **Figure 5A**, both IL-17A and IL-1β significantly increased IL-19 and IL-24 mRNA expression compared to the unstimulated group. TNF, on the other hand, was only a significant inducer of IL-24 (**Figure 5A**). Additionally, we observed an intrinsic higher expression of IL-19 and IL-24 in psoriatic skin fibroblasts compared to skin fibroblasts from healthy controls (**Figure 5A**).

To further explore the induction of IL-19 and IL-24 by IL-17A, healthy and psoriasis skin fibroblasts were co-cultured with CD4+CD45RO+CD14-CD25low/int T memory cells for 72 hours with or without stimulation. Neutralizing antibodies for IL-17A, TNF or the combination were added to these co-cultures. **Figure 5B** showed that anti-IL-17A treatment significantly reduced mRNA expression of IL-19 and IL-24 compared to either the isotype antibody or anti-TNF. In contrast, anti-TNF treatment only significantly reduced expression of IL-19 but not of IL-24 (**Figure 5B**). No additive effect was shown when both IL-17A and TNF were neutralized compared to anti-IL-17 treatment alone. In line with the mRNA expression data, ELISA data showed that both anti-IL-17A and anti-TNF significantly reduced the protein levels of IL-19 in the co-culture supernatants with anti-IL-17 more potent than anti-TNF, but without additive effect when treatment was combined (**Figure 5C**). However, anti-IL17A and anti-TNF both significantly reduced the levels of IL-8, with additive effect when combined (**Figure 5C**). Also in these skin fibroblast-T cell co-cultures, IL-19 and IL-24 mRNA expression as well as protein levels of IL-19 and IL-8 were all lower when skin fibroblast were derived from healthy controls compared to patients with psoriasis (**Figure 5B,C**). This indicates an intrinsic higher expression of both cytokines in psoriasis lesions.

IL-17A-induced expression of IL-19 was also checked in healthy human skin primary keratinocytes. Expression of the IL-17 receptor, IL-17RA and IL-17RC, was confirmed in these keratinocytes (**Supplementary Figure S4A**). IL-17A stimulation of human keratinocytes resulted in increased IL-19 mRNA expression. On the other hand, inhibition of IL-17A in keratinocyte-T cell co-cultures reduced IL-19 mRNA expression (**Supplementary Figure S4B**). Overall, these data indicate that IL-17A induces IL-19 and IL-24 expression in human skin resident cells such as fibroblasts and keratinocytes, and IL-17A blockade reduces their expression.

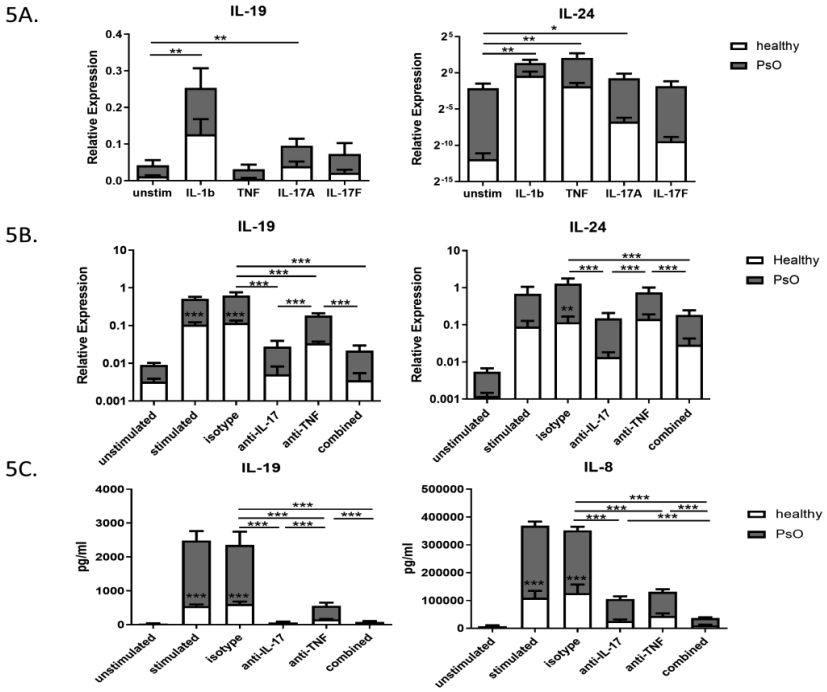



**Figure 5. IL-17A induces IL-19 and IL-24 expression in human skin resident cells such as fibroblasts and keratinocytes, and IL-17A blockade reduces their expression. (A)** IL-19 and IL-24 mRNA expression in fibroblasts of healthy donors (n=6) and psoriasis patients (n=4) without stimulation or stimulated with IL-1β, TNFα, IL-17A and IL-17F for 24 hours. **(B)** Healthy control skin fibroblasts and psoriatic fibroblasts (n=6 each) were co-cultured with unstimulated or anti-CD3/anti-CD28 stimulated memory CD4+ T cells from healthy controls for 72 hours. IL-19 and IL-24 mRNA expression in these co-cultures without treatment or treated with an anti-IL-17A antibody, anti-TNF antibody, their combination or an isotype control antibody. **(C)** Levels of human IL-19 and IL-8 protein in the supernatant of the above- mentioned co-cultures without treatment or treated with an anti-IL-17A antibody, anti-TNF antibody, their combination or an isotype control antibody. Data are shown as means ± SEMs. * $P$<0.05, ** $P$<0.01, and *** $P$<0.001.

## IL-17A neutralization reduces IL-19 and cell proliferation in psoriatic skin lesions

Gene expression data from psoriatic lesional skin, psoriatic non-lesional skin, and healthy skin, obtained in clinical trials with anti-IL-17 biologics in psoriasis, were analyzed using the Genomic Spatial Event (GSE) database. As shown in **Figure 6A**, IL-17A, IL-19, and IL-24 were all significantly higher in psoriatic

lesional skin compared to either psoriatic non-lesional skin or healthy skin. This corroborated our findings of a higher expression of IL-19 and IL-24 in psoriatic skin fibroblasts compared to healthy fibroblasts (**Figure 5**). Furthermore, Ki-67, was significantly increased in psoriatic lesional keratinocytes (**Figure 6A**). Significant upregulation of IL-17A, IL-19, IL-24, and Ki-67 in psoriasis was confirmed in another independent dataset (**Supplementary Figure S5A**).

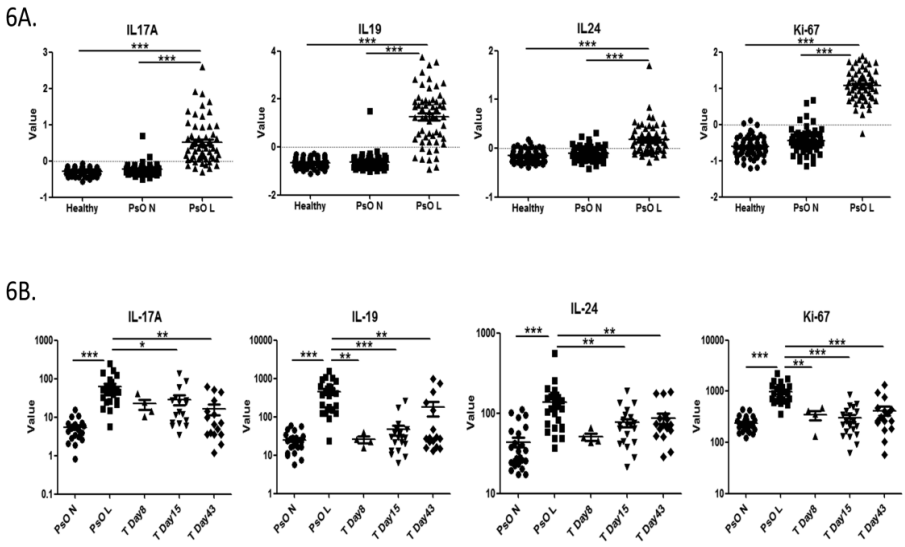



**Figure 6. Expression of human IL-17A, IL-19, IL-24 and Ki-67 in psoriatic lesions and the effects of anti-IL-17RA therapy. (A)** Expression of IL-17A, IL-19, IL-24 and Ki-67 in lesional and non-lesional skin biopsies from 58 psoriasis patients and in healthy skin biopsies from 64 normal controls in data set GSE13355. **(B)** Expression of IL-17A, IL-19, IL-24 and Ki-67 in lesional and non-lesional skin biopsies from 25 psoriasis patients before anti-IL-17RA treatment, 8, 15 and 43 days after treatments in data set GSE53552. Data are shown as means ± SEMs. * $P$<0.05, ** $P$<0.01, and *** $P$<0.001.

In a separate dataset, in which an IL-17 receptor A (IL-17RA) antibody was used in the treatment of psoriasis, a significant reduction of the expression of IL-19, IL-24, and Ki-67 in psoriatic lesional skin was found, reducing their expression levels close to those of non-lesional skin after treatment (**Figure 6B**). Similar significant down-regulation of IL-19 and Ki-67 was confirmed in another study using anti-IL-17A treatment (**Supplementary Figure S5B**). To summarize, multiple gene expression data of psoriatic patients before and after

targeting the IL-17 pathway support our data that IL-17A regulates IL-19 expression in fibroblasts and keratinocytes.

## DISCUSSION

In the present study, we showed that IL-10 neutralization enhanced skin inflammation, thickness and scaling in the IMQ-induced psoriasis mouse model beyond day 5, via upregulation of the IL-17/IL-19 axis. IL-17A induced IL-19 and IL-24 expression in human dermal fibroblasts and epidermal keratinocytes, and IL-17A neutralization reduced the expression of both cytokines. Gene array expression data also show high expression of IL-17A, IL-19, IL-24 and proliferation marker Ki-67 in psoriatic skin lesions, and that anti-IL-17 therapy reduced their expression. In addition to keratinocytes, dermal fibroblasts, through interaction with immune cells and cytokines such as IL-17A and TNF in psoriasis, can be a major source of IL-19 and IL-24 that contribute to perpetuation of psoriatic symptoms such as keratinocyte proliferation and acanthosis.



In psoriasis, recombinant human IL-10 treatment has been demonstrated to improve psoriatic symptoms in clinical trials (20-23). Similarly, in the IMQ-induced psoriasis mouse model, a subset of IL-10-producing B cells was identified, and adoptive transfer of these IL-10-producing B cells reduced disease severity (24). In contrast, as shown by our and other groups, IL-10 neutralization or IL-10 deficiency induced persistent psoriasis-like inflammation after IMQ application (25). In psoriatic skin, macrophages and DC both can be producers as well as direct target cells of IL-10 (28). Frequency of Th17 cells can also be directly controlled by IL-10 and vice versa (29,30).

IL-10 belongs to the cytokine family of IL-19, IL-20, IL-22 and IL-24 and are located in the same cluster on chromosome 1 (31,32). Although IL-10 is the only anti-inflammatory cytokine in this family, simultaneous induction of IL-10 and the IL-20 subfamily has been observed in monocytes by stimulants (33). IL-10 production during inflammation acts as a natural counter-balance to limit the side effects of inflammation. Without this Yin-Yang dynamic equilibrium, inflammation will be skewed towards uncontrolled harmful diseases. For instance, in psoriasis, low levels of IL-10 have been reported in comparison to other inflammatory skin conditions, while contrarily, enhanced expression of IL-

23/IL-17 pathway cytokines has been widely confirmed in psoriatic lesions (9,34,35).

Overexpression of IL-19 and IL-24 has also been observed in psoriatic skin and both induce keratinocyte hyper-proliferation in a reconstituted human skin model (36-38), suggesting a pathogenic role in psoriasis. Myeloid cells are producers of both cytokines, and keratinocytes are also potential producers (31,32,39). Recently, IL-19 was suggested as an important mediator of the IL-23/IL-17 cascade in psoriasis, and IL-17A-induced expression of IL-19 in keratinocytes amplifies keratinocyte responses via auto-paracrine regulation (14). Here we confirmed the induction of IL-19 by IL-17A in human keratinocytes and extended to show that IL-17A neutralization reduced IL-19 in human keratinocyte-T cell co-cultures (**Supplementary Figure S4B**). In addition, our study provided evidence that dermal fibroblasts also produced IL-19 and IL-24 in response to IL-1β and IL-17A, and, when co-cultured with activated memory T cells, fibroblasts produced significant protein levels of IL-19 (**Figure 5A-C**). Synergistic induction of IL-20 subfamily cytokines by IL-1β and IL-17A has also been observed in recent publications (40). Interestingly, in psoriatic fibroblast-T cell co-cultures, higher levels of IL-19 was observed in comparison to healthy fibroblast co-cultures, further supporting the contribution of psoriatic fibroblasts to local IL-19 production. Compared to epidermal keratinocytes, dermal fibroblasts are positioned to encounter more frequently with inflammatory cells including T cells as most infiltrating T cells accumulate in the dermis (**Supplementary Figure S3A**). Therefore, in psoriasis, dermal fibroblasts could be an important local source of IL-20 subfamily cytokines and contribute to keratinocyte hyper-proliferation through a paracrine mechanism.

Discordant regulation of IL-20 in contrast to IL-19 and IL-24 has been found in our study. This could be due to different immune and resident cell types as potential cellular sources for these cytokines (32). Like skin fibroblasts and keratinocytes, other tissue cells such as endothelial cells and fibroblast-like synovial cells can also produce IL-20 subfamily cytokines (32,41). Nevertheless, whether blocking the IL-20 subfamily cytokines will be viable options in psoriasis treatments still warrants further research.

In summary, we show that IL-10 regulates the expression of cytokines related to the IL-23/IL-17 axis via IL-19 and IL-24 influencing a.o. skin thickness and scaling. These data give further insight into the cytokine network in the stromal milieu of psoriasis plaques. Strategies to upregulate local or systemic production of IL-10 in patients with psoriasis could help increasing the effectiveness of current therapies.

## DATA AVAILABILTIY STATEMENT

All datasets generated in this study are provided in the article/supplementary material.



## CONFLICTS OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## AUTHOR CONTRIBUTIONS

XX contributed to the study design, performed experiments and wrote the manuscript. EF, PA, and AM performed experiments and revised the manuscript. PL and LB supported experiments and revised the manuscript. EP contributed to the study design and revised the manuscript. EL designed the study and revised the manuscript.

## FUNDING

The current project is funded by the Departments of Rheumatology and Dermatology, Erasmus MC, University Medical Center Rotterdam. X. Xu is supported by a scholarship under China State Scholarship Fund (CSC No.201406100056).

## Reference


1. Nestle FO, Kaplan DH, Barker J. Psoriasis. *N Engl J Med* 2009; 361:496-509.
2. Greb JE, Goldminz AM, Elder JT, et al. Psoriasis. *Nat Rev Dis Primers* 2016; 2:16082.
3. Griffiths CE, Barker JN. Pathogenesis and clinical features of psoriasis. *Lancet* 2007; 370:263-71.
4. Nickoloff BJ. The Cytokine Network in Psoriasis. *Arch Dermatol* 1991; 127:871-84.
5. Johansen C, Usher PA, Kjellerup RB, et al. Characterization of the interleukin-17 isoforms and receptors in lesional psoriatic skin. *Br J Dermatol* 2009; 160:319-24.
6. Soderstrom C, Berstein G, Zhang W, et al. Ultra-Sensitive Measurement of IL-17A and IL-17F in Psoriasis Patient Serum and Skin. *AAPS J* 2017; 19:1218-22.
7. Lowes MA, Kikuchi T, Fuentes-Duculan J, et al. Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. *J Invest Dermatol* 2008; 128:1207-11.
8. Ortega C, Fernández-A S, Carrillo JM, et al. IL-17-producing CD8+ T lymphocytes from psoriasis skin plaques are cytotoxic effector cells that secrete Th17-related cytokines. *J Leukoc Biol* 2009; 86:435-43.
9. Cai Y, Shen X, Ding C, et al. Pivotal role of dermal IL-17-producing γδ T cells in skin inflammation. *Immunity* 2011; 35:596-610.
10. Krueger JG, Brunner PM. Interleukin-17 alters the biology of many cell types involved in the genesis of psoriasis, systemic inflammation and associated comorbidities. *Exp Dermatol* 2018; 27:115-23.
11. Wang CQF, Akalu YT, Suarez-Farinas M, et al. IL-17 and TNF synergistically modulate cytokine expression while suppressing melanogenesis: potential relevance to psoriasis. *J Invest Dermatol* 2013; 133:2741-52.
12. Hawkes JE, Yan BY, Chan TC, et al. Discovery of the IL-23/IL-17 Signaling Pathway and the Treatment of Psoriasis. *J Immunol* 2018; 201:1605-13.
13. Li HH, Lin YC, Chen PJ, et al. Interleukin-19 upregulates keratinocyte growth factor and is associated with psoriasis. *Br J Dermatol* 2005; 153(3):591-5.
14. Witte E, Kokolakis G, Witte K, et al. IL-19 is a component of the pathogenetic IL-23/IL-17 cascade in psoriasis. *J Invest Dermatol* 2014; 134:2757-67.
15. Konrad RJ, Higgs RE, Rodgers GH, et al. Assessment and Clinical Relevance of Serum IL-19 Levels in Psoriasis and Atopic Dermatitis Using a Sensitive and Specific Novel Immunoassay. *Sci Rep* 2019; 9:5211.
16. van der Fits L, Mourits S, Voerman JS, et al. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J Immunol* 2009; 182:5836-45.
17. Vinter H, Iversen L, Steiniche T, et al. Aldara-induced skin inflammation: studies of patients with psoriasis. *Br J Dermatol* 2015; 172:345-53..

18. van Hamburg JP, Asmawidjaja PS, Davelaar N, et al. Th17 cells, but not Th1 cells, from patients with early rheumatoid arthritis are potent inducers of matrix metalloproteinases and proinflammatory cytokines upon synovial fibroblast interaction, including autocrine interleukin-17A production. *Arthritis Rheum* 2011; 63:73-83.
19. Van Belle AB, de Heusch M, Lemaire MM, et al. IL-22 is required for imiquimod-induced psoriasiform skin inflammation in mice. *J Immunol* 2012; 188:462-9.
20. Asadullah K, Sterry W, Stephanek K, et al. IL-10 is a key cytokine in psoriasis. Proof of principle by IL-10 therapy: a new therapeutic approach. *J Clin Invest* 1998; 101:783-94.
21. Friedrich M, Döcke WD, Klein A, et al. Immunomodulation by interleukin-10 therapy decreases the incidence of relapse and prolongs the relapse-free interval in Psoriasis. *J Invest Dermatol* 2002; 118:672-7.
22. Reich K, Garbe C, Blaschke V, et al. Response of psoriasis to interleukin-10 is associated with suppression of cutaneous type 1 inflammation, downregulation of the epidermal interleukin-8/CXCR2 pathway and normalization of keratinocyte maturation. *J Invest Dermatol* 2001; 116:319-29.
23. McInnes IB, Illei GG, Danning CL, et al. IL-10 improves skin disease and modulates endothelial activation and leukocyte effector function in patients with psoriatic arthritis. *J Immunol* 2001; 167:4075-82.
24. Yanaba K, Kamata M, Ishiura N, et al. Regulatory B cells suppress imiquimod-induced, psoriasis-like skin inflammation. *J Leukoc Biol* 2013; 94:563-73.
25. Jin SP, Koh SJ, Yu DA, Kim MW, Yun HT, Lee DH, Yoon HS, Cho S, Park HS. Imiquimod-applied Interleukin-10 deficient mice better reflect severe and persistent psoriasis with systemic inflammatory state. *Exp Dermatol* 2018; 27:43-49.
26. Kimball AB, Kawamura T, Tejura K, et al. Clinical and immunologic assessment of patients with psoriasis in a randomized, double-blind, placebo-controlled trial using recombinant human interleukin 10. *Arch Dermatol* 2002; 138:1341-6.
27. Saxena A, Khosraviani S, Noel S, et al. Interleukin-10 paradox: A potent immunoregulatory cytokine that has been difficult to harness for immunotherapy. *Cytokine* 2015; 74:27-34.
28. Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. *Nat Rev Immunol* 2010; 10:170-81.
29. Huber S, Gagliani N, Esplugues E, et al. Th17 cells express interleukin-10 receptor and are controlled by Foxp3$^-$ and Foxp3+ regulatory CD4+ T cells in an interleukin-10-dependent manner. *Immunity* 2011; 34:554-65.
30. Shimizu T, Kamata M, Fukaya S, et al. Anti-IL-17A and IL-23p19 antibodies but not anti-TNFα antibody induce expansion of regulatory T cells and restoration of their

suppressive function in imiquimod-induced psoriasiform dermatitis. *J Dermatol Sci* 2019; 95:90-8.

31. Rutz S, Wang X, Ouyang W. The IL-20 subfamily of cytokines--from host defence to tissue homeostasis. *Nat Rev Immunol* 2014; 14:783-95.
32. Sabat R. IL-10 family of cytokines. *Cytokine Growth Factor Rev* 2010; 21:315-24.
33. Wolk K, Kunz S, Asadullah K, et al. Cutting edge: immune cells as sources and targets of the IL-10 family members? *J Immunol* 2002; 168:5397-402.
34. Nickoloff BJ, Fivenson DP, Kunkel SL, et al. Keratinocyte interleukin-10 expression is upregulated in tape-stripped skin, poison ivy dermatitis, and Sezary syndrome, but not in psoriatic plaques. *Clin Immunol Immunopathol* 1994; 73:63-8.
35. Mussi A, Bonifati C, Carducci M, et al. IL-10 levels are decreased in psoriatic lesional skin as compared to the psoriatic lesion-free and normal skin suction blister fluids. *J Biol Regul Homeost Agents* 1994; 8:117-20.
36. Otkjaer K, Kragballe K, Funding AT, et al. The dynamics of gene expression of interleukin-19 and interleukin-20 and their receptors in psoriasis. *Br J Dermatol* 2005; 153:911-8.
37. Rømer J, Hasselager E, Nørby PL, et al. Epidermal overexpression of interleukin-19 and -20 mRNA in psoriatic skin disappears after short-term treatment with cyclosporine a or calcipotriol. *J Invest Dermatol* 2003; 121:1306-11.
38. Sa SM, Valdez PA, Wu J, et al. The effects of IL-20 subfamily cytokines on reconstituted human epidermis suggest potential roles in cutaneous innate defense and pathogenic adaptive immunity in psoriasis. *J Immunol* 2007; 178:2229-40.
39. Yano S, Banno T, Walsh R, et al. Transcriptional responses of human epidermal keratinocytes to cytokine interleukin-1. *J Cell Physiol* 2008; 214:1-13.
40. Bertelsen T, Iversen L, Johansen C. I-Kappa-B-Zeta regulates interleukin-17A/tumor necrosis factor-alpha mediated synergistic induction of interleukin-19 and interleukin-20 in humane keratinocytes. *Ann Dermatol* 2021; 33:122-130.
41. Sakurai N, Kuroiwa T, Ikeuchi H, et al. Expression of IL-19 and its receptors in RA: potential role for synovial hyperplasia formation. *Rheumatology (Oxford)* 2008; 47:815-20.

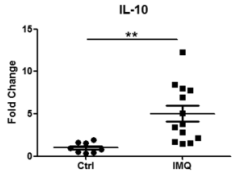
**Supplementary tables**

**Table S1. Primer sequences used in this study.**

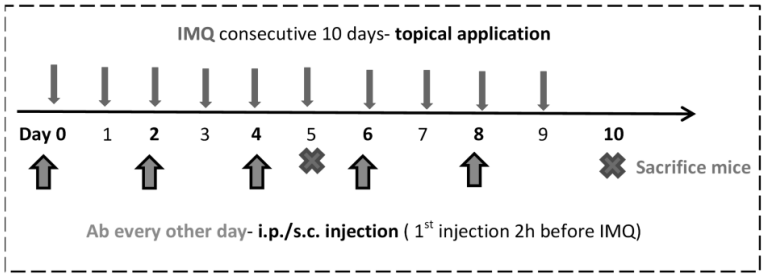
| Name | Species | Sequence |
|---|---|---|
| mIL-23p19 F | Mouse | CACCTCCCTACTAGGACTCAGC |
| mIL-23p19 R | Mouse | TGGGCATCTGTTGGGTCT |
| mIL-22 F | Mouse | TTTCCTGACCAAACTCAGCA |
| mIL-22 R | Mouse | CTGGATGTTCTGGTCGTCAC |
| mIL-17A F | Mouse | TTTTCAGCAAGGAATGTGGA |
| mIL-17A R | Mouse | TTCATTGTGGAGGGCAGAC |
| mIL17F F | Mouse | CAAGAAATCCTGGTCCTTCG |
| mIL17F R | Mouse | GAGCATCTTCTCCAACCTGAA |
| mIL-19 F | Mouse | TGGAGAACCTCAGGAGCATT |
| mIL-19 R | Mouse | GAATGTCAGCAGGTTGTTGG |
| mIL-24 F | Mouse | AGAACCAGCCACCTTCACAC |
| mIL-24 R | Mouse | GTGTTGAAGAAAGGGCCAGT |
| mCXCL2 F | Mouse | AAAATCATCCAAAAGATACTGAACAA |
| mCXCL2 R | Mouse | CTTTGGTTCTTCCGTTGAGG |
| mKC (CXCL1) F | Mouse | GACTCCAGCCACACTCCAAC |
| mKC (CXCL1) R | Mouse | TGACAGCGCAGCTCATTG |
| mGAPDH F | Mouse | AGCTTGTCATCAACGGGAAG |
| mGAPDH R | Mouse | TTTGATGTTAGTGGGGTCTCG |
| IL-19 F | Human | GGAGACTCTGCAGATCATTAAGC |
| IL-19 R | Human | GATCCTTGAACACCCTGTCC |
| IL-24 F | Human | GAAGAATTGAGGCTGCTTGG |
| IL-24 R | Human | GAGGGCAGAAGGGTCTGG |
| HPRT F | Human | TGACCTTGATTTATTTTGCATACC |
| HPRT R | Human | CGAGCAAGACGTTCAGTCCT |

## Supplementary Figures

S1A




S1B




| Groups: | Abbreviation |
|---|---|
| Group1- Control | Ctrl |
| Group2- IMQ + Saline (s.c.) | IMQ |
| Group3- IMQ +Dexa (s.c.) | Dex |
| Group4- IMQ + Isotype (i.p.) | Iso |
| Group5- IMQ + IL-10 Ab (i.p.) | aIL-10 |

**Figure S1. Experimental design of anti-IL-10 therapy in the IMQ-induced psoriasis mouse model.** (A) Expression of IL-10 in lesional skin after topical Aldara application in the IMQ-induced mouse model at day 5. (B) Experimental design and groups of the anti-IL-10 treatment experiments in IMQ-induced psoriasis mouse model sacrificing mice at days 5 and 10. Data are shown as means ± SEMs. ** $P$<0.01.

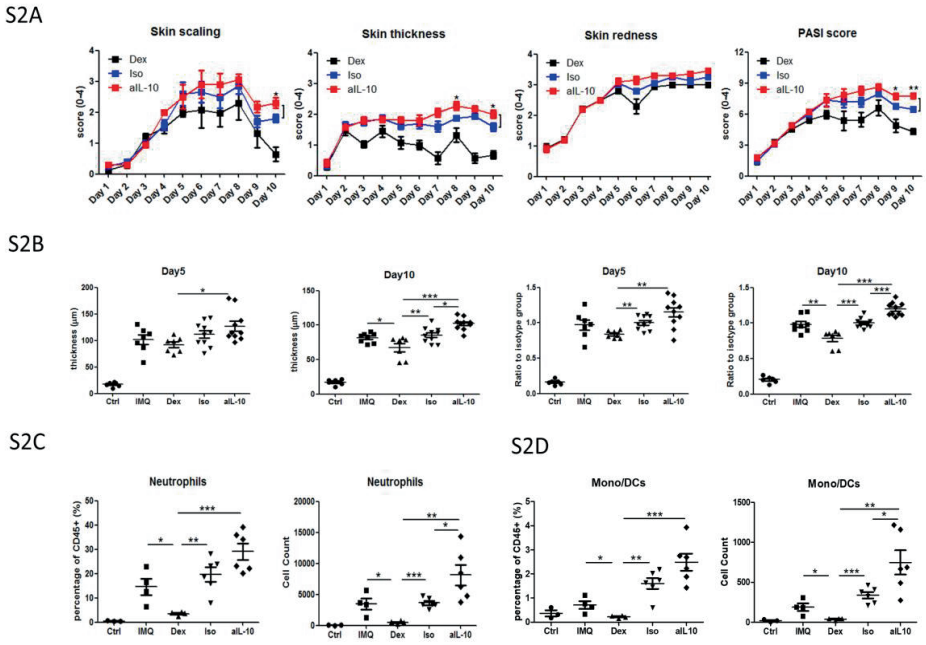



**Figure S2. Kinetic information of macroscopic and histologic scores and infiltration of myeloid cells after anti-IL-10 treatment in the IMQ-induced mouse model.** (A) Time-course follow-up of local PASI scores, including skin scaling, thickness and redness, in anti-IL-10, isotype control antibody and dexamethasone-treated groups during 10 days of Aldara application. (B) Summary of days 5 and 10 epidermal thickness and thickness ratios in all groups including saline-treated IMQ group. (c&d) Summary of percentages and cell numbers of neutrophils (C) and monocyte-derived dendritic cells (D) in about 1 $cm^2$lesional digested skin from all groups including saline-treated IMQ group at day10. Data are shown as means ± SEMs. * $P$<0.05, ** $P$<0.01, and *** $P$<0.001.

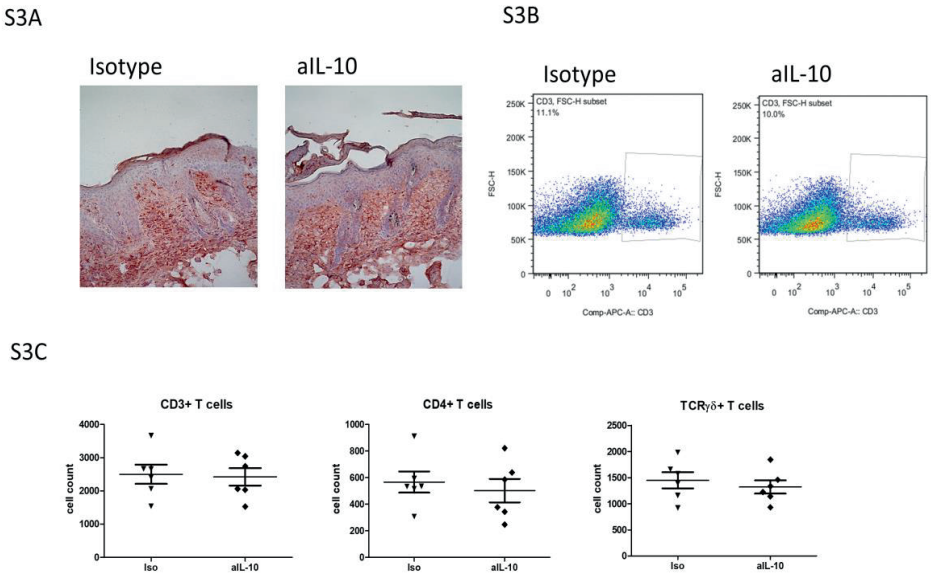



**Figure S3. T cell subsets infiltration in IMQ-applied skin after IL-10 neutralization compared to isotype antibody group.** (A) Representative CD3+ IHC staining in anti-IL-10 and isotype antibody groups 10 days after IMQ application. (B) Representative flow cytometry staining of CD3+ T cells among CD45+ immune cells in anti-IL-10 and isotype antibody groups 10 days after IMQ application. (C) Cell number summary of infiltrating CD3+ T cells, CD3+CD4+ T cells, and CD3+TCRγδ+ T cells in anti-IL-10 and isotype antibody groups (n=6 each) 10 days after IMQ application.

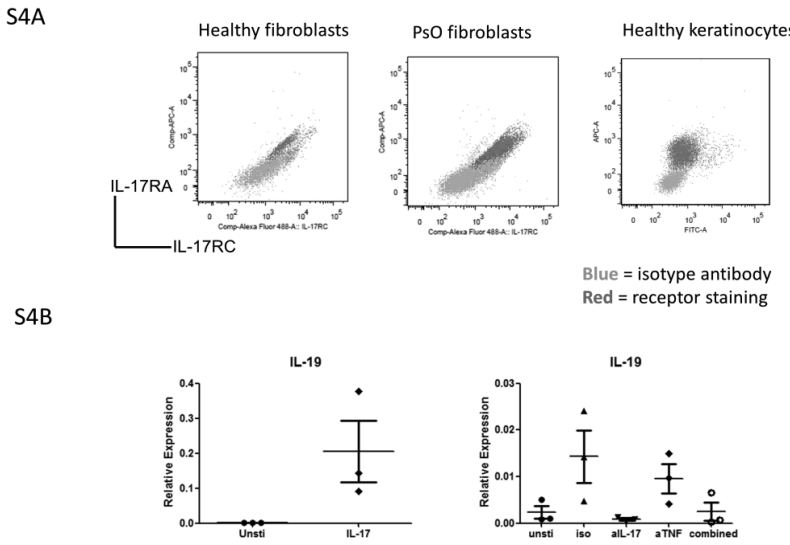



**Figure S4. Fibroblasts and keratinocytes express IL-17RA and IL-17RC and respond to IL-17A stimulation.** (A) Representative staining of IL-17RA and IL-17RC compared to isotype antibody controls on healthy and psoriatic skin fibroblasts, as well as healthy skin keratinocytes. (B) mRNA expression of IL-19 in keratinocytes stimulated with IL-17A for 24 hrs (left panel) and in anti-CD3/anti-CD28 stimulated co-cultures of keratinocytes and sorted healthy CD4+ T memory cells in which neutralizing antibodies against IL-17A, TNF, the combination of these antibodies or an isotype control antibody was added (right panel).

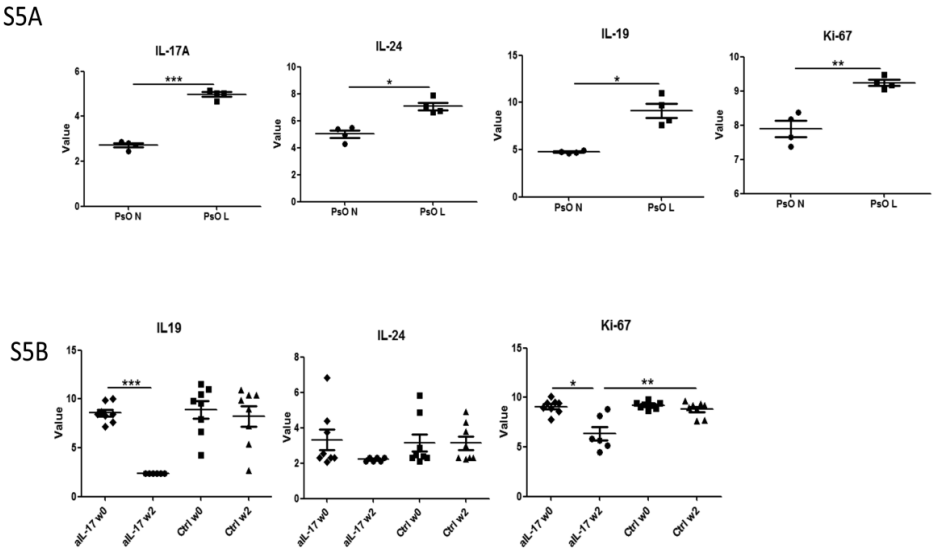



**Figure S5. Expression of IL-19, IL-24 and Ki-67 in psoriatic lesions and the effect of anti-IL-17A antibody treatment.** (A) Expression of IL-17A, IL-19, IL-24 and Ki-67 in lesional and non-lesional skin biopsies from 4 psoriasis patients in dataset GSE50790. (B) Normalization of IL-19, IL-24 and Ki-67 in lesional biopsies from psoriasis patients before (n=8) and after (n=6) 2-week anti-IL-17A treatment with placebo (n=8 each) treatment controls in dataset GSE31652. Data are shown as means ± SEMs. * $P$<0.05, ** $P$<0.01, and *** $P$<0.001.

# 4

## Dual neutralization of IL-17A and TNFα shows additive suppressive effects on proinflammatory cytokines and MMPs in a human model system of Hidradenitis Suppurativa

X. Xu, K.R. van Straalen, K. Dudink, N. Davelaar, A.M.C. Mus,

E. Lubberts*, E.P. Prens*

**Both senior authors contributed equally*

Manuscript in submission

## Abstract

### *Purpose*

Hidradenitis suppurativa (HS) is a recurrent, debilitating skin disease with limited therapeutic options. The purpose of this study is to identify the source and targets of IL-17A in HS lesions and to investigate the potential interaction with TNF in the pathogenesis of HS.

### *Methods*

Expression of proinflammatory cytokines, chemokines and MMPs in lesional HS skin were assessed using RT-qPCR. Flow cytometry was used to identify IL-17A, IL-22, TNF and IL-17RA/RC positive cells. Interaction between IL-17A and TNF were investigated by neutralisation studies of these cytokine in an in vitro human model system of HS.

### *Results*

Proinflammatory cytokines, including IL-17 and IL-1 family members were elevated in HS lesion compared to healthy skin. A significant enrichment of IL-17A+, TNF+ and IL-22+ CD4+, and IL-17A+, TNF+ CD8+ T cells was found locally but not systemically in HS. Both IL-17RA, and in particular IL-17RC, were significantly upregulated in lymphocytes, monocytes, and neutrophils from HS skin relative to healthy blood. Also, HS skin fibroblasts were IL-17RA and IL-17RC positive. Interestingly, neutralizing IL-17A in addition to TNF in a human model system of HS has additive suppressive effects on the production of IL-6, IL-8, IL-beta and MMP-1 compared to single anti-cytokine treatment.

### *Conclusions*

Here we show enrichment of IL-17A, TNF and IL-22 positive cells in HS lesional skin and found lymphocytes, myeloid and stromal cells as IL-17A target cells in HS. Targeting both IL-17A and TNF show better therapeutic effects in a human model system of HS compared to single cytokine blockade.

## Introduction

Hidradenitis suppurativa (HS) is a recurrent, chronic skin disease involving primarily the inverse body areas [1,2]. Inflammatory nodules and abscesses are the typical acute clinical manifestations [3,4]. These lesions are characterized by a highly mixed immune cell infiltrate and upregulation of a multitude of different cytokines and chemokines (such as IL-17A, IL-1β, TNFα, IL-6, CXCL8, CCL20). Tunnel formation and tissue destruction are prominent characteristics of chronic disease potentially driven by matrix metalloproteinases (MMPs). A strong IL-23/IL-17 signature has been consistently found in lesional skin regardless of disease severity or duration [5-7]. The capacity of IL-17A to induce many of the aforementioned downstream inflammatory markers characteristic of HS lesions makes it a potential pivotal player in HS pathophysiology. Nonetheless, in depth analysis of the IL-17A-producing as well as IL-17 receptor- expressing target cells involved in HS lesions remains scarce [10,11]. Despite the lack of in-depth knowledge of the role of IL-17 in HS, anti-IL-17 is currently under investigation in two ongoing phase 3 clinical HS trials (NCT02421172, NCT03248531). Anti-IL-17 could be an interesting addition to the biologic arsenal for this disease, which is currently limited to adalimumab (anti-TNFα) with a response rate of 45-50% [12]. Additive and synergistic effects of IL-17A and TNF have been shown on keratinocytes in vitro and in other immune-mediated inflammatory diseases [13-15]. This suggests that combining anti-IL-17A with adalimumab (anti-TNFα) could increase treatment efficacy [12,16,17].

This study aims to generate a more in depth understanding of the IL-17 pathway in HS by: 1) screening expression profiles of HS-associated cytokines, chemokines and MMPs in HS skin; 2) identifying IL-17A-producing and IL-17 receptor (IL-17RA/IL-17RC) expressing cells in HS skin lesions; and 3) analyzing the potential therapeutic benefits of anti-IL-17A in comparison to and combined with anti-TNF in a co-culture system of IL-17R positive HS skin

fibroblasts and sorted memory CD4+ T cells.

## Materials and methods

### Patient characteristics

Skin samples of 18 HS patients undergoing excision surgery at the Department of Dermatology, Erasmus MC, University Medical Center Rotterdam, The Netherlands were obtained. Patients did not use any biologics <2 months prior to surgery. The opt-out principle of the Erasmus MC, University Medical Center Rotterdam, allows the use of surgical discard for research purposes without the need for additional informed consent. Skin samples from 8 patients were used for RT-qPCR analysis of HS-associated cytokines, chemokines and MMPs. Samples of 10 patients were used to assess identifying IL-17A-producing and IL-17 receptor (IL-17RA/IL-17RC) expressing cells by flow cytometry.

Healthy skin samples (n=8) were obtained from abdominoplasty and breast reduction surgery in the Sint Franciscus Hospital in Rotterdam, The Netherlands. Healthy controls reported no DMARD or biologic use and no family history of HS. Written informed consent was provided by all healthy controls.

In addition, blood from HS patients (not paired with skin) and healthy volunteers was collected to serve as control for flow cytometry staining. Ethical review board approval was provided by the IRB Erasmus University Medical Center Rotterdam (reference NL45264.078.13).

### Flow cytometry

Flow cytometry was used to identify TNF, IL-22, IL-17A-producing and IL-17 receptor (IL-17RA/IL-17RC) expressing cells in HS skin lesions. Skin samples were cut into small pieces and digested with 50µg/mL Liberase DH (dispase high, Sigma-Aldrich) overnight at 40C and an additional 2 hours at 370C. 0.01%

DNase I (Sigma-Aldrich) was added for the last 15 minutes during digestion at 370C. Single cells were vigorously resuspended and filtered through 70µm meshes. Parts of the cell pellets were stimulated with 50 ng/ml phorbol myristate acetate (PMA) (Sigma-Aldrich), 500 ng/ml ionomycin and Golgistop (BD Biosciences) for 4 hours and intracellular staining was performed as previously published [14]. Standard surface staining was performed for IL-17RA and IL-17RC.

Flow cytometry of healthy skin samples, which are known to be almost devoid of T cells, yielded low T cell numbers (supplementary Fig. S1). As infiltrating dermal T cells are derived from the circulation, peripheral blood from non-matched HS patients (HS blood, n=5) and healthy donors (healthy blood, n=5) was included as controls. Red blood cells were lysed prior to surface and intracellular staining. BD LSRFortessa flow cytometer (BD Biosciences) was used to analyze stained samples and FlowJo software (TreeStar) was used to process results.



The following antibodies were used during staining. From BD Biosciences: CD8-PE-CF594 (clone RPA-T8), CD19-PE Cy7 (clone SJ25C1), CD45-PE-CF594 (clone HI30), CD14-APC H7 (clone MϕP9). From BioLegend: CD15-AF700 (clone W6D3), CD16-BV785 (clone 3G8), IFN-γ-AF488 (clone 4S.B3), TNFα-BV421 (clone MAb11), IL-17RA-AF647 (clone BG/hIL17AR), IL-17RA isotype-AF647 (clone MOPC-21). From eBioscience: IL-17A-PE (clone eBio64DEC17), IL-22-eF660 (clone 22URTI). From Sony Biotechnology: CD45-PerCP Cy5.5 (clone HI30), CD3-BV785 (clone OKT3), CD4-BV711 (clone OKT4). From R&D systems: IL-17RC-AF488 (clone 309822), IL- 17RC isotype-AF488 (clone 133303).

To compare expression levels among different cell types between groups, the mean fluorescent index (MFI) of IL-17RA or IL-17RC was divided by the MFI of the corresponding isotype controls and expression ratios were calculated for each sample (with expression ratios of 1 indicating negative

expression).

**Cell sorting**

To obtain memory CD4+ T cells (CD4+CD25low/intCD14-CD45RO+) for co-culture experiments, healthy peripheral blood mononuclear cells were surface stained and memory CD4+ T cells were sorted with FACSAria cell sorter (BD Biosciences). Purity of obtained cell populations was ⩾98%. The following antibodies were used: CD45RO-PerCP Cy5.5 (clone UCHL1), CD14-APC H7 (clone MϕP9), both from BD Biosciences, CD25-PE Cy7 (clone BC96, BioLegend), CD4-FITC (clone RPA-T4, Sony Biotechnology).

**Cell cultures**

HS fibroblasts were isolated and cultured as described previously [18]. Cultured HS fibroblasts were passaged to improve purity with passages 3-8 used in experiments. After reaching 90% confluence, HS fibroblasts were seeded 1.0×104 per well in 96-well flat bottom culture plates. After overnight incubation, fibroblasts were stimulated with IL-1β (0.01 ng/ml), TNFα (5 ng/ml), IL-17A (50 ng/ml), or IL-17F (500 ng/ml), all from R&D systems, for 24 hours in DMEM medium with 0.5% AB serum and 100 U/ml penicillin/streptomycin.

In co-cultures, overnight seeded HS fibroblasts were cocultured with 2.5×104 per well sorted memory CD4+ T cells in complete IMDM medium containing 10% fetal calf serum (Invitrogen), 100 U/ml penicillin/streptomycin, 2 mM L-Glutamine (both from Lonza) and 50 μM β-mercapto-ethanol (Merck). Soluble anti-CD3 (300 ng/ml) / anti-CD28 (400 ng/ml) (both from Sanquin, Amsterdam, The Netherlands) were added in co-cultures for 72 hours. To neutralize cytokines in co-cultures, 100 μg/ml anti-IL-17 (secukinumab, Novartis), 1μg/ml anti-TNF (adalimumab, AbbVie) or their combination were used. An isotype IgG1κ (Sigma-Aldrich) was included as control. The functionality and specificity of these antibodies are shown in supplementary Figure S4b.

**Enzyme-linked immunosorbent assay (ELISA)**

Concentrations of IFN-γ, IL-6 and IL-8 in culture supernatants were measured with Invitrogen ELISA kits. Levels of IL-17A, TNFα, MMP-1 and MMP-3 were measured with R&D systems ELISA Duoset kits. All procedures were performed according to manufacturers' instructions.

**Reverse transcription and real-time polymerase chain reaction (RT-PCR)**

RNA was isolated with Total RNA Miniprep Kit (Sigma Aldrich). Subsequently cDNA was synthesized with 10U/µl Superscript II after treatment with 0.1 U/µl DNAse (both from Invitrogen). RT-PCR was performed with ViiA7 sequence detection system (Life Technologies). Probes were chosen from the universal probe library (Roche Applied Science) and primers were designed using ProbeFinder software. Gene expression data was normalized to house-keeping gene hypoxanthine-guanine phosphoribosyltransferase (HPRT). Primer sequences are available upon request.



**Statistical analysis**

Statistical differences were determined with paired or unpaired student's t test. All data analyses were performed with GraphPad Prism V5 and P-values <0.05 were considered as significant.

## Results

### Dysregulation of proinflammatory cytokines, chemokines, and MMPs in HS skin

Lesional biopsies from 8 HS patients (Hurley stage I n=3, Hurley stage II n=5) were compared with skin of 8 non-matched healthy controls (Table 1). Lesional HS skin showed significantly higher expression of IL-1β, IL-6, and IL-8 compared with healthy skin (Fig. 1a). All assessed IL-23/IL-17 axis cytokines (IL-23p19, IL-22, IL-17A, and IL-17F) and IL-17C were significantly increased in HS lesions (Fig. 1b). In addition, significantly elevated expression of IL-36α,

IL-36γ and IL-36 receptor antagonist (IL-36RN), were found in HS lesional skin, while the expression of the anti-inflammatory cytokine IL-37 was significantly decreased compared with healthy skin (Fig. 1c). Relative to control skin, all analysed MMPs showed significantly increased expression in HS lesional skin (Fig. 1d). In addition, all assessed chemokines were significant upregulated in HS lesional skin compared with healthy control skin (Fig. 1e).

Figure 1

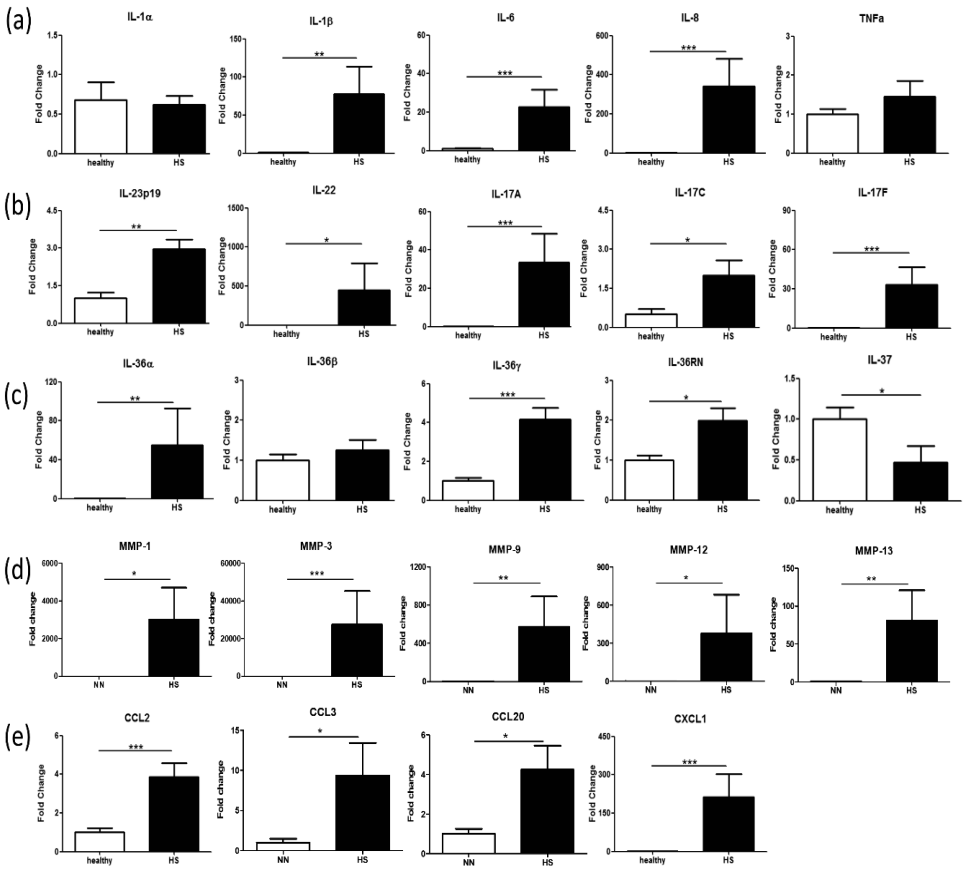



**Figure 1**. Expression levels of cytokines, chemokines and MMPs in HS lesional skin and healthy skin. mRNA levels of (a) inflammatory cytokines, (b) IL-23/IL-17 axis related and IL-17 family cytokines, (c) IL-36 family cytokines, (d) MMPs and (e) chemokines in lesional HS skin and healthy skin controls (n=8 each). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

**Increased frequency of IL-17A+ and IL-22+ CD4+, and IL-17A+ CD8+ T cells in HS skin compared to peripheral blood**

Single cells were isolated from lesional skin of 10 HS patients (Hurley stage II n=7 and III n=3) and compared with non-matched HS blood (n=5) and healthy blood (n=5), (Table 1).

Representative staining results of IL-17A+, IFN-γ+, TNF+, and IL-22+ CD4+ and CD8+ T cells, can be found in Figures 2a and 2c respectively. Lesional HS skin showed a significantly higher proportion of IL-17A+ among CD4+ T cells (13.09±1.77%, mean±SEM) than HS blood (1.24±0.42%, p=0.0004) and healthy blood (0.45±0.20%, p=0.0002) (Fig. 2b). Similarly, a higher percentage of CD8+ T cells were IL-17A+ in HS skin (6.98±1.74%) compared with HS blood (0.07±0.01%) and healthy blood (0.07±0.02%), both p=0.0007 (Fig. 2d).

Comparable proportions of IFN-γ+ CD4+ and IFN-γ+ CD8+T cells were observed in HS skin, HS blood, and healthy blood. Both percentages of TNFα+ CD4+ and TNFα+ CD8+ T cells were significantly higher in HS skin compared with healthy blood (respectively 22.70±4.48% versus 6.97±2.63%, p=0.0343 and 17.23±3.21% versus 6.41±1.68%, p=0.041).

A significantly greater proportion of CD4+ T cells was IL-22+ in HS lesional skin (3.21±0.63%) relative to HS blood (0.56±0.09%) and healthy blood (0.34±0.04%), respectively p=0.013 and p=0.0036 (Fig. 2b). None of the three groups showed credible populations of IL-22+ CD8+ T cells (Fig. 2c).

**Table 1. HS patients' characteristics**

| | **HS patients** | | | **Controls** | | |
|---|---|---|---|---|---|---|
| | **Skin samples** | | **Blood samples** | **Skin samples** | **Blood samples** | |
| **Analysis** | RT-PCR | FC | FC | RT-PCR | FC cytokine staining | FC receptor staining |
| | n=8 | n=10 | n=5 | n=8 | n=5 | n=10 |
| **Female**, *n (%)* | 5 (63%) | 5 (50%) | 2 (40%) | | 2 (40%) | 5 (50%) |
| **Age**, *mean ±SD* | 43.0 ± 14.8 | 38.3 ± 11.2 | 51.0 ± 5.5 | | 48.8 ± 14.5 | 45.0 ± 12.1 |
| **BMI**, *mean ±SD* | 32.2 ± 8.3 | 30.6 ± 4.1 | 30.0 ± 6.5 | NA | NA | NA |
| **Current- or ex-smoker**, *n (%)* | 6 (75%) | 9 (90%) | 4 (80%) | NA | NA | NA |
| **Hurley stage** | | | | | | |
| Stage I, *n (%)* | 3 (26%) | 0 (0%) | 1 (20%) | | | |
| Stage II, *n (%)* | 5 (63%) | 7 (70%) | 3 (60%) | | | |
| Stage III, *n (%)* | 0 (0%) | 3 (30%) | 1 (20%) | | | |
| **Location** | | | | | | |
| Axilla, *n (%)* | 5 (63%) | 5 (50%) | | | | |
| Inguinal, *n (%)* | 3 (27%) | 3 (30%) | | | | |
| Gluteal, *n (%)* | 0 | 2 (20%) | | | | |
| Abdomen, *n (%)* | | | | 5 (62%) | | |
| Mammary, n (%) | | | | 3 (38%) | | |

**SD: standard deviation, FC: flow cytometry. * missing =3.**

Figure 2

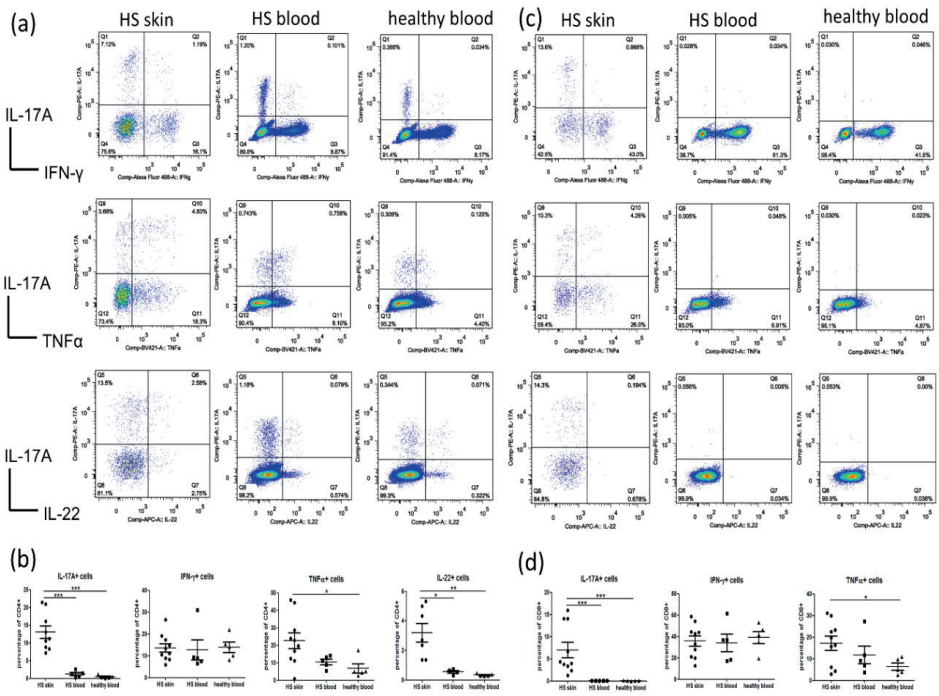



**Figure 2**. Enhanced accumulation of IL-17A+, TNFα+, IL-22+ CD4+ and IL-17+, TNFα+ CD8+ T cells in lesional HS skin compared to peripheral blood. Representative IL-17A, IFN-γ, TNFα, and IL-22 staining results among CD4+ (a) and CD8+ (c) T cells in HS skin (n=10) and peripheral blood of HS patients or healthy donors (n=5 each). Summary of IL-17A, IFN-γ, TNFα, and IL-22 staining results among CD4+ (b) and CD8+ (d) T cells in above samples. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

## Expression of IL-17RA and IL-17RC on lymphocytes, myeloid immune cells and fibroblasts in HS skin

Expression of IL-17RA and IL-17RC was measured on inflammatory cells from HS skin (n=10) and healthy blood (n=10) using flow cytometry (Table 1). Representative staining results from HS skin are shown in supplementary Figure S2a.

In HS skin, the MFI of IL-17RA was significantly higher than isotype control

on CD4+ T cells (p=0.0008), CD8+ T cells (p=0.0025) and CD19+ B cells (p=0.0388), Fig. 3a. Interestingly, CD4+ and CD8+ T cells and CD19+ B cells from HS skin showed a significantly higher MFI of IL-17RC than isotype control (p=0.0005, p=0.0019,and p=0.0088, respectively). Similar to HS skin, expression of IL-17RA and IL-17RC was found on the limited number of CD4+ and CD8+ T cells obtained from healthy skin (supplementary Fig. S3a).

Among myeloid cells, a significantly higher MFI of both IL-17RA and IL-17RC was present on CD14+ monocytes, CD15+CD16+ neutrophils, and CD117+FcεR+ mast cells in HS skin (Fig. 3b).

In healthy blood, a significantly elevated MFI of IL-17RA was found on all above cell types except mast cells (Fig. 3a,b), which was due to negligible numbers of mast cells in healthy blood (data not shown). Uniquely, in healthy blood IL-17RC was not expressed on CD4+ and CD8+ T cells (Fig. 3a,b). In contrast, monocytes and neutrophils were IL-17RC positive and IL-17RC was only slightly expressed on B cells (Fig. 3a,b).

Interleukin-17RA was universally present on all tested lymphocytes and myeloid cells with expression ratios all around 2 (Fig. 3c). Similarly, IL-17RC was expressed on all tested cells with significantly higher expression of IL-17RC on monocytes and neutrophils when compared with CD4+ (respectively p=0.0214 and p=0.0023) and CD8+ T cells (respectively p=0.0090 and p=0.0026), Figure 3c.

Lesional HS skin showed a significantly higher expression of IL-17RC on CD4+ and CD8+ T cells, B cells, monocytes, and neutrophils compared with healthy blood (Fig. 3d). In contrast, the IL-17RA expression on T and B cells was similar between HS skin and healthy blood (supplementary Fig. S2b).

Figure 3

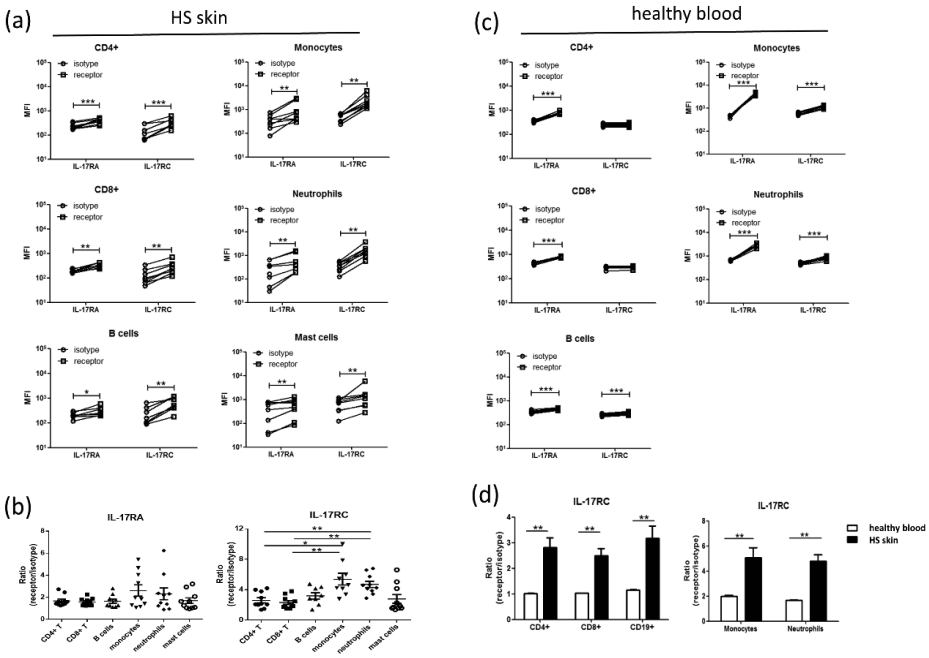



**Figure 3**. Enhanced expression of IL-17RA and IL-17RC on immune cells in lesional HS skin compared to peripheral blood. Mean fluorescence intensity (MFI) of IL-17RA and IL-17RC compared to corresponding isotypes on lymphocyte subsets and myeloid cell types in HS skin (a) and healthy peripheral blood (c) (n=10 each). (b) relative expression ratios of IL-17RA and IL-17RC across various lymphoid and myeloid cell subsets in HS skin. (d) relative expression ratios of IL-17RC on lymphoid and myeloid cell subsets in HS skin compared to healthy peripheral blood. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

## Additive effect of individual or dual neutralization of IL-17A and TNFα in a human ex vivo HS fibroblast - T cell co-culture system

Fibroblasts cultured from HS skin lesions showed IL-17RA and IL-17RC expression (supplementary Fig S4a). To demonstrate functionality of the IL-17RA/RC complex, fibroblasts were stimulated with IL-17A and IL-17F, which resulted in significantly upregulated protein levels of IL-6 and IL-8 in the culture supernatant. Stimulation with IL-17A but not IL-17F induced a significant

increase in levels of MMP-1 and MMP-3 compared to medium control (Fig. 4a). In addition, both IL-1β and TNFα were potent inducers of the above-mentioned mediators (Fig. 4a).

Co-cultures were created with HS derived fibroblasts and healthy peripheral blood derived anti-CD3/anti-CD28 activated memory CD4+ T cells (CD4+CD45RO+CD14-CD25low/int). These memory CD4+ T cells showed upregulated expression of IL-17RA and IL-17RC when co-cultured with skin fibroblasts. Anti-CD3/anti-CD28 activation further increased the expression of both IL-17RA and IL-17RC (supplementary Fig. S3b).

Figure 4

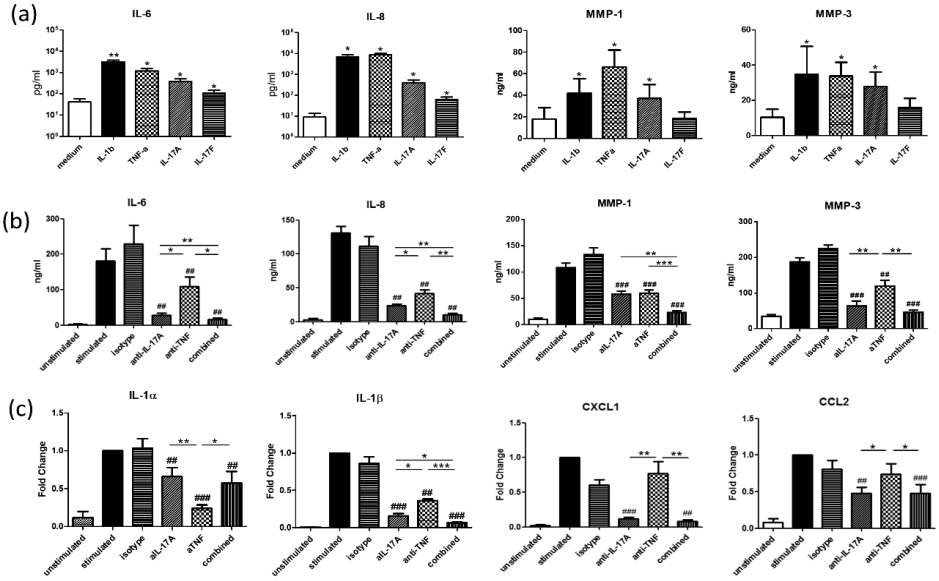



**Figure 4**. IL-17A neutralization reduces inflammatory cytokines, chemokines and MMPs in HS fibroblast-T cell co-cultures. (a) IL-1β, TNFa, IL-17A and IL-17F induced secretion of inflammatory cytokines (IL-6 and IL-8) and MMPs (MMP-1 and -3) in HS skin fibroblast cultures (n=6). (b) Anti-IL-17A, anti-TNF or their combination inhibited secretion of inflammatory cytokines (IL-6 and IL-8) and MMPs (MMP-1 and -3) and (c) mRNA expression of inflammatory cytokines (IL-1α and IL-1β) and chemokines (CXCL1 and CCL2) in HS skin fibroblast-T cell co-cultures (n=6). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ## $p < 0.01$, ### $p < 0.001$.

Neutralizing IL-17A or TNFα significantly reduced the levels of IL-6, IL-8, MMP-1, and MMP-3 in co-culture supernatants compared with isotype antibody controls (Fig. 4b). Combination treatment of anti-IL-17A and anti-TNFα showed significantly stronger inhibition of the levels of IL-6, IL-8 and MMP-1 compared with either anti-IL-17A or anti-TNFα alone (Fig. 4b). The expression of IL-1α and IL-1β mRNA was significantly reduced after neutralization of IL-17A (respectively p=0.0062 and p=0.0001) or TNFα (respectively p=0.0007 and p=0.0058) compared to isotype control. Remarkably, neutralization of anti-TNFα led to a stronger suppression of IL-1α mRNA whereas anti-IL-17A led to a stronger suppression of IL-1β mRNA (Fig. 4c). Combining anti-IL-17A and anti-TNFα treatment significantly suppressed the mRNA expression of IL-1β compared with either antibody alone (Fig. 4c). Anti-IL-17A but not anti-TNFα was found to significantly inhibit the mRNA expression of CXCL1 (respectively p=0.0007) and CCL2 (respectively p=0.0049) compared with isotype control (Fig. 4c).



**Discussion**

In this study, upregulation of a broad range of chemokines, MMPs and proinflammatory cytokines such as IL-1 family members and IL-23-IL-17 immune pathway related cytokines in HS lesional skin was found. Moreover, IL-17-producing and target cells were identified in HS lesional skin and in blood of HS patients compared with healthy controls. Interaction of IL-17A with TNF activity was shown in a human ex vivo model of HS showing that combination therapy of neutralizing IL-17A and TNF resulted in a significantly stronger effect on the suppression of the proinflammatory cytokines IL-6, IL-8 and IL-1beta and the proteinase MMP1 compared to anti-TNF alone.

Screening of spontaneous cytokine production by HS lesional skin identified clear immune dysregulation with significantly upregulated cytokines from the IL-1 family and the IL-23/IL-17 axis, the chemokines CCL2, CCL3, CCL20, and

CXCL1, and MMP-1, -3, -9, -12 and -13 similar to previous findings [19,25,26]. Interestingly, TNFα mRNA expression was not significantly different between HS lesions and healthy skin. This can be explained by the fact that TNFα mRNA expression and TNFα protein production do not correlate well in HS skin as observed in our earlier publication [17]. Lesional skin showed a significant upregulation of IL-1β but not of IL-1α compared with healthy skin, as has been previously reported [19,20]. These decreased or non-elevated IL-1α levels in lesional HS skin have previously been found and could indicate consumption of IL-1α and preferential intracellular translocation of IL-1α at sites of inflammation.

Our results support the importance of the IL-23/IL-17 axis in HS pathogenesis with significantly elevated levels of IL-23p19, IL-22, IL-17A, and IL-17F [6,27]. Moreover, IL-17C, which is mostly produced by activated epithelial cells, including keratinocytes, was significantly upregulated in HS lesions, as previously shown [28]. Upregulation of IL-17C and IL-8 together with IL-36γ contribute to the strong neutrophilic nature of HS.

Metalloproteinases (MMPs) are known to facilitate epithelial-mesenchymal transition. In HS lesions they may allow the epithelium from ruptured hair follicles or cyst walls to become invasive in the microenvironment and to start tunnel formation [29,30]. As depicted in Figure 1d, we found upregulation of MMP-1, -3, -9, -12 and MMP-13 mRNA in HS lesional skin. From our ex vivo stimulation experiments using HS fibroblasts, IL-17A could, comparable to IL-1beta and TNF, directly induce the production of IL-6, IL-8, and MMP-1 and MMP-3, further supporting the role of IL-17A in HS pathogenesis (Fig. 4a).

Not merely overexpression of IL-17 but also an imbalance of IL-17A producing T helper 17 cells (Th17) and regulatory T cells (Treg) has been suggested to contribute to HS pathogenesis [5]. Our data confirm the enrichment of IL-17A+ CD4+ and IL-17A+ CD8+ T cells in HS skin lesions compared with peripheral blood. This indicates that multiple cell sources

contribute to local IL-17A production in HS lesions, maintaining a pro-inflammatory environment. Recently, a study in psoriatic arthritis revealed that CD4+ but not CD8+ T cells secreted IL-17A upon anti-CD3/anti-CD28 activation, although both were IL-17A+ during intracellular staining with PMA/Ionomycin stimulation [15]. Therefore, the exact role of CD8+ T cells in IL-17 production in HS requires further research. We also found enrichment of IL-22+ CD4+ T cells in HS skin compared with peripheral blood [5,31]. These results indicate specific local accumulation of IL-17A+ and IL-22+ CD4+ T cells in HS skin lesions. Since the IL-22 family is considered the driving force of epidermal hyperplasia in psoriasis, the presence of IL-22+ T cells in HS lesions might explain the observed psoriasiform hyperplasia in the overlying epidermis in HS [28,32].



Apart from IL-17-producing cells, less is known regarding IL-17 target cells in HS [32]. IL-17RA is ubiquitously expressed on almost every cell and IL-17RC is predominantly expressed on stromal cells and myeloid cells [11,34]. We show that the expression of IL-17RA is present on lymphocytes and myeloid cells from both HS skin and healthy blood. In contrast, the expression of IL-17RC was significantly higher in CD4 and CD8 positive cells from HS skin but not in healthy blood. Since both T cell subsets express IL-17RA, this indicate that CD4+ and CD8+ T cells are also target cells for IL-17A and IL-17F in HS skin. Therefore, IL-17RC might serve as another potential target for HS treatment.

Although IL-17RA and IL-17RC expression was present across different immune cells in HS skin, both IL-17RA and IL-17RC were expressed at higher levels in monocytes and neutrophils compared to other leukocyte subsets. This suggests that monocytes and neutrophils are better suited to respond to IL-17A and IL-17F stimulation and to contribute to the feed forward loop of inflammation in HS lesions. Overall, the multitude of immune cells in HS lesions expressing IL-17RA and the upregulation of IL-17RC on diverse immune cells in HS skin highlight the sensitivity of local lesional skin to IL-17A stimulation

underscoring its key role in HS pathogenesis. However, it should be noted that resident cell types such as keratinocytes, endothelial cells and fibroblasts, are direct targets of IL-17A and IL-17F and contribute to the inflammation loop and tunnel formation in HS [30,31].

We have shown that HS fibroblasts are IL-17RA and IL-17RC positive and will directly respond to IL-17A in HS by producing inflammatory cytokines and MMPs (Fig. 4a) [19]. When co-cultured with anti-CD3/anti-CD28 activated memory CD4+ T cell, HS fibroblasts produced significantly more inflammatory cytokines, chemokines and MMPs (Fig. 4b and c), suggesting a positive feed-forward loop that might play a role in the development of chronic inflammation in HS, something we have observed previously in inflammatory arthritis [15,18]. This HS fibroblasts-T cell co-culture system provides a simplified, additional ex vivo model to the HS skin explant model reported earlier [17].

Anti-TNF therapy has been approved for HS patients and although a clinical response can be observed, only 45-50% of the HS patients respond to this therapy [12]. Therefore, there is an enormous unmet medical need. In our human ex vivo model system of HS, anti-IL-17A and anti-TNF antibody therapy showed overlapping as well as complementary differential effects on the expression and levels of proinflammatory cytokines, chemokines, and MMPs. For instance, anti-TNF was more potent in reducing IL-1α, while anti-IL-17A was better at inhibiting IL-1β, CXCL1, and CCL2. Both therapies suppressed IL-6, IL-8, MMP-1 and MMP-3, although at a different level. Interestingly, combining anti-TNF with anti-IL-17 achieved better reduction of IL-6, IL-8 and MMP-1 compared to single antibody treatment. In light of the ca. 50% response rate to anti-TNF monotherapy, the potentially synergistic effects of anti-IL-17 therapy and anti-TNF-therapy could indicate a use for a combination of biologics in HS patients. Moreover, the significant reduction in MMP-1 when combining anti-IL-17 therapy and anti-TNF-therapy could suggest a role for combined treatment prior to or at the early stages of tunnel formation. Increased

risks of infection and other potential side effects should be duly noted when considering clinical implementation of this combination therapy.

We acknowledge the limitations of our study. Only very few immune cells could be isolated from healthy skin samples and therefore no trustworthy staining results could be achieved for IL-17A and IL-17RA/IL-17RC positive cells. Therefore, HS lesional skin was compared to peripheral blood, as immune cells from peripheral blood migrate quickly into acute skin lesions to form the inflammatory infiltrate. Interestingly, with the lack of significant differences found between HS blood and healthy blood, and the significant differences seen between HS skin and peripheral blood leukocytes, our results highlight the compartmentalization of the immune response in HS [22].

**Conclusions**

In this study, we showed the overexpression of MMPs, IL-1 family and IL-23/IL-17 axis related cytokines in HS lesions compared with healthy skin. In addition, significant enrichment of IL-17A+ and IL-22+ CD4+, and IL-17A+ CD8+ T cells was found in HS lesional skin compared to blood of HS patients or healthy donors. In addition, we showed for the first time the upregulation of IL-17RC expression on lymphocytes in HS lesions. Finally, we showed an interaction between IL-17A and TNF activity as neutralizing both cytokines showed the best results in suppressing IL-6, IL-8, IL-1beta and MMP-1 in our ex vivo HS fibroblast-T cell co-cultures.



## References

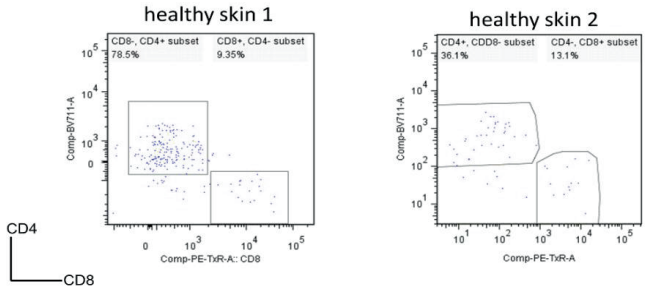
1. Jemec GB. Clinical practice. Hidradenitis suppurativa. N Engl J Med. 2012;366:158-64.
2. Sabat R, Jemec GBE, Matusiak L, et al. Hidradenitis suppurativa. Nat Rev Dis Primers. 2020;6:18.
3. Kurzen H, Kurokawa I, Jemec GB, et al. What causes hidradenitis suppurativa? Exp Dermatol. 2008;17:455-6.
4. Vinkel C, Thomsen SF. Hidradenitis Suppurativa: Causes, Features, and Current

Treatments. J Clin Aesthet Dermatol. 2018;11:17-23.
5. Moran B, Sweeney CM, Hughes R, et al. Hidradenitis Suppurativa Is Characterized by Dysregulation of the Th17:Treg Cell Axis, Which Is Corrected by Anti-TNF Therapy. J Invest Dermatol. 2017;137:2389-95.
6. Schlapbach C, Hänni T, Yawalkar N, et al. Expression of the IL-23/Th17 pathway in lesions of hidradenitis suppurativa. J Am Acad Dermatol. 2011;65:790-8.
7. Thomi R, Schlapbach C, Yawalkar N, et al. Elevated levels of the antimicrobial peptide LL-37 in hidradenitis suppurativa are associated with a Th1/Th17 immune response. Exp Dermatol. 2018;27:172-7.
8. Kelly G, Hughes R, McGarry T, et al. Dysregulated cytokine expression in lesional and nonlesional skin in hidradenitis suppurativa. Br J Dermatol. 2015;173:1431-9.
9. Musilova J, Moran B, Sweeney CM, et al. Enrichment of Plasma Cells in the Peripheral Blood and Skin of Patients with Hidradenitis Suppurativa. J Invest Dermatol. 2020;140:1091-4.e2.
10. Cua DJ, Tato CM. Innate IL-17-producing cells: the sentinels of the immune system. Nat Rev Immunol. 2010;10:479-89.
11. Jin W, Dong C. IL-17 cytokines in immunity and inflammation. Emerg Microbes Infect. 2013;2:e60.
12. Kimball AB, Okun MM, Williams DA, et al. Two phase 3 trials of adalimumab for hidradenitis suppurativa. N Eng J Med. 2016;375:422-434.
13. Moran EM, Mullan R, McCormick J, et al. Human rheumatoid arthritis tissue production of IL-17A drives matrix and cartilage degradation: synergy with tumour necrosis factor-alpha, Oncostatin M and response to biologic therapies. Arthritis Res Ther. 2009;11:R113.
14. Noack M, Beringer A, Miossec P. Additive or Synergistic Interactions Between IL-17A or IL-17F and TNF or IL-1β Depend on the Cell Type. Front Immunol. 2019;10:1726.
15. Xu X, Davelaar N, Mus AM, et al. Interleukin-17A Is Produced by CD4+ but Not CD8+ T Cells in Synovial Fluid Following T Cell Receptor Activation and Regulates Different Inflammatory Mediators Compared to Tumor Necrosis Factor in a Model of Psoriatic Arthritis Synovitis. Arthritis Rheumatol. 2020;72:1303–13.
16. Thorlacius L, Theut Riis P, Jemec GBE. Severe hidradenitis suppurativa responding to treatment with secukinumab: a case report. Br J Dermatol. 2018;179:182-5.
17. Vossen ARJV, Ardon CB, van der Zee HH, et al. The anti-inflammatory potency of biologics targeting tumour necrosis factor-α, interleukin (IL)-17A, IL-12/23 and CD20 in hidradenitis suppurativa: an ex vivo study. Br J Dermatol. 2019;181:314-23.
18. van Hamburg JP, Asmawidjaja PS, Davelaar N, et al. Th17 cells, but not Th1 cells, from patients with early rheumatoid arthritis are potent inducers of matrix metalloproteinases and proinflammatory cytokines upon synovial fibroblast interaction, including autocrine interleukin-17A production. Arthritis Rheum. 2011;63:73-83.
19. Witte-Händel E, Wolk K, Tsaousi A, et al. The IL-1 Pathway Is Hyperactive in Hidradenitis Suppurativa and Contributes to Skin Infiltration and Destruction. J Invest Dermatol. 2019;139:1294-305.
20. Ardon CB, Wang C, Prens EP, et al. Noninvasive assessment of cytokine and antimicrobial peptide levels in hidradenitis suppurativa using transdermal analysis patches. Br J Dermatol. 2021;184:343-5

21. Gottlieb A, Natsis NE, Kerdel F, et al. A Phase II Open-Label Study of Bermekimab in Patients with Hidradenitis Suppurativa Shows Resolution of Inflammatory Lesions and Pain. J Invest Dermatol. 2020;140:1538-45.e2.
22. Kanni T, Tzanetakou V, Savva A, et al. Compartmentalized Cytokine Responses in Hidradenitis Suppurativa. PLoS One. 2015;10:e0130522.
23. Kanni T, Argyropoulou M, Spyridopoulos T, et al. MABp1 Targeting IL-1α for Moderate to Severe Hidradenitis Suppurativa Not Eligible for Adalimumab: A Randomized Study. J Invest Dermatol. 2018;138:795-801.
24. van der Zee HH, Laman JD, de Ruiter L, et al. Adalimumab (antitumour necrosis factor-α) treatment of hidradenitis suppurativa ameliorates skin inflammation: an in situ and ex vivo study. Br J Dermatol. 2012;166:298-305.
25. Balato A, Caiazzo G, Di Caprio R, et al. Response to: 'Interleukin-36 in hidradenitis suppurativa: evidence for a distinctive proinflammatory role and a key factor in the development of an inflammatory loop'. Br J Dermatol. 2018;178:807.
26. Hessam S, Sand M, Gambichler T, et al. Interleukin-36 in hidradenitis suppurativa: evidence for a distinctive proinflammatory role and a key factor in the development of an inflammatory loop. Br J Dermatol. 2018;178:761-7.
27. Vossen ARJV, van Straalen KR, Florencia EF, et al. Lesional Inflammatory Profile in Hidradenitis Suppurativa Is Not Solely Driven by IL-1. J Invest Dermatol. 2020;140:1463-6.e2.
28. Navrazhina K, Frew JW, Krueger JG. Interleukin 17C is elevated in lesional tissue of hidradenitis suppurativa. Br J Dermatol. 2020;182:1045-7.
29. Sanchez J, Le Jan S, Muller C, et al. Matrix remodelling and MMP expression/activation are associated with hidradenitis suppurativa skin inflammation. Exp Dermatol. 2019;28:593-600.
30. Vossen ARJV, van der Zee HH, Prens EP. Hidradenitis Suppurativa: A Systematic Review Integrating Inflammatory Pathways Into a Cohesive Pathogenic Model. Front Immunol. 2018;9:2965.
31. Hotz C, Boniotto M, Guguin A, et al. Intrinsic Defect in Keratinocyte Function Leads to Inflammation in Hidradenitis Suppurativa. J Invest Dermatol. 2016;136:1768-80.
32. van der Zee HH, de Ruiter L, Boer J, et al. Alterations in leucocyte subsets and histomorphology in normal-appearing perilesional skin and early and chronic hidradenitis suppurativa lesions. Br J Dermatol. 2012;166:98-106.
33. Fletcher JM, Moran B, Petrasca A, et al. IL-17 in inflammatory skin diseases psoriasis and hidradenitis suppurativa. Clin Exp Immunol. 2020;201:121-34.
34. Gaffen SL. Structure and signalling in the IL-17 receptor family. Nat Rev Immunol. 2009;9:556-67.

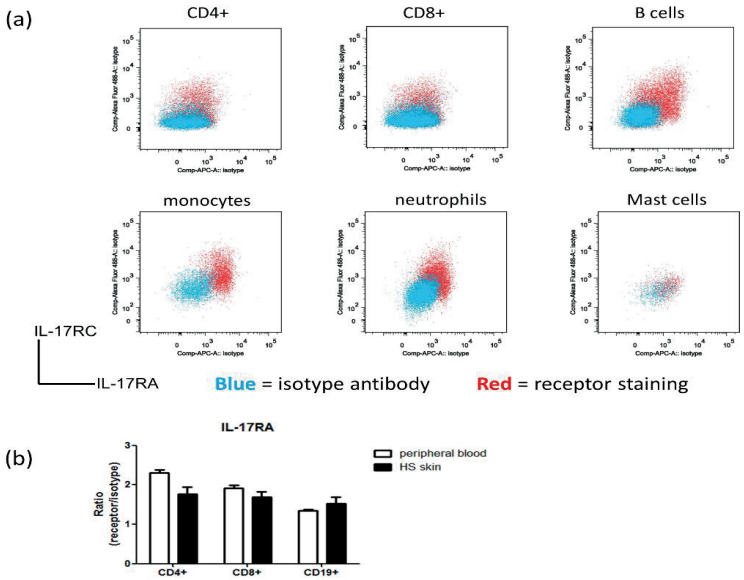
# Supplementary figure legends

supplementary figure 1



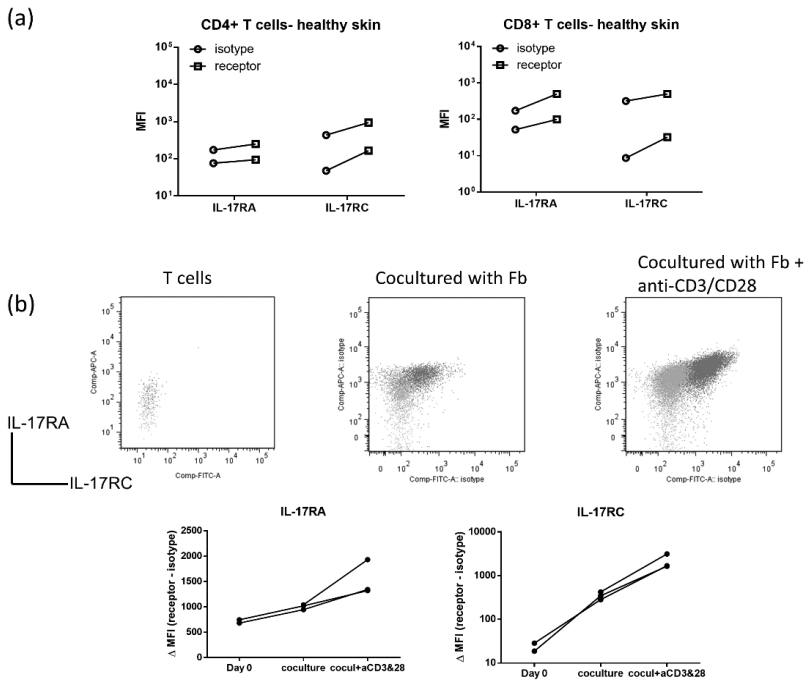

**Supplementary Figure S1**. Low numbers of CD4+ and CD8+ T cells in healthy skin. Representative staining results of CD4+ and CD8+ T cells among CD45+CD3+ T cells in healthy skin (n=2).

supplementary figure 2



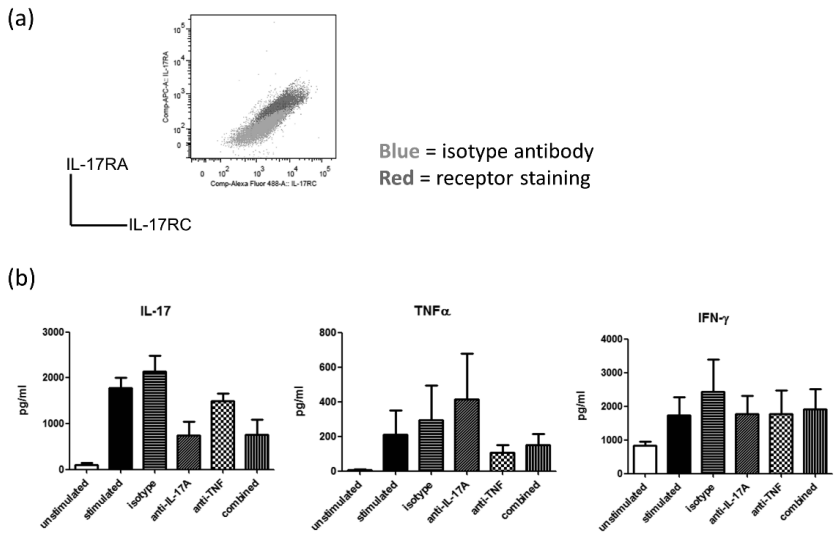

**Supplementary Figure S2**. Expression of IL-17RA and IL-17RC on various immune cells in HS skin. (a) representative flow cytometry staining results of IL-17RA and IL-17RC on CD4+ and CD8+ T cells, B cells, monocytes, neutrophils and mast cells in HS skin. (b) relative expression ratio of IL-17RA on CD4+ and CD8+ T cells, CD19+ B cells in HS skin compared to healthy peripheral blood (n=10 each).

supplementary figure 3




**Supplementary Figure S3**. Expression of IL-17RA and IL-17RC on T cells in healthy skin and their modulation during *ex vivo* co-cultures with skin fibroblasts. (a) expresion of IL-17RA and IL-17RC on CD4+ and CD8+ T cells in healthy skin compared to isotype controls (n=2). (b) representative staining of IL-17RA/RC on CD4+CD45RO+ T cells before and after co-cultures with skin fibroblasts with/without anti-CD3/anti-CD28 stimulation. Summary of relative expression of IL-17RA and IL-17RC on these CD45+ T cells before and after *ex vivo* co-cultures (n=3).

supplementary figure 4




**Supplementary Figure S4.** Expression of IL-17RA/RC on HS fibroblasts and IL-17A and TNF neutralization in HS fibroblast-T cell co-cultures (a) representative flow cytometry staining result of IL-17RA and IL-17RC on HS fibroblasts isolated from HS skin lesions. (b) protein levels of IL-17A, TNFα and IFN-γ in supernatants of HS fibroblast-T cell co-cultures with addition of anti-IL-17A, anti-TNF, their combination or isotype antibody (n=6).

# 5

# Tyk2, but not Lck, inhibition attenuates dermal inflammation and skin scaling but not epidermal hyperplasia in the imiquimod-induced psoriasis mouse model

Xiaofei Xu, Errol Prens, Patrick Asmawidjaja,

Anne-Marie Mus, Erik Lubberts

Manuscript in preparation

## Abstract

Psoriasis is a chronic inflammatory skin disease characterized by typical red, scaly papules and plaques. Abnormal activation of immune cells, especially the IL-23/IL-17 pathway, are central in psoriasis disease pathogenesis. Tyrosine kinases such as the Janus kinase family (including Tyk2) and the Src kinase family (including Lck) are involved in signaling pathways of immune cell functions. In this study, a Tyk2 and a Lck inhibitor were used to examine the influence of these kinase pathways on psoriasis using the imiquimod-induced psoriasis-like mouse model. Tyk2 but not Lck inhibition significantly reduced skin scaling and both had no effect on skin thickness and redness. In addition, Tyk2, but not Lck inhibition, prevented dermal accumulation of immune cells, such as T cells, monocytes and neutrophils, and reduced the expression of Th17 related cytokines as well as chemokines. Interestingly, both kinase inhibitors suppressed IL-23 receptor (IL-23R) expression. These data show that Tyk2, but not Lck is involved in dermal inflammation and scaling in the IMQ-induced psoriasis model, which corresponds with the positive results in clinical trials.

## Introduction

Psoriasis is a chronic autoimmune skin disease affecting around 2 to 3% of the whole population[1,2]. Immune dysregulation is found to play a pivotal role in psoriasis pathogenesis[3]. Aberrant expression of IL-23/IL-17 pathway has been discovered in psoriatic lesions, and recent clinical successes of antibodies targeting these cytokines further confirmed the contribution of this pathway[4-7]. However, treatments with neutralizing antibodies, which need to be injected subcutaneously, are expensive, and are restricted to patients with moderate to severe psoriasis. Therefore, there is still a need to search for more alternatives for oral administration, and small molecule inhibitors might be a plausible option.

Tyrosine kinases are intracellular enzymes that phosphorylate tyrosine molecules of downstream targets, and function as mediators to relay signal transduction from cell surface receptors. Increasing evidence indicates that abnormal activation of tyrosine kinases contributes to various inflammatory skin diseases including psoriasis[8]. Non-receptor tyrosine kinases are kinases without a direct extracellular receptor, but are often coupled to other surface receptors to transmit extracellular signals. Among them, the Jak-family kinases and the Src-family kinases are the most widely studied.

Tyk2 is an important member of the Janus kinase family, is ubiquitously expressed in all tissues and mediates IL-12/IL-23 induced T cell responses[9,10]. Recent evidence showed that Tyk2 inhibitor reduced IL-23 induced T cell activation and IL-23-mediated diseases such as psoriasis and spondyloarthritis[11,12].

Lck is one of the Src kinase family, and plays an important role in T cell receptor (TCR) signaling. TCR dependent anti-CD3 induced T cell proliferation and IL-2 production were reported to be dampened by Lck inhibition[13]. Therefore, it is of great interest to evaluate the potential of Tyk2 and Lck inhibition in psoriasis treatment. Therefore, we used the imiquimod (IMQ)-induced psoriasis-like mouse model which was previously established in our

lab and, like psoriasis is driven by the IL-23/IL-17 axis[14]. Treatments with either Tyk2 or Lck inhibitors were examined and standard psoriasis treatments with corticosteroid β-methasone were included as positive controls.

## Methods

### *IMQ-induced psoriasis mouse model*

BALB/C mice (8-11 week-old) received daily topical applications of 62.5mg 5% Aldara (3M Pharmaceuticals) on the shaved back skin. Control mice were treated with petrolatum (Fagron). Daily evaluation of local psoriasis area and severity index (PASI) including skin thickness, redness and scales was described previously[14]. 70 mg/kg body weight of Tyk2 inhibitor, 45 mg/kg body weight of Lck inhibitor or 5 mg/kg body weight of dexamethasone was intraperitoneally injected daily. Both inhibitors were kindly provided by Dr. R. Buijsman and Dr. G. Zaman, Netherlands Translational Research Center (NTRC), Oss, The Netherlands. On day five of IMQ induction, mice were sacrificed for analysis. Animal experiments were approved by the Erasmus Medical Center animal welfare committee according to the Dutch/European law.

### *Histology and Immunohistochemistry*

Skin biopsies were snap-frozen in TissueTek (Bayer) and sections were cut with a Leica cryostat. CD3 antibody (clone KT3, dilution 1:3,), CD4 antibody (GK1.5, dilution 1:1), CD11c antibody (clone N418, dilution 1:3) and Gr-1 antibody (clone RB6-8C5, dilution 1:300) were used for IHC staining. All primary antibodies were from Bioceros B.V.. H&E and IHC stainings were performed as described earlier[14].

Images were analyzed with LAX V4.12 program (Leica microsystems) or NDP.view2 (Hamamatsu photonics). To measure epidermal thickness, each section was semiquantified by counting the layers of keratinocytes at two different points in the epidermis and the average values were used to reflect overall epidermal thickness.

***Reverse transcription and real-time polymerase chain reaction (RT-PCR)***

RNA was isolated with TRIzol (Thermo Fisher) or Total RNA Miniprep Kit (Sigma-Aldrich). cDNA was synthesized with Superscript II after DNase treatment (both from Invitrogen). RT-PCR was performed with ViiA7 sequence detection system (Life Technologies). Gene expression data were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in mouse samples. Primer sequences are provided in supplementary data.

***Statistical analysis***

Statistical differences were determined with unpaired student's t test. All statistical analyses were performed with GraphPad Prism V5 and P-values <0.05 were considered as significant.

## Results

***Tyk2 inhibition reduces skin scaling but not thickness in IMQ-induced psoriasis-like model***

Tyk2 inhibition significantly reduced skin scaling but not the clinical skin thickness and redness in the IMQ-induced psoriasis model, leading to no significant difference in PASI score. In comparison, β-methasone inhibited both skin scaling and thickness but not skin redness, while Lck inhibition showed no effect on all of these symptoms (**Fig. 1, Suppl fig 1**). To further confirm these findings, H&E staining was performed to measure epidermal thickness and representative staining results are shown in **figure 1B**. Similar to the earlier clinical findings, measuring epidermal thickness revealed that only β-methasone treatment significantly reduced epidermal thickness (**Fig. 1C**).

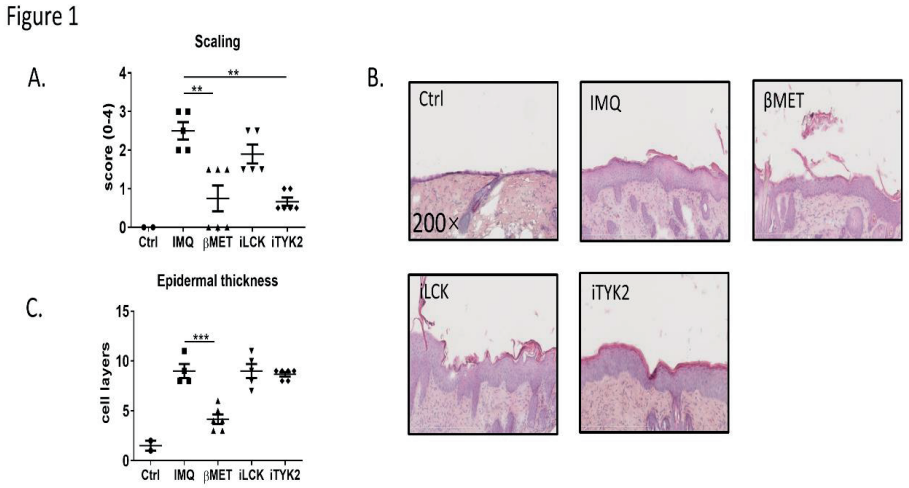



**Figure 1. Tyk2 inhibition reduced scaling but not skin thickness in psoriasis-like mouse model.** (A) skin scaling in IMQ-induced psoriasis-like skin with or without treatments. (B) representative H&E staining results and (C) epidermal thickness in IMQ-induced psoriasis-like skin with or without treatments. βMET, iLCK, and iTYK2 indicate β-methasone treatment, Lck and Tyk2 inhibition, respectively. N=5/6 per group. Normal mouse skin without IMQ application were included as controls (Ctrl, n=2). All staining results were observed with 200× magnification. ** p<0.01, *** p<0.001.

Epidermal levels of expression of keratins and anti-microbial peptides such as psoriasin (S100A7) serve as bio-markers in psoriasis[15,16]. In lesional skin, mRNA expression of keratin16 and keratin 17 was only significantly reduced by β-methasone treatment, but not by Tyk2 or Lck (**Fig. 2A**), which is in line with our clinical and histological observation that Tyk2 and Lck inhibition didn't improve skin thickness in this model. However, and on the contrary, the anti-microbial peptide S100A7A was significantly down-regulated by both β-methasone and Tyk2 (**Fig. 2B**). Taken together, our results show that Tyk2 inhibition improved skin scaling but not thickness in the IMQ-induced psoriasis model.

Figure 2

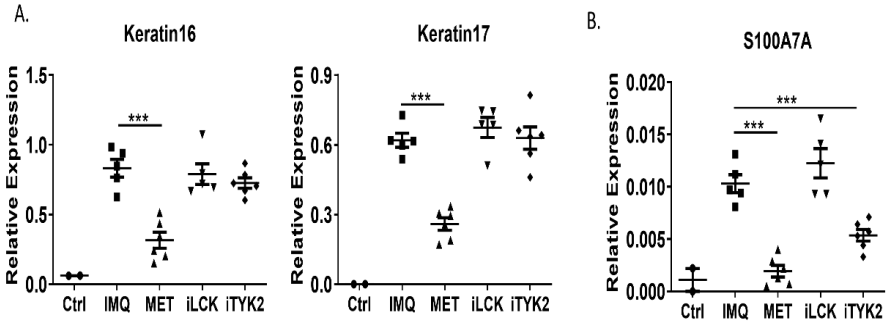



**Figure 2. Tyk2 inhibition reduced antimicrobial peptide but not keratin 16 and keratin 17 mRNA expression in psoriasis-like mouse model.** Expression of keratin16, keratin17 (A) and S100A7A (B) in IMQ-induced psoriasis-like skin with or without treatments. *** p<0.001.

### *Tyk2 inhibition reduces immune cells infiltration in psoriasis-like skin in IMQ-induced model*

IHC staining was performed to examine skin infiltration of CD3+ and CD4+ T cells, CD11c+ monocytes, and Gr-1+ neutrophils. Representative staining results are shown in **figure 3A**. Staining scores are summarized in **figure 3B**. Here we found that β-methasone significantly reduced the infiltraion of CD3+ and CD4+ T cells, monocyte and neutrophils in psoriasis-like skin. Tyk2 inhibition also significantly down-regulated skin infiltration of above cell types (**Fig. 3B**). Lck inhibition didn't affect dermal infiltration of immune cells in psoriasis-like skin (**Fig. 3B**). In summary, Tyk2 but not Lck inhibition significantly reduced immune cell infiltration in IMQ-induced psoriasis-like model.

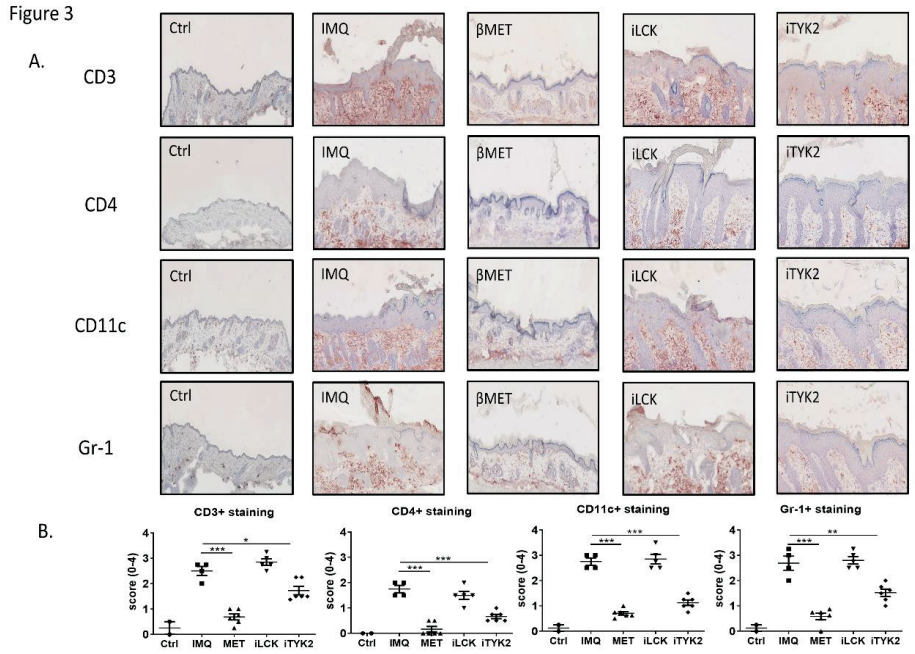



**Figure 3. Tyk2 inhibition reduced immune cell infiltration in psoriasis-like mouse model.** Representative IHC staining (A) and score results (B) including CD3+, CD4+, CD11c+ and Gr-1+ staining in IMQ-induced psoriasis-like skin with or without treatments. βMET, iLCK, and iTYK2 indicate β-methasone treatment, Lck and Tyk2 inhibition, respectively. Normal mouse skin without IMQ application were included as controls (Ctrl). All staining results were observed with 200× magnification.

### *Tyk2 inhibition reduces cytokines and chemokines in IMQ-induced psoriasis-like model*

To further investigate the relation between the degree of infiltration of skin immune cells and proinflammatory cytokines/chemokines, expression of various cytokines and chemokines were analyzed in IMQ-induced skin lesions. Comparable to β-methasone treatment, Tyk2 inhibition significantly down-regulated mRNA expression of IL-23/IL-17 axis-related cytokines including IL-23p19, IL-22, and IL-17A (**Fig. 4A**). Other inflammatory cytokines such as IL-12p35, IL-6 and TNFα were also significantly or strongly suppressed by Tyk2 inhibition and β-methasone treatment (**Fig. 4A**). Interestingly, T helper cell 2

cytokine, IL-4, and anti-inflammatory cytokine, IL-10, were both significantly reduced by Tyk2 inhibition, similar to β-methasone treatment, indicating broad suppression of local skin inflammation (**Fig. 4A**). Consistent with cytokine results, β-methasone treatment and similarly Tyk2 inhibition both significantly

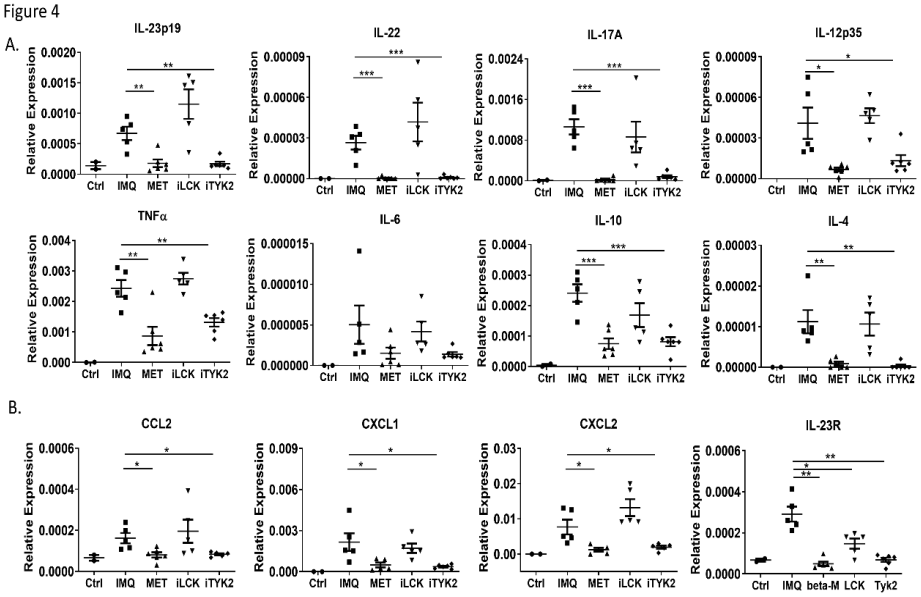



Figure 4. **Tyk2 inhibition reduced skin inflammation such as cytokine and chemokine expression in psoriasis-like mouse model.** mRNA expression of various cytokines (A), chemokines and IL-23R (B) in IMQ-induced psoriasis-like skin. βMET, iLCK, and iTYK2 indicate β-methasone treatment, Lck and Tyk2 inhibition, respectively. N=5/6 per group. Normal mouse skin without IMQ application were included as controls (Ctrl, n=2). * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

suppressed mRNA expression of chemokines such as CCL2, CXCL1, and CXCL2 (**Fig. 4B**). Lck blockade showed no inhibition of all above cytokines and chemokines, except a down-regulation of IL-23 receptor expression (**Fig. 4A&B**). Taken together, our data showed that Tyk2, but not Lck, inhibition significantly reduced expression of cytokines and chemokines in psoriasis-like lesions, further supporting earlier observations of reduced immune cell infiltration in lesional skin.

## Discussion

Our results show that Tyk2 inhibitor suppressed expression of diverse cytokines including the IL-23/IL-17 pathway and reduced accumulation of immune cells such as CD4+ T cells, CD11c+ monocytes, Gr-1+ neutrophils in psoriasis lesions. These results corroborate findings from other groups in which an IL-23 induced psoriasis model was used instead of the imiquimod-induced model as in our study[11]. Furthermore, several phase II and phase III clinical trials reported promising results of Jak1/Tyk2 and selective Tyk2 inhibition in patients with moderate-to-severe psoriasis[17-19]. Suppression of IL-23/IL-17 pathway and immune cell recruitment was also supported in these trials[17,18]. However, contrary to our findings, in Jak1/Tyk2 inhibitor clinical trials, epidermal thickness was observed to be reduced. This might be a result of dual Jak1/Tyk2 inhibition instead of single Tyk2 inhibition in our case. Similar to our observations, a separate study found that epidermal thickness was improved with a Jak1/Tyk2 inhibitor, but not in Tyk2 mutant mice[20]. Moreover, according to earlier experiences, peak inflammation at day 5 of imiquimod-induced psoriasis-like model was chosen as the endpoint, which should be optimal for observation of epidermal thickness differences.

Lck inhibitor, to our surprise, reduced neither skin inflammation nor epidermal thickness in imiquimod-induced psoriasis-like model. Though expression of IL-23 receptor was reduced after Lck inhibition, general experimental psoriatic symptoms were not improved with Lck treatment. This might be because Lck inhibition preferentially regulated TCR-dependent T activation but not non-TCR-dependent phorbol 12-myristate 13-acetate/interleukin-2 (IL-2)-induced T cell proliferation[13]. In imiquimod-induced psoriasis-like model, strong local predominant innate inflammation and cytokine production might bypass TCR-dependent activation and promote cytokine-centered non-TCR-dependent activation. Therefore, this phenomenon renders Lck inhibition ineffective.

In summary, our data show that Tyk2, but not Lck, inhibition reduced skin

scaling and dermal inflammation in imiquimod-induced psoriasis-like model. However, in our experimental model epidermal thickness was not improved by Tyk2 inhibition. This points either to a deficiency in the IMQ model in not being capable of capturing the full potential of Tyk2 inhibtion as seen in clinical trials with the selective Tyk2 inhibitor deucravacitinib or that our Tyk2 inhibitor is less potent than deucravacitinib.

## Acknowledgement

We would like to thank Dr. R. Buijsman and Dr. G. Zaman, Netherlands Translational Research Center (NTRC), Oss, The Netherlands, for kindly providing both Tyk2 and Lck inhibitors.

## Reference

1. Nestle FO, Kaplan DH, Barker J. Psoriasis. **N Engl J Med**. 2009 Jul 30;361(5):496-509.

2. Greb JE, Goldminz AM, Elder JT, Lebwohl MG, Gladman DD, Wu JJ, Mehta NN, Finlay AY, Gottlieb AB. Psoriasis. **Nat Rev Dis Primers**. 2016 Nov 24;2:16082.

3. Griffiths CE, Barker JN. Pathogenesis and clinical features of psoriasis. **Lancet**. 2007 Jul 21;370(9583):263-271.

4. Johansen C, Usher PA, Kjellerup RB, Lundsgaard D, Iversen L, Kragballe K. Characterization of the interleukin-17 isoforms and receptors in lesional psoriatic skin. **Br J Dermatol**. 2009 Feb;160(2):319-24.

5. Di Cesare A, Di Meglio P, Nestle FO. The IL-23/Th17 axis in the immunopathogenesis of psoriasis. **J Invest Dermatol**. (2009) 129:1339-50.

6. Amin M, Darji K, No DJ, Wu JJ. Review of phase III trial data on IL-23 inhibitors tildrakizumab and guselkumab for psoriasis. **J Eur Acad Dermatol Venereol**. 2017 Oct;31(10):1627-1632.

7. Farahnik B, Beroukhim K, Nakamura M, Abrouk M, Zhu TH, Singh R, Lee K, Bhutani T, Koo J. Anti-IL-17 Agents for Psoriasis: A Review of Phase III Data. **J Drugs Dermato**l. 2016 Mar;15(3):311-6.

8. Szilveszter KP, Németh T, Mócsai A. Tyrosine Kinases in Autoimmune and Inflammatory Skin Diseases. **Front Immunol**. 2019 Aug 9;10:1862.

9. Shimoda K, Kato K, Aoki K, Matsuda T, Miyamoto A, Shibamori M, et al. Tyk2 plays a restricted role in IFN alpha signaling, although it is required for IL-12-mediated T cell function. **Immunity**. (2000) 13:561-71.

10. Karaghiosoff M, Neubauer H, Lassnig C, Kovarik P, Schindler H, Pircher H, et al. Partial impairment of cytokine responses in Tyk2-deficient mice. **Immunity**. (2000) 13:549-60.

11. Ishizaki M, Muromoto R, Akimoto T, Sekine Y, Kon S, Diwan M, et al. Tyk2 is a therapeutic target for psoriasis-like skin inflammation. **Int Immunol**. (2014)26:257-67.

12. Gracey E, Hromadová D, Lim M, Qaiyum Z, Zeng M, Yao Y, Srinath A, Baglaenko Y, Yeremenko N, Westlin W, Masse C, Müller M, Strobl B, Miao W, Inman RD. TYK2 inhibition reduces type 3 immunity and modifies disease progression in murine spondyloarthritis. **J Clin Invest**. 2020 Apr 1;130(4):1863-1878.

13. Hanke JH, Gardner JP, Dow RL, Changelian PS, Brissette WH, Weringer EJ, Pollok BA, Connelly PA. Discovery of a novel, potent, and Src family-selective tyrosine kinase inhibitor. Study of Lck- and FynT-dependent T cell activation. **J Biol Chem**. 1996 Jan 12;271(2):695-701.

14. van der Fits L, Mourits S, Voerman JS, Kant M, Boon L, Laman JD, Cornelissen F, Mus AM, Florencia E, Prens EP, Lubberts E. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. **J Immunol**. 2009 May 1;182(9):5836-45.

15. Leigh IM, Navsaria H, Purkis PE, McKay IA, Bowden PE, Riddle PN. Keratins (K16 and K17) as markers of keratinocyte hyperproliferation in psoriasis in vivo and in vitro. **Br J Dermatol**. 1995 Oct;133(4):501-11.

16. Ekman AK, Vegfors J, Eding CB, Enerbäck C. Overexpression of Psoriasin (S100A7) Contributes to Dysregulated Differentiation in Psoriasis. **Acta Derm Venereol**. 2017 Apr 6;97(4):441-448.

17. Papp K, Gordon K, Thaci D, Morita A, Gooderham M, Foley P, et al. Phase 2 trial of selective tyrosine kinase 2 inhibition in psoriasis. **N Engl J Med**. (2018)379:1313–21.

18. Page KM, Suarez-Farinas M, Suprun M, Zhang W, Garcet S, Fuentes-Duculan J, Li X, Scaramozza M, Kieras E, Banfield C, Clark JD, Fensome A, Krueger JG, Peeva E. Molecular and Cellular Responses to the TYK2/JAK1 Inhibitor PF-06700841 Reveal Reduction of Skin Inflammation in Plaque Psoriasis. **J Invest Dermatol**. 2020 Aug;140(8):1546-1555.e4.

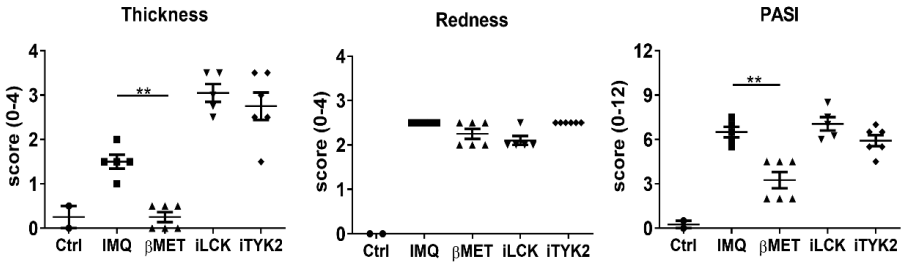
19. Forman SB, Pariser DM, Poulin Y, Vincent MS, Gilbert SA, Kieras EM, Qiu R, Yu D, Papacharalambous J, Tehlirian C, Peeva E. TYK2/JAK1 Inhibitor PF-06700841 in Patients with Plaque Psoriasis: Phase IIa, Randomized, Double-Blind, Placebo-Controlled Trial. **J Invest Dermatol**. 2020 Dec;140(12):2359-2370.e5.

**20.** Works MG, Yin F, Yin CC, Yiu Y, Shew K, Tran TT, Dunlap N, Lam J, Mitchell T, Reader J, Stein PL, D'Andrea A. Inhibition of TYK2 and JAK1 ameliorates imiquimod-induced psoriasis-like dermatitis by inhibiting IL-22 and the IL-23/IL-17 axis. **J Immunol**. 2014 Oct 1;193(7):3278-87.

## Supplementary figure



Supplementary figure 1




Supplementary figure 1. **Tyk2 inhibition showed no effects on skin thickness, redness and total PASI scores in psoriasis-like mouse model.**

**Supplementary table**

| Gene | | Primers |
|---|---|---|
| mGAPDH | Forward | AGCTTGTCATCAACGGGAAG |
| | Reverse | TTTGATGTTAGTGGGGTCTCG |
| mKeratin16 | Forward | AGCAGGAGATCGCCACCTA |
| | Reverse | AGTGCTGTGAGGAGGAGTGG |
| mKeratin17 | Forward | CCGAGATTGGTACCAGAAGC |
| | Reverse | AGGATGCTGGCATTGTCC |
| mS100a7a | Forward | GCCTCGCTTCATGGACAC |
| | Reverse | CGGAACAGCTCTGTGATGTAGT |
| mIL23p19 | Forward | CACCTCCCTACTAGGACTCAGC |
| | Reverse | TGGGCATCTGTTGGGTCT |
| mIL22 | Forward | TTTCCTGACCAAACTCAGCA |
| | Reverse | CTGGATGTTCTGGTCGTCAC |
| mIL17A | Forward | TTTTCAGCAAGGAATGTGGA |
| | Reverse | TTCATTGTGGAGGGCAGAC |
| mIL12p35 | Forward | GAGACTTCTTCCACAACAAGAGG |
| | Reverse | CAGGGTCATCATCAAAGACG |
| mTNFa | Forward | CCACGTCGTAGCAAACCAC |
| | Reverse | TTTGAGATCCATGCCGTTG |
| mIL6 | Forward | ATCAGGAAATTTGCCTATTGAAA |
| | Reverse | CCAGGTAGCTATGGTACTCCAGA |
| mIL10 | Forward | CAGAGCCACATGCTCCTAGA |
| | Reverse | TGTCCAGCTGGTCCTTTGTT |
| mIL4 | Forward | CATCGGCATTTTGAACGAG |
| | Reverse | GACGTTTGGCACATCCATCT |
| mCCL2 | Forward | CATCCACGTGTTGGCTCA |
| | Reverse | GATCATCTTGCTGGTGAATGAGT |
| mCXCL1 | Forward | GACTCCAGCCACACTCCAAC |
| | Reverse | TGACAGCGCAGCTCATTG |
| mCXCL2 | Forward | AAAATCATCCAAAAGATACTGAACAA |
| | Reverse | CTTTGGTTCTTCCGTTGAGG |
| mIL23R | Forward | CCAAGTATATTGTGCATGTGAAGA |
| | Reverse | AGCTTGAGGCAAGATATTGTTGT |

# 6

## Differential expression of IL-17RC on synovial T cells of patients with rheumatoid arthritis compared to psoriatic arthritis: searching for potential regulation mechanisms

Xiaofei Xu, Nadine Davelaar, Anne-Marie Mus, Patrick S. Asmawidjaja,

Marijn Vis, Radjesh J. Bisoendial,

Errol P. Prens, Erik Lubberts

Manuscript in preparation

## Abstract

A different role for the IL-17 pathway between rheumatoid arthritis (RA) and psoriatic arthritis (PsA) has been suggested. Mostly because clinical trials with IL-17A neutralizing antibodies in patients with RA were discontinued due to lower than expected efficacy. In contrast, biologicals targeting the IL-17 pathway is approved for clinical treatment in patients with PsA. Here, we examined whether there is a difference in the synovial cytokine profile, and in expression and regulation of IL-17RA and IL-17RC on synovial immune cells between RA and PsA. We found much overlap in cytokine expression levels in the synovium fluid from patients with RA compared to PsA, except for IL-6, IL-1beta, IL-10, RANKL, OPG, and PTH, which were significantly higher in RA. In addition, the composition of synovial T cell subsets was similar between PsA and RA. Interestingly, the expression of IL-17RC on synovial CD4+ and CD8+ T cells and on synovial B cells was significantly higher in RA compared to PsA. Furthermore, T cell receptor (TCR) activation increased the intensity of IL-17RA and IL-17RC expression, although the effect was higher for IL-17RA. In contrast, cytokine stimulation or stromal cell interaction increased significantly the intensity of IL-17RC expression. Taken together, we found differential expression and regulation of IL-17RC on synovial lymphoid cells between RA and PsA, indicating differences in IL-17 target cells between these diseases. Further analysis is needed to examine the functionality of these findings and to further explore the regulation of the IL-17 receptor family in the different types of inflammatory arthritis.

## Introduction

IL-17A is the most widely studied IL-17 family member, which consist of IL-17A to IL-17F. IL-17A was first discovered in a rodent T cell hybridoma and later confirmed to be mainly produced by T cells in both rodents and humans (1). Similar to the cytokines, the IL-17 receptor family (IL-17R) consists of IL-17RA through IL-17RE. IL-17A functions through the heterodimer receptor IL-17RA and IL-17RC (1).

IL-17A/IL-17R pathway has long been associated with the pathogenesis of arthritis (2,3). Early studies showed that high levels of IL-17A and enhanced percentages of IL-17A+ T cells were present in the synovium and synovial fluid from patients with RA (2). Various arthritis animal models also supported the pivotal importance of IL-17A/IL-17R pathway in RA pathogenesis, and targeting this pathway promised new therapeutic options (3,4).

Development of IL-17A/IL-17R neutralizing biologics put this idea to test and several proof-of-concept phase I clinical trials reassured the treatment benefits of IL-17A neutralizing antibodies in RA (5,6). However, during subsequent phase II clinical trials, IL-17A neutralizing antibodies fell short of expected therapeutic efficacy in RA, although improvements in the disease activity score in 28 joints (DAS28) were observed (7-9). Further phase III trials found significant but modest effects with IL-17A neutralization in RA, but no superior benefits were reported in comparison to other treatments (10-12). More contradicting was the findings that no therapeutic efficacy was observed with IL-17RA blockade in RA (13,14). On the contrary, in psoriatic arthritis (PsA), biologicals targeting the IL-17A/IL-17R pathway showed more favorable therapeutic results and subsequently were approved for clinical usage (15). To decipher the discrepancy between RA and PsA, we collected cells from synovial fluid of RA and PsA patients and studied the expression and the modulation of IL-17A, IL-17RA and IL-17RC with focus on T cells using flow cytometry.

## Methods

### *Patients*

Synovial fluid (SF) of active PsA and RA patients (n=10 each) were collected. RA patients' age 52.70±5.22, female:male ratio 7:3, PsA patients' age 51.44±4.11, female:male ratio 5:7. All diagnoses were performed by rheumatologists according to classification criteria. This study is part of the Dutch South-West Psoriatic Arthritis Register (DEPAR) study and is approved by medical ethics review board of Erasmus Medical Center Rotterdam.

### *Flow cytometry and cell sorting*

Cell pellets from SF were stained for surface markers following standard operating procedures. For intracellular staining, cells were stimulated for 4 hours (hrs) with 50 ng/ml phorbol myristate acetate (PMA) (Sigma-Aldrich), 500 ng/ml ionomycin and Golgistop (BD Biosciences). Afterwards, following manufacturer's instructions, cells were stained for surface markers and Fixable Viability Dye eF506 (eBioscience 65-0866-14). Subsequently, cells were fixed with 2% paraformaldehyde in phosphate buffered saline (PBS) and permeabilized with 0.5% saponin buffer (0.5% BSA, 0.05% NaN3 in PBS). CD4+CD45RO+CD25$^{low/int}$CD14- T memory cells were sorted from buffy coats. LSR II flow cytometer (BD Biosciences) was used to analyze samples and FlowJo software (TreeStar) was used to process results. Cells were sorted with FACSAria cell sorter (BD Biosciences) and purity of obtained cell populations was $\geqslant$ 98%.

### *Flow cytometry antibodies*

Following antibodies were used during staining. From BD Biosciences: CD8-PE-CF594 (clone RPA-T8), TCRγδ-PE Cy7 (clone 11F2), CD45-PE-CF594 (clone HI30), CD14-APC H7 (clone MϕP9), CD45RO-PerCP Cy5.5 (clone UCHL1). From BioLegend: CD25-PE Cy7 (clone BC96), IL-17RA-AF647 (clone BG/hIL17AR), IL-17RA isotype-AF647 (clone MOPC-21). From eBioscience:

IL-17A-PE (clone eBio64DEC17). From Sony Biotechnology: CD45-PerCP Cy5.5 (clone HI30), CD3-BV785 (clone OKT3), CD4-BV711 (clone OKT4), CD4-FITC (clone RPA-T4). From R&D systems: IL-17RC-AF488 (clone 309822), IL-17RC isotype-AF488 (clone 133303).

***Cell culture***

Sorted T cells were seeded $1\times10^5$ per well in 96-well round bottom culture plates and stimulated with 0.3 µg/ml soluble anti-CD3 and 0.4 µg/ml soluble anti-CD28 (both from Sanquin, Amsterdam, The Netherlands) for 3 days in IMDM (Lonza) supplemented with 10% fetal calf serum (Invitrogen), 100 U/ml penicillin/streptomycin, 2 mM L-Glutamine (both from Lonza) and 50 µM β-mercapto-ethanol (Merck). For cytokine stimulation, 10 ug/mL IL-6, 2 ug/mL IL-23 or their combination was used in addition to anti-CD3/anti-CD28 activation. **Fibroblast-like synovial cells (**FLS) was isolated and cultured as described previously (16). After reaching 90% confluence, allogeneic FLS were seeded $1.0\times10^4$ per well in 96-well flat bottom culture plates and after overnight incubation, $2.5\times10^4$ T cells per well were added with/without soluble anti-CD3/anti-CD28 as above.

***Legendplex cytokine analysis***

Synovial fluid from 21 RA and 24 PsA patients were analyzed for cytokines and bone markers using Legendplex kits (BioLegend) according to manufacturer's recommendations.

***Statistical analysis***

Data are shown as mean ± standard error (SEM). Statistical differences were determined with paired or unpaired student's *t* test. All data analyses were performed with GraphPad Prism V5 and P-values <0.05 were considered as significant.

## Results

### *Overlapping but differential cytokine levels in synovial fluid of RA compared to PsA*

Synovial fluid (n=21 respectively 24) from RA and PsA patients were analyzed for cytokines and bone markers. Compared to PsA, significantly higher levels of IL-1 β , IL-6, and IL-10 were found in RA, while cytokines such as TNF, IFNɣ, IL-17A, and IL-17F were similar between the two diseases (**Figure 1)**. Moreover, osteoprotegrin (OPG), RANK ligand (RANKL), and parathyroid hormone (PTH) were all significantly higher in RA compared to PsA, but levels of osteopontin (OPN) were not different. Taken together, these data suggest that, overlapping but differential cytokine levels exist in RA and PsA, and a greater dysregulation of cytokines might contribute to RA pathogenesis.

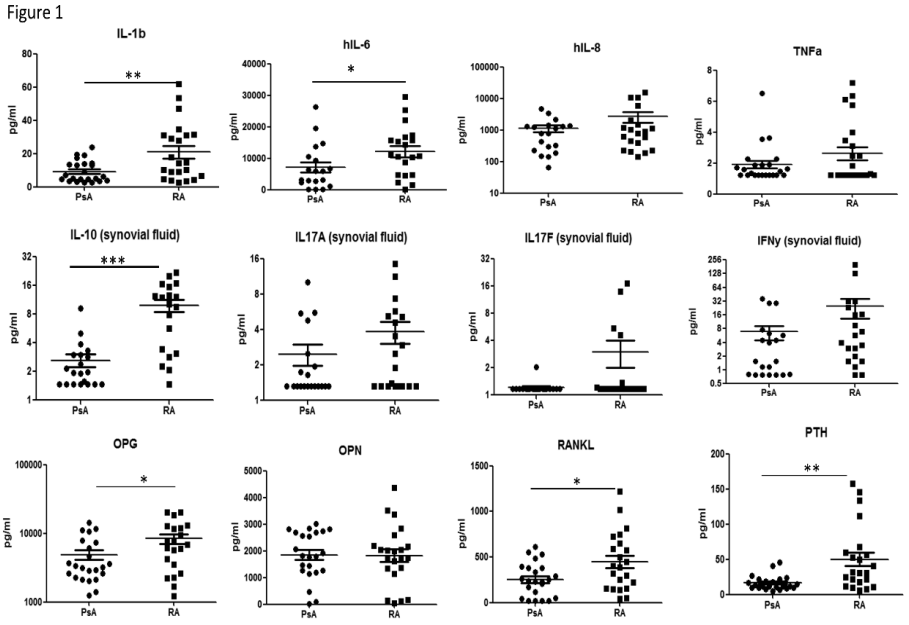



**Figure 1. Overlapping but differential cytokine levels in PsA and RA SF.** Levels of various cytokines and bone markers in synovial fluid of PsA and RA patients. Osteoprotegrin (OPG), osteopontin (OPN), and parathyroid hormone (PTH). * $p<0.05$, ** $p<0.01$, and *** $p<0.001$.

***Similar T cell composition, but differential IL-17A/IL-17R expression in RA compared to PsA***

Cell pellets collected from synovial fluid (SF) of RA and PsA patients (n=10 each) were analyzed with flow cytometry. As shown in **Figure 2**, percentages of CD3+ T cells were comparable between RA and PsA SF (RA 23.26±4.53% (mean±SEM) versus PsA 21.90±5.97%). Similarly, comparing SF from patients with RA versus PsA, no significant differences were found for the percentages of T cell subsets such as CD4+ (RA 54.99±3.79% versus PsA 62.82±2.77%), CD8+ (RA 39.55±3.78% versus PsA 29.54±3.12%) and γδT cells (RA 2.70±0.99% versus PsA 2.31±0.57%) (**Figure 2**). Among myeloid cells, neutrophils (RA 60.00±4.15% versus PsA 45.55±7.02%), eosinophils (RA 0.72±0.34% versus PsA 2.65±0.99%), monocytes (RA 6.50±2.42% versus PsA 8.62±2.36%) and mast cells (RA 0.74±0.26% versus PsA 2.30±0.84%) were all similar in SF from RA compared to PsA (**Figure 2**).

Figure 2

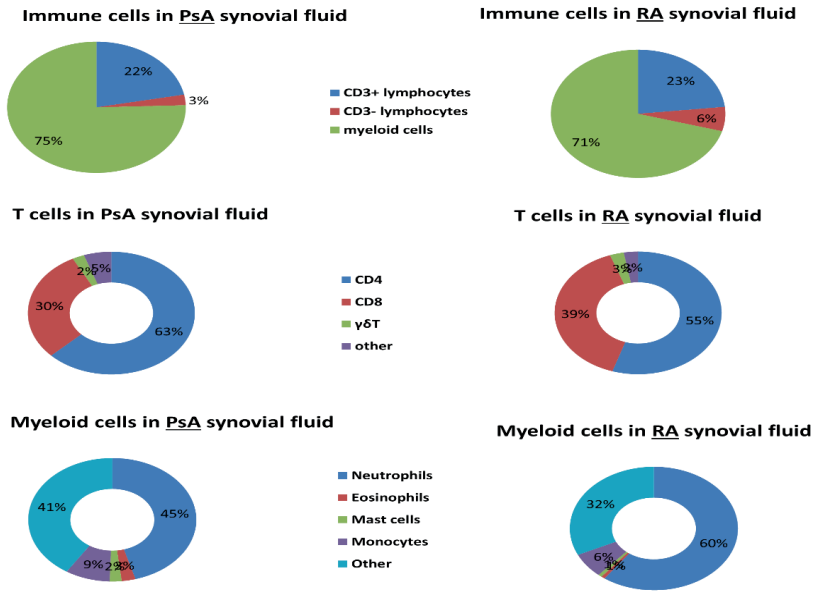



**Figure 2. Similar cell composition in PsA SF and higher IL-17R on T cells in RA SF.** Cell percentage results of various immune cells including lymphocytes such as CD3+ T cells and CD4+, CD8+, γδT cell subsets and myeloid cells in PsA and RA SF (n=10 each).

Percentages of IL-17A+ cells in SF were also similar between RA and PsA (RA 1.16±0.34% versus PsA 0.90±0.18%) (**supplementary Figure 1**). However, among CD8+ T cells, percentages of SF IL-17A+ cells were significantly higher in PsA (1.31±0.29%) compared to RA (0.32±0.10%) (**supplementary Figure 1**).

To identify the expression of the IL-17 receptor subunits IL-17RA and IL-17RC, subunit specific antibodies and their isotype controls were used. Representative IL-17RA and IL-17RC staining results on SF lymphoid and myeloid cells from patients with RA versus PsA are shown in **supplementary Figure S2**. To compare between groups, IL-17RAIL-17RC double positive results were summarized in **Figure 3**. Among the different myeloid cells examined, such as neutrophils, eosinophils, monocytes and mast cells, showed a 70-100% IL-17RAIL-17RC double positivity in the RA and PsA SF samples (**Figure 3A**). However, among lymphoid cells, almost none of T cell subsets and B cells were IL-17RAIL-17RC double positive in PsA. Interestingly, the majority of the SF RA samples showed IL-17RAIL-17RC double positivity for CD4+, CD8+, B cells and gd T cells (Figure 3, Supple Fig 2).

To further analyze the IL-17 receptor expression between groups, results were normalized by dividing the mean fluorescence intensity (MFI) of target antibodies with their corresponding isotype controls. As shown in **Figure 3B**, when comparing RA SF to PsA, trends of enhanced expression of IL-17R subunit IL-17RA was observed on both CD4+ and CD8+ T cells. Interestingly, as summarized in **Figure 3C**, significant up-regulation of IL-17R subunit IL-17RC were found on these two T cell subtypes in RA SF compared to PsA (**Figure 3C**). Together, our data show that expression of IL-17A and the IL-17 receptor subunit IL-17RC on T cells were differently regulated in RA and PsA SF.

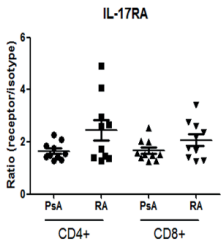
Figure 3

A

| | | Synovial fluid ( PsA ) (n=10) | Synovial fluid ( RA ) (n=10) |
|---|---|---|---|
| | | IL-17RA & IL-17RC | IL-17RA & IL-17RC |
| Lymphoid | CD4+ T cells | 1/10 | 7/10 |
| | CD8+ T cells | 0/10 | 7/10 |
| | γδ T cells | 0/7 | 5/7 |
| | B cells | 0/9 | 6/9 |
| Myeloid | Neutrophils | 7/10 | 10/10 |
| | Eosinophils | 9/9 | 10/10 |
| | Mast cells | 9/10 | 10/10 |
| | Monocytes | 10/10 | 10/10 |

Number in each cell of the table indicates the positive prevalence of IL-17RA & IL-17RC in that cell subsets, and deep colors mean a positive prevalence of larger than 50%.

B




C

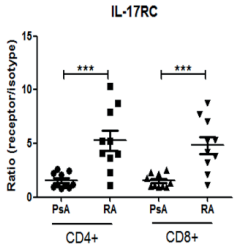



**Figure 3. Higher expression of IL-17R, particularly IL-17RC, on lymphoid cells in RA SF compared to PsA.** (A) summary of IL-17RA+IL-17RC double positivity in various lymphocytes such as CD3+, CD4+, CD8+, γδT cell subsets and myeloid cells in PsA and RA SF (n=10-12). (B) Expression of IL-17RA and IL-17RC was normalized to isotype antibody staining, and compared between PsA and RA SF among CD4+ and CD8+ T cells. *** p<0.001.

### *TCR activation, cytokine stimulation and cellular contacts regulate IL-17R expression on T cells*

To understand the regulation of IL-17R subunit expression of IL-17RA and IL-17RC, CD4+CD45RO+CD25$^{low/int}$CD14- T memory cells were sorted from buffy coats. Changes of IL-17R subunit expression were calculated by subtracting MFI results of receptor stainings from those of isotype controls. After 3 days of anti-CD3/anti-CD28 activation, expression levels of both IL-17RA and IL-17RC were significantly up-regulated (**Figure 4A** and **supplementary Figure S3A**). Moreover, in addition to anti-CD3/anti-CD28 activation, cytokine stimulation with IL-23, IL-6 or their combination further enhanced expression of IL-17RC but not that of IL-17RA (**Figure 4B** and **supplementary Figure S3B**). Percentages of IL-17R+ (IL-17RA/RC) cells were significantly increased with

IL-6 or combined IL-6/IL-23 stimulation, and higher levels of IL-6 were found in RA SF compared to PsA SF (**supplementary Figure S3B** and **Figure 1**). Furthermore, T cells cocultured with fibroblast-like synovial cells (FLS) greatly up-regulated IL-17RC expression, while activation with anti-CD3/anti-CD28 greatly increased the expression of IL-17RA (**Figure 4C** and **supplementary Figure S3C**). Collectively, our data suggest that the expression of IL-17R subunits IL-17RA and IL-17RC on T cells could be modulated by anti-CD3/anti-CD28 activation, IL-6/IL-23 cytokine stimulation and/or T cell-fibroblast contacts.

Figure 4

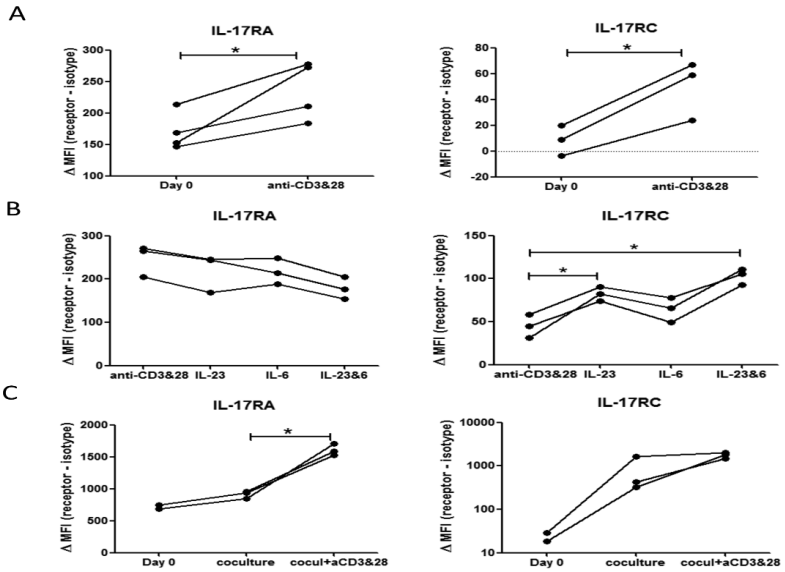



**Figure 4. TCR activation, cytokine stimulation and stromal cell contacts regulate expression of IL-17R on CD4+ T cells.** (A) CD4+CD45RO+ T memory cells (n=3-4) were sorted from buffy coats, and stimulated with anti-CD3/anti-CD28 for 3 days. Expression of IL-17RA and IL-17RC was studied on these T cells before and after TCR stimulation with subtraction of isotype staining background. (B) Besides anti-CD3/anti-CD28 activation, IL-23, IL-6 or their combination were added to stimulate T cells. 3 days later, expression of IL-17RA and IL-17RC was checked. (C) Expression of IL-17RA and IL-17RC on sorted CD4+ T memory cells at day0 or co-cultured with SF FLS with/without anti-CD3/anti-CD28 activation at day4. * $p < 0.05$.

## Discussion

In animal and in vitro experiments, the IL-17A/IL-17R pathway has been demonstrated to contribute to the pathogenesis of arthritis (2,3). However, clinical trials targeting this pathway revealed that neutralzing the acitivty of the IL-17A/IL-17R axis achieved limited efficacy in RA compared to PsA (7-12). This indicates that the underlying regulation of the IL-17 pathway may be different in RA and PsA. Here, our data support this hypothesis, and show that the both IL-17A and the IL-17R subunit IL-17RC were differentially expressed in SF lymphoid cells from RA and PsA. Al though the percentages of IL-17A+ CD4+ T cells were similar in these two diseases, significantly higher IL-17A+ CD8+ T cells were found in PsA compared to RA, which is in line with findings of other groups (17,18). Moreover, the expression of IL-17RC was higher on SF derived CD4+ and CD8+ T cells in RA compared to PsA. Our data also suggest that this is the case for SF derived B cells and gdTcells in RA compared to PsA. Our data further show that TCR activation, cytokine stimulation and cellular contacts influence the expression of IL-17RA and/or IL-17RC.

The dilemma of targeting IL-17 pathway in RA was nicely reviewed in a recent publication (19). New discoveries in the field of IL-17 and IL-17R biology may help to better understand the regulation mechanisms of this pathway. Our group recently reported that in PsA SF, although the percentages of IL-17A+ CD8+ T cells were higher, TCR activation didn't induce meaningful amounts of IL-17A secretion from SF derived CD8+ T cells compared to CD4+ T cells (16). Therefore, the higher percentages of IL-17A+ CD8+ T cells observed in PsA compared to RA still need to be further investigated with other techniques. Similarly, new findings of IL-17RD as a functional receptor subunit of IL-17A also complicated the scope of the IL-17 pathway (20-22). Whether IL-17RD was differently expressed in RA and PsA posted another question to be answered. Besides, results supporting the existence of soluble IL-17R subunits are accumulating in other diseases (2), and whether/how they function as

regulators of IL-17 pathway is unknown.

To summarize, our data show that IL-17A and in particular IL-17RC are differently regulated in SF derived lymphoid cells from patients with RA compared to PsA. TCR activation, cytokine stimulation and cellular contacts influence the expression of the IL-17R subunits IL-17RA and IL-17RC. Future research is needed to decipher the biological dynamics of the IL-17 pathway regulation. A deeper understanding of the IL-17R positive cells in disease settings and their contribution to the disease pathogenesis will help to increase personalized medicine targeting this immune pathway in inflammatory arthritis and other disorders.

## Reference

1. Gaffen SL. Structure and signalling in the IL-17 receptor family. **Nat Rev Immunol**. 2009 Aug;9(8):556-67.

2. Robert M, Miossec P. IL-17 in Rheumatoid Arthritis and Precision Medicine: From Synovitis Expression to Circulating Bioactive Levels. **Front Med** (Lausanne). 2019 Jan 14;5:364.

3. Lubberts E. The IL-23-IL-17 axis in inflammatory arthritis. **Nat Rev Rheumatol**. 2015 Jul;11(7):415-29.

4. Kugyelka R, Kohl Z, Olasz K, Mikecz K, Rauch TA, Glant TT, Boldizsar F. Enigma of IL-17 and Th17 Cells in Rheumatoid Arthritis and in Autoimmune Animal Models of Arthritis. **Mediators Inflamm**. 2016;2016:6145810.

5. Genovese MC, Van den Bosch F, Roberson SA, Bojin S, Biagini IM, Ryan P, Sloan-Lancaster J. LY2439821, a humanized anti-interleukin-17 monoclonal antibody, in the treatment of patients with rheumatoid arthritis: A phase I randomized, double-blind, placebo-controlled, proof-of-concept study. **Arthritis Rheum**. 2010 Apr;62(4):929-39.

6. Hueber W, Patel DD, Dryja T, Wright AM, Koroleva I, Bruin G, Antoni C, Draelos Z, Gold MH; Psoriasis Study Group, Durez P, Tak PP, Gomez-Reino JJ; Rheumatoid Arthritis Study Group, Foster CS, Kim RY, Samson CM, Falk NS, Chu DS, Callanan D, Nguyen QD; Uveitis Study Group, Rose K, Haider A, Di Padova F. Effects of AIN457, a fully human antibody to interleukin-17A, on psoriasis, rheumatoid arthritis, and uveitis. **Sci Transl Med**. 2010 Oct 6;2(52):52ra72.

7. Tlustochowicz W, Rahman P, Seriolo B, Krammer G, Porter B, Widmer A, Richards HB. Efficacy and Safety of Subcutaneous and Intravenous Loading Dose Regimens of Secukinumab in Patients with Active Rheumatoid Arthritis: Results from a Randomized Phase II Study. **J Rheumatol**. 2016 Mar;43(3):495-503.

8. Mease PJ, Jeka S, Jaller JJ, Kitumnuaypong T, Louthrenoo W, Mann H, Matsievskaia G, Soriano ER, Jia B, Wang C, Nie J, Hsia E. CNTO6785, a Fully Human Antiinterleukin 17 Monoclonal Antibody, in Patients with Rheumatoid Arthritis with Inadequate Response to Methotrexate: A Randomized, Placebo-controlled, Phase II, Dose-ranging Study. **J**

**Rheumatol**. 2018 Jan;45(1):22-31.

9. Burmester GR, Durez P, Shestakova G, Genovese MC, Schulze-Koops H, Li Y, Wang YA, Lewitzky S, Koroleva I, Berneis AA, Lee DM, Hueber W. Association of HLA-DRB1 alleles with clinical responses to the anti-interleukin-17A monoclonal antibody secukinumab in active rheumatoid arthritis. **Rheumatology** (Oxford). 2016 Jan;55(1):49-55.

10. Tahir H, Deodhar A, Genovese M, Takeuchi T, Aelion J, Van den Bosch F, Haemmerle S, Richards HB. Secukinumab in Active Rheumatoid Arthritis after Anti-TNFα Therapy: A Randomized, Double-Blind Placebo-Controlled Phase 3 Study. **Rheumatol Ther**. 2017 Dec;4(2):475-488.

11. Dokoupilová E, Aelion J, Takeuchi T, Malavolta N, Sfikakis PP, Wang Y, Rohrer S, Richards HB. Secukinumab after anti-tumour necrosis factor-α therapy: a phase III study in active rheumatoid arthritis. **Scand J Rheumatol**. 2018 Jul;47(4):276-281.

12. Blanco FJ, Möricke R, Dokoupilova E, Codding C, Neal J, Andersson M, Rohrer S, Richards H. Secukinumab in Active Rheumatoid Arthritis: A Phase III Randomized, Double-Blind, Active Comparator- and Placebo-Controlled Study. **Arthritis Rheumatol**. 2017 Jun;69(6):1144-1153.

13. Martin DA, Churchill M, Flores-Suarez L, Cardiel MH, Wallace D, Martin R, Phillips K, Kaine JL, Dong H, Salinger D, Stevens E, Russell CB, Chung JB. A phase Ib multiple ascending dose study evaluating safety, pharmacokinetics, and early clinical response of brodalumab, a human anti-IL-17R antibody, in methotrexate-resistant rheumatoid arthritis. **Arthritis Res Ther**. 2013 Oct 25;15(5):R164.

14. Pavelka K, Chon Y, Newmark R, Lin SL, Baumgartner S, Erondu N. A study to evaluate the safety, tolerability, and efficacy of brodalumab in subjects with rheumatoid arthritis and an inadequate response to methotrexate. **J Rheumatol**. 2015 Jun;42(6):912-9.

15. Blauvelt A, Chiricozzi A. The Immunologic Role of IL-17 in Psoriasis and Psoriatic Arthritis Pathogenesis. **Clin Rev Allergy Immunol**. 2018 Dec;55(3):379-390.

16. Xu X, Davelaar N, Mus AM, Asmawidjaja PS, Hazes JMW, Baeten DLP, Vis M, Bisoendial RJ, Prens EP, Lubberts E. Interleukin-17A Is Produced by CD4+ but Not CD8+ T Cells in Synovial Fluid Following T Cell Receptor Activation and Regulates Different

Inflammatory Mediators Compared to Tumor Necrosis Factor in a Model of Psoriatic Arthritis Synovitis. **Arthritis Rheumatol**. 2020 Aug;72(8):1303-1313.

17. Menon B, Gullick NJ, Walter GJ, Rajasekhar M, Garrood T, Evans HG, Taams LS, Kirkham BW. Interleukin-17+CD8+ T cells are enriched in the joints of patients with psoriatic arthritis and correlate with disease activity and joint damage progression. **Arthritis Rheumatol**. 2014 May;66(5):1272-81.

18. Steel KJA, Srenathan U, Ridley M, Durham LE, Wu SY, Ryan SE, Hughes CD, Chan E, Kirkham BW, Taams LS. Polyfunctional, Proinflammatory, Tissue-Resident Memory Phenotype and Function of Synovial Interleukin-17A+CD8+ T Cells in Psoriatic Arthritis. **Arthritis Rheumatol**. 2020 Mar;72(3):435-447.

19. Taams LS. Interleukin-17 in rheumatoid arthritis: Trials and tribulations. **J Exp Med**. 2020 Mar 2;217(3):e20192048.

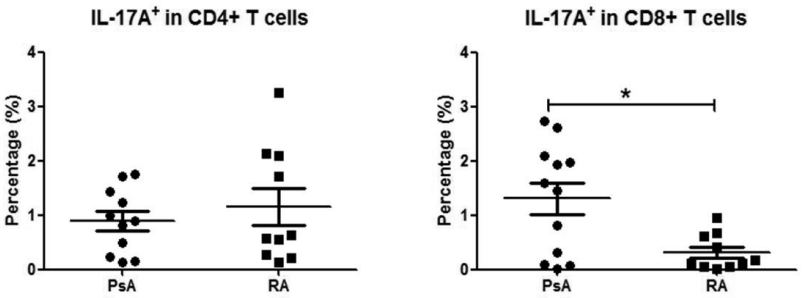
20. Rong Z, Wang A, Li Z, Ren Y, Cheng L, Li Y, Wang Y, Ren F, Zhang X, Hu J, Chang Z. IL-17RD (Sef or IL-17RLM) interacts with IL-17 receptor and mediates IL-17 signaling. **Cell Res**. 2009 Feb;19(2):208-15.

21. Mellett M, Atzei P, Horgan A, Hams E, Floss T, Wurst W, Fallon PG, Moynagh PN. Orphan receptor IL-17RD tunes IL-17A signalling and is required for neutrophilia. **Nat Commun**. 2012;3:1119.

22. Su Y, Huang J, Zhao X, Lu H, Wang W, Yang XO, Shi Y, Wang X, Lai Y, Dong C. Interleukin-17 receptor D constitutes an alternative receptor for interleukin-17A important in psoriasis-like skin inflammation. **Sci Immunol**. 2019 Jun 7;4(36):eaau9657.

## Supplementary figures

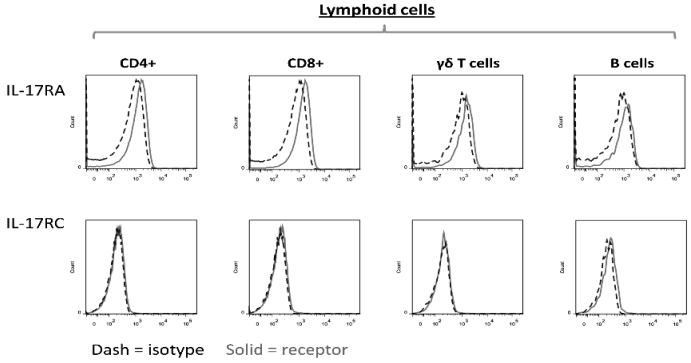
Supplementary Figure S1



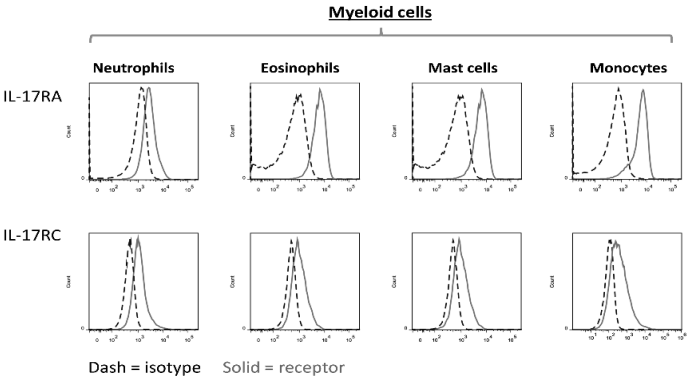

**Supplementary figure 1. Higher percentages of IL-17A+ in CD8+ T cells in RA SF compared to PsA.** Summary IL-17A+ staining results of CD4+ and CD8+ T cells in RA and PsA SF (n=10-12). * p<0.05.

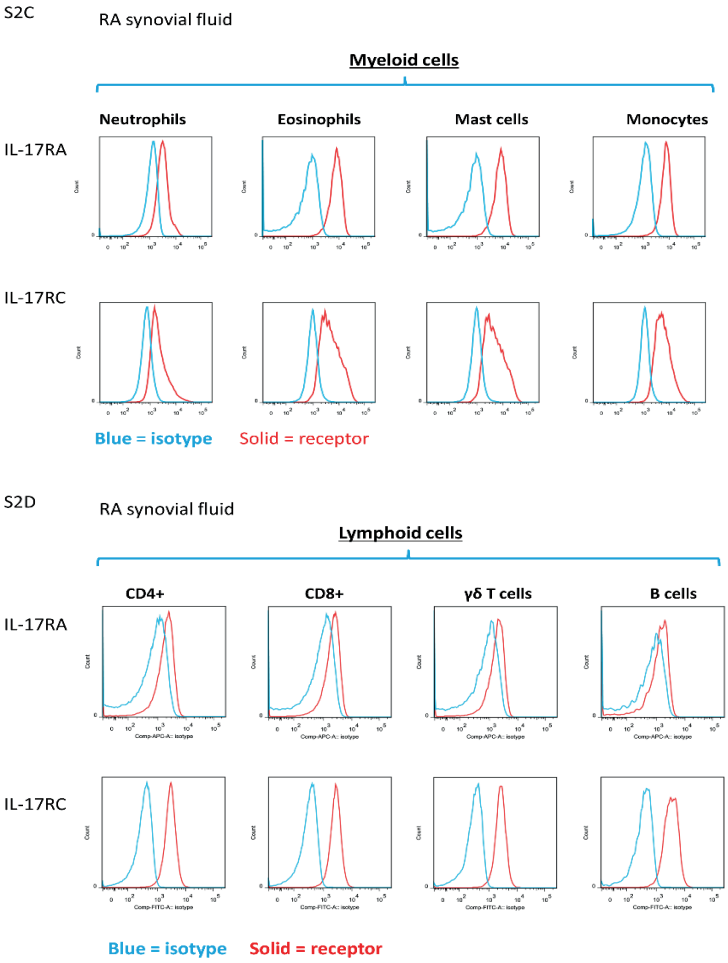
Supplementary Figure S2

S2A PsA synovial fluid

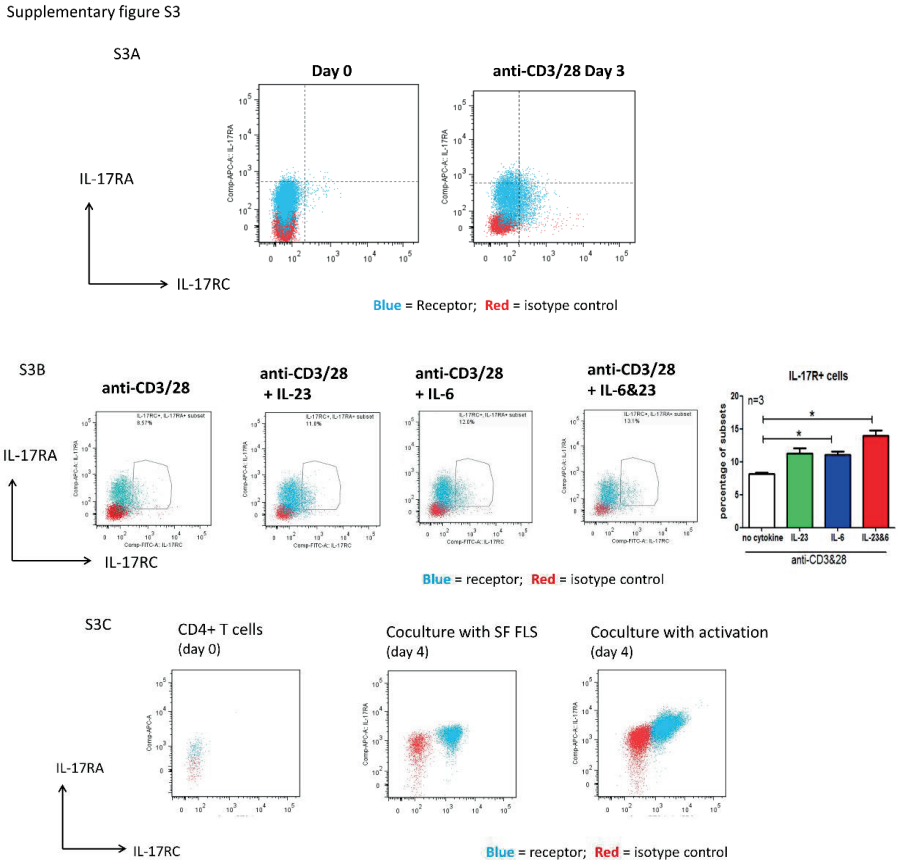



S2B PsA synovial fluid






**Supplementary figure 2. Higher expression of IL-17RC on lymphocytes in RA SF compared to PsA.** Representative staining results of IL-17RA and IL-17RC on various cell types in RA and PsA SF (n=10-12). IL-17RA or IL-17RC stainings were indicated as red solid lines, while isotype antibodies for each receptor antibody were included and labeled as black dash lines or blue solid lines, respectively.




**Supplementary figure 3. TCR activation, cytokine stimulation and cellular contacts up-regulated IL-17R expression on CD4+ T memory cells.** (A) representative staining results of IL-17RA and IL-17RC on sorted CD4+CD45RO+ T memory cells before and after 3 days' anti-CD3/anti-CD28 activation. (B) representative staining results of IL-17RA and IL-17RC on CD4+CD45RO+ T memory cells after 3 days' anti-CD3/anti-CD28 activation with or without stimulation of IL-23, IL-6 or their combination, and summary of IL-17R+ (IL-17RA+/RC+) results (n=3). (C) representative staining results of IL-17RA and IL-17RC on CD4+CD45RO+ T cells before and after 4 days' co-cultures with SF FLS with/without anti-CD3/anti-CD28 activation (n=3). * $p<0.05$.

# 7

## Biologics in immune-mediated inflammatory diseases

Xiaofei Xu, Koen Dudink, Errol P. Prens, Erik Lubberts

**Manuscript in preparation**

## Biologics in immune-mediated inflammatory diseases (IMID)

During the past two decades, advents of biological treatments targeting specific immune pathways have revolutionized therapeutic options in the management of IMID. These biological disease-modifying antirheumatic drugs (DMARDs) also shed light on the diverse contribution of molecular and cellular mechanisms in IMID pathogenesis such as psoriasis, hidradenitis suppurativa (HS), rheumatoid arthritis (RA) and psoriatic arthritis (PsA). Hereby, we will focus our discussion on the common and different therapeutic effects of targeting TNF, IL-23/IL-17 axis, IL-1 and IL-6 in these IMID diseases. A summary of currently available biologics targeting these pathways in psoriasis, HS, RA and PsA can be found in **table 1**.

**Table 1. Summary of biologics in IMID with focus on joint and skin**

| Targeting pathway | Biologics | IMID Diseases | | | |
|---|---|---|---|---|---|
| | | RA | PsA | PsO | HS |
| **TNF** | Adalimumab | + | + | + | + |
| | Infliximab | + | + | + | * |
| | Golimumab | + | + | | |
| | Certolizumab pegol | + | + | + | |
| | Etanercept | + | + | + | * |
| **IL-17** | Secukinumab | | + | + | * |
| | Ixekizumab | | + | + | |
| | Bimekizumab | | | + | |
| | Brodalumab | | | + | |
| **IL-23** | Guselkumab | | + | + | |
| | Tildrakizumab | | | + | |
| | Risankizumab | | | + | |
| **IL-1** | Anakinra | + | | Pustular* | * |
| | AMG 108 | Phase II | | | |
| **IL-6** | Tocilizumab | + | | Pustular* | |
| | Clazakizumab | Phase II | Phase II | | |

+ approved in clinic * multiple case reports

**Tumor necrosis factor (TNF) inhibitors**

TNF is mainly produced by immune cells, particularly by activated macrophages and lymphocytes, although production by stromal cells such as fibroblasts, adipocytes and endothelial cells were also reported (1). TNF exists in two different forms, secreted or membrane-bound (2). Secreted TNF functions through the receptor TNFR1, which contributes importantly to autoimmune reactions (2). Membrane-bound TNF can function through both receptor TNFR1 and TNFR2, where TNFR2 signaling is involved in would healing (2).

TNF is a master regulator of gene expression in a cell-specific manner. For instance, we and others found that TNF induces large amounts of cytokines such as IL-6 and IL-8 and tissue-degrading MMPs in synovial fibroblast-like synoviocytes (FLS) and skin fibroblasts (3,4, **chapter 2-4**). In contrast, cytokine induction such as IL-6 in monocytes and neutrophils by TNF is lower or limited (2). This might be attributed to the sustained NFκB signaling induced by TNF in stromal cells compared to better fine-tuned NFκB activation in immune cells (2). Furthermore, TNF contributes significantly to the recruitment and local survival of immune cells during inflammation (2).

Anti-TNF biologics are among the first approved immunomodulatory monoclonal antibodies in the treatment of IMID. Currently available TNF targeting biologics include adalimumab, infliximab, golimumab, certolizumab pegol, and etanercept, a receptor fusion protein (5) (**table 1**). Besides golimumab, all other anti-TNF biologics have been approved as clinical therapies for psoriasis, RA and PsA. Golimumab is currently approved for the treatment of moderate to severe RA and PsA. To date, adalimumab remains the only approved biological agent for the treatment of moderate to severe HS (6). The effectiveness of other TNF biologics on HS is being actively tested, and several clinical trials show promising results with anti-TNF treatments in HS (7). In psoriasis, neutralizing TNF lead to reduction of Th17 cells at the site of

inflammation and downregulation of Th17 cytokines (8). However, in around 1-5% of patients with RA or Crohn diseases, psoriasis develops paradoxically in early uninvolved skin after receiving anti-TNF biologics (9). This calls for further dissection of the pro-inflammatory and anti-inflammatory properties of TNF in immune reactions. Interestingly, it is suggested that TNF might be able to enhance IL-10 expression via the regulation of PD-1 and PD-L1 axis during viral infections, although a direct correlation between TNF and IL-10 was not supported in patients' blood sample analysis (10).

Moreover, limitations with TNF blockade in the IMID treatments should also be acknowledged, and clinical benefits versus entailed side effects with potential TNF depletion needs to be considered. These adverse effects include opportunistic infections, gradual generation of anti-drug antibodies, and low rates of disease remission (2). To reduce immunogenicity, induction of autoantibodies against TNF by active immunization is being tested for its treatment efficacy (11). To increase efficacy, biologics are being developed and evaluated that have multiple targets, such as bispecific antibodies targeting both TNF and IL-17A, TNF and vascular endothelial growth factor, or TNF and MMP-14 (2). Another approach is targeting primarily the pathogenic TNFR1 and sparing the homeostatic TNFR2. Currently, several TNFR1-specific antibodies have been developed, and their therapeutic potentials in IMID will be exciting to witness in the near future (2).

**IL-17 inhibitors**

The IL-17 family in humans comprises IL-17A, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F (12). IL-17E is also known as IL-25 (12). Among them, IL-17A and IL-17F show the closest homology (around 50%) and consist as a homodimer (IL-17A/A or IL-17F/F) or as a heterodimer (IL-17A/F) (12). The most studied member is IL-17A, which shares the functional heterodimer receptor IL-17RA/RC with IL-17F and IL-17A/F (12). Recently, IL-17RD, forming a

heterodimer with IL-17RA, is also reported to be a functional receptor for IL-17A (13,14). IL-17A is mainly produced by immune cells including CD4+ Th17 cells, CD8+ T cells, γδ T cells and type III innate lymphoid cells (ILC3) (12). Elevated expression of IL-17A or increased recruitments of IL-17A+ cells are widely observed in psoriasis, HS, RA and PsA (15,16). Compared to IL-17F, IL-17A is a more potent inducer of many other cytokines (such as TNF, IL-6, IL-20 subfamily), various chemokines (such as IL-8, CXCL1, CCL2) and MMPs in skin fibroblasts and synovial FLS, as observed in our own studies as well as from other groups (17,18, **chapter 2-4**). Critically, the release of IL-17A cytokines perpetuate autoimmune responses by establishing a positive-feedforward loop between Th17 cells and local stromal cells (19). IL-17A and the downstream-induced cytokines such as IL-20 subfamily contribute to keratinocytes hyperplasia observed in psoriasis and to a lesser extent in HS (20,21, **chapter 3**). Production of MMPs induced by IL-17A is hypothesized to play an important role in tunnel formation in HS and cartilage degradation in RA and PsA (22,23, **chapter 2&4**).

Current clinically approved IL-17 pathway-targeting biologics include secukinumab, ixekizumab, both neutralizing IL-17A, bimekizumab, targeting both IL-17A and IL-17F, and brodalumab, which blocks the receptor IL-17RA (24). The clinical intended diseases (among RA, PsA, psoriasis and HS) are till now psoriasis and PsA for secukinumab, ixekizumab, and psoriasis for bimekizumab and brodalumab (**table 1**). Anti-IL-17 treatments prove to be a success in psoriasis and PsA, especially regarding the improvement of skin symptoms. Furthermore, clinical efficacy in suppressing joint inflammation is comparable to anti-TNF therapy in PsA (25). Remarkably, although a significant increase of IL-17 was observed in RA patients, the efficacy of IL-17 targeting biologics failed to achieve satisfactory therapeutic results during clinical trials (26). Heterogeneity of IL-17 expression in RA synovitis may be the underlying reason for insufficient anti-IL-17 responses (26). Nevertheless, it should be

pointed out that, significant improvements were reported in a subgroup of RA patients with high levels of C-reactive protein (27). Therefore, immunological stratification may help to identify potential RA patients who can benefit from IL-17 biologics. Therefore, as was nicely reviewed in a recent publication, IL-17 targeting strategy should not be completely excluded from the choice of RA treatments (26). To our surprise, enhanced expression of IL-17 receptor, in particular IL-17RC, was observed on RA, but not PsA synovial lymphocytes (**chapter 6**). Whether this IL-17R upregulation is conducive to disappointing anti-IL-17 results in RA still needs further research. Clinical trials of targeting IL-17A are actively ongoing in HS, and reported successes in case studies showed that anti-IL-17 treatment may be beneficial and effective in HS (28). Similar to RA, an upregulation of IL-17R, in particular IL-17RC, was observed in lymphocytes from HS lesional skin (**chapter 4**). The biological consequences of this upregulation are currently under investigation.

**IL-23 inhibitors**

IL-23 is a heterodimeric cytokine composed of the IL-23p19 subunit and the IL-12p40 subunit. It is mainly produced by myeloid cells such as activated dendritic cells, macrophages or monocytes (29). Similarly, the functional IL-23 receptor is a heterodimer and is composed of IL-23R and IL-12Rβ1. Polymorphisms of IL-23R, also known as PSORS7, have been linked to disease susceptibility in psoriasis and PsA (30). The pathogenic role of IL-23 in IMID has been attributed to its involvement in Th17 maintenance and expansion. Differentiation of Th17 cells is initiated by Th17 transcription factor RORγt, which is promoted by IL-6 and TGF-β cytokines. However, only with stimulation of IL-23, Th17 cells stabilize RORγt expression and function properly to secret Th17 cytokines such as IL-17A, IL-17F and IL-22 (31). Similar effects of IL-23 on ILC3 cells have been described as in Th17 cells (32).

Currently, several antibodies targeting the IL-23p19 subunit are clinically

available including guselkumab, tildrakizumab, and risankizumab (**table 1**) (24). Guselkumab is licensed for the treatment of psoriasis and PsA, while tildrakizumab and risankizumab are approved for psoriasis therapy. All anti-IL-23p19 treatments achieved high efficacy in alleviating skin symptoms in psoriasis and PsA (33,34). Surprisingly, their efficacy in relieving joint symptoms are short of the successes observed in skin, though still effective in PsA (35). This highlights the differential role of IL-23 in skin compared to joint pathogenesis. Gene expression studies of paired skin and synovium from PsA patients suggest that IL-23 and IL-12/IL-23p40 is homogeneously expressed in lesional skin, but their expression is heterogeneous in paired synovial tissues and selective in different PsA patients (36). These differences might account for the observed differential efficacy in skin and joint treatments with anti-IL-23 biologics.

In contrast to psoriasis and PsA, treatment with IL-23 antibody guselkumab didn't show significant improvement in RA patients with inadequate responses to methotrexate (37). Ustekinumab, the IL-12/IL-23p40 neutralizing antibody, is approved for the treatment of psoriasis and PsA among other IMID. However, as guselkumab, ustekinumab showed limited reduction of disease activities in RA patients (37). Cytokine expression studies with *ex vivo* transplants showed that unlike skin lesions in psoriasis and inflamed synovium in PsA, the inflammatory condition of RA synovium is distinctive (38). Furthermore, in response to anti-TNF, infliximab, divergent changes of gene expression are observed in psoriasis, PsA and RA (39). HS treatment with IL-23 targeting biologics are only found in occasional case reports, mostly following psoriasis treatment regimen (40). The exact efficacy of anti-IL-23 in HS still needs proof from large-scale clinical research, which is currently under way (7).

IL-23 intracellular signaling involves activation of the Janus kinase (JAK) family members of tyrosine kinases, JAK2 and TYK2 (29). Small molecules targeting the JAK family have been approved as treatment options for

autoimmune and allergic diseases, for example, tofacitinib, baricitinib, and upadacitinib (41). Among them, only tofacitinib has been approved for use in both RA and PsA, while baricitinib and upadacitinib have been approved only for RA (41). These JAK inhibitors target multiple JAK kinases. Currently, selective inhibitors of the JAK family are under intensive research and show promising results in clinical trials such as TYK2 inhibitors (41,42). Interestingly, using the imiquimod-induced psoriasis-like mouse model, we found that TYK2 inhibition attenuated significantly skin inflammation but was less effective in normalizing skin thickness (**chapter 5**). Collectively, current study results of small molecule inhibitors suggest that targeting these enzymes is applicable in the treatment of IMID. However, therapeutic efficacy and selection for specificity to limit side effects will be two trading-off points that warrants detailed consideration.

**IL-1 inhibitors**

The IL-1 family comprises 11 members with IL-1α, IL-1β, IL-18, and IL-33 the most widely studied (43). Besides eliciting inflammation, IL-1 family cytokines have been shown to influence naïve T cell differentiation and subsequent maintenance of phenotypes (43). For instance, IL-1β contribute to the differentiation of Th17 cells together with IL-6 and TGF-β (44). IL-18 is reported to induce Th1 cells and IFN-γ production together with IL-12 and IL-15, while in contrast, IL-33 promotes Th2 responses and IL-4 secretion (43). IL-1α and IL-1β share the same heterodimer receptor IL-1 receptor type I (IL-1R1) and IL-1R accessory protein (IL-1RAP). IL-1β is mainly produced by activated monocytes and macrophages, while IL-1α and IL-1β can also be expressed by keratinocytes and endothelial cells besides myeloid cells (43). IL-1 receptor antagonist (IL-1Ra), a naturally secreted member of the IL-1 cytokine family, can bind to the IL-1 receptor without signaling and thereby inhibit IL-1 function. Anakinra, a slightly modified version of IL-1Ra, is approved for the treatment of

patients with RA among other indications (**table 1**). However, the therapeutic efficacy of anakinra in RA is rather disappointing with less clinical improvements and higher disease scores compared to TNF biologics (45). Similar to the clinical results of IL-1Ra, antibodies targeting IL-1R such as AMG 108 was found not as effective as other biological treatments in RA (**table 1**) (43). Nevertheless, IL-1 inhibition might be a more promising target in skin diseases with massive infiltration of neutrophils such as pustular psoriasis and HS. Compared to plaque psoriasis, pustular psoriasis shows higher expression of cytokines IL-1, IL-36 and chemokines CXCL1, CXCL2, CXCL8, all of which are chemotactic for neutrophils (46). Successful treatment of pustular psoriasis with anakinra has been observed in sporadic case reports (47). However, scientifically designed clinical trials will be needed to objectively evaluate the benefits of IL-1 inhibitors in treating pustular psoriasis. Similarly, in HS skin lesions, higher expression of IL-1 family cytokines and enhanced infiltration of neutrophils are present compared to healthy skin controls (48, **chapter 4**). This provides a good rationale for testing the efficacy of anakinra as a therapy in HS. Results from a small HS patients group showed that 78% (7 of 9) of the treatment group achieved clinical response at 12 weeks compared to 30% (3 of 10) of the placebo group (49). As in pustular psoriasis, large scale random controlled clinical trials will be necessary to objectively evaluate the efficacy of IL-1 inhibitors in HS.

**IL-6 inhibitors**

IL-6 is synthesized and secreted by both immune cells (such as monocytes, T cells) and stromal cells (such as fibroblasts, endothelial cells) (50, **chapter 2&4**). It binds to the IL-6Rα chain, which leads to the dimerization of gp130 and intracellular signal transduction by gp130 (50). IL-6 can signal via the classical signaling pathway, in which IL-6 binds to the IL-6R on the cell membrane or via the trans-signaling pathway, where soluble IL-6R binds IL-6 and forms a

complex with gp130 on the cell membrane to signal. Trans-signaling is possible on cells without membrane expression of IL-6R, but these cells should express gp130. Increased expression of IL-6, which is acknowledged as an inflammatory cytokine, has been found in many IMID including psoriasis, PsA and RA (51,52). Higher expression of IL-6 in HS skin lesions compared to healthy skin was confirmed in our study (**chapter 4**). IL-6, one of the cytokines involved in Th17 differentiation, can in synergy with TNF and IL-17A, be a potent stimulator of other cytokines (53). The involvement of IL-6 in the positive feed-forward loop in skin or synovial fibroblasts co-cultures with T cells has been observed across psoriasis, HS, PsA and RA (19, **chapter 2-4**).

Tocilizumab, an IL-6R antibody, has been approved for RA treatments (**table 1**). Unexpectedly, however, tocilizumab is not effective in treating skin symptoms in psoriasis or PsA (54). Contrarily, like TNF biologics in RA and IBD treatments, tocilizumab induced paradoxical psoriasis in some patients with no previous history of the disease (55). Similar ineffectiveness regarding skin diseases in PsA was observed for clazakizumab, an anti-IL-6 monoclonal antibody (56). A possible mechanism accounting for this phenomenon is that IL-6 inhibition may lead to compensatory upregulation of other cytokines such as TNF (57). Recent studies suggest that IL-6 can also act as an anti-inflammatory cytokine, and inhibit IL-1 and TNF via regulation of IL-1Ra and IL-10 (58). Another hypothesis is that IL-6 bound to membrane IL-6R (classical pathway) has mainly a regenerative and protective role, while IL-6 bound to soluble IL-6R (trans-signaling) mediates inflammation (50). Interestingly, although plaque psoriasis seems not to benefit from IL-6 inhibitors, pustular psoriasis, on the contrary, may respond well to anti-IL-6 therapy as described in some case reports (59,60). Whether the indicated efficacy of anti-IL-6 biologics in pustular psoriasis holds true in a wider population still needs more clinical research.

## Summary

Diverse cell types and cytokines are conducive to IMID pathogenesis, and differential contribution of innate and adaptive immunity are observed in IMID such as psoriasis, HS, RA and PsA. Compartmentalization of inflammatory responses in skin (such as psoriasis, HS) and joint (such as RA, PsA) seems to favor contributions of different cells and diverse pathways. The surging usage of biologics targeting specific pathway provides unique opportunities to observe their individual contributions to the disease pathology. In psoriasis and PsA, biologics targeting the IL-23/IL-17 pathway are likely superior to anti-TNF in alleviating skin symptoms, while in RA, TNF blockade showed more consistent improvements in treating joint diseases (25,26). In PsA itself, IL-23 biologics achieved better therapeutic results in treating skin compared to joint symptoms, and a stronger IL-17 gene signature is observed in skin compared to synovium (33-35,38). Even within the same disease, different subtypes may be caused by divergent immune dysregulation. For instance, subgroups of RA patients with high levels of C-reactive protein may potentially benefit from IL-17 biologics, while RA patients with low IL-17 expression may not respond to this therapy (26,61). Pustular psoriasis shows a more dominant presence of neutrophils and IL-1 family cytokines and will potentially respond better to IL-1 blockade than other subtypes of psoriasis (46,47). Therefore, immune stratification will not only help to shed light on the pathophysiology of IMID but also further improve targeted therapeutic effects in the personalized medicine era.

## Reference

1. Sedger LM, McDermott MF. TNF and TNF-receptors: From mediators of cell death and inflammation to therapeutic giants - past, present and future. Cytokine Growth Factor Rev. 2014 Aug;25(4):453-72. doi: 10.1016/j.cytogfr.2014.07.016.
2. Kalliolias GD, Ivashkiv LB. TNF biology, pathogenic mechanisms and emerging therapeutic strategies. Nat Rev Rheumatol. 2016 Jan;12(1):49-62. doi: 10.1038/nrrheum.2015.169.
3. Choi HM, Oh DH, Bang JS, Yang HI, Yoo MC, Kim KS. Differential effect of IL-1β and

TNFα on the production of IL-6, IL-8 and PGE2 in fibroblast-like synoviocytes and THP-1 macrophages. Rheumatol Int. 2010 Jun;30(8):1025-33. doi: 10.1007/s00296-009-1089-y.

4. Paulissen SM, van Hamburg JP, Davelaar N, Asmawidjaja PS, Hazes JM, Lubberts E. Synovial fibroblasts directly induce Th17 pathogenicity via the cyclooxygenase/prostaglandin E2 pathway, independent of IL-23. J Immunol. 2013 Aug 1;191(3):1364-72. doi: 10.4049/jimmunol.1300274.
5. Monaco C, Nanchahal J, Taylor P, Feldmann M. Anti-TNF therapy: past, present and future. Int Immunol. 2015 Jan;27(1):55-62. doi: 10.1093/intimm/dxu102.
6. Kim ES, Garnock-Jones KP, Keam SJ. Adalimumab: A Review in Hidradenitis Suppurativa. Am J Clin Dermatol. 2016 Oct;17(5):545-552. doi: 10.1007/s40257-016-0220-6.
7. Sabat R, Jemec GBE, Matusiak Ł, Kimball AB, Prens E, Wolk K. Hidradenitis suppurativa. Nat Rev Dis Primers. 2020 Mar 12;6(1):18. doi: 10.1038/s41572-020-0149-1.
8. Balato A, Schiattarella M, Di Caprio R, Lembo S, Mattii M, Balato N, Ayala F. Effects of adalimumab therapy in adult subjects with moderate-to-severe psoriasis on Th17 pathway. J Eur Acad Dermatol Venereol. 2014 Aug;28(8):1016-24. doi: 10.1111/jdv.12240.
9. Mylonas A, Conrad C. Psoriasis: Classical vs. Paradoxical. The Yin-Yang of TNF and Type I Interferon. Front Immunol. 2018 Nov 28;9:2746. doi: 10.3389/fimmu.2018.02746.
10. Schönrich G, Raftery MJ. The PD-1/PD-L1 Axis and Virus Infections: A Delicate Balance. Front Cell Infect Microbiol. 2019 Jun 13;9:207. doi: 10.3389/fcimb.2019.00207.
11. Durez P, Vandepapeliere P, Miranda P, Toncheva A, Berman A, Kehler T, Mociran E, Fautrel B, Mariette X, Dhellin O, Fanget B, Ouary S, Grouard-Vogel G, Boissier MC. Therapeutic vaccination with TNF-Kinoid in TNF antagonist-resistant rheumatoid arthritis: a phase II randomized, controlled clinical trial. PLoS One. 2014 Dec 17;9(12):e113465. doi: 10.1371/journal.pone.0113465.
12. McGeachy MJ, Cua DJ, Gaffen SL. The IL-17 Family of Cytokines in Health and Disease. Immunity. 2019 Apr 16;50(4):892-906. doi: 10.1016/j.immuni.2019.03.021.
13. Mellett M, Atzei P, Horgan A, Hams E, Floss T, Wurst W, Fallon PG, Moynagh PN. Orphan receptor IL-17RD tunes IL-17A signalling and is required for neutrophilia. Nat Commun. 2012;3:1119. doi: 10.1038/ncomms2127.
14. Su Y, Huang J, Zhao X, Lu H, Wang W, Yang XO, Shi Y, Wang X, Lai Y, Dong C.

Interleukin-17 receptor D constitutes an alternative receptor for interleukin-17A important in psoriasis-like skin inflammation. Sci Immunol. 2019 Jun 7;4(36):eaau9657. doi: 10.1126/sciimmunol.aau9657.

15. Schett G, McInnes IB, Neurath MF. Reframing Immune-Mediated Inflammatory Diseases through Signature Cytokine Hubs. N Engl J Med. 2021 Aug 12;385(7):628-639. doi: 10.1056/NEJMra1909094.
16. Moran B, Sweeney CM, Hughes R, Malara A, Kirthi S, Tobin AM, Kirby B, Fletcher JM. Hidradenitis Suppurativa Is Characterized by Dysregulation of the Th17:Treg Cell Axis, Which Is Corrected by Anti-TNF Therapy. J Invest Dermatol. 2017 Nov;137(11):2389-2395. doi: 10.1016/j.jid.2017.05.033.
17. Chang SH, Dong C. A novel heterodimeric cytokine consisting of IL-17 and IL-17F regulates inflammatory responses. Cell Res. 2007 May;17(5):435-40. doi: 10.1038/cr.2007.35.
18. Wright JF, Guo Y, Quazi A, Luxenberg DP, Bennett F, Ross JF, Qiu Y, Whitters MJ, Tomkinson KN, Dunussi-Joannopoulos K, Carreno BM, Collins M, Wolfman NM. Identification of an interleukin 17F/17A heterodimer in activated human CD4+ T cells. J Biol Chem. 2007 May 4;282(18):13447-55. doi: 10.1074/jbc.M700499200.
19. Lubberts E. Th17 cytokines and arthritis. Semin Immunopathol. 2010 Mar;32(1):43-53. doi: 10.1007/s00281-009-0189-9.
20. Sa SM, Valdez PA, Wu J, Jung K, Zhong F, Hall L, Kasman I, Winer J, Modrusan Z, Danilenko DM, Ouyang W. The effects of IL-20 subfamily cytokines on reconstituted human epidermis suggest potential roles in cutaneous innate defense and pathogenic adaptive immunity in psoriasis. J Immunol. 2007 Feb 15;178(4):2229-40. doi: 10.4049/jimmunol.178.4.2229. Erratum in: J Immunol. 2007 Jun 1;178(11):7487.
21. Hotz C, Boniotto M, Guguin A, Surenaud M, Jean-Louis F, Tisserand P, Ortonne N, Hersant B, Bosc R, Poli F, Bonnabau H, Thiébaut R, Godot V, Wolkenstein P, Hocini H, Lévy Y, Hüe S. Intrinsic Defect in Keratinocyte Function Leads to Inflammation in Hidradenitis Suppurativa. J Invest Dermatol. 2016 Sep;136(9):1768-1780. doi: 10.1016/j.jid.2016.04.036.
22. Chabaud M, Garnero P, Dayer JM, Guerne PA, Fossiez F, Miossec P. Contribution of interleukin 17 to synovium matrix destruction in rheumatoid arthritis. Cytokine. 2000 Jul;12(7):1092-9. doi: 10.1006/cyto.2000.0681.
23. Jiang SW, Whitley MJ, Mariottoni P, Jaleel T, MacLeod AS. Hidradenitis Suppurativa: Host-Microbe and Immune Pathogenesis Underlie Important Future Directions. JID Innovations (2021);1:100001 doi:10.1016/j.xjidi.2021.100001.

24. Liu T, Li S, Ying S, Tang S, Ding Y, Li Y, Qiao J, Fang H. The IL-23/IL-17 Pathway in Inflammatory Skin Diseases: From Bench to Bedside. Front Immunol. 2020 Nov 17;11:594735. doi: 10.3389/fimmu.2020.594735.
25. Smolen JS, Mease P, Tahir H, Schulze-Koops H, de la Torre I, Li L, Hojnik M, Sapin C, Okada M, Caporali R, Gratacós J, Goupille P, Liu Leage S, Pillai S, Nash P. Multicentre, randomised, open-label, parallel-group study evaluating the efficacy and safety of ixekizumab versus adalimumab in patients with psoriatic arthritis naïve to biological disease-modifying antirheumatic drug: final results by week 52. Ann Rheum Dis. 2020 Oct;79(10):1310-1319. doi: 10.1136/annrheumdis-2020-217372.
26. Taams LS. Interleukin-17 in rheumatoid arthritis: Trials and tribulations. J Exp Med. 2020 Mar 2;217(3):e20192048. doi: 10.1084/jem.20192048.
27. Burmester GR, Durez P, Shestakova G, Genovese MC, Schulze-Koops H, Li Y, Wang YA, Lewitzky S, Koroleva I, Berneis AA, Lee DM, Hueber W. Association of HLA-DRB1 alleles with clinical responses to the anti-interleukin-17A monoclonal antibody secukinumab in active rheumatoid arthritis. Rheumatology (Oxford). 2016 Jan;55(1):49-55. doi: 10.1093/rheumatology/kev258.
28. Casseres RG, Prussick L, Zancanaro P, Rothstein B, Joshipura D, Saraiya A, Turkowski Y, Au SC, Alomran A, Abdat R, Abudu M, Kachuk C, Dumont N, Gottlieb AB, Rosmarin D. Secukinumab in the treatment of moderate to severe hidradenitis suppurativa: Results of an open-label trial. J Am Acad Dermatol. 2020 Jun;82(6):1524-1526. doi: 10.1016/j.jaad.2020.02.005.
29. Tang C, Chen S, Qian H, Huang W. Interleukin-23: as a drug target for autoimmune inflammatory diseases. Immunology. 2012 Feb;135(2):112-24. doi: 10.1111/j.1365-2567.2011.03522.x.
30. Greb JE, Goldminz AM, Elder JT, Lebwohl MG, Gladman DD, Wu JJ, Mehta NN, Finlay AY, Gottlieb AB. Psoriasis. Nat Rev Dis Primers. 2016 Nov 24;2:16082. doi: 10.1038/nrdp.2016.82.
31. McGeachy MJ, Cua DJ. Th17 cell differentiation: the long and winding road. Immunity. 2008 Apr;28(4):445-53. doi: 10.1016/j.immuni.2008.03.001.
32. Cua DJ, Tato CM. Innate IL-17-producing cells: the sentinels of the immune system. Nat Rev Immunol. 2010 Jul;10(7):479-89. doi: 10.1038/nri2800. Epub 2010 Jun 18. Erratum in: Nat Rev Immunol. 2010 Aug;10(8):611. Erratum in: Nat Rev Immunol. 2010 Jul;10(7):following 489.
33. Gooderham MJ, Papp KA, Lynde CW. Shifting the focus - the primary role of IL-23 in psoriasis and other inflammatory disorders. J Eur Acad Dermatol Venereol. 2018

Jul;32(7):1111-1119. doi: 10.1111/jdv.14868.

34. Nerviani A, Boutet MA, Tan WSG, Goldmann K, Purkayastha N, Lajtos TA, Hands R, Lewis M, Kelly S, Pitzalis C. IL-23 skin and joint profiling in psoriatic arthritis: novel perspectives in understanding clinical responses to IL-23 inhibitors. Ann Rheum Dis. 2021 May;80(5):591-597. doi: 10.1136/annrheumdis-2020-218186.
35. McGonagle D, Watad A, Sharif K, Bridgewood C. Why Inhibition of IL-23 Lacked Efficacy in Ankylosing Spondylitis. Front Immunol. 2021 Mar 19;12:614255. doi: 10.3389/fimmu.2021.614255.
36. Nerviani A, Boutet MA, Tan WSG, Goldmann K, Purkayastha N, Lajtos TA, Hands R, Lewis M, Kelly S, Pitzalis C. IL-23 skin and joint profiling in psoriatic arthritis: novel perspectives in understanding clinical responses to IL-23 inhibitors. Ann Rheum Dis. 2021 May;80(5):591-597. doi: 10.1136/annrheumdis-2020-218186.
37. Smolen JS, Agarwal SK, Ilivanova E, Xu XL, Miao Y, Zhuang Y, Nnane I, Radziszewski W, Greenspan A, Beutler A, Baker D. A randomised phase II study evaluating the efficacy and safety of subcutaneously administered ustekinumab and guselkumab in patients with active rheumatoid arthritis despite treatment with methotrexate. Ann Rheum Dis. 2017 May;76(5):831-839. doi: 10.1136/annrheumdis-2016-209831.
38. Belasco J, Louie JS, Gulati N, Wei N, Nograles K, Fuentes-Duculan J, Mitsui H, Suárez-Fariñas M, Krueger JG. Comparative genomic profiling of synovium versus skin lesions in psoriatic arthritis. Arthritis Rheumatol. 2015 Apr;67(4):934-44. doi: 10.1002/art.38995.
39. Rosenberg A, Fan H, Chiu YG, Bolce R, Tabechian D, Barrett R, Moorehead S, Baribaud F, Liu H, Peffer N, Shealy D, Schwarz EM, Ritchlin CT. Divergent gene activation in peripheral blood and tissues of patients with rheumatoid arthritis, psoriatic arthritis and psoriasis following infliximab therapy. PLoS One. 2014 Oct 21;9(10):e110657. doi: 10.1371/journal.pone.0110657.
40. Casseres RG, Kahn JS, Her MJ, Rosmarin D. Guselkumab in the treatment of hidradenitis suppurativa: A retrospective chart review. J Am Acad Dermatol. 2019 Jul;81(1):265-267. doi: 10.1016/j.jaad.2018.12.017.
41. Fragoulis GE, McInnes IB, Siebert S. JAK-inhibitors. New players in the field of immune-mediated diseases, beyond rheumatoid arthritis. Rheumatology (Oxford). 2019 Feb 1;58(Suppl 1):i43-i54. doi: 10.1093/rheumatology/key276.
42. Armstrong A, Gooderham M, Warren RB, et al. POS1042 efficacy and safety of deucravacitinib, an oral, selective tyrosine kinase 2 (tyk2) inhibitor, compared with placebo and apremilast in moderate to severe plaque psoriasis: results from the phase

3 poetyk pso-1 study. Annals of the Rheumatic Diseases 2021;80:795-796.

43. Sims JE, Smith DE. The IL-1 family: regulators of immunity. Nat Rev Immunol. 2010 Feb;10(2):89-102. doi: 10.1038/nri2691.
44. Chung Y, Chang SH, Martinez GJ, Yang XO, Nurieva R, Kang HS, Ma L, Watowich SS, Jetten AM, Tian Q, Dong C. Critical regulation of early Th17 cell differentiation by interleukin-1 signaling. Immunity. 2009 Apr 17;30(4):576-87. doi: 10.1016/j.immuni.2009.02.007.
45. Mertens M, Singh JA. Anakinra for rheumatoid arthritis: a systematic review. J Rheumatol. 2009 Jun;36(6):1118-25. doi: 10.3899/jrheum.090074.
46. Johnston A, Xing X, Wolterink L, Barnes DH, Yin Z, Reingold L, Kahlenberg JM, Harms PW, Gudjonsson JE. IL-1 and IL-36 are dominant cytokines in generalized pustular psoriasis. J Allergy Clin Immunol. 2017 Jul;140(1):109-120. doi: 10.1016/j.jaci.2016.08.056.
47. Tauber M, Viguier M, Alimova E, Petit A, Lioté F, Smahi A, Bachelez H. Partial clinical response to anakinra in severe palmoplantar pustular psoriasis. Br J Dermatol. 2014 Sep;171(3):646-9. doi: 10.1111/bjd.13012.
48. Witte-Händel E, Wolk K, Tsaousi A, Irmer ML, Mößner R, Shomroni O, Lingner T, Witte K, Kunkel D, Salinas G, Jodl S, Schmidt N, Sterry W, Volk HD, Giamarellos-Bourboulis EJ, Pokrywka A, Döcke WD, Schneider-Burrus S, Sabat R. The IL-1 Pathway Is Hyperactive in Hidradenitis Suppurativa and Contributes to Skin Infiltration and Destruction. J Invest Dermatol. 2019 Jun;139(6):1294-1305. doi: 10.1016/j.jid.2018.11.018.
49. Tzanetakou V, Kanni T, Giatrakou S, Katoulis A, Papadavid E, Netea MG, Dinarello CA, van der Meer JWM, Rigopoulos D, Giamarellos-Bourboulis EJ. Safety and Efficacy of Anakinra in Severe Hidradenitis Suppurativa: A Randomized Clinical Trial. JAMA Dermatol. 2016 Jan;152(1):52-59. doi: 10.1001/jamadermatol.2015.3903. Erratum in: JAMA Dermatol. 2017 Sep 1;153(9):950.
50. Schaper F, Rose-John S. Interleukin-6: Biology, signaling and strategies of blockade. Cytokine Growth Factor Rev. 2015 Oct;26(5):475-87. doi: 10.1016/j.cytogfr.2015.07.004.
51. Hirano T, Matsuda T, Turner M, Miyasaka N, Buchan G, Tang B, Sato K, Shimizu M, Maini R, Feldmann M, et al. Excessive production of interleukin 6/B cell stimulatory factor-2 in rheumatoid arthritis. Eur J Immunol. 1988 Nov;18(11):1797-801. doi: 10.1002/eji.1830181122.
52. Grossman RM, Krueger J, Yourish D, Granelli-Piperno A, Murphy DP, May LT, Kupper

TS, Sehgal PB, Gottlieb AB. Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proc Natl Acad Sci U S A. 1989 Aug;86(16):6367-71. doi: 10.1073/pnas.86.16.6367.

53. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol. 2014 Sep 4;6(10):a016295. doi: 10.1101/cshperspect.a016295.
54. Blauvelt A. IL-6 Differs from TNF-α: Unpredicted Clinical Effects Caused by IL-6 Blockade in Psoriasis. J Invest Dermatol. 2017 Mar;137(3):541-542. doi: 10.1016/j.jid.2016.11.022.
55. Wendling D, Letho-Gyselinck H, Guillot X, Prati C. Psoriasis onset with tocilizumab treatment for rheumatoid arthritis. J Rheumatol. 2012 Mar;39(3):657. doi: 10.3899/jrheum.111166.
56. Mease PJ, Gottlieb AB, Berman A, Drescher E, Xing J, Wong R, Banerjee S. The Efficacy and Safety of Clazakizumab, an Anti-Interleukin-6 Monoclonal Antibody, in a Phase IIb Study of Adults With Active Psoriatic Arthritis. Arthritis Rheumatol. 2016 Sep;68(9):2163-73. doi: 10.1002/art.39700.
57. Fritz Y, Klenotic PA, Swindell WR, Yin ZQ, Groft SG, Zhang L, Baliwag J, Camhi MI, Diaconu D, Young AB, Foster AM, Johnston A, Gudjonsson JE, McCormick TS, Ward NL. Induction of Alternative Proinflammatory Cytokines Accounts for Sustained Psoriasiform Skin Inflammation in IL-17C+IL-6KO Mice. J Invest Dermatol. 2017 Mar;137(3):696-705. doi: 10.1016/j.jid.2016.10.021.
58. Steensberg A, Fischer CP, Keller C, Møller K, Pedersen BK. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. Am J Physiol Endocrinol Metab. 2003 Aug;285(2):E433-7. doi: 10.1152/ajpendo.00074.2003.
59. Younis S, Rimar D, Slobodin G, Rosner I. Tumor necrosis factor-associated palmoplantar pustular psoriasis treated with interleukin 6 blocker. J Rheumatol. 2012 Oct;39(10):2055-6. doi: 10.3899/jrheum.120304.
60. Rueda-Gotor J, González-Gay MA, Blanco Alonso R, Gonzalez-Vela C, Lopez-Obregon C, González-López MA. Successful effect of tocilizumab in anti-TNF-α-induced palmoplantar pustulosis in rheumatoid arthritis. Joint Bone Spine. 2012 Oct;79(5):510-3. doi: 10.1016/j.jbspin.2012.06.010.
61. Robert M, Miossec P. IL-17 in Rheumatoid Arthritis and Precision Medicine: From Synovitis Expression to Circulating Bioactive Levels. Front Med (Lausanne). 2019 Jan 14;5:364. doi: 10.3389/fmed.2018.00364.

# 8

# General discussion

Immune-mediated inflammatory diseases (IMID) is an umbrella term used to describe a group of diseases characterized by chronic inflammation in diverse organs. Though a definitive etiology is still elusive, IMID share similar features of molecular and cellular immune dysregulation. The focus of this thesis is on the IL-17A/IL-17R pathway in IMID, including psoriasis, hidradenitis suppurativa as templates of skin conditions, and rheumatoid arthritis, psoriatic arthritis as examples of joint diseases. Using patients' samples, the contribution of this pathway was studied in psoriatic arthritis (PsA, **chapter 2**), hidradenitis suppurativa (HS, **chapter 4**), and rheumatoid arthritis (RA, **chapter 6**). Using the imiquimod-induced psoriasis-like mouse model, the pathological roles of the IL-17A-IL-19 axis and tyrosine kinase 2 (Tyk2) in psoriasis were investigated (**chapter 3 & 5**). With these experiments, we have shown that IL-17A/IL-17R pathway is conducive to IMID pathogenesis with diverse effects at the cellular, tissue and clinical levels with therapeutic potentials. In this chapter the findings in the thesis are discussed and some future perspectives in research designs are presented.

**Cellular sources of IL-17A expression in IMID**

Diverse cell types have been reported to produce IL-17A, including CD4+, CD8+, γδ T cells, and type 3 innate lymphoid cells (ILC3) (1). In different IMID, contributions of these cells are distinct. For instance, IL-17A+ CD8+ T cells are more predominantly found in PsA synovial fluid than in RA, and their percentages also correlate with the disease severity of PsA patients (2). In **chapter 2**, we confirmed this finding and extended that sorted CD4+ T cells, but not CD8+, from PsA synovial fluid produced IL-17A upon *ex vivo* anti-CD3/CD28 activation. The production of IL-17A by CD8+ T cells can be induced by phorbol myristate acetate (PMA)/ionomycin, which is commonly used as a T cell activator in flow cytometry. Anti-CD3/CD28 activation, which mimics the

process of T cell receptor/costimulatory pathway activation, is potentially the *in vitro* method to reflect more genuinely *in vivo* production of IL-17A compared to PMA/ionomycin stimulation. Interestingly, it was reported that *in vitro* PMA/ionomycin stimulation of ILC3 failed to induce IL-17A expression in spondyloarthritis (3). This raises the question which cell types are the main *in vivo* producers of IL-17A locally. CD4+ and CD8+ T cells can both be potential sources of IL-17A in IMID, as we have found in PsA synovial fluid and HS skin lesions (**chapter 2 & 4**). However, in peripheral blood of PsA and HS patients, the presence of IL-17A+ CD8+ T cells seem negligible (2, **chapter 4**). Therefore, their respective contribution to IL-17A production *in vivo* still warrants further research.

## IL-17A versus IL-17F in IMID

Both IL-17A and IL-17F are secreted by T helper 17 cells (Th17). Our preliminary data showed that, in PsA synovial fluid, only a portion of the IL-17A+ CD4+ T cells are IL-17F+ during intracellular staining (**figure 1**).

**Figure 1. PsA synovial fluid** – intracellular staining

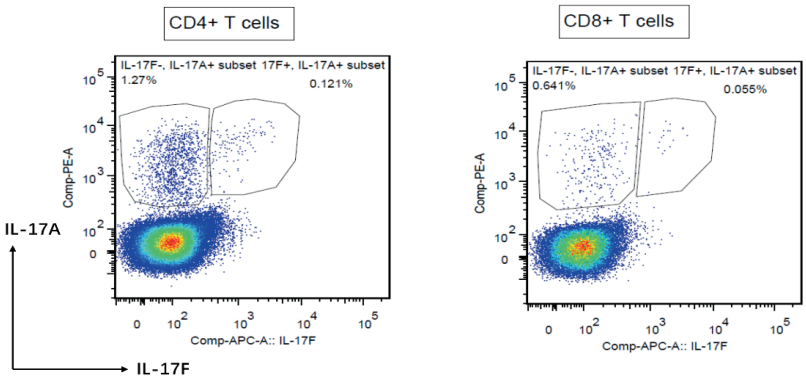



**Figure 1. Representative staining results of IL-17A+ and IL-17F+ among CD4+ and CD8+ T cells in PsA synovial fluid.**

In an experimental autoimmune encephalomyelitis (EAE) model, expression of IL-17F was also found in a subpopulation of IL-17A+ cells (4). This suggests that cellular sources of IL-17F are more restricted compared to IL-17A. Whether the differential expression of IL-17A and IL-17F in CD4+ T cells represents different Th17 populations is still not very well understood. Nevertheless, different transcriptional regulations of IL-17A and IL-17F have been suggested. During *in vitro* Th17 cell development, both RORα and RORγ are responsible for IL-17A induction, while RORα is dispensable for IL-17F production (5). In a B cell-T cell coculture system, prostaglandin E2 (PGE2) increases IL-17A concentration, but inhibits IL-17F secretion (6). Moreover, our and others' studies show that IL-17F is less potent than IL-17A in induction of cytokines, chemokines, and matrix metalloproteinases (MMPs) during *in vitro* stimulation (1, **chapter 3**). Interestingly, in a head-to-head clinical trial for psoriasis, bimekizumab, an anti-IL-17A and anti-17F bispecific antibody, showed superior therapeutic effects compared to secukinumab anti-IL-17A alone (7). This indicates that, in psoriasis, both IL-17A and IL-17F contribute to disease development. Synergy with TNF has been reported for both cytokines. TNF potentiates the proinflammatory effects of IL-17A and IL-17F, while IL-17A and IL-17F can induce TNF receptor II expression (8). This may help explain the observed better therapeutic results when both cytokines were blocked compared to anti-IL-17A alone.

Other studies suggest a differential role of IL-17A and IL-17F in IMID. In fungal proteinase (FAP)-induced acute asthma model and dextran sulfate sodium-induced colitis model, reduced symptoms were observed in IL-17F, but not IL-17A, knock-out mice (4). Similarly, in a toluene diisocyanate-induced asthma model, neutralization of IL-17F, but not IL-17A, suppressed airway hyper reactivity (9). It seems that, in mucosal inflammation such as lung and intestine, IL-17F production by CD4+ T cells plays a more inflammatory role

than IL-17A. However, it should be noted that, in an ovalbumin-induced chronic asthma model, IL-17A knock-out, but not IL-17F knock-out, attenuated eosinophil accumulation in bronchoalveolar lavage fluid compared to wild-type mice (4). Whether these differential effects observed for IL-17A and IL-17F are a site-specific result or an individual disease entity still needs future research. Another key aspect that needs further consideration is the expression of the IL-17 receptor (IL-17R). IL-17A and IL-17F signal both through the heterodimer IL-17R that consists of IL-17RA and IL-17RC. However, in humans, IL-17A binds IL-17RA with higher affinity than IL-17F, while both cytokines bind comparably to IL-17RC (10). How this differential binding between IL-17A and IL-17F translates to clinical relevance is still beyond our understanding.

**IL-17 receptor (IL-17RA/RC) in IMID**

The IL-17 receptor (IL-17R) functions as a heterodimer of IL-17RA and IL-17RC to mediate IL-17A/A, IL-17A/F and IL-17F/F signaling. While IL-17RA expression is almost ubiquitous, expression of IL-17RC is high in epithelial cells but low in hematopoietic cells (10). In synovial tissues, immunohistological staining reveals that IL-17RA is present mostly in the sublining, while IL-17RC is expressed in intimal lining layer (11). Currently, limited data are available regarding the regulation of IL-17R expression. In this thesis, expression of the IL-17 receptor (IL-17R), both IL-17RA and IL-17RC, was studied by flow cytometry in HS (**chapter 4**), RA and PsA (**chapter 6**). Up-regulation of IL-17R, in particular IL-17RC, was observed in lymphocytes from RA and HS, but not from PsA (**chapter 4 & 6**). T cell activation (with anti-CD3/anti-CD28), cytokine stimulation (IL-6, IL-23), and cellular contacts with stromal cells (fibroblasts) might influence the expression of IL-17R (**chapter 6**). The clinical relevance of differential IL-17R regulation still warrants further studies. Mathematic modeling of IL-17 family cytokines and IL-17R binding might help to understand in this respect, as shown in the modeling of differential effects of anti-TNF biologics in

RA and systemic inflammatory response syndrome (12).

Recently, IL-17RD, forming a heterodimer with IL-17RA, has also been reported to be a functional receptor for IL-17A (13). Knockout of IL-17RD down-regulated IL-17A stimulated neutrophilia in mice, but expression of IL-17A-induced IL-6 and CXCL1 was enhanced (14). This indicates, contrary to IL-17RC, that IL-17RD mediates differential functions of IL-17A. Therefore, it would be interesting to screen the expression profile of IL-17RD compared to IL-17RC and decipher their respective properties during IL-17A mediated inflammation. Soluble IL-17R has also been observed. Downregulation of soluble IL-17RA is reported in alveolar Echinococcosis patients (15). Soluble form of IL-17RC has been suggested due to alternative RNA splicing (10). How the soluble forms of IL-17R regulate IL-17 levels and modulate disease pathogenesis still awaits exploration. Recently, soluble IL-17RD has been found to exacerbate collagen-induced arthritis via enhancement of the TNF inflammatory response (16). This further solidifies the speculation that, during IL-17 inflammatory cascades, IL-17RD signaling induces different responses from IL-17RC signaling.

**Combination therapies in IMID**

Biologics targeting the IL-17A/IL-17R pathway have achieved great success in treating IMID, as discussed in **chapter 7**. For instance, anti-IL-17 neutralizing antibodies proved successful in PsA, psoriasis and ankylosing spondylitis treatments (17). Indication extension into other IMID is currently under clinical evaluation such as in HS. However, one surprise was the unexpected low to non-responsiveness of RA patients during IL-17 blockade trials (18). Though a portion of RA patients with high C reactive protein (CRP) levels achieved benefits from anti-IL-17 biologics, the overall response was modest compared to conventional therapies (18,19). In **chapter 6**, the expression profile of IL-17A/IL-17R pathway was explored in RA and PsA synovial fluid. We found lower

percentages of IL-17A+ CD8+ T cells in synovial fluid of patients with RA, while on the contrary, higher expression of IL-17R, in particular IL-17RC, were found on synovial lymphocytes in RA compared to PsA. How this differential expression of the IL-17 pathway is related to the different therapeutic results is beyond the scope of our studies. However, a better understanding of these differences may shed light on more specific targeting of the IL-17 pathway in the future.

Distinctive strengths of biologics with different modes of action should be evaluated to tailor for the needs of individual patients. Here, we studied the differential therapeutic benefits of IL-17 biologics compared to TNF blockade. Using an *ex vivo* T cell-fibroblast co-culture system, we found that in IMID such as PsA and HS, anti-IL-17 (secukinumab) seemed to show better inhibition of inflammatory cytokines such as IL-6 (**chapter 2 & 4**). Contrarily, in PsA but not HS, anti-TNF (adalimumab) showed stronger suppression of MMPs (**chapter 2 & 4**). Considering the lack of efficiency or fading efficacy during IMID treatments, the possibility of combination therapy should be explored to achieve long-lasting benefits for the patients.

Another therapeutic arsenal for IMID that is under fast development are small molecule inhibitors. For instance, the Janus kinase (JAK) family including JAK1, 2, 3 and TYK2 are widely researched target for IMID. Both selective and nonselective JAK inhibitors are available to treat IMID (20). Tofacitinib, a nonselective JAK inhibitor, has been approved for treatments of RA, PsA, ulcerative colitis, and polyarticular juvenile idiopathic arthritis (20,21). Adverse effects related to cardiovascular problems were observed in RA patients (21), and a safety review for JAK inhibitors in inflammatory disorders has been started by the European Medicines Agency. Deucravacitinib, a TYK2-selective inhibitor, is currently under evaluation for treatment of moderate-to-severe psoriasis by Food and Drug Agency. In **chapter 5**, using the imiquimod-induce psoriasis-like mouse model, we found that TYK2 inhibitors partially suppressed

skin inflammation, but didn't improve keratinocyte hyperproliferation. Whether this result is specifically related to the mouse disease model or translatable in clinical therapy still warrants future research. Additionally, the safety profile of TYK2 inhibitors should be closely followed as for other JAK inhibitors, particularly in cardiovascular diseases.

**Therapeutic potential of IL-20 subfamily in IMID**

In psoriasis, aberrant expression of IL-17A and IL-22 contributed to the induction of IL-20 subfamily cytokines such as IL-19 and IL-24, which in turn were conducive to keratinocyte hyperproliferation (**chapter 3**). Therefore, the IL-20 subfamily may be a potential therapeutic target in treating IMID with features of acanthosis such as psoriasis and HS (22). However, it was reported that IL-24 expression formed a negative feedback loop to downregulate IL-17A signaling (23,24). In a Th17-induced uveitis model, silencing of IL-24 aggravated disease while IL-24 treatment ameliorated disease (23). Similarly, IL-19 reportedly plays a protective role in colitis and IL-19 knockout mice exhibited higher levels of inflammatory cytokines in DSS-induced colitis model (24). Therefore, it would be wise to further investigate the relationships between IL-17 pathway and IL-20 subfamily cytokines, before delving into their neutralization in IMID.

**Fibroblasts as therapeutic targets in IMID**

Fibroblasts are important participants in immune reactions. In response to IL-17A, skin fibroblasts (from psoriasis and HS patients) and synovial fibroblasts (from PsA patients) produced inflammatory cytokines such as IL-6, IL-8 and matrix metalloproteinases (MMPs), which feed-forward the inflammatory loop (**chapter 2-4**). Using the fibroblast- T cell co-culture system, biologics targeting IL-17A and TNF were evaluated for their differential effects in PsA, psoriasis and HS (**chapter 2-4**). Fibroblasts are abundant, and actively interact with

immune cells in the pathogenesis of IMID. Fibroblast like synoviocytes (FLS) in RA were reportedly epigenetically imprinted with an inflammatory phenotype (25). However, how to harness this aberrant profile and target the FLS without causing widespread side effects still elude our understanding. Better identification of FLS subsets might hold the keys for future therapeutic success. For instance, in synovium, cadherin 11 (CDH11) is regarded as a surface marker for FLS from the synovial lining (25). Heterogeneity of FLS is observed in the sublining layer, and different subsets identified using diverse markers have been proposed by several groups (25). Currently, whether these FLS subsets are true subsets with a fixed phenotype or plastic modifications due to local microenvironment is unclear. Some believe that the FLS subsets are a spreading continuum that reflect the local influences of inflammation (25). Similarly, several types of skin dermal fibroblasts have been defined by their spatial location. The papillary fibroblasts are located close to the basement membrane and epidermis, while reticular fibroblasts reside within the lower reticular dermis (26). These different skin fibroblasts reportedly secret distinct extracellular matrix, with papillary, but not reticular, fibroblasts supporting the formation of basement membrane (26). Search for definite markers describing human skin fibroblast subsets has just started, and single cell sequencing analysis may help to unravel the heterogeneity of skin fibroblasts. Therefore, identification and characterization of FLS and dermal fibroblast subsets will contribute to our understanding of their involvements in joint and skin inflammatory diseases.

**Main findings**

The current thesis delves into the role of IL-17A/IL-17R pathway in PsA, RA, psoriasis and HS. IL-17A-producing T cells and IL-17RA/IL-17RC expression on immune cells were studied in these diseases. Fibroblasts and keratinocytes are important responder cells to IL-17A, IL-17F and IL-17AF stimulation, and they participate actively in the feedback loop of IL-17A-induced inflammation.

The main findings are summarized as follows:

1. In PsA synovial fluid, CD4+, but not CD8+, T cells secrete IL-17A upon *ex vivo* stimulation with anti-CD3/anti-CD28, though both are IL-17+ when stimulated with PMA/ionomycin during flow cytometry intracellular staining. (**chapter 2**).
2. Using the T cell-fibroblast co-culture system, overlapping and differential effects of anti-IL-17A and anti-TNF are observed in IMID such as PsA, psoriasis and HS. (**chapter 2, 3, 4**).
3. Overexpression of IL-17R, in particular IL-17RC, is found on lymphocytes of HS skin and RA synovial fluid, but not in PsA synovial fluid (**chapter 4 & 6**).
4. Excessive IL-17A (and IL-17F) in imiquimod-induced psoriasis-like mouse model induces skin fibroblasts to produce IL-20 subfamily cytokines such as IL-19 and IL-24, which contribute to keratinocyte hyperproliferation. Neutralization of IL-17A suppresses IL-19 and IL-24 expression and normalizes keratinocytes proliferation in psoriasis. (**chapter 3**)
5. Inhibition of tyrosine kinase 2 (Tyk2) but not lymphocyte-specific protein tyrosine kinase (Lck) reduces skin inflammation in imiquimod-induced psoriasis-like mouse model. However, Tyk2 suppression doesn't improve keratinocyte acanthosis in this imiquimod mouse model. (**chapter 5**)
6. Expression of IL-17R, the IL-17RA/RC heterodimer, on T cells may be enhanced by anti-CD3/anti-CD28 activation, cytokine stimulation and cellular contacts with fibroblasts (**chapter 6**).

**Proposed future experiments**

1. Considering that PMA/ionomycin but not anti-CD3/anti-CD28 activation induced CD8+ T cells to secrete IL-17A (and IL-17F potentially), it would be interesting to analyze and compare the gene expression pattern of CD4+ and CD8+ T cells stimulated with PMA/ionomycin and anti-CD3/anti-CD28.
2. Overexpression of IL-17 induced IL-19 and IL-24 production, and subsequent keratinocyte hyperproliferation in imiquimod-induced psoriasis-like model. Whether IL-19 and IL-24 neutralization benefits psoriatic symptoms can be evaluated with the imiquimod-induced model, and blockades at early and later time-points should also be taken into consideration.
3. Upregulation of IL-17R, in particular IL-17RC, was found on lymphocytes in HS skin and RA synovial fluid. Further elucidation of IL-17R regulation can be studied with *in vitro* T cell activation, cytokine stimulation and co-cultures with stromal cells.
4. Expression of IL-17RD should be profiled using flow cytometry in HS, psoriasis skin, and RA, PsA synovial fluid.
5. Use gene expression profiling to phenotypically characterize HS, psoriasis skin fibroblasts, and RA and PsA fibroblast-like synoviocytes (FLS). This may potentially help to understand the different subsets of fibroblasts in each disease. The main question is whether these differences contribute to the diverse anti-IL-17 and anti-TNF responses in *ex vivo* T cell-fibroblast co-culture system and potentially correlate to clinical therapeutic effects.
6. Tyk2 but not LCK inhibition improves skin inflammation in imiquimod-induced psoriasis-like mouse model. *In vitro* inhibition of T cells and T cells co-cultured with skin fibroblasts may facilitate understanding the differential effects of these two inhibitors.

## Reference


1. McGeachy MJ, Cua DJ, Gaffen SL. The IL-17 Family of Cytokines in Health and Disease. Immunity. 2019 Apr 16;50(4):892-906. doi: 10.1016/j.immuni.2019.03.021.
2. Menon B, Gullick NJ, Walter GJ, Rajasekhar M, Garrood T, Evans HG, Taams LS, Kirkham BW. Interleukin-17+CD8+ T cells are enriched in the joints of patients with psoriatic arthritis and correlate with disease activity and joint damage progression. Arthritis Rheumatol. 2014 May;66(5):1272-81. doi: 10.1002/art.38376.
3. Blijdorp ICJ, Menegatti S, van Mens LJJ, van de Sande MGH, Chen S, Hreggvidsdottir HS, Noordenbos T, Latuhihin TE, Bernink JH, Spits H, Rogge L, Baeten DLP, Yeremenko NG. Expansion of Interleukin-22- and Granulocyte-Macrophage Colony-Stimulating Factor-Expressing, but Not Interleukin-17A-Expressing, Group 3 Innate Lymphoid Cells in the Inflamed Joints of Patients With Spondyloarthritis. Arthritis Rheumatol. 2019 Mar;71(3):392-402. doi: 10.1002/art.40736.
4. Yang XO, Chang SH, Park H, Nurieva R, Shah B, Acero L, Wang YH, Schluns KS, Broaddus RR, Zhu Z, Dong C. Regulation of inflammatory responses by IL-17F. J Exp Med. 2008 May 12;205(5):1063-75. doi: 10.1084/jem.20071978.
5. Yang XO, Pappu BP, Nurieva R, Akimzhanov A, Kang HS, Chung Y, Ma L, Shah B, Panopoulos AD, Schluns KS, Watowich SS, Tian Q, Jetten AM, Dong C. T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. Immunity. 2008 Jan;28(1):29-39. doi: 10.1016/j.immuni.2007.11.016.
6. Melton AC, Melrose J, Alajoki L, Privat S, Cho H, Brown N, Plavec AM, Nguyen D, Johnston ED, Yang J, Polokoff MA, Plavec I, Berg EL, O'Mahony A. Regulation of IL-17A production is distinct from IL-17F in a primary human cell co-culture model of T cell-mediated B cell activation. PLoS One. 2013;8(3):e58966. doi: 10.1371/journal.pone.0058966.
7. Reich K, Warren RB, Lebwohl M, Gooderham M, Strober B, Langley RG, Paul C, De Cuyper D, Vanvoorden V, Madden C, Cioffi C, Peterson L, Blauvelt A. Bimekizumab versus Secukinumab in Plaque Psoriasis. N Engl J Med. 2021 Jul 8;385(2):142-152. doi: 10.1056/NEJMoa2102383.
8. Robert M, Miossec P. IL-17 in Rheumatoid Arthritis and Precision Medicine: From Synovitis

Expression to Circulating Bioactive Levels. Front Med (Lausanne). 2019 Jan 14;5:364. doi: 10.3389/fmed.2018.00364.

9. Chen R, Zhang Q, Chen S, Tang H, Huang P, Wei S, Liang Z, Chen X, Tao A, Yao L. IL-17F, rather than IL-17A, underlies airway inflammation in a steroid-insensitive toluene diisocyanate-induced asthma model. Eur Respir J. 2019 Apr 4;53(4):1801510. doi: 10.1183/13993003.01510-2018.
10. Ho AW, Gaffen SL. IL-17RC: a partner in IL-17 signaling and beyond. Semin Immunopathol. 2010 Mar;32(1):33-42. doi: 10.1007/s00281-009-0185-0.
11. van Baarsen LG, Lebre MC, van der Coelen D, Aarrass S, Tang MW, Ramwadhdoebe TH, Gerlag DM, Tak PP. Heterogeneous expression pattern of interleukin 17A (IL-17A), IL-17F and their receptors in synovium of rheumatoid arthritis, psoriatic arthritis and osteoarthritis: possible explanation for nonresponse to anti-IL-17 therapy? Arthritis Res Ther. 2014 Aug 22;16(4):426. doi: 10.1186/s13075-014-0426-z.
12. Jit M, Henderson B, Stevens M, Seymour RM. TNF-alpha neutralization in cytokine-driven diseases: a mathematical model to account for therapeutic success in rheumatoid arthritis but therapeutic failure in systemic inflammatory response syndrome. Rheumatology (Oxford). 2005 Mar;44(3):323-31. doi: 10.1093/rheumatology/keh491.
13. Rong Z, Wang A, Li Z, Ren Y, Cheng L, Li Y, Wang Y, Ren F, Zhang X, Hu J, Chang Z. IL-17RD (Sef or IL-17RLM) interacts with IL-17 receptor and mediates IL-17 signaling. Cell Res. 2009 Feb;19(2):208-15. doi: 10.1038/cr.2008.320.
14. Mellett M, Atzei P, Horgan A, Hams E, Floss T, Wurst W, Fallon PG, Moynagh PN. Orphan receptor IL-17RD tunes IL-17A signalling and is required for neutrophilia. Nat Commun. 2012;3:1119. doi: 10.1038/ncomms2127.
15. Lechner CJ, Grüner B, Huang X, Hoffmann WH, Kern P, Soboslay PT. Parasite-specific IL-17-type cytokine responses and soluble IL-17 receptor levels in Alveolar Echinococcosis patients. Clin Dev Immunol. 2012;2012:735342. doi: 10.1155/2012/735342.
16. Liu S, Fu Y, Mei K, Jiang Y, Sun X, Wang Y, Ren F, Jiang C, Meng L, Lu S, Qin Z, Dong C, Wang X, Chang Z, Yang S. A shedding soluble form of interleukin-17 receptor D exacerbates collagen-induced arthritis through facilitating TNF-α-dependent receptor

clustering. Cell Mol Immunol. 2021 Aug;18(8):1883-1895. doi: 10.1038/s41423-020-00548-w.

17. Blauvelt A, Chiricozzi A. The Immunologic Role of IL-17 in Psoriasis and Psoriatic Arthritis Pathogenesis. Clin Rev Allergy Immunol. 2018 Dec;55(3):379-390. doi: 10.1007/s12016-018-8702-3.
18. Taams LS. Interleukin-17 in rheumatoid arthritis: Trials and tribulations. J Exp Med. 2020 Mar 2;217(3):e20192048. doi: 10.1084/jem.20192048.
19. Burmester GR, Durez P, Shestakova G, Genovese MC, Schulze-Koops H, Li Y, Wang YA, Lewitzky S, Koroleva I, Berneis AA, Lee DM, Hueber W. Association of HLA-DRB1 alleles with clinical responses to the anti-interleukin-17A monoclonal antibody secukinumab in active rheumatoid arthritis. Rheumatology (Oxford). 2016 Jan;55(1):49-55. doi: 10.1093/rheumatology/kev258.
20. Spinelli FR, Meylan F, O'Shea JJ, Gadina M. JAK inhibitors: Ten years after. Eur J Immunol. 2021 Jul;51(7):1615-1627. doi: 10.1002/eji.202048922.
21. El Jammal T, Sève P, Gerfaud-Valentin M, Jamilloux Y. State of the art: approved and emerging JAK inhibitors for rheumatoid arthritis. Expert Opin Pharmacother. 2021 Feb;22(2):205-218. doi: 10.1080/14656566.2020.1822325.
22. Stenderup K, Rosada C, Worsaae A, Clausen JT, Norman Dam T. Interleukin-20 as a target in psoriasis treatment. Ann N Y Acad Sci. 2007 Sep;1110:368-81. doi: 10.1196/annals.1423.039.
23. Chong WP, Mattapallil MJ, Raychaudhuri K, Bing SJ, Wu S, Zhong Y, Wang W, Chen Z, Silver PB, Jittayasothorn Y, Chan CC, Chen J, Horai R, Caspi RR. The Cytokine IL-17A Limits Th17 Pathogenicity via a Negative Feedback Loop Driven by Autocrine Induction of IL-24. Immunity. 2020 Aug 18;53(2):384-397.e5. doi: 10.1016/j.immuni.2020.06.022.
24. Azuma YT, Matsuo Y, Nakajima H, Yancopoulos GD, Valenzuela DM, Murphy AJ, Karow M, Takeuchi T. Interleukin-19 Is a Negative Regulator of Innate Immunity and Critical for Colonic Protection. J Pharmacol Sci. 2011;115(2):105-111. doi: 10.1254/jphs.10R02CR.
25. Nygaard G, Firestein GS. Restoring synovial homeostasis in rheumatoid arthritis by targeting fibroblast-like synoviocytes. Nat Rev Rheumatol. 2020 Jun;16(6):316-333. doi:

10.1038/s41584-020-0413-5.

26. Ghetti M, Topouzi H, Theocharidis G, Papa V, Williams G, Bondioli E, Cenacchi G, Connelly JT, Higgins CA. Subpopulations of dermal skin fibroblasts secrete distinct extracellular matrix: implications for using skin substitutes in the clinic. Br J Dermatol. 2018 Aug;179(2):381-393. doi: 10.1111/bjd.16255.

## Summary

The focus of this thesis is on the IL-17A/IL-17R pathway in immune-mediated inflammatory diseases. IL-17A-producing T cells were studied in psoriatic arthritis (PsA, **chapter 2**), hidradenitis suppurativa (HS, **chapter 4**), and rheumatoid arthritis (RA, **chapter 6**). Using flow cytometry, CD4+IL-17A+ and CD8+IL-17A+ T cells were found in PsA synovial fluid and HS skin lesions, while CD4+ T cells were IL-17A+ in RA synovial fluid rather than CD8+ T cells. Interestingly, sorted CD4+ T cells, but not CD8+, from PsA synovial fluid secrete IL-17A upon ex vivo anti-CD3/CD28 activation (**chapter 2**). Whether the same holds true for CD8+ T cells in HS skin lesion still awaits future research, partly due to the limited number of T cells in HS skin biopsies.

Expression of the IL-17 receptor (IL-17R), that consist as a heterodimer of IL-17RA and IL-17RC, were studies in RA, PsA (**chapter 6**) and HS (**chapter 4**). Up-regulation of IL-17R, in particular IL-17RC, was observed in synovial fluid and skin derived lymphocytes from RA and HS, respectively, but not from PsA. T cell activation, cytokine stimulation, and cellular contacts with stromal cells might influence the expression of IL-17R (**chapter 6**). The clinical relevance of differential IL-17RA/IL-17RC regulation still warrants further studies.

The pathological roles of IL-17A and tyrosine kinase 2 (Tyk2) in psoriasis were investigated using the imiquimod-induced psoriasis mouse model (**chapter 3 & 5**). Aberrant expression of IL-17A contributed to the induction of IL-20 family cytokines such as IL-19 and IL-24, which in turn were conducive to keratinocyte hyperproliferation (**chapter 3**). Tyk2 inhibitor down-regulated IL-17A expression and attenuated skin inflammation. However, skin acanthosis was not improved by Tyk2 inhibition, which suggests a decoupled process between inflammation and keratinocyte proliferation in this psoriasis model (**chapter 5**).

Fibroblasts are important participants in immune reactions. In response to IL-17A, skin fibroblasts (from psoriasis and HS patients) and synovial fibroblasts

(from PsA patients) produced inflammatory cytokines such as IL-6, IL-8 and matrix metalloproteinases (MMPs), inducing a proinflammatory feed-forward loop (**chapter 2-4**). Using the fibroblast-T cell co-culture system, biologics targeting IL-17A and TNF were evaluated for their differential effects in PsA, psoriasis and HS (**chapter 2-4**). A better inhibition of proinflammatory cytokines such as IL-6 by anti-IL-17A and a better reduction of MMPs such as MMP-1 by anti-TNF were generally found in this co-culture system across diseases. Whether these observations can be translated into clinical relevance still needs further studies.

In summary, the current thesis delves into the role of IL-17A/IL-17R pathway in PsA, RA, psoriasis and HS. CD4+ T cells are probably the main *in vivo* IL-17A-producing T cells in psoriatic arthritis. Expression of IL-17R, in particular IL-17RC, were upregulated on lymphocytes in RA synovial fluid and HS skin. Skin fibroblasts and fibroblast-like synoviocytes are important responding cells to IL-17A stimulation, and they participate actively in the feed-forward loop of IL-17A-induced inflammation. Inhibition of IL-17A and TNF shows overlapping and yet distinct results in a T cell-fibroblast co-culture system. Dual targeting of both cytokines might confer additive or synergistic therapeutic effects.

## Samenvatting

De focus van dit proefschrift ligt op de IL-17A/IL-17R route in immuun gemedieerde ontstekingsziekten. IL-17A-producerende T-cellen werden onderzocht bij artritis psoriatica (PsA, **hoofdstuk 2**), hidradenitis suppurativa (HS, **hoofdstuk 4**) en reumatoïde artritis (RA, **hoofdstuk 6**). Met behulp van flowcytometrie werd aangetoond dat zowel CD4+ als CD8+ T-cellen in PsA-synoviale vloeistof en HS-huidlaesies IL-17A+ zijn, terwijl in RA-synoviale vloeistof alleen CD4+ T-cellen IL-17A+ waren maar niet de CD8+ T-cellen. Interessant is dat alleen gesorteerde CD4+ T-cellen, maar niet CD8+ cellen, uit PsA synoviale vloeistof IL-17A produceren na ex vivo anti-CD3/CD28 activering (**hoofdstuk 2**). Of hetzelfde geldt voor CD8+ T-cellen in HS-huidlaesie, moet nog verder onderzocht worden.

Expressie van de IL-17 receptor (IL-17R), die bestaat als een heterodimeer van IL-17RA en IL-17RC, werd onderzocht in RA, PsA (**hoofdstuk 6**) en HS (**hoofdstuk 4**). Verhoging van de expressie van IL-17R, in het bijzonder IL-17RC, werd waargenomen in gewrichtsvloeistof en van huid afgeleide lymfocyten van respectievelijk RA en HS, maar niet van PsA. T-cel activering, cytokinestimulatie en cellulaire contacten met stromale cellen kunnen de expressie van IL-17R beïnvloeden (**hoofdstuk 6**). De klinische relevantie van deze differentiële IL-17RA/IL-17RC regulatie rechtvaardigt verder onderzoek.

De pathologische rol van IL-17A en tyrosine kinase 2 (Tyk2) bij psoriasis werd onderzocht met behulp van het imiquimod-geïnduceerde psoriasis muismodel (**hoofdstuk 3 & 5**). Afwijkende expressie van IL-17A droeg bij aan de inductie van IL-20 familie cytokines zoals IL-19 en IL-24, die op hun beurt bevorderlijk waren voor hyperproliferatie van keratinocyten (**hoofdstuk 3**). Het gebruik van een Tyk2-remmer verlaagde de IL-17A-expressie en verminderde de huidontsteking. Huid acanthose werd echter niet verbeterd door Tyk2-remming, wat wijst op een ontkoppeld proces tussen ontsteking en proliferatie van keratinocyten bij Tyk-2 remming in dit psoriasismodel (**hoofdstuk 5**).

Fibroblasten zijn belangrijke deelnemers aan immuunreacties. Als reactie op IL-17A, produceerden huidfibroblasten (van psoriasis- en HS-patiënten) en synoviale fibroblasten (van PsA-patiënten) inflammatoire cytokines zoals IL-6, IL-8 en matrixmetalloproteïnasen (MMP's), waardoor een pro-inflammatoire feed-forward-cyclus werd geïnduceerd (**hoofdstuk 2-4**). Met behulp van het fibroblast-T-cel-co-cultuur systeem werden biologics, die gericht zijn op IL-17A en TNF, geëvalueerd op hun differentiële effecten in PsA, psoriasis en HS (**hoofdstuk 2-4**). Een betere remming van pro-inflammatoire cytokines zoals IL-6 door anti-IL-17A en een betere reductie van MMP's zoals MMP-1 door anti-TNF werden in dit co-cultuursysteem gevonden voor PsA. In zowel het co-cultuursysteem voor PsA als HS, werd er een betere onderdrukking van pro-inflammatoire cytokines als MMPs gevonden na combinatie behandeling van anti-IL-17A en anti-TNF. Of deze observaties vertaald kunnen worden naar klinische relevantie zal verder onderzocht moeten worden.

Samenvattend gaat het huidige proefschrift in op de rol van de IL-17A/IL-17R-route in PsA, RA, psoriasis en HS. CD4+ T-cellen zijn waarschijnlijk de belangrijkste in vivo IL-17A-producerende T-cellen bij artritis psoriatica. Expressie van IL-17R, in het bijzonder IL-17RC, werd verhoogd gevonden op lymfocyten in RA-synoviale vloeistof en HS-huid. Huidfibroblasten en fibroblastachtige synoviocyten zijn belangrijke cellen die reageren op IL-17A-stimulatie en ze nemen actief deel aan de feed-forward-cyclus van door IL-17A geïnduceerde ontsteking. Remming van IL-17A en TNF vertoont overlappende en verschillende resultaten in een T-cel-fibroblast-co-cultuursysteem. Dubbele targeting van beide cytokinen kan additieve of synergetische therapeutische effecten hebben in diverse immune gemedieerde ontstekingsziekten.

## Curriculum Vitae

Xiaofei Xu was born on May 2nd 1986 in Laizhou, Shandong Province, P.R. China. From 2005-2011, he studied medical imaging (Radiology) as a bachelor student in Shandong University. The program training included clinical imaging techniques such as ultrasound, computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET). From 2011-2014, he studied immunology as a master student in Fudan University, Shanghai. Research projects involved the role of chemerin, a chemokine, in mouse models of systemic lupus erythematosis (SLE), psoriasis and colitis. From 2014-2015, he studied tumor immunology as a PhD study at Erasmus Medical Center (EMC), Rotterdam.

In 2015, he transferred to the joint program of dermatology/rheumatology/immunology at EMC Rotterdam. From 2015-2019, during the joint PhD program, he studied the pathologic role of IL-17/IL-17R pathway in immune-mediated inflammatory diseases (IMID) such as psoriasis, hidradenitis suppurativa (HS), psoriatic and rheumatoid arthritis. This thesis is a summary of the work performed during this period.

From 2019-2021, he worked as a researcher at Quantum Hi-Tech (Guangdong) Biological Co. Ltd in Guangdong, China. The job focused on the immunological effects of prebiotics using mouse models and in vitro immune cell cultures. From 2021 till now, he worked as a scientist at Crown Bioscience in Suzhou, China. The current job involves establishing IMID disease models and conducts studies for clients.

# PhD Portfolio

**Xiaofei Xu**
Departments of Dermatology, Rheumatology and Immunology

Research school: postgraduate school of molecular medicine

PhD period: September 2015- August 2019
1st Promoter: Prof.dr. E.P. Prens
2nd Promoter: Dr. E. Lubberts

**1.PhD training**

| | year | workload (ETCS) |
|---|---|---|
| **Courses:** | | |
| Statistics R course | 2017 | 1.8 ECTS |
| Advanced immunology | 2017 | 3 ECTS |
| Flow cytometry training | 2017 | 1 ECTS |
| Laboratory animal science | 2014 | 3 ECTS |
| **International Conferences** | | |
| Oral presentations | | |
| EWRR annual meeting, Lyon, France | 2019 | 1 ECTS |
| Erasmus MC Molmed Day | 2019 | 1 ECTS |
| ACR annual meeting, San Diego, USA | 2017 | 1 ECTS |
| NVR annual meeting, Arnhem, The Netherlands | 2017 | 1 ECTS |
| Poster and pitch presentations | | |
| EWRR in Geneva, Switzerland | 2018 | 1 ECTS |
| Erasmus MC Molmed Day (elevator pitch) | 2018 | 1 ECTS |
| Psoriasis from gene to clinic in London, UK | 2017 | 1 ECTS |
| BSI/NVvI annual meeting in Liverpool, UK | 2016 | 1 ECTS |
| NVvI congress in Efteling-Kaatsheuvel | 2014 | 1 ECTS |
| Erasmus MC Molmed Day | 2014-2019 | 3 ECTS |
| Other | | |
| Immunology Journal Club | 2015-2019 | 2 ECTS |

**2. Teaching**

**Supervising bachelor's internship study**

Bachelor student HLO Rotterdam "Biology & Medical Laboratory researches"
2017/2018 (6 months) 10 ECTS

Bachelor student HLO Rotterdam "Biology & Medical Laboratory researches"
2017/2018 (8 months) 10 ECTS

# Publications:

## This thesis

IL-17A is produced by synovial fluid CD4+ but not CD8+ T cells after TCR activation and regulates different inflammatory mediators compared to TNF in a synovitis model of psoriatic arthritis.
**Xiaofei Xu**, Nadine Davelaar, Anne-Marie Mus, Patrick S. Asmawidjaja, Marijn Vis, Radjesh J. Bisoendial, Errol P. Prens, Erik Lubberts.
Arthritis Rheumatol. 2020 Aug;72(8):1303-1313

Interleukin-17A drives IL-19 and IL-24 expression in skin stromal cells regulating keratinocyte proliferation.
**Xiaofei Xu**, Errol Prens, Edwin Florencia, Pieter Leenen, Luis Boon, Patrick Asmawidjaja, Anne-Marie Mus, Erik Lubberts.
Front Immunol. 2021 Sep 20;12:719562

Dual neutralization of IL-17A and TNFα shows additive suppressive effects on proinflammatory cytokines and MMPs in a human model system of Hidradenitis Suppurativa.
**X. Xu**, K.R. van Straalen, K. Dudink, N. Davelaar, A.M.C. Mus, E. Lubberts*, E.P. Prens*.
In submission

Molecular and cellular signatures in immune-mediated inflammatory diseases related to joint and skin disorders: similarities and differences.
**Xiaofei Xu**, Koen Dudink, Errol Prens, Erik Lubberts.
Manuscript in preparation

Tyk2, but not Lck, inhibition attenuates psoriasis-like inflammation in imiquimod-induced mouse model.
**Xiaofei Xu**, Errol Prens, Patrick Asmawidjaja, Anne-Marie Mus, Erik Lubberts.
Manuscript in preparation

Differential expression of IL-17A/IL-17R pathway on T cells with rheumatoid arthritis compared to psoriatic arthritis: tentative searching for potential regulation mechanisms.
**Xiaofei Xu**, Nadine Davelaar, Anne-Marie Mus, Patrick S. Asmawidjaja, Marijn Vis, Radjesh J. Bisoendial, Errol P. Prens, Erik Lubberts.
Manuscript in preparation

## Others

Systematic Characterization and Longitudinal Study Reveal Distinguishing Features of Human Milk Oligosaccharides in China.
Wu J, Wu S, Huo J, Ruan H, **Xu X**, et al.
Curr Dev Nutr. 2020; 4(8): nzaa113

Ultraviolet B irradiation induces skin accumulation of plasmacytoid dendritic cells: a possible role for chemerin.
Yin Q*, **Xu X***, Lin Y, et al.
Autoimmunity, 2014; 47(3):185-92.

Chemerin aggravates DSS-induced colitis by suppressing M2 macrophage polarization.
Lin Y*, Yang X*, Yue W, **Xu X**, et al.
Cell & Mol Immunol. 2014; 11(4):355-66.

Characterizing the contrast of white matter and grey matter in high-resolution phase difference enhanced imaging of human brain at 3.0 T.
Yang L, Wang S, Yao B, Li L, **Xu X**, et al.
Eur Radiol. 2015; 25(4):1068-76.