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UNDERSTANDING INFLAMMATORY MECHANISMS IN RHEUMATIC DISEASES

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To my Family

"The best scientist is open to experience and begins with romance – The Idea that anything is possible"

Ray Bradbury

ABSTRACT

Rheumatoid arthritis (RA), Psoriasis (Ps) and Psoriasis arthritis (PsA) are chronic inflammatory autoimmune disorders, where primary targets are peripheral joints, skin and skin/joints respectively. Both innate and adaptive immunity play a role in disease initiation and progression.

B cell selection processes were studied by using a VDJ replacement mouse strain ACB (anti-C1 B cell mouse strain), which spontaneously produces anti-C1 antibodies. C1 is one of the major, well-defined immunodominant epitopes on CII molecule. This model allowed for the first time to understand B cell tolerance mechanisms to CII, a matrix protein. We demonstrated that C1-specific B cells are neither negatively selected nor functionally anergized. Thus, this study contributed to better understanding of autoimmunity and pathogenesis of human RA. Tolerance mechanisms toward CII were explored using the classical collagen induced arthritis mouse (CIA) model. Interestingly, ACB mice were protected from arthritis development despite having elevated auto-antibodies in the sera. Introducing a mutation in the *Ncf1* gene leading to ROS deficiency initiated arthritis that was associated with enhanced germinal centre (GC) formation, increased T cell responses and epitope-spreading of the CII-specific antibody repertoire. Hence, ROS mediated auto-B cell tolerance mechanisms might have important implications for understanding the epitope spreading events leading to onset of RA.

A new mouse model of Ps and PsA in mice triggered by previously regarded non-pathogenic mannan from *Saccharomices cerevisiae* was characterised. A new pathogenic pathway driven by macrophages and γδ T cells secreting IL-17A was demonstrated. Moreover, cutaneous and articular inflammation in mice was significantly increased under reduced oxidative environment. This novel Ps and PsA model could be extremely useful for testing new therapeutics for Ps and PsA patients. Different scoring techniques for Ps and PsA were evaluated in mice, in order to better assess disease severity for skin and joint inflammation in mannan induced model. This method will be most valuable to quantify disease activity for testing novel therapeutics.

LIST OF PUBLICATIONS

I. **Pathogenic Autoreactive B Cells Are Not Negatively Selected toward Matrix Protein Collagen II**
Cao D, **Khmaladze I*,** Jia H, Bajtner E, Nandakumar KS, Blom T, Mo JA, Holmdahl R.
J Immunol. *2011; 1;187(9):4451-8. doi: 10.4049/jimmunol.1101378*

II. **Lack of Reactive Oxygen Species Promotes Development of Arthritis in Autoreactive IgG Heavy Chain Knock-in Mice**
Khmaladze I, Saxena A, Nandakumar KS, Holmdahl R.
Athritis & Rheumatology *(under revision)*

III. **Mannan Induces ROS-Regulated, IL-17A–Dependent Psoriasis Arthritis-Like Disease In Mice**
Khmaladze I, Kelkka T, Guerard S, Wing K, Pizzolla A, Saxena A, Lundqvist K, Holmdahl M, Nandakumar KS, and Holmdahl R.
PNAS 2014; *111 (35) E3669–E3678, doi: 10.1073/pnas.1405798111*

IV. **Mannan Induced Psoriasis and Psoriasis Arthritis-like Disease in Mice.**
Khmaladze I, Holmdahl R, Nandakumar KS.
Manuscript (Method protocol)

*** Co-first Author**

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LIST OF ABBREVIATIONS

Ab	Antibody
ACPA	Anti-Citrulinated Protein Antibodies
APC	Antigen Presenting Cell
BCR	B-Cell Receptor
BM	Bone Marrow
CAIA	Collagen Antibody Induced Arthritis
CD	Cluster of Differentiation
CFA	Complete Freund`s Adjuvant
CIA	Collagen Induced Arthritis
CII	Collagen Type II
CSR	Class Switch Recombination
GC	Germinal Centre
HLA	Human Leucocyte Antigen
IL	Interleukin
Ig	Immunoglobulin
LPS	Lipopolysaccharide
MHC	Major Histocompatibility Complex
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NCF	Neutrophil Cytosolic Factor
NOX2	NADPH oxidase 2
Ps	Psoriasis
PsA	Psoriasis Arthritis
RA	Rheumatoid Arthritis
ROS	Reactive Oxygen Species
TCR	T-Cell Receptor
Th	T-helper cell
TLR	Toll-Like Receptor

INTRODUCTION

1 THE IMMUNE SYSTEM

The immune system organization is dependent on two-protection systems: **non-specific responses** (I) comprising anatomical and physiological barriers as the first line of defence and (II) innate immunity as the second line; **specific responses**-involving (III) adaptive (acquired) immunity [1] as summarized in figure 1. The interaction between these components of the immune system favours guarding of our body from invading/foreign microorganisms such as viruses, bacteria, fungi and protozoa [2].

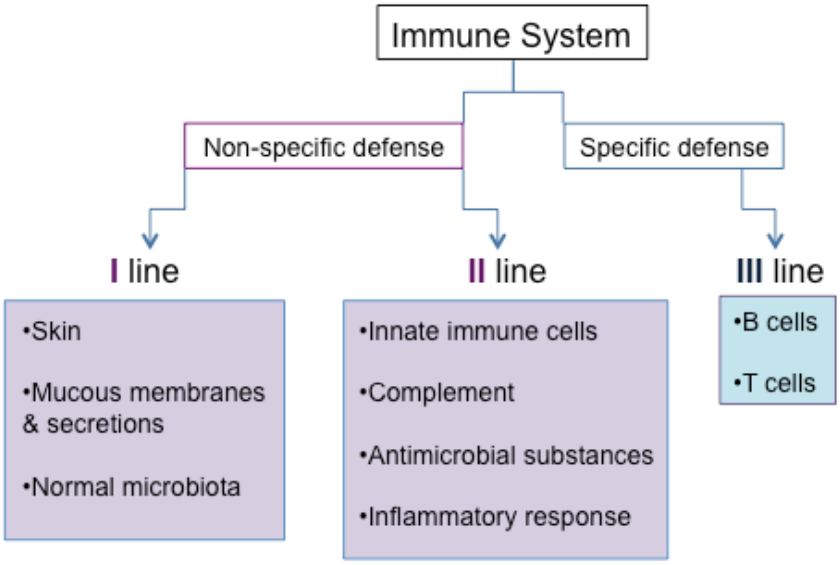

Figure 1. *Schematic representation of the immune system with non-specific and specific defence mechanisms*

1.1 NON-SPECIFIC RESPONSES

1.1.1 First line of defense

Major anatomical barriers to microorganisms are the **skin** and the **mucous membranes** of the respiratory, gastrointestinal (GUT) system that are able to trap microbes and prevent their passage into our body. A variety of physiological **secretions** as tears, saliva or nasal secretions protect natural openings. The protective roles of the "normal" **microbiota** (microbial flora) which are growing on the skin and in the mouth, gastrointestinal tract, and other areas of the body do not cause disease, while their growth is kept under control by the host's defence mechanisms. When the growth of the "normal" microbial flora is suppressed, foreign, disease causing agents may be able to enter, infect and initiate the disease [1, 2].

1.1.2 Second line of defense

Innate immunity, an ancient component of the immune system, represents a non-specific mechanism of the host defence, existing in all the multicellular organisms, while acquired immune system developed relatively later in only "higher" vertebrates. The most important cell types involved in innate protection system comprised of **innate immune cells** such as macrophages that detect, track, engulf, and kill the invading bacteria and viruses as well as infected host cells and other debris; neutrophils, dendritic cells, eosinophils, mast cell, natural killer (NK) cells and natural killer T cells. To supplement immune responses against microorganisms, additional help from humoral components is provided. For example: **complement** proteins and **anti-microbial peptides** including defensins [1]. The most essential feature of the innate immunity is an immediate responsiveness to the microbial invasion, which is achieved through microbial components, designated as pathogen-associated molecular patterns (PAMPs). TLRs are a type of *pattern recognition receptor* (PRR) recognizing PAMPs [3]. PAMP-TLR interactions initiate intracellular signaling cascades, leading to the expression of different inflammatory molecules and thereby orchestrating the early defense response to infections [4]. TLRs are divided into sub-families depending on their recognition motifs. TLR1, TLR2, TLR4 and TLR6 recognize lipids, whereas TLR3, TLR7, TLR8 and TLR9 recognize nucleic acids [4]. **Inflammation** can be another non-specific defence mechanism that helps to prevent infectious agents spreading in the body. The inflammatory response involves redness (erythema), swelling (edema) and pain (dolor). But in certain conditions, inflammatory reactions can lead to tissue damage and disease induction.

1.2 SPECIFIC RESPONSES

1.2.1 Third Line: Adaptive immunity

1.2.2 B cells

B cell discovery took place in the mid-1960s and early 1970s using animal models. Max Cooper and Robert Good suggested a cell type in the chicken bursa of Fabricius, which is responsible for immunoglobulin (antibody) production. By using murine transplant models it was demonstrated that bone marrow (BM) derived cells mediate antibody responses [5].

1.2.2.1 B cell development

B cell development takes place in the BM, via a diverse repertoire of VDJ and VJ rearrangements encoding the BCR (B-cell receptor). The recombinase activating genes 1 and 2 (RAG1/2) join a D and J_H segment at the Ig heavy chain (IgH) locus, followed by V_H to DJ_H rearrangement [6, 7] at **pro-B** cell stage of B cell development. Productively recombined V_HDJ_H at IgH, pairs with surrogate light chain (λ5-Vpre-B) and forms the **pre-BCR**. This stage is called as large pre-BCR stage. The pre-BCR complex includes the Ig-α and Ig-β cell signaling components to the cell surface [8]. As a next step, light chain rearrangement occurs as part of the small pre-B cell stage. Successfull light chain rearrangement at either the Ig kappa (κ) or lambda (λ) light chain locus gives rise to the expression of a complete BCR, denoted as immature B cell stage, **Imm-B** (Figure 2) [8] Most of the B cell differentiation and Ig gene rearrangement data were obtained from mouse studies, but human B cell development is markedly similar. As in mice, human B cells arise in the fetal liver or BM and are continuously generated throughout life [9].

There are at least 10 different transcription factors regulate B cell development at early stages. Among them, EBF, E2A and Pax5 are important in B-cell linage commitment and differentiation. Pax 5 can activate the genes, which are required for B-cell development and repress the once which are critical in non-B cell lineage cells. As a fact, Pax5 deficiency in mice leads to B-cell developmental arrest at the stage of transition from DJ to VDJ rearrangement [10].

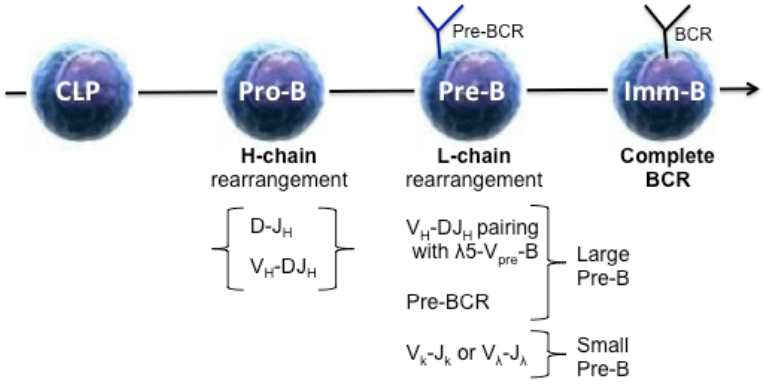

Figure 2. *B-cell developmental stages in the bone marrow from CLP (common lymphoid progenitor) to ImmB (immature B cell) subsets (Adapted and modified from source: Tucker W. Lebien. 2008. Blood) [11].*

1.2.2.2 B cell subsets

B cells can be separated into two lineages: B-1, "innate-like" and B-2. It still remains unclear whether B-1 and B-2 cells are derived from a common progenitor [12]. Murine **B-1** cells are derived primarily from the fetal liver and the self-renewal occurs largly in the periphery [13, 14]. In contrast, **B-2** cells arise mostly from the bone marrow and are produced constitutively. Peritoneal B-1 cells are subdivided into two subsets: the B-1a (CD5+) and B-1b (CD5−), where **B-1a** cells produce so called "natural antibodies" providing protection towards bacterial infections, while **B-1b** cells contribute to long-term "adaptive antibody" responses to polysaccharides and other T cell–independent type 2 antigens during infection [15]. Notably, **MZB** cells are a unique population of murine splenic B cells residing (at least in mice) within the marginal zone of the splenic white pulp [16]. **FOB** cells are recirculating and are found in the blood and secondary lymphoid organs. Besides, FO and MZB cells display different signaling characteristics of BCR and serve distinct functions [16]. Cytokine milleu, BCR specificity and competition with pre-existing mature B cells are decisive factors for mature B cell subsets to dictate which B cell subsets they should enter. **B-10** cells are specific IL-10-producing subset having regulatory function mediated by IL-10 (Figure 3). B10 cells share surface markers with other B cell subsets but currently there is no cell surface/intracellular marker, which is unique to B10 cells [17].

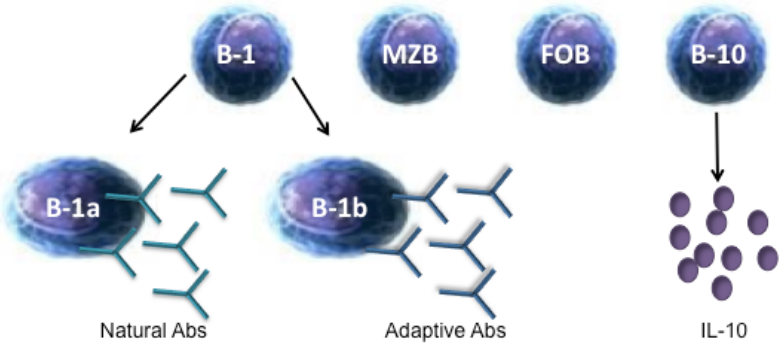

Figure 3. *B cell subsets: B1 (B-1a, B-1b), MZB (Marginal Zone B cells), FOB (Follicular B cells), B-10 cells*

1.2.2.3 B cell selection in the periphery

After leaving the bone marrow, "transitional B cells" (T1 and T2) (immature B cells), respond to follicular dendritic cells (FDCs) bound T cell–dependent antigens. They get activated (Act-B), proliferate, and either differentiate into short lived plasma cells

(SL-PC), (extrafollicular origin) secreting low affinity abtibodies or enter GC reactions. Proliferating B cells move to the borders of B cell and T cell zones in the secondary lymphid organs (lymph nodes and spleen). These structures are called germinal centers (GCs); where T-cell dependent (TD) immune responses take place, which includes affinity maturation, memory B cell pool formation and long-lived plasma cell (LL-PC) generation [18, 19] (Figure 4). Commitment to the PC fate involves the expression of B lymphocyte induced maturation protein 1 (Blimp1), which extinguishes the mature B cell gene expression program [20]. Blimp1 initiates the PC transcriptional program and represses both Bcl6 and Pax5 [21]. Antigen specifc antibodies derived from long-lived plasma cells travel to the bone marrow and stay there for the lifetime without any need for self-replenishment [22, 23]. GC formation requires cognate interactions between activated CD4 T cells and antigen-presenting B cells, which includes MHC class II-restricted presentation by the B cell, costimulation via CD40-CD40L and certain cytokines such as IL-21. A very important gene activated in GC B cells is activation-induced deaminase (AID), which induces point mutations in Ig V regions [24]. This is called as somatic hypermutation (SHM) and as a result clonal variants of GC B cells with altered antigen affinity and specificity are formed [25]. Higher affinity BCR bearing B cells for antigen are positively sellected, whereas those with lower affinity die by apoptosis [26]. AID also mediates class switch recombination (CSR) [24] (Figure 4).

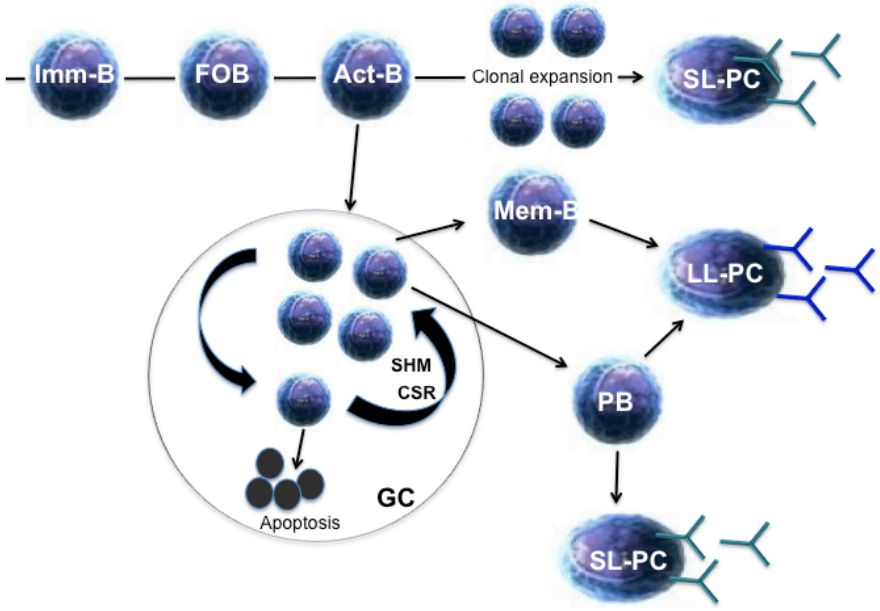

Figure 4. *B cell sellection in the peripheral lymphoid organ, GC and non-GC reaction. Imm-B (immature B cell), FOB (follicular B cell), Act-B (activated B cell), SL-PC (short-lived plasma cell), Mem-B (memmory B cell), LL-PC (long lived plasma cell) and PB (plasma blast).*

1.2.3 T cells

To eliminate viral, bacterial or parasitic infections, T cels mediate adaptive immunity processes. Foreign or self-Ag recognition by T cells is based on the recognition through T cell receptor (TCR) of antigenic peptides which are presented by MHC molecules present on Ag-presenting cells (APC) [27] where the recognition of self-Ag can lead to induction of different autoimmune inflammatory diseases. T cell-mediated responses include 1) a primary response by naive T cells, 2) effector functions by activated T cells and 3) persistence of Ag-specific memory T cells. The activated T cells clonally expand and perform effector functions such as cell-mediated cytotoxicity and secretion of different cytokines. Infected or malignant cells bearing the Ag are directly lysed by cytotoxic CD8+ T cells [28], while CD4+ T helper cells can produce cytokines that act toxic to the target cells or able to stimulate other T cell effector functions or B cell antibody production [29]. Naive T cells live for a short period and effector cells disappear at the end of the immune response. Memory T cells can survive for many years and upon activation can respond immediately in peripheral tissues or undergo activation and proliferation in lymphoid organs to mount a secondary immune response to already encountered Ag [30].

2 AUTOIMMUNITY

Autoimmunity is a failure of the immune response enabling recognition of self-cells and tissues and as a result an aberrant immune response is formed towards own constituent parts of the body. The diseases, arising from such pathogenic immune grounds are referred as autoimmune diseases.

2.1 AUTOIMMUNE DISEASES

There are large number of immune disorders recognized as autoimmune diseases. A German-American immunologist, Ernst Wibetsky co-autored a paper in 1957, where the first formulation of autoimmune disease criteria's, denoted as "Witebsky`s postulates" were published [31]. A recent modification to these standards were accepted in 1994 [32] as follows: a) Direct evidence from transfer of pathogenic antibody or pathogenic T cells; b) Indirect evidence based on reproduction of the autoimmune disease in experimental animals; c) Circumstantial evidence from clinical clues [32].

2.2 AUTOIMMUNE DISEASE CLASSIFICATION

Autoimmune diseases are classified as **organ-specific** (localized) as in *Celiac disease, Multiple sclerosis*, *Type 1 Diabetes*, *Ulcerative Colitis* or **systemic** (involving several organs) as in *Systemic Lupus erythematosus*, *Rheumatoid Arthritis, Scleroderma*, *Sjogren`s Syndrome*. However, in certain conditions, organ specific disorder could be provoked by systemic autoimmunity [33].

CHAPTER I

3 RHEUMATOID ARTHRITIS (RA)

3.1 OVERVIEW

Rheumatoid arthritis (RA) is an autoimmune, polygenic, systemic inflammatory disorder affecting up to 1% of the worldwide population [34]. Many different extra-articular body tissues and organs are involved in the disease pathogenesis [35], but particularly, peripheral (synovial) joints are disturbed. This inflammatory disease causes pain and if not adequate care/treatment is provided, pathologic condition can get worsened, leading to loss of joint function and mobility.

3.1.1 Clinical symptoms

RA affected joint is symptomized with painful (dolour), stiff, swollen (edema), red (erythema) joints. Stiffness, lasting for over an hour in particular during morning, is also called as morning stiffness. Diseased joints are mainly symmetrically affected but at an initial phase of the disease it can be asymmetric too. In figure 5, arthritis affected hind paws in animal model of RA disease is shown.

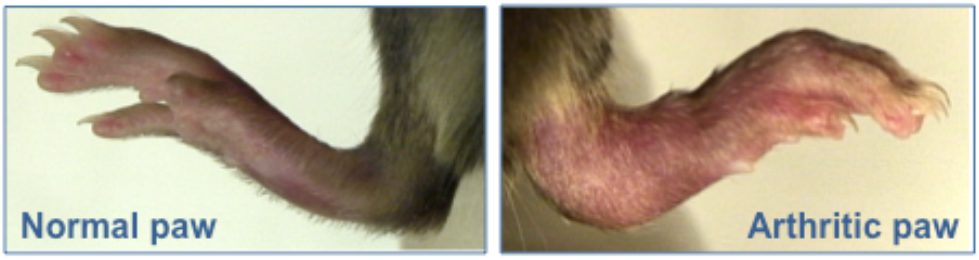

Figure 5. *Representative images of a naïve and arthritic hind paws of mice.*

3.1.2 RA joint

Arthritic joint is characterised by synovial inflammation (inflamed capsule around the joint), excess synovial fluid and pannus formation (fibrous tissue development) in the joint cavity [36]. Severe joint inflammation can lead to joint architecture distruction and ankylosis (fusion) of the joints. Arthritic mouse ankle joint histopathology is shown in figure 6.

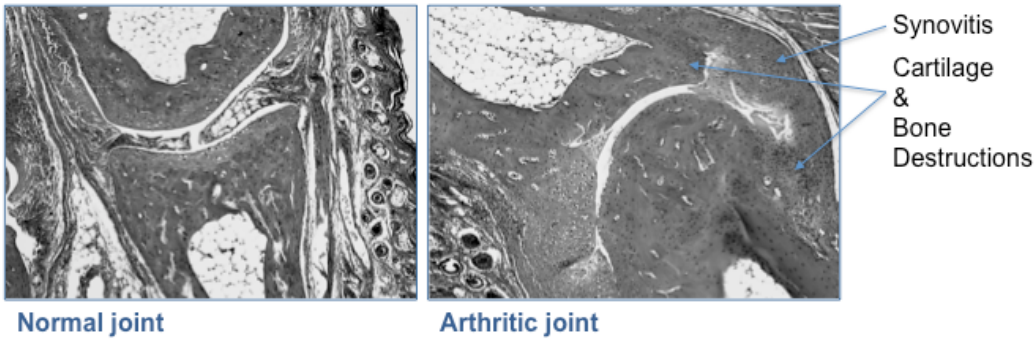

Figure 6. *Histopathology of a normal and arthritis diseased hind paw ankle joint.*

3.1.3 Epidemiology

The overall prevalence of RA worldwide is approximately 0.5% to 1% but there is a large regional variation towards disease prevalence. Disease incidence seems to be highest in Pima Indians (5.3%) and Chippewa Indians (6.8%), and lowest in people from China and Japan (0.2%-0.3%), suggesting a possible role for specific risk factors such as genes and environmental triggers [37-39].

3.1.4 Genetic susceptibility

3.1.4.1 MHC genes

Major histocompatibility complex (MHC) is the only region of the genome, which has been steadily shown to be associated with RA. In the HLA class II region are the HLA-DR, -DP and -DQ loci encoding the α and β chains of the various HLA class II molecules [40, 41]. In 1976 Stastny reported an association of RA with HLA-Dw4 allele [42] but later on it was investigated that the different HLA-DR4 alleles are not equally associated with RA. Gregerson and co-workers suggested 'SE' hypothesis, which is an unifying hypothesis for the association of different HLA-DRB1 specificities with RA. The alleles carrying this nucleotide sequence are DRB1*0401, *0404, *0405, *0408, *0101, *0102, *1402, *09 and *1001, where *0401 and *0404 are the predominant RA associated alleles in Caucasians. In contrast, there are other alleles that are negatively associated with RA and therefore have a protective role (DRB1*0103, *0402, *0802, *1302) [43].

Furthemore, there is a hierarchy of strength of the association of the different SE-positive HLA-DRB1 alleles and RA. For example, the DRB1*0401/*0404 heterozygote genotype is strongly associated with early disease onset and a more

severe disease phenotype than either DRB1*0401 or DRB1*0404 homozygosity [40].

3.1.4.2 Non-MHC genes

There are number of non-HLA gene single nucleotide polymorphisms (SNPs) associated with RA [44]. The gene for the protein tyrosine phosphatase non-receptor type 22 (***PTPN22***) encodes the cytoplasmic lymphoid specific phosphatase (Lyp), which is a powerful inhibitor of T-cell activation [45]. A functional variant of PTPN22 has been recently shown to be associated with diffenet autoimmune disorders including RA. Additionally, correlation between PTPN22 and heavy cigarette smoking was shown [46]. Cytotoxic T lymphocyte-associated antigen 4 (**CTLA-4**) is important for down regulation of T-cell activation and CTLA-4 gene polymorphisms have been implicated as risk factors for rheumatoid arthritis (RA), specifically in Chinese Han population [47]. The genome wide association studies on RA patients revealed a linkage with peptidyl arginine deiminase 4 (**PADI4**) genes. Case-control association studies and mRNA stability assays reported the association of PADI4 gene with RA in Korean and Japanese populations [48]. A meta-analysis showed a positive association between PADI4 and RA not only in the Japanese population but also in populations of European descent [49]. A polymorphism in chemokine receptor expressed by Th17 cells (***CCR6)*** correlated with expression level of *CCR6* mRNA and with the presence of IL-17 cytokine in the sera of RA patients, highlighting the importance of the Th17 pathway in RA pathogenesis [50]. TNF receptor-associated factor 1 (TRAF1), is encoded by the ***TRAF1*** gene. TRAF1 mediate the signal transduction through various receptors of the TNFR superfamily. A common genetic variant at the *TRAF1-C5* locus on chromosome 9 is associated with an increased risk of anti-CCP-positive rheumatoid arthritis [51]. Recent meta-analysis study show interferon regulatory factor 5 (***IRF5***) rs2004640, rs729302 and rs752637 polymorphisms association with RA susceptibility in Europeans and Asians [52]. The signal transducer and activator of transcription 4 (**STAT4**) is a critical molecule for the development of the Th1 cells. STAT4-mediated signaling promoted the production of autoimmune-associated components, which are implicated in the pathogenesis of autoimmune diseases, such as rheumatoid arthritis [53]. The Fc gamma receptor 3A (**FCGR3A**) polymorphism can predict response to biologic therapy in patients with rheumatoid arthritis as investigated by a meta-analysis [54]. Genotyping for these polymorphisms might be a good tool for personalized treatment. A large UK cohort study shows a strong association of ***CD40*** gene, coding for the

costimulatory protein CD40 on antigen presenting cells, with susceptibility to RA [55]. Carriage of chemokine (C-C motif) ligand 21 (***CCL21***) risk alleles was associated with premature mortality in inflammatory polyarthritis, independently of anti-CCP antibody and SE status [56].

3.1.5 Risk factors

Varieties of risk factors have been identified as possible triggers of arthritis disease. RA is an ***age*** related disorder. It can occur at any age from childhood to old age, but usual onset is between 30 - 50 years of life. ***Gender*** has an importance in disease susceptibility; women are more prone to develop RA than men. It has been assumed that RA was linked to infections but Scher and colleagues show for the first time an association of a specific ***microbe*** to RA. Expansion of intestinal *Prevotella copri,* which is an intestinal gram-negative bacteria, correlated with an enhanced susceptibility to arthritis [57]. There have been several reports indicating that some periodontal pathogens such as *Porphyromonas gingivalis* are possible cause of RA [58]. ***Smoking*** contributes up to 25% of the population with a burden of developing RA. The risk is dose related, stronger in males and especially stronger for anti-citrullinated peptide antibody positive (ACPA+) RA through an interaction with the shared epitope. The disease suusceptibility varies, which is much higher (35%) for ACPA+ RA group and up to 55% in individuals with two copies of the HLA-DRB1 SE [59, 60]. Other associations are high ***coffee*** consumption and ***obesity***, which may also increase RA risk [61]. ***Stress*** might exacerbate autoimmune disease by amplifying pro-inflammatory cytokine production [62]. Interestingly, occupational exposures, for example ***silica***, have been associated with RA and to other autoimmune diseases. Silica is cytotoxic and produces inflammation in the lungs causing loss of self-tolerance and production of autoantibodies [63].

3.1.6 Diagnosis and threatment

According to 2010 ACR/EULAR classification criteria, RA is diagnosed based on following measurements: a) JOINT INVOLVEMNT (by counting the number of small and large joints affected); b) SEROLOGICAL FINDINGS (measuring RF and ACPA levels); c) ACUTE PHASE REACTANTS (CRP and ESR levels); d) DURATION OF CLINICAL SYMPTOMS [37].

3.1.6.1 First line "fast acting" drug therapy

Non-steroidal anti-inflammatory drugs (**NSAID**-s) are used as first line medications to relieve pain and swelling by reducing the inflammation. Patients' response to different NSAID medications vary. NSAIDs block the production of prostaglandins (PGs) accomplished by inhibiting the activity of the enzyme cyclooxygenase (COX) [64]. Inhibition of COX-2 by NSAIDs blocks PG production at the site of inflammation, while inhibition of COX-1 can block PGs in other tissues, for example in gastro-duodenal mucosa, potentially leading to common side effects such as bleeding and gastrointestinal ulceration [65]. **Corticosteroid** medications are more potent than NSAIDs in reducing inflammation and in restoring joint mobility and function [66]. Corticosteroids are used during severe flares of arthritis and are mainly given intra-articularly (IA).

3.1.6.2 Second line "slow acting" drug therapy

First line medication is helpful to relieve joint inflammation and pain but they do not prevent disease progression. To better control the inflammatory processes, disease modifying anti-inflammatory drugs (**DMARD**-s) are used. Treatment with these "slow-acting" drugs will potentially take some time for its effectivness. Immunosuppressive medicines are powerful in suppressing the body's immune system [67]. A list of immunosuppressive drugs include cyclosporins, cyclophosphamide, myocrisin (gold injections), hydroxychloroquine, leflunomide, methotrexate, mycophenolate and sulfasalazine. Because of potentially serious side effects, immunosuppressive medicines (other than methotrexate) are generally used for those patients who have very aggressive disease [68].

3.1.6.3 Biological drug therapies

In recent years, new drug therapies (biologics) have been developed to target

individual molecules. This type of therapy is more efficient than conventional DMARDs and is only given to DMARD therapy non-responders or to the patients with severe side effects. Biological therapies are often given in combination with methotrexate [67]. Anti–tumor necrosis factor (**anti-TNF**) therapies with adalimumab (a full monoclonal antibody), etanercept and infliximab (a chimeric monoclonal antibody) therapies have been routinely used as a treatment option of rheumatoid arthritis (RA) patients. However, about 30% of the patients abandon treatment with anti-TNF therapy within a year due to either side effects or the inefficacy of the treatment [69]. The second option is an alternative anti-TNF therapy [70-72] or **anti-CD20** therapy with Rituximab (RTX), a chimeric monoclonal antibody against the B lymphocyte surface marker CD20, introduced in 2006 for the medication of RA patients who have failed one or more anti-TNF therapies. RTX has been shown to be effective in different clinical trials [73, 74] and has been proven to cause circulating B cell depletion through antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity and apoptosis [75]. Abatacept, a human cytotoxic T-lymphocyte antigen (CTLA)-4 and Fc-IgG1 fusion protein that blocks the costimulatory signal between CD28 and CD80/CD86 is approved for the treatment of active RA patients [76]. A humanised monoclonal antibody- Tocilizumab targeting the interleukin-6 receptor (**anti-IL-6R**) is another treatment option for RA. Tocilizumab blocks the downstream effects of IL-6 by affecting the function of neutrophils, T cells, B cells, monocytes, and osteoclasts and thereby the inflammatory cascade in RA [76].

Blocking of complement-C5a, chemokines-CCL2, CCR1, cytokines-IL-10, IFN-beta, anti-CD4, anti-CD52 are experimental targeted therapies that are under consideration to be implicated in RA pathogenesis [77].

Despite having so many treatment options, there is no cure for the disease. Moreover, all these treatments are associated with many side effects as infections. The sites of infections associated with biological therapy are respiratory tract infections including *pneumonia*, septic arthritis, skin and soft tissue infections, and urinary tract infections. TNF plays an important role in the host defense mechanism against intracellular pathogens and consequently anti-TNF therapy is associated with increased risk of infection with intracellular micro-organisms such as *Mycobacterium tuberculosis*, *Listeria monocytogenes* and *Legionella pneumophila* [78, 79].

4 AUTO-ANTIGENS IN RA

A variety of different autoantigens has been described to be associated with human RA by identifying the reactivity to those antigens in the sera and synovial fluid. Most of these autoantigens can not be associated with RA patogenesis due to little experimental evidence or clinical observations. Autoantigens can be categorized as **JOINT-associated** autoantiges such as CII (Collagen type II), proteoglycans as well as HCgp-39 (human chondrocyte glycoprotein 39) [80] and **NON-JOINT-associated** autoantigens including HSPs (heat shock proteins), citrulinated fillagrin (post-translationaly altered proteins) and ubiquitously expressed proteins such as GPI6 (Glucoso-6-phosphate isomerase), P205 (contains an 11 aminoacid stretch identical to a sequence (278-288) located in the CH2 domain of immunoglobulin G (this domain contains the major epitopes of rheumatoid factors) and BiP (HSPs secreted duing stress) [80].

4.1 COLLAGEN TYPE II (CII), THE C1 EPITOPE

Collagen type II (CII) is a major autoantigen in animal model of RA and belong to fibrilar forming collagen group together with type I, II, V and XI collagen. CII consists of 3 pro-alpha chains, which are assembled into triple helical molecule. After specific cleaving of non-helical ends with proteinases, triple helix is assembled into fibrils (Figure 7) [81].

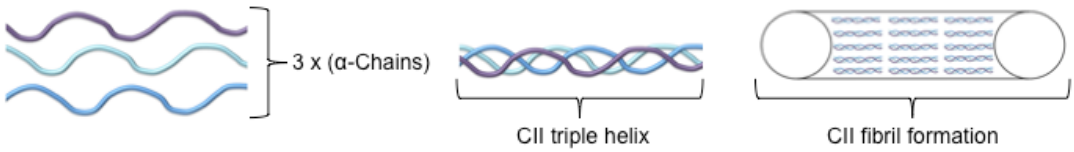

Figure 7. *Simple illustration of CII fibril formation.*

Anti-CII monoclonal antibodies are capable of initiating arthritis in animal model of rheumatoid arthritis independent of B and T cells during the effector phase of arthritis [82]. There are different immunodominant epitopes on triple helical CII, recognized by autoreactive B cells.

The dominant and most characterized CII epitopes in CIA model are C1, U1, J1 and F4, and the monoclonal antibodies specifically recognising these epitopes are denoted as CB20 (IgG1) and CIIC1 (IgG2a), UL-1 (IgG2b), M2139 (IgG2b) and CIIF4 (IgG2a) respectively [83]. These antibodies are not cross-reacting with other collagen types or the denatured CII. The structural integrity of CII epitopes for antibody recognition is very important. CIA is only induced by immunization with triple helical CII and not by the denatured one [84, 85].

Interestingly, not all the CII specific antibodies are having pathogenic capacity. Antibodies to C1, J1 and U1 epitops are arthritogenic, while CIIF4 antibody was shown to negatively associate with the CAIA disease model of arthritis. Pathogenic monoclonal antibodies have different degradative capacities *in vitro*. As an example, CII-C1 has destructive effect on cartilage synthesis and dissorganization of CII fibrils. UL-1 can induce inflammation-independent proteoglycan depletion *in vitro*, whereas M2139 induces thickening and aggregation of CII fibrils and abnormal morphology in chondrocytes [86-88].

Among C1 epitope specific monoclonal antibody library, 40% of them are mainly recognizing certain amino acid sequence: GARGLTGROGDA (O = hydroxyproline) located at position 358–369 on CII in mice and human [89, 90]. Moreover, the arginine residues of C1 can be enzymatically modified by peptidyl arginine deiminase (PAD) enzymes to citrulline, and autoantibodies to citrullinated C1 have also been detected in RA [91].

Earlier studies have shown that C1-specific antibodies (such as the well-characterized, germline-encoded, CB20) were obtained from mice after the primary immunization with CII [92, 93].

5 ANIMAL MODELS OF RA

To identify the susceptible genes and pathological pathways in autoimmune RA, different animal models are used. By altering animal housing conditions, one can identify various environmental factors triggering disease and its development. There are several important criteriae of selecting animal models for the disease studies: a) a capacity to predict different therapeutic agents in humans, b) easy to perform, c) reproducibility of the data, d) duration of the test period and e) similarity to human disease pathogenesis. Mouse models of arthritis are classified as spontaneous and induced.

5.1 SPONTANEOUS

Spontaneous models of arthritis do not require any external trigger for disease induction. **SKG** - a mouse model of spontaneous arthritis caused by a ζ-chain–associated protein kinase 70 (ZAP-70) mutation, which spontaneously develop into chronic arthritis. Synovial inflammation in affected joints resembles rheumatoid arthritis with an accompanying infiltration of $CD4^+$ T cells with eroded cartilage and bone and mounting of autoantibodies including rheumatoid factor [94]. **MRL/l** mice spontaneously develop arthritis similar to human rheumatoid arthritis with synovitis and/or arthritis, presence of circulating IgM rheumatoid factor (RF) and demonstrable synovial and/or joint pathology [95]. **TNFα Tg**-mouse over-expresses human TNF-alpha and develops an erosive polyarthritis which has many characteristics of rheumatoid arthritis in patients. The TNFα-Tg mice are very useful tools to understand pathogenic mechanisms of arthritis and to evaluate the efficacy of novel therapeutic strategies for rheumatoid arthritis [96]. **IL-1Ra k/o** mice bred on the BALB/cA background, spontaneously develops inflammatory arthritis with many features resembling rheumatoid arthritis (RA) in humans [97]. **IL-6R Tg** mice have a homozygous mutation in the gp130 IL-6 receptor subunit and show enhanced signal transduction and STAT3 activation and develop RA-like joint disease [98]. In **K/BxN** mice, the expression of both the T cell receptor transgene *KRN* and the MHC class II molecule Ag7, results in the development of spontaneous arthritis [99]. The development of disease requires the presence of T and B lymphocytes and is dependent on the MHC class II molecule I-A(g7). B cell activation by antigen and an additional CD40-CD40 ligand interaction was found to give rise to production of

autoantibodies. Glucose-6-phosphate isomerase was identified as the target of the autoantibodies; moreover, the transgenic T cells were demonstrated to exhibit a dual specificity for both bovine RNase and glucose-6-phosphate isomerase [100]. **ACB** mice, an IgH chain knock in mouse strain, spontaneously produce anti-C1 abs but do not develop spontaneous arthritis [101] and are relatively resistant to CIA. Only when ACB mice are bred to B10.Q/*Ncf1*** mice [a mutation in the *Ncf1* gene in the B10Q mice impairs expression of the *Ncf1* gene and totally blocks the function of the NOX2 complexes) [102] brakedown of CIA resistance is operating during late phase of the disease, which was associated with epitope spreading on CII matrix. (Paper II).

5.2 INDUCED

In **K/BxN** serum transfer model, arthritis disease is serum transferable to normal recipients, which enables the examination of the pathogenic mechanisms of joint inflammation and destruction. Recent studies also suggest the importance of the innate immune system and its machinery such as complement components, Fc receptors and neutrophils, which are indispensable for disease induction [100]. **GPI** (Glucoso-6-Phosphate Isomerase)-induced arthritis is based on immunization with recombinant human G6PI, which results in polyarthritis that is dependent on MHC II and mouse genetic background [103]. Mice H-2^q and H-2^p MHC haplotypes are more prone to disease development [104]. In **PgIA** model of arthritis, immunization with chondroitinase ABC-digested fetal human cartilage proteoglycan and Freund's complete adjuvant induces polyarthritis and ankylosing spondylitis in female BALB/c mice. Clinicaly evident swelling and redness and histologicaly observed synovial inflammation of the paws was associated with mononuclear cell infiltration and perivascular concentration and occlusion of small vessels. Development of this arthritis was dependent on cell-mediated and humoral immunity to the immunizing antigen [105]. **COMPIA**-is a cartilage oligomeric matrix protein (COMP) induced arthritis, where immunization with rat COMP induced a severe, chronic, relapsing arthritis with a female preponderance in mice. This disease is strain dependent with high susceptibility in C3H.NB mice but not in B10.P mice, although they share the same MHC haplotype. Both H-2^q and H-2^p MHC haplotypes develop COMPIA. Interestingly, the transfer of anti-COMP serum was found to induce arthritis in naive mice. This model also provides a useful tool to study the pathogenesis of RA [106]. **ZIA** is an experimental animal model for human RA induced by injection of zymosan

into knee joints of mice. Within 7 days after intra-articular injection, a chronic inflammatory arthritis with mononuclear cell infiltration, synovial hyperplasia and pannus formation was observed [107].

5.2.1 CIA

Collagen induced arthritis (**CIA**) is the most commonly used mouse model of rheumatoid arthritis, in which mice are immunized with CII in complete Freund´s adjuvant (CFA) at the base of the tail on day 0 and 5 weeks later a booster injection of CII in incomplete Freund´s adjuvant (IFA) is given. CIA model is shown to be dependent on T cells, APCs and B cells - particularly in the production of autoreactive antibodies toward auto-antigen CII. T and B cell deficient mice did not develop CIA [108-111]. Similar to human RA, CIA disease development is associated with MHC class II haplotypes. Mice having MHC H-2^q haplotype are most susceptible for arthritis [112]. Similar to human arthritic joint, CIA paws are characterized with synovial hyperproliferation, cartilage and bone destruction [113]. The severity of joint destruction is dependent on tested mouse strain.

5.2.2 CAIA

Collagen antibody induced arthritis (**CAIA**), is commonly used animal model of arthritis, depicting the effector phase of arthritis. Autoreactive CII reactive antibodieas are generated from B cell hybridomas from mice previously immunized with CII [114, 115]. Monoclonal CII-reactive antibodies are injected as a single dose or in the cocktail form using different concentrations depending on the mouse background used [114]. Within a week after antibody passive transfer, mice are boosted i.p. with bacterial lipopolysaccharide (LPS). The aim of LPS administration is to enhance the severity of the disease. The whole disease course lasts for 3 to 5 weeks. Contrary to CIA, CAIA can be induced in T and B cell deficient mice but the role of Fc gamma receptors and complement is crucial [116, 117]. Evenmore, for successful disease induction one need to consider monoclonal antibody specificity, isotype, concentration, antibody cocktail composition and the strain of the mice [114, 118].

6 B CELL ROLE IN RA

6.1 B CELL TOLERANCE CHECKPOINTS

B cells play an important role in RA pathogenesis in many different aspects. B cell depletion therapies in humans [119] have shifted attention from macrophages and T cells towards B cells role in autoimmune diseases.

In healthy individuals, most autoreactive B cells are eliminated from the body by two main mechanisms [120, 121]. A central B-cell tolerance checkpoint taking place in the bone marrow and removes the majority of autoreactive B cells expressing polyreactive phenotype. If some autoreactive B cells escape the central tolerance mechanisms, then a peripheral B-cell tolerance checkpoint takes over to further eliminate autoreactivity at emigrant/transitional B cell stage before they enter into the long-lived mature B cell pool. A large number of autoreactive B cells in different compartments in RA pathogenesis indicate that both central and peripheral B-cell tolerance checkpoints are not operating optimally and are defective in RA [121]. In untreated RA patients, the frequency of autoreactive transitional B cells in the blood was increased 3.4 - fold compared to control group, highlighting the inability of the immune system in central and peripheral lymphoid organs to remove polyreactive B cells [121].

There are 3 possible functions of B cells to contribute in RA pathogenesis: **Autoantibody production** - where B cells are the source of the rheumatoid factors, anti-CII and anti-citrullinated protein antibodies, which contribute to immune complex formation and complement activation in the joints. **Antigen presentation** - where B cells can contribute to T cell activation through expression of co-stimulatory molecules. **Cytokine secretion** - where the chemokines and cytokines secreted can promote leukocyte infiltration into the joints, angiogenesis and synovial hyperplasia.

6.2 AUTOANTIBODY PRODUCTION

In RA, variable number of autoantigens are recognized by autoantibodies. The most studied autoantibodies are RFs (autoantibodies directed to the Fc portion of IgG) and ACPAs (anti-citrullinated protein antibodies). Autoreactive response towards self-IgG

usually occurs independent of T cell help and outside the GCs, while the response to citrulinated antigens develop through GC responses by acquiring T cell help. As it was shown, extrafollicular B cell responses are mainly regulated by TLR engagenment [122], but SHM and CSR of Ig genes, which mainly characterize T cell-dependent GC responses can also occur extrafollicularly upon TLR signaling. RF clones from RA patients are in fact somatically mutated compared to healthy subjects [123]. Although T cells are not required for the extrafollicular responses, they still help in amplifying and sustaining the chronic autoantibody production via CD40L and interleukin (IL)-21 signaling [122]. In contrast, immune response to citrullinated antigens is regulated via autoreactive T cells within established GC reactions. ACPA response is strongly associated with HLA DR alleles [124] and represent switched IgG-s generated from joint-derived B cells of RA patients [125].

6.3 ANTIGEN PRESENTATION

B cells act as competent antigen presenting cells (APCs) to prime T cells and to develop memory CD4+ T-cell pool. B cells can selectively take up an antigen for presentation. As studies show, particularly RF+ B cells are important antigen presenting cells, where they can bind to antigen-Ig immune complexes via their surface Ig receptors specific to RF. After antigen processing, B cells then present peptides to T cells thereby inducing both T-cell activation and T-cell help [126].

A good experimental evidence of T cell response dependency on B cell in RA synovium comes from Takemura et al. study, where it was shown that anti-CD20 antibody treatment in RA synovial tissue xenotransplated SCID (immunodeficient) mice led to disruption of GCs, loss of follicular dendritic cell (FDC) networks, and impairment of T cell activation with a reduced production of T cell-derived cytokines [127]. It is tempting to speculate that there is a local cross-talk between citrullinated peptide-reacting B cells, which are functioning as APC for citrullinated peptide-specific synovial T cells, based on the recent investigation where citrulinated peptide reactive B cells have been found to be enriched in RA joints [125].

Morover, B cells can stimulate pathogenic T-cell responses via cytokine secretion. B cells are a major source of IL-6 in the chronic phase of RA [128]. Mice, which were deficient in IL-6 production had mild arthritis severity together with impaired CD4+

T cell produced IL-17 levels [129]. Notably, IL-6 inhibition increases the frequency of T-regs in both experimental and rheumatoid arthritis [130]. Recently, IL-6 is in fact been acknowledged as a major regulator of maintaining the balance between Th17 cells and T-regs. In turn, Th17 cells and their derived cytokines can promote B-cell proliferation, differentiation, CSR and antibody production *in vivo* [131], which suggest that there might be a positive feedback loop between T and B cells potentially involved amplifying inflammatory responses. As shown, B-cell depletion affects both B cell and Th17 responses *in vitro* [132].

6.4 CYTOKINE PRODUCTION

Accumulated data on B cell studies in autoimmune conditions indicate that B cells in RA can directly contribute to the local production of variable proinflammatory cytokines involved in the disease pathogenesis. B cells are major source for receptor activator nuclear factor kappa B ligand (RANKL) in the rheumatoid environment, suggesting their involvement in osteoclastogenesis. Latest reports have also shown that CD19+ B cells from both RA patients and healthy individuals are competent of producing IL-17A [133], possibly involved in different aspects of inflammation and bone damage. Evenmore, B cells infiltrating in the inflamed synovium may induce cytokine synthesis in the synovial fibroblasts through the production of IL-36α [134]. This study suggests tissue infiltrating B cells can interact not only with haematopoietic cells but also with the local stroma through paracrine mechanisms.

CHAPTER II

7 PSORIASIS (PS)

7.1 OVERVIEW

Psoriasis (Ps) is a common, chronic, immune-mediated, skin inflammatory disease recognized since early times when psoriasis, leprosy and other inflammatory skin disorders were erroneously thought to be the same condition [135]. Hippocrates was one of the first authors to write descriptions about skin disorders. He used the word *lopoi* to describe the dry, scaly and disfiguring eruptions of psoriasis, leprosy and other inflammatory skin disorders [136]. Psoriasis skin disorder affects up to 3% of worldwide population with equal sex distribution [137]. Disease development is higher in American and Canadian populations compared to Africans and Asians with a prevalence of 4.6-4.7% to 0.4-0.7% respectively [138]. Ps is considered to be organ-specific autoimmune disease and it is possible to study its immunopathology and genomic features due to its occurrence in the accessible organ [139]. Psoriasis is a complex multifactorial syndrome, where different environmental triggers initiate the disease in genetically prone individuals. Additionally, patients with Ps disease have other disorders involving musculoskeletal structures, cardiovascular system, eye and the gut [140, 141].

7.2 PS CLINICOPATHOLOGY

Ps disease is usually manifested as erythematous, thickened plaques with silvery scales as shown in figure 1.

Figure 1. *Clinical phenotypes of Ps in mannan-induced Ps-like disease in mice.*

Histopathologically, Ps is characterized by thickening of epidermis represented as acanthosis due to increased proliferation of keratinocytes with elongated rete ridges (ERR) (in humans only) protruded downward into the dermis. Incomplete maturation of epidermal keratinocytes results in abnormal retention of nuclei in the stratum corneum, denoted as parakeratosis (Figure 2) and inflammatory infiltrates in the epidermis (EPI). The dermis of the skin usually consists of DC, MF, Neutrophils and T cells [142]. Erythema of the Ps skin lesions is due to increased dilation of elongated blood vessels in the papillary dermal region. Moreover, endothelial cells are also activated in the Ps lesions via ICAM-1 (intracellular adhesion molecule), VCAM-1 (vascular cell adhesion molecule) and E-selectin (CD62E) molecules [139].

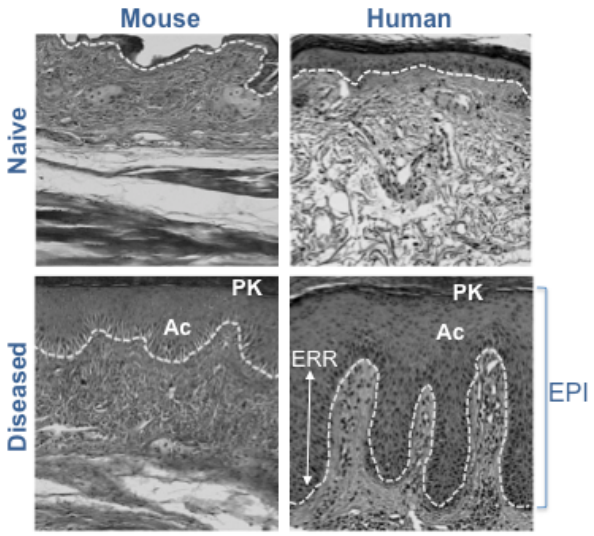

Figure 2. *Ps histopathology. PK (parakeratosis), Ac (acanthosis), ERR (elongated rete ridges), dashed line indicates the border between epidermis (EPI) and the dermis.*

7.3 PS GENETICS

Ps disease susceptibility is complex. Population based studies showed higher disease inheritance in first/second degree relatives compared to the general population [143]. However, psoriasis propensity is never 100% among monozygotic twins, which argues for the influence of environmental factors on disease manifestation. Classic genome wide linkage analysis has mapped PSORS1 (psoriasis susceptibility 1) locus as a major genetic determinant [144]. PSORS1 is located on chromosome 6 in the MHC spanning within the class I telomeric region of HLA-B. It accounts for up to 50% of the inheritability of psoriasis [145]. HLA-Cw6, an associated variant of HLA-C is the susceptibility allele within PSOPS1 region [146]. Presence of HLA-Cw*0602 is associated with early onset and more severe Ps and it is found in 100% of patients with

guttate psoriasis [143, 147]. A genome-wide association study has identified sequence variants in the gene IL-23R and its ligand IL-12B conferring protection against psoriasis [148]. During inflammation, regulation of gene-expression network is controlled by microRNAs (miRNAs) via interfering with key inflammatory checkpoints [149]. It was shown that a distinct miRNA expression profile exists in psoriasis skin compared to healthy skin. For example, miR-203, miR-125b, miR-424 and miR-99a regulate keratinocyte proliferation and differentiation, whereas miR-21 is up regulated in psoriatic skin and suppresses T cell apoptosis [149-152]. Suppression of miR-31 (a miRNA overexpressed in psoriasis keratinocytes) in psoriasis skin alleviated inflammation by interfering with the cross talk between the keratinocytes and immune cells [153].

7.4 TRIGGERS

Any person can develop psoriasis but certain factors might increase the risk of triggering the disease in individuals having genetic predisposition as discussed previously. ***Infections:*** Various microorganisms such as bacteria (*Streptococcus pyogenes*, *Staphylococcus aureus),* fungi (Malassezia, *Candida albicans*) and viruses (papillomaviruses, retroviruses, endogenous retroviruses) are associated with triggering and/or exacerbation of psoriasis skin lesions [154, 155]. Skin infections are rare in psoriasis patients but infected tonsils might result in psoriatic lesions in the skin. ***Stress:*** Development and exacerbation of psoriasis can be influenced by emotional stress. "Stress responders" in psoriasis patients are considerably high ranging from 37% to 78% [156]. ***Smoking:*** Based on recent meta-analysis, smoking is identified as an independent risk factor for the development of psoriasis and patients with established psoriasis continue to smoke more than patients without psoriasis [157]. ***Obesity:*** In 1986, a Scandinavian study revealed a higher prevalence of obesity in psoriatic women than in healthy controls [158]. Neimann et al demonstrated that the risk of obesity was higher in patients with severe psoriasis than in those with moderate disease [159]. ***Medications****:* Surprisingly, few currently used medications such as Lithium (prescribed for bipolar disorder), anti-malarial agents (AMs) and Non-steroidal anti-inflammatory drugs (NSAIDs) can provoke or induce psoriasis symptoms [154, 160].

7.5 CLINICAL VARIANTS

7.5.1 Cutaneous manifestations

Psoriasis can manifest into two different forms viz., cutaneous and extracutaneous. In cutaneous psoriasis different patterns are recognised depending on clinical manifestations and body part involvement [161]: **Chronic Plaque Ps** (psoriasis vulgaris) (CPP), which is the most common variant of the disease affecting approximately 85-90% of psoriasis patients [162] characterized by erythematous plaques with adherent silvery scale in the skin. Usually the scalp, elbows, knees and lumbosacral areas are involved [163]. Less common variants are ***Guttate Ps*** *(GP)* [164], ***Pustular Ps*** *(PS)* [165], ***Inverse Ps*** *(IP),* and ***Erythrodermic Ps*** *(EP),* (exfoliative psoriasis) [166]. Among extra-cutaneous forms, ***Nail psoriasis*** *(NP)* and ***Psoriasis arthritis*** *(PsA)* are recognized (Figure 3).

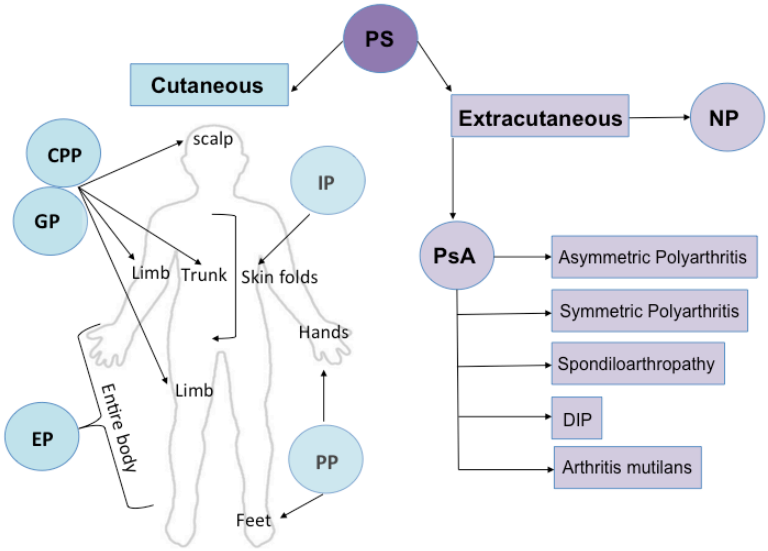

Figure 3. *Clinical variants of psoriasis (Ps). CPP (chronic plaque ps), GP (guttate Ps), EP (erythrodermic Ps), IP (inverse Ps), PP (pustular Ps), PsA (psoriasis arthritis) and NP (nail Ps).*

7.5.2 Extracutaneous manifestations

7.5.2.1 Nail psoriasis (NP)

Nail involvement in psoriasis patients was observed in 80% of the cases [167] affecting the nail matrix, bed, plate and the hyponychium (epithelium between the nail bed and nail plate). Psoriatic nail changes may include the following features: Pitting, Subungual hyperkeratosis/dystrophy, Oil spots and Onycholysis [163].

7.5.2.2 Psoriatic arthritis (PsA)

Psoriasis arthritis (PsA) is an inflammatory disease with an additional involvement of joints. It is a sero-negative arthritis and occurs in 6 to 26% of CPP patients [168]. In 1973, Moll and Wright described five distinct patterns of PsA based on clinical presentations and distribution of the joint disease [169] as summarized in Figure 3: ***Asymmetric polyarthritis*** is the most common form affecting the distal joints in an asymmetric fashion involving only few joints (oligoarthritis). ***Symmetric polyarthritis*** is the peripheral joint disease, which is symmetrically distributed. ***Spondyloarthropathy,*** including sacroiliitis and ankylosis spondylitis. ***Predominant DIP*** involves distal interphalangeal joints. ***Arthritis mutilans*** with distal joint desorption.

In recent years, extensive research provided novel insights into the mechanisms, which underlie and link both the skin and joint inflammation in PsA. As documented, the main pathological changes in PsA are associated with skin inflammation, synovial inflammation in the affected joints, enthesitis (enthesial inflammation), tenosynovitis (tendon sheath inflammation) and bone abnormalities. Until recently it was thought that psoriasis and psoriasis arthritis susceptible genes are the same but recent studies have shown that there are distinct genetic make-up between these two diseases. The gene MICA*002 is more specific to PsA than Ps. HLA-B38 and HLA-B39 are associated with peripheral PsA and HLA-B27 is more frequently identified in PsA with spondylitis [170]. Psoriatic arthritis patients with HLA-B27 or DQB1*02 were shown to have an increased risk of developing the most severe form of psoriatic arthritis as arthritis mutilans [171].

7.6 DIAGNOSIS

The diagnosis of psoriasis is mainly based on physical examination involving following criteria: a) Skin examination/inspection, determination of disease-involved sites and nail involvement, which is more common in psoriatic arthritis 2) A history of previous psoriasis or a family history of psoriasis. 3) Skin punch biopsy, which is a simple and effective way to confirm the diagnosis [163].

Psoriasis severity can be categorized according to the **P**soriasis **A**rea and **S**everity **I**ndex (**PASI**) or the **P**hysicians **G**lobal **A**ssessment (PGA) [172]. PASI measures the

sum of both the severity of psoriasis and the percentage involvement of each body region with a 4-point scoring system. The intensity/severity of redness, thickness and scaling of the psoriasis is assessed as none (0), mild (1), moderate (2), severe (3) or very severe (4). The PGA measures psoriasis on a 7-point scale from clear (0) to very severe (6).

It is of crucial importance to diagnose PsA as early as possible because early recognition of the disease will lead to treatment and consequently this might reduce irreversible joint damages. The most widely used **C**l**AS**sification criteria for **P**soriatic **AR**thritis **(CASPAR)** diagnostic system is applied for patients having inflammatory articular disease involving joints, spine or enthesis and ≥3 points using CASPAR system [173] is considered as positive identification as described in table 1.

(CASPAR) Cl**AS**sification criteria for **P**soriatic **AR**thritis

CATEGORY	POINTS
Current Ps / personal Ps/ family history of Ps	2 (current) 1 (personal / family)
Ps nail dystrophy on current examination (onycholysis, pitting, hyperkeratosis)	1
Negative for RF	1
Current dactylitis / history of dactylitis recorded by a rheumatologist	1
Radiographic evidence of juxtaarticular new bone formation	1

Table 1. ***ClASsification criteria for Psoriatic ARthritis (CASPAR)*** *[173].*

7.7 TREATMENT

There is no cure for psoriasis disease and all the available treatment options are aimed only to control the severity of the disease. These treatments are divided into 5 main categories: ***Topical*** treatment agents (lotion, gel, cream and ointment) are mainly used when Ps affected body surface area is less then 10%. This first line of medication can possibly be used as a monotherapy or in combination with other treatment options such as phototherapy and systemic medication [174]. ***Intralesional steroid injection*** is used to deliver the medications into skin lesions, to provide prolonged therapy and thereby minimizing the adverse effects of systemic therapy [163]. ***Phototherapy*** is usually used for both extensive and moderate Ps diseases. It consists of ultraviolet B (UVB) radiations, where NB-UVB (Narrow-Band UVB) may provide a faster rate of remission. Also, Ultraviolet A (UVA), which penetrates deeper into the skin and is mostly combined with the drug Psoralen (acting as a photosensitizer and increases the

local effect of UVA-treatment), so called PUVA treatment [163]. Patients with moderate to severe Ps, when more than 10% of body surface area is affected, and/or non-responders to topical/phototerapy are subjected to **systemic treatment** with methotrexate, acitretin or cyclosporine, or in combination with **biologics** including anti–tumor necrosis factor (**anti-TNF**) therapies, such as adalimumab (a full monoclonal antibody), etanercept and infliximab. Secukinumab, a recombinant, high-affinity, fully human immunoglobulin monoclonal antibody that selectively neutralizes interleukin-17A showed the efficacy and safety in two randomized, phase 3 trials in patients with moderate-to-severe plaque psoriasis [175]. Another human monoclonal antibody Brodalumab, against interleukin-17 receptor A (IL17RA), was tested in a phase 2, randomized, double-blind, placebo-controlled study and showed significantly improved response among patients with psoriatic arthritis [176]. Also, alefacept (a fully human LFA3-IgG1 fusion protein targeting CD2) and ustekinumab (a fully human mAb targeting p40 subunit of IL12/IL-23) may be used in the combination therapy [177].

8 IMMUNOPATHOLOGY OF PS AND PSA

It is well acknowledged that both innate and adaptive immunity play a functional role in psoriasis pathology and in their interactions with keratinocytes.

8.1 NON-IMMUNE CELLS

Keratinocytes have a key function in balancing skin homeostasis. They serve as sentinels of the skin and protect our body against invading pathogens. Keratinocyte activation via TLR/NLR leads to predominant Th1-type immune responses with type I interferons (IFNs) secretion [178]. Keratinocytes may have anti-microbial activity by producing anti-microbial peptides (AMPs) like, psoriasin (S100A7), HBD-s (human β-defensin-2 and human β-defensin-3) and LL-37 (Cathelicidin) [179, 180]. Keratinocytes can secrete pro-inflammatory cytokines and chemokines such as IL-1, IL-6, CXCL8, CXCL10 and CCL20 in response to different cytokine stimulations produced by innate and adaptive cells and additionally secreted anti-microbial peptides, which form chemotactic gradients to attract immune cells into the skin tissue [180]. Furthermore, keratinocytes express MHC class II molecules and might act as non-professional antigen-presenting cells (**APCs**) [181].

8.2 INNATE IMMUNITY

8.2.1 Monocytes/macrophages

Monocytes/macrophages (Mo/MF) are divided into three major groups based on their functional properties: M1 macrophages, M2 macrophages and wound–healing macrophages [182], where M1 macrophages play an important role in both acute and chronic inflammation of the skin [183-185]. Psoriatic skin contains a large number of MF-secreting pro-inflammatory cytokines such as IL-6, IL-12 and IL-23 as described previously [186, 187]. Also, MF is the major source of TNF-α, which is involved in the activation of IL-17A driven inflammatory pathways and consequently triggering Ps/PsA like-inflammation in mice [185].

8.2.2 Neutrophils and mast cells

Neutrophils and mast cells play an important role in Ps and PsA pathogenesis. In mannan induced Ps/PsA model, granulocyte infiltration was observed in the psoriatic

skin and peritoneal cavity. Anti-Ly6G treatment had suppressing effect on both skin and joint lesions [185]. Neutrophils together with mast cells are normally found in the infiltrations of psoriatic plaques, where mast cells and neutrophils but not the T cells are the main source for IL-17 secretion in the human skin. IL-17 (+) mast cells and neutrophils are found at higher densities than IL-17 (+) T cells in psoriasis lesions [188]. But it is still debatable whether positive staining for IL-17 in these cell types is due to its secretion or uptake.

8.2.3 Dendritic cells

Dendritic cells (DC) are another sentinels of the immune system that bridge innate and adaptive immunity. They are normally found in both the layers of the skin: LCs (Langerhans cells) in the epidermis and, myeloid DC (mDC) and plasmacytoid DC (pDC) in the dermis [189]. In the dermis an increased number of CD11c+ mDC s were found that are secreting pro-inflammatory IL-12 and IL-23 cytokines. These mDC might be the immigrant cells derived from circulating DC precursors that are migrated and trapped in the skin in response to chemo-attraction [189] induced by AMPs and chemokines produced by keratinocytes [180]. pDC cell numbers are also significantly increased in Ps skin. They are mainly activated via TLR signalling. pDC produces large amount of IFN-α in response to self-DNA-LL37 complexes targeting TLR9, or self-RNA-LL37 complexes recognizing TLR7 and TLR8. Self-DNA/RNA fragments itself are released by the dying cells in the skin [190, 191].

8.2.4 γδ T cells

γδ T cells have important role in skin inflammation. They provide protection towards skin invading agents through production of IFNγ and IL-17. Mouse IL-17-producing γδ T cells were shown to be important in imiquimod (IMQ)-induced Ps and mannan-induced Ps/PsA models [185, 192]. In IMQ-model, opposing effects of IL-15 and IL-15Rα were shown in the psoriasiform skin inflammation, where IL-15 was responsible for the expansion of IL-17-producing γδ (and αβ) T cell populations and inhibited by keratinocyte-derived soluble IL-15 receptor antagonist [193]. Even more, CCR6 was required for epidermal trafficking of γδ-T cells in the IL-23-induced model of psoriasiform dermatitis [194]. Recent investigations showed that IL-23 from Langerhans cells was necessary for the development of IMQ-induced Ps-like dermatitis by induction of IL-17A-producing γδ T Cells [195].

8.3 ADAPTIVE IMMUNITY

8.3.1 T cells

T cells are considered to be the major mediators of Ps based on previous studies, which demonstrated lymphocyte inhibition and T cell–specific immunosuppressive (cyclosporine) treatments led to clinical improvement of the patients [196, 197]. Ps was shown to be Th1 driven pathology but later on another cytokine was discovered to be crucial for disease development. This cytokine has a p19 protein paired to IL-12 p40 subunit (p40 is a shared subunit between IL-12 and IL-23 cytokines) and forms a new cytokine called IL-23. In the psoriatic skin, levels of IL-23 p19 and p40 subunits but not IL-12 p35 subunit are increased [198]. Depending on the cytokine milieu, T cells can be profiled differently. IL-23 was shown to activate T cells that expressed different cytokine profile such as IL-17A and IL-17F termed as Th17 cells and IL-22 expressing T cell subsets called Th22. Both Th17 and Th22 cells are influenced by the cytokine IL-23, which is required for their expansion and maintenance, which were shown to be involved in Ps pathogenesis [199, 200]. Thus IL-23-mediated Ps-like inflammation in the skin is IL-17A dependent [201], where IL-17A and other Th17 effector cytokines can lead to keratinocyte activation and further production of inflammatory mediators, all of which help in maintaining the psoriatic lesions. There are different models of Ps available to understand disease immune mechanisms. IL-22 overexpressing transgenic mouse has aberrant skin phenotypes mimicking psoriasis [202]. It was shown that in the absence of IL-22, IL-23-mediated dermal inflammation is reduced [203]. Recently it was shown in the IMQ-induced psoriasiform skin inflammation model, skin pathologies were almost absent after daily applications of imiquimod in the IL-22-deficient mice and also in mice treated with blocking anti-IL-22 Abs [204]. A novel type of Th cells, designated as Th9, was identified recently but little information is available about these cells in humans. Recently, Schlapbach et al., showed that most of the memory Th9 cells are skin-tropic or skin-resident. IL-9-producing T cells were increased in the skin lesions of psoriasis, suggesting that these cells may contribute to human inflammatory skin disease. They also demonstrated that IL-9 was necessary for efficient production of IFN-γ, IL-9, IL-13 and IL-17 by skin-tropic T cells. Authors suggest that human Th9 cells may have protective function in the skin, but anomalous activation of these cells may contribute to skin inflammatory diseases [205].

9 ANIMAL MODELS OF PS AND PSA

9.1 SPONTANEOUS MODELS

Various attempts have been made by researchers to establish animal models of cutaneous and articular inflammation resembling Ps and PsA to mimic human diseases. There are a number of spontaneous models available such as Flaky skin mice [206], which have a spontaneous mutation inducing increased squamous proliferation but without T-cell infiltration and keratinocyte hyperproliferation [207]. Similarly, spontaneous mutation in CPD (Chronic Proliferative Dermatitis) (*Sharpin*^{cpdm}/*Sharpin*^{cpdm}) mice induces skin lesions at the age of 5 to 6 weeks [208]. These lesions are characterized by epidermal hyperplasia, hyper- and parakeratosis and necrotic keratinocytes. Dermis and epidermis were infiltrated by granulocytes and macrophages [208] and skin inflammation was mainly dependent on Th2 cytokines (IL-4, IL-5, IL-13) but responded to IL-12 treatment [209]. Another spontaneous model is aging male DBA/1 mice, which develops spontaneous arthritis with some shared features of PsA in humans such as dactylitis, ankylosing enthesitis and onychoperisotitis [210] (Figure 4).

9.2 GENETICALLY ENGINEERED MODELS

A transgenic mouse model, designated as K5.Stat3C is a genetically manipulated strain, where signal transducer and activator of transcription (STAT) 3 was constitutively activated in basal keratinocytes under the control of the keratin 5 promoter (K5.Stat3C) [211]. Ps-like skin inflammation in this model was dependent on keratinocytes and T cells [211]. CD18 β2 Integrin hypomorphic mice have a decreased expression of common β2 –chain of lymphocyte integrin adhesion molecules. On PL/J strain background PL.129S7-*Itgb2*$^{(tm1Bay)}$ mice develop Ps-like skin inflammation with infiltrating lymphocytes having non-psoriasiform epidermal hyperplasia and also lacking hyper-proliferative keratinocytes [212, 213]. JunB/c-Jun epidermal inducible double knockout mouse represents another model, where targeting of epidermal keratinocytes led to Ps-like phenotypes and arthritic lesions. In contrast to the skin phenotype, development of arthritis required adaptive immune cells and signalling through tumour necrosis factor receptor 1 (TNFR1). Thus, epidermal alterations were sufficient to initiate both cutaneous and articular diseases [214]. Another PsA model by

Bardos et al described and characterized PsA-like disease in “humanized” (HLA transgenic) mice lacking their own major histocompatibility complex (MHC). Animals of 4 transgenic lines (HLA–DR2.Ab^0, DR4.Ab^0, DQ6.Ab^0, and DQ8.Ab^0) developed severe PsA-symptoms such as hyperkeratosis and parakeratosis, nail deformities and bone desorption associated with significantly fewer CD4+ cells in the peripheral blood and, reduced NK cell activity compared to disease resistant HLA–DR3.Ab^0 transgenic mice [215]. Mice overexpressing VEGF epidermally via K14 promoter develops Ps-like inflammation including vascular, epidermal and inflammatory features with an increased infiltration of mast cells in the upper dermis, and increased leukocyte rolling and adhesion in the post-capillary skin venules [216]. Epidermal specific deletion of the IKK2, which is a catalytic subunit of the IκB kinase complex (necessary for NF-κB activation through pro-inflammatory signals) causes Ps-like cutaneous inflammation similar to human Ps, including dependency on TNF signalling but independent of alpha beta T-cell-mediated inflammatory responses [217]. Keratin 14 promoter AR gene (K14-ARGE) has been shown to induce an early-onset and severe skin pathology characterized by hyperkeratosis with focal parakeratosis, acanthosis, lymphocyte and neutrophilic infiltration and vasodilation, which suggests that aberrant epidermal expression of AR might play a critical role in the development of psoriatic lesions [218]. In the follow up study by Cook et al., involucrin enhancer/promoter-dependent expression of human AR (INV-AR) in the supra-basal epidermis of transgenic mice also depicted the Ps-like phenotype. Histopathologically, INV-AR mouse also showed epidermal hyperkeratosis, parakeratosis, acanthosis, an exaggerated dermal vasculature and, infiltrated neutrophils and CD3(+) T lymphocytes in the lesions [219]. Interestingly, young K14-ARGE transgenic mice displayed synovitis with lymphocyte infiltration, increased vascularization and enhanced deposition of fibrous matrix in the knee synovium, while INV-AR transgenic mice knee joint did not have any of these abnormalities. Thus, epidermal AR expression is a possible mediator of cutaneous inflammation and also a potential trigger of both cutaneous psoriasis and psoriatic arthritis (Figure 4).

9.3 INDUCED MODELS

Imiquimod (IMQ), a topical application targeting TLR7/8 can induce and exacerbate IL-23/IL-17 axis dependent psoriasis in mice. IMQ-induced inflamed scaly skin lesions resemble plaque type psoriasis showing increased epidermal proliferation, abnormal

differentiation, CD4+ T cells, CD11c+ dendritic cells, plasmacytoid dendritic cells and neutrophils infiltration along with neo-angiogenesis. Also, epidermal expression of IL-23, IL-17A and IL-17F, and an increase in splenic Th17 cells were noted [220]. Recently, we have developed a new disease model, in which a single intraperitoneal injection of *Saccharomyces cerevisiae* mannan induced psoriasis and psoriasis arthritis-like symptoms in mice (Paper III & IV). Reactive oxygen species determined the nature of the developed disease. Erythema and edema of joints start one-day post-injection, reaching maximum severity on days 4-5, while skin scaling appears from day 3. This model depicts inflammatory phase of psoriasis arthritis, where macrophages and γδ-T cells, and IL-17A are the major contributors. Treatment with clodronate liposomes, anti-Ly6G and neutralization of IL-17A and TNF-α either completely blocked or significantly attenuated both joint and skin inflammation [185].

9.4 HUMAN SKIN TRANSPLANT MODELS

In human skin transplant models, so-called xenotransplantation models, skin biopsy from Ps patient or *in vitro* cultured skin is transplanted into the mice. As a recipient strain for human skin transplants, severe combined immuno-deficient (SCID) and AGR129 mice are used [221, 222]. In these mice, grafted tissues were well tolerated and psoriatic characteristics were maintained for several months in the transplants [222]. Boyman et al. demonstrated that human uninvolved psoriatic skin grafted onto AGR129 mice spontaneously developed psoriatic plaques without injection of activated immune cells or any other exogenous factors [221]. Grafted skin histopathology was comparable to Ps lesions from the same patient [222] (Figure 4).

9.5 IN VITRO MODELS

An alternative approach to study Ps pathogenesis is by using *in vitro* models. However, contrary to *in vivo* models they have limitations. In the *in vitro* system epidermal keratinocytes obtained either from psoriatic or healthy individuals are grown at an air-liquid interface where they differentiate and mimic the morphology of the stratified squamous epidermis. Keratinocytes are then treated with different factors (growth factors/cytokines) to develop psoriatic epidermis characteristics. This system can be used for studying keratinocyte behaviour towards different stimulation agents [223] (Figure 4).

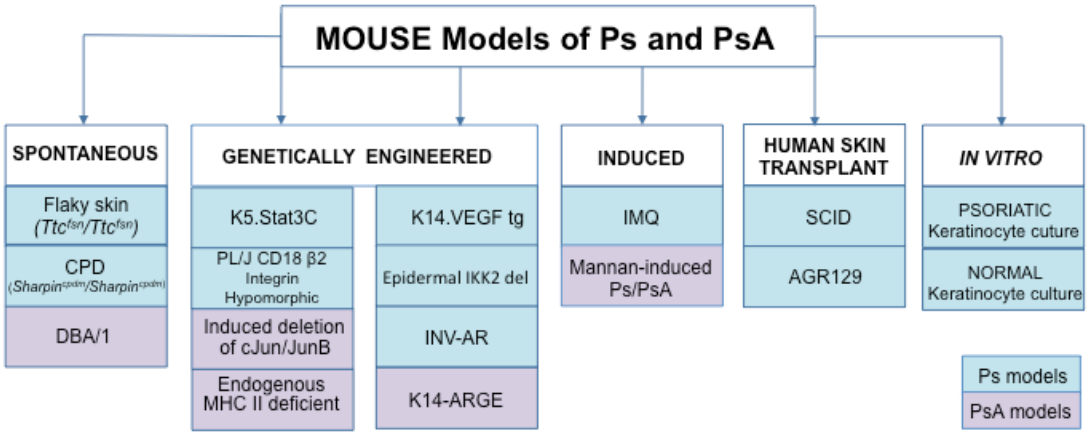

Figure 4. *Mouse models of Ps and PsA grouped as spontaneous, genetically engineered, induced, human skin transplant and in vitro models.*

10 ROS IN PSORIASIS

10.1 OVERVIEW

Reactive Oxygen Species and reactive nitrogen species (ROS/RNS) describe oxygen derived reactive molecules, free radicals and nitrogen-containing oxidants with one or more uncoupled electrons. ROS generation as a by product occurs with mitochondria, peroxisomes, cytochrome *P*-450 and other cellular elements, however, the phagocyte nicotinamide adenine dinucleotide phosphate (NADPH oxidase) was the first identified example of a system that generates ROS not as a by product, but rather as the primary function of the enzyme system. The phagocyte NOX family of NADPH oxidases consist of NOX complexes (NOX1-5) and dual oxidase complexes (DUOX1-2) [224]. These enzymes are participating in reactive oxygen species (ROS) generation by transporting electrons across the plasma membrane. It was originally thought that phagocytic cells were responsible only for ROS production as part of the defence mechanism against invading pathogens but later it has also been demonstrated that ROS have a role in cell signalling including apoptosis, gene expression and in the activation of cell signalling cascades [225]. Overproduction or inadequate removal of ROS can result in oxidative stress leading to cell and tissue pathological changes due to damages in lipids, proteins and DNA [226]. In 1957, Berendes et al. [227] described a rare syndrome, triggered by pyogenic infections, now referred as chronic granulomatous disease (CGD) [228] caused by the absence of respiratory burst in the phagocytes of CGD patients. Patients with this genetic disorder suffer from life-threatening infections [229]. It was found that in CGD patients, all the subunits in NOX complexes have mutations [230] [231]. The accepted view from early studies is that an insufficient anti-oxidant system together with increased levels of ROS leads to pathological changes in cells and tissues, as in inflammatory skin conditions. Oxidative stress is believed to be a key factor in the pathogenesis of psoriasis [226]. It is well evidenced that polymorphonuclear (PMN) infiltrates are present in the dermis of psoriatic lesions [232], where many factors could play a role in chemo-attraction of PMN into psoriatic lesions. According to one theory, ROS discharged by keratinocytes, fibroblasts and endothelial cells have chemotactic effects on neutrophils [233] and accumulation of neutrophils in psoriatic lesions may cause abundant superoxide production during phagocytic processes [234].

10.2 THE PHAGOCYTE NADPH COMPLEX

In a resting condition, the catalytic core of the phagocyte NADPH complex is composed of an **enzymatic part** - flavocytochrome b558, which consists of membrane integrated glycoprotein, gp91phox (NOX2) and p22phox protein. They jointly make up the central component of NADPH oxidase complex (Figure 1A). The phagocyte NADPH complex also contain four cytosolic components denoted as *Ncf1* (p47phox), *Ncf2* (p67phox), *Ncf4* (p40phox) and the small G-protein Rac1 or Rac2 [235], which play a regulatory role (Figure 5A). In the resting state, this cytosolic Phox-protein complex is inactive due to *Ncf1* auto-inhibited conformation. Upon activation (exposure to microbes or inflammatory mediators), there is an exchange of GDP for GTP on Rac leading to its activation. Additionally, the p47phox becomes heavily phosphorylated releasing the auto-inhibitory confirmation, which enables the whole Phox-protein translocation and binding to flavocytochrome b558 [236]. The active enzyme complex transports electrons from cytoplasmic NADPH to extracellular or phagosomal oxygen to generate the ROS superoxide (O2−) molecules (Figure 5B).

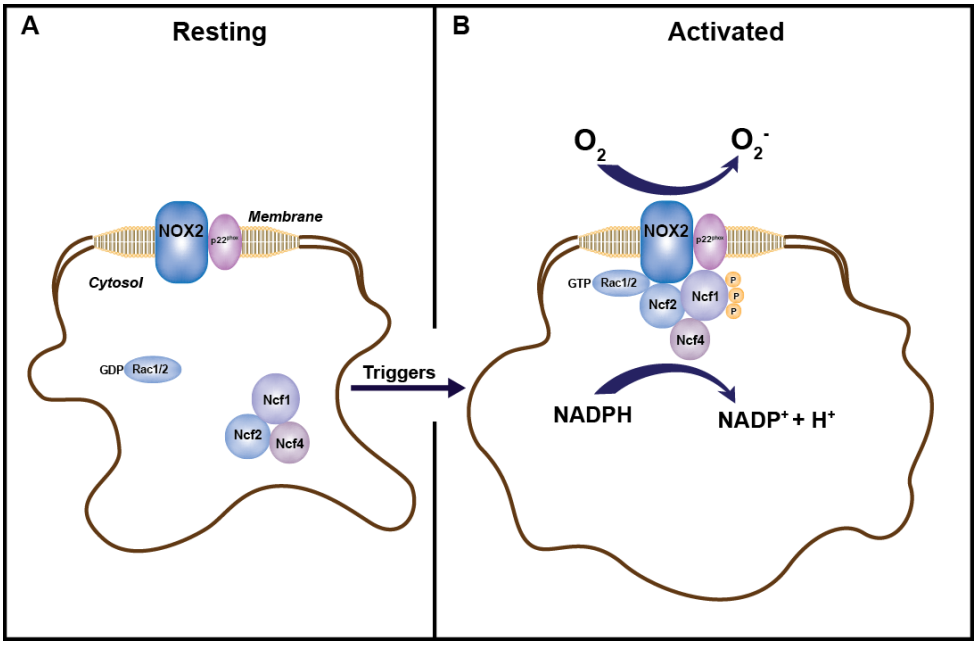

Figure 5. *NADPH complex consists of membrane flavocytochrome b558 (NOX2 and p22phox protein), cytosolic components Ncf1, Ncf2, Ncf4 and the small G-protein Rac1 or Rac2 (A). Upon activation, there will be GDP exchange to GTP on Rac leading to its activation. Phosphorylated Ncf1 releases the auto-inhibitory confirmation and enables the whole complex translocation to flavocytochrome b558. The active enzyme complex transports electrons from cytoplasmic NADPH to extracellular or phagosomal oxygen to generate ROS.*

10.3 ROS/RNS GENERATION

ROS and RNS are derived from the Nox/Duox enzymes. Superoxide (**O2^{-}**) is generated by the Nox enzymes and can be converted to hydrogen peroxide (**H_2O_2**), either spontaneously or by the action of superoxide dismutase (SOD), or alternatively can react with nitric oxide (**NO**) to produce peroxynitrite (**ONOO^{-}**). H_2O_2 generated by the Duox enzymes or by dismutation of O^{2-} can be scavenged by the antioxidants catalase (CAT) or glutathione peroxidase (GPx) and form water (**H_2O**) and oxygen (O_2); be partially reduced to generate hydroxyl radical (**OH**) by the metal (Fe^{3+}) catalyzed Haber-Weiss and Fenton reactions or react with chloride in a reaction catalyzed by myeloperoxidase (MPO) resulting in the formation of hypochlorous acid (**HOCl**) [237](Figure 6).

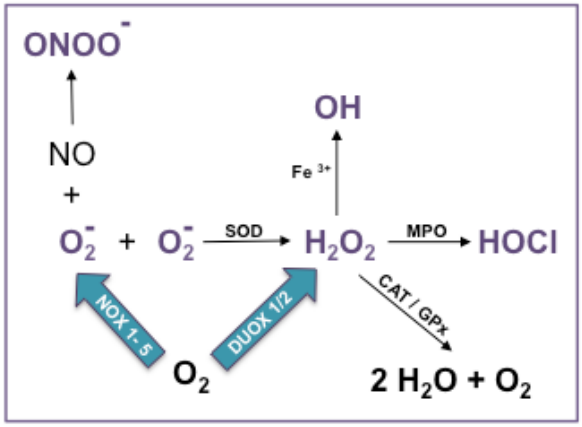

Figure 6. *Schematic representation of ROS/RNS generation in macrophages, where superoxide (**O2^{-}**), nitric oxide (**NO**), peroxynitrite (**ONOO^{-}**), water (**H_2O**), hydroxyl radical (**OH**) and (**HOCl**) are formed by the action of NOX1-5, DUO1/2 enzymes, antioxidants as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), myeloperoxidase (MPO) and the metal (Fe^{3+}).*

10.4 ROS PROTECTION

There are a number of clinical and experimental observations that implicate immuno-regulatory role of ROS in inflammatory diseases in contrast to traditional view of damaging effects of ROS. As described previously, CGD patients are more prone to development of different autoimmune diseases. Hyung-Ran Kim et al demonstrated that elevated levels of ROS due to defects in GPx1 and catalase (Cat) in $GPx1^{-/-}$ × $Cat^{-/-}$ mice were resistant to Dextran Sodium Sulfate (DSS)-induced colitis. Additionally, administration of n-acetyl cysteine (NAC) reduced Treg functions and

made GPx1$^{-/-}$ × Cat$^{-/-}$ mice susceptible to DSS-induced colitis [238]. Similarly, GPx1 deficiency in mice attenuated allergen-induced airway inflammation by suppressing Th2 and Th17 cell development [239]. In another independent study by Won et al it was shown that ablation of Prx II (non-enzymatic cellular anti-oxidant, peroxiredoxin) in mice attenuates colitis by increasing Treg function [240]. Most recently, Tiago Rodrigues-Sousa et al suggested lowered ROS association to severe chronic DSS-Induced Colitis in Ncf1/p47^{phox}-mutant mice, mediated by local accumulation of peroxynitrites, pro-inflammatory cytokines and lymphocytes, and systemic immune deregulation similar to CGD [241]. Based on all these recent studies, ROS level is suspected to be associated with T cell /Treg responsiveness. Previously, reduced ROS levels were shown to be associated with autoimmune arthritis and encephalomyelitis using autoimmune disease prone *Ncf1* gene mutated mouse strain, in which *Ncf1* gene encoding the p47^{phox} subunit of the NOX2 complex was dysfunctional leading to significantly lowered ROS production [102]. *Ncf1* was identified as a regulator of autoimmune arthritis in rodents and [242] as later studies showed decreased ROS levels were associated with an increased number of cell surface thiol groups on T cells, which enhanced their arthritogenicity significantly [243]. Even more, macrophage-specific ROS production was suppressing T cell responses and thereby arthritis development [244]. Hypo-functional Tregs in reduced ROS conditions have been suggested before [245] and recently Hyung-Ran Kim et al demonstrated Treg hyper-functionality in elevated levels of ROS in IMQ-induced PD (Psoriasis dermatitis) model. Treg hyper-functionality to high levels of ROS is suggested to operate as a compensatory mechanism to overcome ROS damaging effect, as Tregs are able to suppress the immune responses, including functions of Th1, Th2, Th17, B, NK cells and DCs and thereby decrease psoriasis. Thus, restoration of impaired Treg functions could be a possible therapeutic strategy for psoriasis [246].

Similarly, successful treatment of psoriasis vulgaris patients by HBOT (hyperbaric oxygen therapy) was demonstrated previously [247]. Furthermore, HBOT attenuated IMQ-induced PD, while NAC (N-acetyl cysteine) aggravated it [246]. It is acknowledged that HBOT increases cellular level of ROS [248]. Recent studies also demonstrated the effect of photo (chemo) therapy in increasing Treg functions and reducing circulating Th17 cells [249]. Since it is known that phototherapy generates ROS levels [250] it allows for the consideration that elevated ROS might be the driving mechanism for Treg hyper-functionality.

11 *NCF1* AS A REGULATOR OF MANNAN-INDUCED PS/PSA IN MICE

Recently, we have developed a new mouse model for psoriasis (Ps) and psoriasis arthritis (PsA)-like disease in mice, where a single injection of mannan i.p. induced an acute disease. We have characterized this model in ROS sufficient and deficient conditions using B10Q (*Ncf1*$^{wt/wt}$) and B10Q.*Ncf1*$^{mlj/mlj}$ mice respectively. We found that disease severity was independent of adaptive immunity and certain acute inflammation-driving cell types [185]. Interestingly, macrophage-produced TNF-α was a trigger factor for γδ T-cell activation and consequent IL-17A production. We have observed that monocyte/macrophage-derived ROS had immuno-regulatory effect in *Ncf1*$^{m1j/m1j.}$MN+ mice, which expresses functional *Ncf1* in macrophages only [185]. Similarly, β1,3-glucan of *Alcaligenes faecalis*-induced ROS from monocytes was also shown to suppress innate inflammation [251]. Moreover, ROS protective function was shown in other human skin diseases, like scleroderma [252]. Importance of macrophages and neutrophils in mannan initiated disease pathogenesis was shown with depletion experiments using clodronate liposomes (CLs) or anti-Ly6G antibodies [185]. Additionally, we measured mannan stimulated ROS/RNS at cell (blood granulocytes) and organ (hind paws) level in B10Q, B10Q.*Ncf1*$^{m1j//m1j}$.MN+ and B10Q.*Ncf1*$^{m1j//m1j}$ mice, where produced ROS correlated with relatively milder peripheral joint arthritis phenotypes, while ROS deficiency promoted a more aggressive disease. Thus, in mannan-induced disease model, increased ROS levels in *Ncf1*$^{wt/wt}$ mice was correlated with partial or complete protection of Ps phenotypes, while ROS deficiency in *Ncf1*$^{mlj/mlj}$ mice was associated with severe Ps disease.

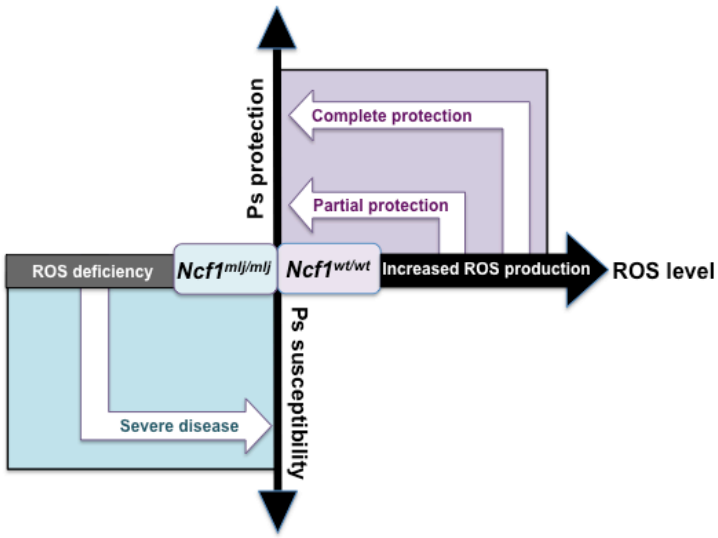

Figure 7. *Ps susceptibility is dependent on ROS levels in mannan-induced disease model.*

Hence, our data show that ROS production by a functional NOX2 is critical for attenuating Ps and accompanying joint phenotypes, but the mechanism whereby ROS protection operates needs to be investigated further in detail. It is most likely to be different from autoimmune arthritis, where αβ T cells play a critical role under the regulatory control of macrophage-produced ROS [244] and also different from IMQ-PD model where ROS mediated prevention of Ps is operating through enhanced IDO (indoleamine 2,3-dioxygenase) expression and Treg functions [246].

Modified version of the above section on Ps (chapter II) is submitted to the ***journal of International Archives of Allergy and Immunology*** *as a review article.*

12 PRESENT INVESTIGATIONS

12.1 STUDY I

Pathogenic autoreactive B cells are not negatively selected toward matrix protein collagen II

We have addressed the importance of B cell tolerance to collagen type II, which is a target in rheumatoid arthritis (RA) and its mouse models. We generated a germline-encoded anti-collagen type II (CII) IgH replacement anti-C1 B cell mouse strain (ACB) to investigate how B cell tolerance to CII is subverted and to further understand pathogenesis of RA. Phenotypic analysis revealed that CII-specific B cells were surprisingly neither deleted nor anergized. Instead, they were readily detected in all lymphoid organs. Spontaneously produced autoantibodies could bind directly to cartilage surface without detectable pathology. However, exaggerated arthritis was seen after injection of anti-CII Abs specific for other epitopes. In addition, Abs from CII-specific hybridomas generated from ACB mice induced arthritis. Interestingly, IgH/L chain sequence data in B cell hybridomas revealed a lack of somatic mutations in auto-reactive B cells. The ACB model provides the first possibility, to our knowledge, to study B cell tolerance to a matrix protein, and the observations made in the study could not be predicted from previous models (Paper I). B cell-reactive epitopes on CII are largely shared between human RA and rodent CII-induced arthritis; this study, therefore, has important implications for further understanding of pathological processes in autoimmune diseases like RA.

12.2 STUDY II

Reactive oxygen species control auto-reactive B cell tolerance in experimental arthritis

Mechanisms underlying the breakdown of B cell tolerance are one of the most important issues in understanding autoimmune diseases, such as rheumatoid arthritis (RA). Using germ line-encoded IgH knock-in mice (B10Q.ACB) having auto-reactive B cells specific to the major C1-epitope of type II collagen (CII), we have shown that CII-reactive B cells are not negatively selected but rather positively selected. We have now observed that these mice are protected from collagen-induced arthritis (CIA). Introducing a mutation in *Ncf1* leading to deficiency of reactive oxygen species (ROS) breaks this strong resistance to CIA. Arthritis in the ROS deficient mice is associated with enhanced germinal centre (GC) formation, increased T cell responses and diverse antibody repertoire against CII. Notably this break of B cells tolerance is independent of FcγRIIb and CD1d influence on autoreactive B cell arthritogenicity. ROS produced by macrophages mediate disease suppressive effect and maintain B cell tolerance. Thus, we have demonstrated a unique mechanism of maintaining tolerance to self and its impact on autoimmune arthritis, which may open up new avenues to better understand RA pathogenesis.

12.3 STUDY III

Mannan induces ROS regulated, IL-17A dependent psoriasis arthritis-like disease in mice

Psoriasis (Ps) and psoriasis arthritis (PsA) are poorly understood common diseases, induced by unknown environmental factors, affecting skin and articular joints. A single i.p. exposure to mannan from *Saccharomyces cerevisiae* induced an acute inflammation in inbred mouse strains resembling human Ps and PsA-like disease, whereas multiple injections induced a relapsing disease. Exacerbation of disease severity was observed in mice deficient for generation of reactive oxygen species (ROS). Interestingly, restoration of ROS production, specifically in macrophages, ameliorated both skin and joint disease. Neutralization of IL-17A, mainly produced by γδT cells, completely blocked disease symptoms. Furthermore, mice depleted of granulocytes were resistant to disease development. In contrast, certain acute inflammatory mediators (C5, Fc receptor III, mast cells, and histamine) and adaptive immune players (αβT and B cells) were redundant in disease induction. Hence, we propose that mannan-induced activation of macrophages leads to TNF-α secretion and stimulation of local γδT cells secreting IL-17A. The combined action of activated macrophages and IL-17A produced *in situ* drives neutrophil infiltration in the epidermis and dermis of the skin, leading to disease manifestations. Thus, our finding suggests a new mechanism triggered by exposure to exogenous microbial components, such as mannan that can induce and exacerbate Ps and PsA.

12.4 STUDY IV

Mannan induced psoriasis and psoriasis arthritis-like disease in mice.

A single intraperitoneal injection of mannan from the bakers yeast, *Saccharomyces cerevisiae* induced psoriasis and psoriasis arthritis-like symptoms in mice. Current protocol describes the induction and evaluation procedures of these clinical phenotypes in detail. Characteristically, arthritis starts with erythema and edema of ankle and wrist joints within 2 days after mannan injection reaching maximum severity on days 4 to 5, while psoriasis skin scaling appears from day 3 onwards, which becomes more scaly over time and eventually peels off entirely. This model depicts an initial phase of inflammatory PsA, where macrophages and γδ-T cells and the secreted IL-17A are the major players in the pathogenic process. Short duration and resolution of inflammation will help for screening various drug candidates targeting the disease process in psoriasis patients.

13 CONCLUDING REMARKS

ACB mouse model of arthritis, provides the first possibility, to study B cell tolerance to a matrix protein and has important implications for further understanding of pathological processes in autoimmune diseases like RA, while B cell-reactive epitopes on CII are largely shared between human RA and rodent CII-induced arthritis. We have demonstrated a unique mechanism of maintaining tolerance to self and its impact on autoimmune arthritis, which helps to understand pathogenic mechanisms in human RA. Moreover, we showed the impact of ROS in auto-reactive B cell selection towards collagen.

Also, we have established and characterized a new model of psoriasis and psoriasis arthritis and suggested a new mechanism triggered by exposure to exogenous microbial components, such as mannan. We have demonstrated that the reduced production of reactive oxygen species helps in more severe disease symptom(s) stimulation, where macrophages and γδ-T cells, and the secreted IL-17A are the major players in the pathogenic process. This is an unique model of psoriasis, which is accompanied by the joint inflammatory disease, where short duration and resolution of inflammation will help in facilitating the screening of various drug candidates targeting the disease process in psoriasis and psoriasis arthritis patients.

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