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UNVEILING THE PROTECTIVE MECHANISMS OF NOX2-DERIVED ROS AGAINST AUTOIMMUNE DISEASES

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THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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Broad sea bears springing fins, and
vast sky breeds soaring feathers.

海阔凭鱼跃 天高任鸟飞

-- 北宋 阮阅

POPULAR SCIENCE SUMMARY OF THE THESIS

The primary function of the immune system is to distinguish self and non-self, allowing it to protect the body from harmful bacteria, viruses, and other foreign pathogens while avoiding damage to healthy own tissues. This process of discrimination is known as tolerance. When tolerance is disrupted, the immune system can attack the body's own tissues, leading to autoimmune diseases. There are many different autoimmune diseases, each with its own set of symptoms and target organs. The causes of them are not fully understood, but scientists believe that a combination of certain genes and environmental triggers lead to their development.

Research has shown that certain genes are associated with an increased risk of developing numerous autoimmune diseases. One of these genes is *NCF1*, which stands for neutrophil cytosolic factor 1. This gene codes for a component of a protein complex that plays an important role in defending against pathogens by producing reactive oxygen species (ROS), which are toxic to harmful organisms. When the *NCF1* gene is defective, it can lead to a malfunctioning of the ROS-producing complex, and this has been linked to an increased risk of developing lupus and rheumatoid arthritis.

Lupus, a Latin word for wolf, was used to name the disease because of the characteristic facial rash that can leave an imprint resembling a wolf bit. It is a chronic and complex autoimmune disease that can affect multiple parts of the body, including the skin, joints, kidneys, lungs, brain, and blood vessels. Rheumatoid arthritis, on the other hand, is characterized by inflammation of joints, typically affecting the small joints of the hands and feet, but capable of targeting almost any joint in the body. Lupus affects about 0.1% of the global population, while rheumatoid arthritis affect about 1%. Both diseases can significantly reduce quality of life and even be life-threatening, and unfortunately, there is currently no cure. Therefore, it is crucial to continue studying the causes of these diseases to improve therapeutic treatments.

As we mentioned earlier, defects in the *NCF1* gene have been associated with the two typical autoimmune diseases. In our research, we studied the underlying mechanisms using animal models. In Study I, we found that NCF1 defects worsened lupus by causing the excessive production and accumulation of a type of immune cells called plasmacytoid dendritic cells (pDCs), which are involved in inflammation. The NCF1 defects also promoted the proinflammatory functions of these cells. However, when we restored the function of NCF1 specifically in pDCs, we were able to improve the symptoms of lupus. In Study II, we discovered that NCF1 defects had an impact on T cells, another type of immune cells that should be trained to target foreign pathogens rather than the body's own cells. Due to the NCF1 defects, the training process was altered, leading to a break in tolerance and the appearance of T cells that target one's own cells. This resulted in arthritis in mice. Finally, in Study III, we investigated the role of NCF4, a protein closely related to NCF1. NCF4 led to a low level of ROS inside the cells when carrying genetic defects. We found that malfunctioning NCF4 promoted the formation of antibody-secreting cells that produce antibodies to self. It also promoted migration of these cells to the joints, leading to joint inflammation and eventually severe arthritis in mice.

In general, our findings provide insight into the protective role of well-functioning NCF1 and NCF4, the two components critical for normal production of ROS, in lupus and arthritis. This knowledge can help develop more targeted and less toxic therapeutic interventions for patients with pertinent genetic abnormalities.

ABSTRACT

Autoimmune diseases, including systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), pose a significant burden on society, affecting a considerable portion of the global population. Both SLE and RA are known to have a strong genetic component, with shared genetic predispositions such as neutrophil cytosolic factor 1 (*NCF1*), which encodes for a subunit of NADPH oxidase 2 (NOX2) complex. Genetic variants of *NCF1* that result in low production of NOX2-derived reactive oxygen species (ROS) have been identified as a significant risk factor for patients with SLE or RA, as well as animal models of these diseases. This finding has challenged the traditional paradigm that ROS are proinflammatory and cause tissue destruction in autoimmune diseases. In line with this, deficiency in another NOX2 subunit, NCF4, has also been linked to RA. However, the regulatory role of NCF1 and NCF4 in SLE and RA remains unclear. Here, we investigated the protective mechanisms of these subunits using mice carrying *Ncf1* defects or *Ncf4* mutation in lupus and arthritis models.

In the first study, we found that mouse $Ncf1^{m1j}$ mutation and human $NCF1^{R90H}$ polymorphism exacerbated disease in lupus models by enhancing the development, accumulation, and function of plasmacytoid dendritic cells (pDCs) as the driver of the type I interferon system. Restoration of NCF1-dependent ROS specifically in pDCs protected against lupus. These findings explained the causative effect of dysfunctional NCF1 in lupus and highlighted the protective role of pDC-derived ROS, providing insights for potential therapeutic strategies in patients with relevant genetic defects.

In the second study, we revealed that the $Ncf1^{m1j}$ mutation contributed to the development of collagen-induced arthritis (CIA) driven by autoreactive T cells. This was due to a decrease in the expression of autoimmune regulator in medullary thymic epithelial cells and B cells, resulting in the release of potentially autoreactive T cells. These results suggested a regulatory role of NCF1 in T cell tolerance and protection against autoimmunity.

In the third study, we discovered that the $Ncf4^{R58A}$ mutation, which results in decreased production of NOX2-derived intracellular ROS, exacerbated CIA by promoting the formation and migration of plasma cells. This led to elevated levels of pathogenic autoantibodies and joint inflammation, ultimately resulting in severe disease. Our findings contribute to a better understanding of the crucial involvement of intrinsically produced NOX2-derived ROS in controlling the autoimmune responses.

Overall, our findings shed light on the mechanisms by which NOX2-derived ROS play a regulatory role in autoimmune diseases.

SCIENTIFIC PAPERS INCLUDED IN THIS THESIS

I. **Luo, H.**, Urbonaviciute, V., Saei, A. A., Lyu, H., Gaetani, M., Végvári, Á., Li, Y., Zubarev, R. A., & Holmdahl, R. (2023). NCF1-dependent production of ROS protects against lupus by regulating plasmacytoid dendritic cell development and functions. *JCI insight*, *8*(7), e164875.

II. Li, Q., Zhong, J., **Luo, H.**, Urbonaviciute, V., Xu, Z., He, C., & Holmdahl, R. (2022). Two major genes associated with autoimmune arthritis, Ncf1 and Fcgr2b, additively protect mice by strengthening T cell tolerance. *Cellular and molecular life sciences : CMLS*, *79*(9), 482.

III. He, C., **Luo, H.**, Coelho, A., Liu, M., Li, Q., Xu, J., Krämer, A., Malin, S., Yuan, Z., & Holmdahl, R. (2022). NCF4 dependent intracellular reactive oxygen species regulate plasma cell formation. *Redox biology*, *56*, 102422.

CONTRIBUTIONS TO OTHER MANUSCRIPTS

I. Vilma Urbonaviciute[†], Laura Romero-Castillo[†], Bingze Xu[†], **Huqiao Luo**, Nadine Schneider, Sylvia Weisse, Nhu-Nguyen Do, Ana Oliveira-Coelho, Gonzalo Fernandez Lahore, Taotao Li, Pierre Sabatier, Christian M Beusch, Johan Viljanen, Roman A Zubarev, Jan Kihlberg, Johan Bäcklund, Harald Burkhardt and Rikard Holmdahl[*]. Novel therapy targeting antigen-specific T cells by a peptide-based tolerizing vaccine against autoimmune arthritis. Under review in *Proceedings of the National Academy of Sciences*. [†]equal contribution.

II. Zhong, J., Li, Q., **Luo, H.**, & Holmdahl, R. (2021). Neutrophil-derived reactive oxygen species promote tumor colonization. *Communications biology*, *4*(1), 865.

III. Amir Ata Saei, Albin Lundin[†], Hezheng Lyu[†], Hassan Gharibi, **Huqiao Luo**, Jaakko Teppo, Xuepei Zhang, Massimiliano Gaetani, Ákos Végvári, Rikard Holmdahl, Steven P. Gygi and Roman A Zubarev[*]. Multifaceted proteome analysis at solubility, redox, and expression dimensions for target identification. Manuscript. [†]equal contribution.

IV. Taotao Li, Changrong Ge, **Huqiao Luo**, Alexander Krämer, Ana Do Carmo Oliveira Coelho, … and Rikard Holmdahl[*]. Citrullinated Type II collagen immunization breaks the physiological T cell tolerance to the immunodominant T cell epitope in P.266E mice. Manuscript.

CONTENTS

LIST OF ABBREVIATIONS

ΔGT	GT deletion
ACPA	Antibodies to citrullinated protein
AIR	Auto-inhibitory region
ANA	Antinuclear antibody
APC	Antigen-presenting cell
ASC	Antibody-secreting cell
BCR	B-cell receptor
CGD	Chronic granulomatous disease
CIA	Collagen-induced arthritis
COL2	Collagen type II
DAMP	Damage-associated molecular pattern
DC	Dendritic cell
FcγR	Fc gamma receptor
GPI	Glucose-6-phosphate isomerase
IC	Immune complex
IFN	Interferon
ISG	Interferon-stimulated gene
LCR	Low-copy repeat
MTEC	Medullary thymic epithelial cell
NET	Neutrophil extracellular trap
NOX	NADPH oxidase
OR	Odds ratio
PDC	Plasmacytoid dendritic cell
PIL	Pristane-induced lupus
PtdIns3P	Phosphatidylinositol 3-phosphate
PX	Phox homology
RA	Rheumatoid arthritis
ROS	Reactive oxygen species
SH3	Src homology 3
SLE	Systemic lupus erythematosus
TCR	T-cell receptor
TLR	Toll-like receptor
Yaa	Y-linked autoimmune accelerating locus

1 INTRODUCTION

1.1 AUTOIMMUNITY AND AUTOIMMUNE DISEASES

The immune system is a complex network of cells, tissues, and molecules that plays a crucial role in protecting the body from pathogenic microorganisms and eliminating abnormal or damaged cells. It consists of two main branches: the innate and adaptive immune system, each with its specific functions, cells, and mechanisms.

The innate immune system mounts a rapid and non-specific response against invading pathogens and damaged cells through a complex interplay of various components, including epithelia, phagocytes, dendritic cells (DCs), natural killer cells, cytokines, and complement proteins. The pathogen-associated molecular patterns and the damage-associated molecular patterns (DAMP) are recognized by a collection of germline-encoded pattern recognition receptors, including Toll-like receptors (TLRs), NOD-like receptors, cytosolic DNA sensors, and lectin receptors (1). Upon ligation of these receptors, the innate immune cells are activated and proceed to engulf and destroy pathogens, coordinate the overall immune response, and provide signals to the adaptive immune system.

The adaptive immune system is distinguished by its high specificity and long-lasting memory. The specificity arises from the unique receptors on the surface of T and B lymphocytes, which are formed through V(D)J recombination, a process where gene segments are randomly combined during the development of T and B cells in the thymus and bone marrow, respectively. This process generates a vast diversity of T-cell receptors (TCRs) and B-cell receptors (BCRs), ensuring that at least a few T and B cells will have receptors capable of binding to any given pathogen, increasing the chances of a successful defense response. T cells are activated by recognizing cognate antigens presented on the surface of antigen-presenting cells (APCs) in the context of a co-stimulatory signal. Activated T cells will proliferate and differentiate into effector cells and memory cells that can carry out specific functions and provide long-lasting protection. Similarly, the humoral immune response is initiated by binding of antigens to specific BCRs on B cells. With the help of T cells, B cells undergo isotype switching and affinity maturation, ultimately differentiating into long-lived plasma cells and memory B cells.

Since the mechanisms for generating TCRs and BCRs do not exclude those specific for self-antigens, the body relies on central and peripheral tolerance to control autoreactivity. Developing T and B cells are screened for self-reactivity in the primary lymphoid organs during negative selection. The cells with high affinity to self-antigens are clonally deleted, receptor edited, or develop into subsets with regulatory properties before they enter the circulation. Nevertheless, some self-reactive cells may escape the described process and reach the periphery. In this case, peripheral tolerance ensures that they are either deleted, suppressed, or become anergic. Such tolerance mechanisms, however, can fail under certain circumstances, leading to autoimmunity in which self-reactive T and B cells are present and autoantibodies are produced. Individuals within such condition may eventually develop autoimmune diseases.

Contemporary theories suggest that autoimmunity arises from a combination of genetic and environmental factors. Although monogenic autoimmune diseases have been identified with mutations in genes such as *AIRE* (2) and *FOXP3* (3), it is more common that multiple risk variants, each explaining only a small portion, additively contribute to genetic susceptibility. These include certain MHC haplotypes and variants of non-MHC genes such as *PTPN22* and *CTLA4* (4). Nevertheless, the concordance of autoimmune diseases in monozygotic twins is only 11-65% (5), suggesting involvement of environmental factors. Infections, exposure to

xenobiotics, and tissue injuries may provide sequestered, cryptic, or neo self-antigens, or activate the immune system through molecular mimicry (4). Recently, the associations between the microbiota, immunomodulation, and autoimmunity have also been proposed (6).

Till now, over 80 different autoimmune diseases are identified, affecting 7-9% global population and causing significant morbidity and mortality (7). Depending on the tissues involved, there are two main categories: organ-specific autoimmune diseases, with Type I diabetes and inflammatory bowel diseases as the typical examples, and systemic autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and Sjögren's syndrome. Although autoimmune diseases are characterized in common by loss of tolerance to self-antigens and tissue damage, they exhibit varied clinical, serological, and histopathological features, on which diagnostic and clinical classifications are based.

Although there is currently no cure for autoimmune diseases, recent advancements in understanding these diseases have led to successful management of symptoms and slowed disease progression through the use of immunosuppressants and biological agents. Further improvements in our knowledge are crucial for developing new predictive, preventive, and therapeutic measures.

1.2 SYSTEMIC LUPUS ERYTHEMATOSUS

SLE is a chronic, relapsing-remitting, systemic autoimmune disease with an overall global incidence of 1.5-11 per 100,000 person-years and prevalence of 0.13-77.13‰ (8). The annual age-standardized mortality rate in SLE patients is around 2.7 per million inhabitants, 2-3 times higher than that in the general population (9). Considerable variation in incidence, prevalence, and disease severity has been observed across different geographic areas, likely due to a combination of factors such as ethnicity, environmental exposures, and socioeconomic status (10). SLE disproportionately affects women of child-bearing age and is a leading cause of death in young females (11). Given the widespread organ damage and high morbidity and mortality, the disease causes vast economic burden on the society and considerably impairs the quality of life of patients (10). Better treatments are therefore urgently needed.

1.2.1 Clinical features and course of SLE

SLE is of vast clinical heterogeneity, ranging from mild fatigue to life-threatening organ failure. The initial presentation of SLE, whether it is a new case or a recurrent flare, is typically characterized by nonspecific symptoms such as low-grade fever, fatigue, weight loss, and arthralgia (12). The musculoskeletal system is frequently involved in SLE, with arthritis and arthralgias reported in almost 90% of patients (13), while cutaneous manifestations, although less common, are more specific to SLE. The most commonly targeted visceral organ in lupus is the kidney, where ICs and overactivated immune cells are accumulated. Up to 50% of patients may develop lupus nephritis, potentially resulting in renal failure and even death. Neurological involvement is reported in 25-75% of SLE patients (12), representing a large variety of complications such as cognitive disorders, stroke, headache, mood disorders, and neuropathies, with unclear cause. Cytopenia, including leukopenia, anaemia, thrombocytopenia, and hypocomplementemia, is common in SLE patients, predisposing them to frequent infections (14). Additionally, SLE can also cause pulmonary, gastrointestinal, cardiac, vascular, ocular, obstetric, and endocrine manifestations (12).

Like other autoimmune diseases, SLE has a preclinical, asymptomatic phase where various autoantibodies appear years before clinical manifestations occur (15). Proinflammatory cytokines such as type I and II interferons (IFNs), IL-6, and TNF may also emerge at this stage (16). Due to the wide range of clinical manifestations, diagnosis is frequently delayed,

and individuals may have experienced joint and skin symptoms for several years. Established SLE is characterized by periods of variable disease activity with unpredictable flares. Prolonged remission and persistently active disease are less common, with the former being associated with fewer and the latter with more severe vascular, renal, and hematological manifestations (17). Over time and with treatment, SLE patients may develop comorbidities such as infections, antiphospholipid syndrome, cardiovascular disease, and malignancies, which increase the disease burden (17).

1.2.2 Immunopathogenesis of SLE

SLE is characterized by the loss of tolerance to ubiquitous self-antigens, high titers of antinuclear autoantibodies, deposition of immune complexes (ICs), and involvement of multiple organs (18). Organ damage can occur as a consequence of chronic and uncontrolled autoimmune responses, or secondarily due to ischemia caused by vasculitis and thrombosis (19).

Patients with SLE exhibit aberrations in their immune system, which include leukocyte overactivation and cytokine overproduction. These abnormalities cause the upregulation of adhesive molecules on the cell surface and the presence of local proinflammatory cytokines, leading to the migration and infiltration of both innate and adaptive immune cells into target organs (18). In active lupus, autoantigen-specific T and B cells are commonly observed at high numbers, possibly due to compromised tolerance checkpoints (20,21). These cells may have amplified antigen receptor signalling, enhancing T cell help and B cell differentiation into plasma cells, subsequently leading to autoantibody production (22). Lupus B cells may also show altered cytokine secretion profiles, increased capacity for antigen processing and presentation, and impaired immunosuppressive function, further augmenting the immune responses (23). Various cytokines and chemokines, such as type I IFNs, IL-6, IL-18, TNF, CCL2, CXCL10, and CXCL16, are associated with lupus activity (22). Upregulation of IFN-stimulated genes (ISGs), a type I IFN signature, is found in the majority of patients with SLE. Plasmacytoid dendritic cells (pDCs), the main producer of type I IFNs, are therefore considered critical in the development of the disease (24).

Tissue injury in lupus is caused by overactivated immune responses and the products of them. ICs, which may accumulate in the kidney, skin, and central nervous system, are central players in tissue injury, leading to lupus nephritis, skin lesions, and central nervous system lupus, respectively (18). Autoantibodies, without forming ICs, can also contribute to tissue injury by directly targeting self-antigens. These include anti-immune-cell antibodies, anti-DNA antibodies that cross-react with antigens in the brain, antiphospholipid antibodies that link to thrombotic events, and antinuclear antibodies (ANA) that bind to ischemic tissues and exacerbate the injury (18,22). Inversible organ damage significantly increases morbidity and mortality in SLE.

1.2.3 Predisposing factors in SLE

Although the cause of SLE remains uncertain, current research suggests that a combination of genetic susceptibility and environmental factors may lead to dysregulation in the immune system, ultimately resulting in the development of SLE. A family-based study estimated that 43.9% of SLE cases are due to genetics, while environmental factors account for the remaining 56.1% (25). The X chromosome and gonadal hormones are also thought to play a role in SLE pathogenesis, as the disease displays significant sex dimorphism with a female to male incidence ratio of 9:1 (15). Estrogen, in particular, has been proposed as a factor that can enhance autoimmunity by upregulating the expression of immunologically relevant molecules, but the exact mechanism of hormone involvement in lupus is still unclear (7,22).

1.2.3.1 Genetic predisposition to SLE

SLE has the highest concordance rates among systemic autoimmune diseases, with monozygotic twins showing pairwise concordance of up to 57% (26).

While deficiencies in complement pathways (e.g., *C1QA*, *C1QB*, *C1QC*), immune clearance (e.g., *DNASE1*), apoptosis (e.g., *FASLG*), DNA damage repair (e.g., *TREX1*), the type I IFN pathway (e.g., *SAMHD1*), and tolerance (e.g., *PRKCD*) can individually lead to SLE (27), the disease is typically the result of a combination of multiple genetic risks, each with a relatively small effect size. More than 60 loci for lupus susceptibility have been identified through candidate gene studies and genome-wide association studies (28), with the HLA region being the most polymorphic. Certain alleles at classical (e.g., *HLA-DRB1*, *HLA-DQB1*, *HLA-DQA1*, *HLA-A*, *HLA-B*, and *HLA-C*) and non-classical HLA loci have been clearly associated with the risk of SLE, and HLA variants are estimated to account for 2.6% of the phenotypic variance in the disease (29).

Non-HLA loci, on the other hand, explain approximately 28% of the phenotypic variance (29). Using genetic association analysis, an increasing number of candidate genes have been linked to SLE (Figure 1), with their involvement confirmed by functional and fine-mapping studies. These genes regulate various pathways, such as NF-κB signaling (e.g., *TNFAIP3*), type I IFN signaling (e.g., *IRF5*, *IRF7*, *TYK2*), apoptosis and immune clearance (e.g., *ATG5*), ICs phagocytosis (e.g., *FCGR2A*, *FCGR3B*), myeloid cell function and signaling (e.g., *ITGAM*), T and B cell function and signaling (e.g., *PTPN22*, *BLK*, *STAT4*), metabolism (e.g., *SMG7*), and oxidative bursts (e.g., *NCF1*, *NCF2*) (28,30,31). Notably, a missense variant of *NCF1* is detected with the largest-ever effect size in the non-HLA loci, while the other loci individually make relatively smaller contributions to the disease risk (Figure 1). To understand the causal association between the *NCF1* variant and the high risk of lupus was the focus of Study I.

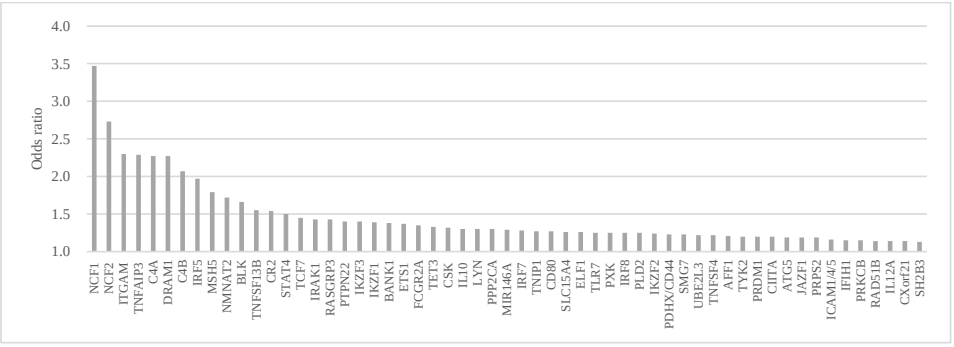

Figure 1. Common SLE susceptibility loci outside the HLA region.

1.2.3.2 Environmental risk factors for SLE

As mentioned previously, a large portion of the risk of SLE is attributable to environmental factors, with ultraviolet radiation being the most recognized. The majority of lupus patients are photosensitive, and exposure to UV light exacerbates skin manifestations and promotes systemic flares, most likely through activation of the type I IFN pathway and accumulation of immune cells (32). However, the pathogenic link between UV light and lupus remains elusive. One hypothesis is that it can cause DNA damage, cell death, and the release of nucleic-acid-associated autoantigens.

Over a hundred drugs have been documented to induce lupus, possibly by altering the functions of innate immune cells, particularly neutrophils, or disrupting T cell tolerance (33). Some drugs, such as procainamide, hydralazine, and TNFα, have a high prevalence of clinical manifestations as well as serological abnormalities. The symptoms of drug-induced lupus tend to be less severe than those of idiopathic SLE and typically resolve after withdrawal of the drug.

Smoking is another well-established environmental factor linked to SLE pathogenesis and production of antiphospholipid (34) and anti-dsDNA autoantibodies (35). Compared to non-smokers, smokers have been found to have a 67% higher risk of lupus, which may persist for several years after smoking cessation (35).

Furthermore, Epstein-Barr virus infection, occupational exposure (e.g., silica), and lifestyle factors (e.g., obesity and psychological stress) have also been linked to an increased risk of lupus, although the association is weaker compared to smoking (15).

1.2.4 Therapeutic interventions of SLE

The overall objectives of SLE treatment are to reduce disease activity, prevent damage accrual, and minimize drug adverse effects. Long-term treatment for SLE primarily involves antimalarials, mainly hydroxychloroquine, while glucocorticoids provide prompt relief of symptoms but may cause various detrimental effects, including irreversible organ damage, if continuously used (36). Immunosuppressive drugs, such as methotrexate, azathioprine, mycophenolate mofetil, and cyclophosphamide (17), are used to facilitate tapering of glucocorticoids and are selected based on the prevailing disease activity and safety concerns. Belimumab, a monoclonal antibody that inhibits BAFF, is effective in preventing organ damage and is particularly considered for patients with persistent disease and general symptoms when first-line treatments fail (37). Anifrolumab, an antagonistic antibody that targets IFNAR1, has recently been approved for use in combination with standard therapy for patients with moderate to severe SLE. More biologics targeting B cells, T cells, IFNs, and Fc gamma receptors (FcγRs) are currently being tested in clinical trials (22).

In the past three decades, the management of SLE has undergone significant advancements, resulting in improved survival rates and better quality of life for patients. However, the heterogeneity of the disease, non-responsiveness to treatment, and the lack of strong scientific evidence for selecting drug combinations have limited the effectiveness of existing therapies. To address these limitations, a more precise characterization of disease phenotypes and a comprehensive understanding of the cellular and molecular pathophysiology of lupus are necessary. These initiatives will improve current therapeutic strategies and potentially lead to more potent and less toxic medications.

1.2.5 Mouse models of lupus

Mouse models of lupus have played a crucial role in advancing our understanding of lupus pathogenesis and have provided valuable preclinical tools for the development and proof of concept of new therapies.

Spontaneous models, such as the NZBWF1 strain, the NZM strain and their congenic derivatives, as well as the MRL-*lpr* and BXSB strains, have been recognized for more than two decades. These strains share common features, including autoantibody production and IC glomerulonephritis development, but differ in gender bias, spectrum of autoantibodies, and organ involvement. Although spontaneous models have the drawback of a long disease incubation time, ranging from 6 to 9 months, they have been extensively used in studies regarding mechanisms of loss of tolerance, impact of sex hormones, and genetics of lupus.

Moreover, they serve as preclinical models for testing various therapeutics that are being taken to clinical trials in lupus, including candidate drugs targeting BAFF (38), IFNs (39), B cells (40), T cells (41), and pDCs (42). However, efficacies in the mouse models do not always translate to efficacy in patients with lupus, likely due to the heterogeneity of human lupus and confounders present in clinics.

Mouse models of induced disease, such as the pristane-induced lupus (PIL) model, reveal the involvement of environmental triggers in lupus. Balb/c mice intraperitoneally receiving pristane, a natural saturated terpenoid alkane, develop lupus-associated autoantibodies, IC glomerulonephritis, and arthritis in a number of months, while pristane-treated B6 mice develop only low-grade autoimmunity (43,44). The PIL model is highly dependent on overproduction of type I IFNs and expression of TLR7 (45), which inspired the establishment of another induced model with epicutaneous application of TLR7 agonists (46). Similar manifestations as PIL are developed four weeks after the cutaneous administration, but less characterization of disease in B6 mice limits the utility of the model. In addition, the graft-versus-host disease is an induced mouse model of lupus specifically for defining the role of T cell alloreactivity in driving B cell responses and IC nephritis (47).

The advent of transgenic and knockin technology has allowed expression or overexpression of certain gene products to define the roles of immunoregulatory factors in immune tolerance and protection against autoimmune development in lupus. Similarly, the techniques of gene knockout, especially conditional knockout, have been extensively used to investigate pathogenic mechanisms in specific cell types and/or at a specific time point. The latest genetic editing technology, CRISPR/Cas9, is useful for introducing specific lupus susceptible single-nucleotide polymorphisms, allowing the study of the impact of the genetic change. These techniques have been combined with spontaneous and induced models and have provided important insights into the effects of lupus-susceptible genes and certain cells in the disease.

1.3 RHEUMATOID ARTHRITIS

RA is a chronic autoimmune and inflammatory joint disease with an estimated incidence of 25–78 per 100,000 person-years and a global prevalence of 0.5-1% (48). Although current treatment strategies have eliminated premature mortality (49), there is still an unmet need to improve treatment outcomes. Effective management requires close monitoring of disease activity and prompt treatment adjustments, which may be hindered by time and resource constraints or patient noncompliance. Uncontrolled disease activity can result in progressive RA, leading to considerable joint damage, dysfunction, work disability, and economic losses. Even with controlled RA, physical performance and mental health may still be affected (50). Therefore, improving the management of RA through a deeper understanding of its pathogenesis and development is essential.

1.3.1 Immunopathogenesis of RA

RA is characterized by inflammation of peripheral joints and bone destruction, caused by immune cell infiltration in synovial tissue, the secretion of proinflammatory cytokines, and the involvement of matrix metalloproteinases. Approximately 40% of RA patients also experience extra-articular manifestations affecting various organs, possibly due to vasculitis (51). Cardiovascular disease is the most common and severe extra-articular manifestation. Secondary Sjögren's syndrome and lymphoma can also occur in some cases of RA. However, such extra-articular manifestations and complications are associated with severe and active RA with a poor prognosis. Nowadays, effective treatments have significantly reduced their occurrence.

The natural course of RA is considered to begin with a susceptible stage, where genetic and environmental factors interact. This progresses to preclinical RA, where neoepitopes are formed through post-translational modifications and activate the adaptive immune system, triggering the production of autoantibodies such as ACPA and rheumatoid factor (52). Autoantibodies can be detectable in circulation several years before the clinical onset of RA and are highly predictive of disease development.

Synovial inflammation, the characteristic of early RA, requires a second hit in addition to the presence of ACPA, such as IC formation and complement activation. This triggers the production of proinflammatory factors by fibroblasts and local APCs. At this stage, there is increased vascular permeability in synovial tissues and an influx of inflammatory cells, mainly $CD4^+$ T cells and macrophages. Synovial T cells receive support for their survival from fibroblast-mediated pro-survival signals (53), potentially leading to prolonged autoimmune responses. Additionally, high levels of matrix metalloproteinases and other autoantibodies such as those against collagen type II (COL2) and glucose-6-phosphate isomerase (GPI) are frequently observed.

The persistence of activated fibroblasts and adaptive immune responses is a key contributor to perpetuating synovitis, eventually leading to cartilage and bone damage, which are the main hallmarks of established RA. Besides fibroblasts and adaptive immune cells, chondrocytes and innate immune cells such as macrophages, neutrophils, and mast cells also play a role in cartilage damage by releasing inflammatory mediators, especially matrix metalloproteinases. Bone erosions are largely caused by osteoclasts, which are induced to mature and activated by RANKL, TNF, and IL-6 secreted from T cells, B cells, macrophages, chondrocytes, and fibroblasts.

Autoimmunity evolves over time during disease progression, from preclinical RA to established RA. It is usually manifested by the expansion of autoreactive T and B cells, epitope spreading, increased inflammation and production of inflammation-triggered antigens, as well as alterations in the glycosylation pattern of autoantibodies (52).

1.3.2 Predisposing factors in RA

The development of RA, like other autoimmune diseases, is linked to a combination of genetics and environmental risk factors. Epigenetic modifications, such as DNA methylation, have also been identified as a potential mechanism by which genetics and environmental factors interact to contribute to the disease. For example, high levels of methylation are associated with seropositive RA in smokers who carry the *HLA-DRB1* risk alleles, but not in non-smokers (54). In addition, RA is a sex-biased disease, with women being affected 2-3 times more often than men (55). While the reason for this bias is unclear, hormonal factors have been proposed to play a role.

1.3.2.1 Genetic predisposition to RA

RA has a strong genetic component, as evidenced by the pairwise concordance of up to 21% in monozygotic twins (26). More than 150 loci have been identified in the disease, with the HLA loci showing the strongest association and conferring a 3-fold increased risk (56,57). Specifically, certain *HLA-DRB1* alleles (e.g., *DRB1**01, *DRB1**04, *DRB1**10) that encode a conserved sequence at residues 70-74 in the third hypervariable region of HLA-DRB1, known as the shared epitope, are linked to seropositive RA (58). Recent studies have also uncovered an association between certain shared-epitope-negative *HLA-DRB1* alleles or alleles of other HLA genes and increased susceptibility (59,60).

In addition, genetic variants in non-HLA genes (e.g., *PTPN22*, *PADI*, *TRAF1-C*, *TNFAIP3*) have been discovered in seropositive RA patients and are estimated to account for approximately 5% of the heritability (57). However, genetic associations outside the HLA loci with seronegative RA require further investigation, given the high prevalence of seropositive RA and the limited discrimination by serology in most studies (56). It is worth noting that although the individual contribution of non-HLA genes to the risk of RA development is low, the combination of multiple genes may increase the overall risk up to 40-fold (57).

1.3.2.2 Environmental risk factors for RA

The low concordance rate of 21% indicates that the development of RA is also strongly influenced by the environment. The most recognized environmental factor with so far the strongest association for seropositive RA is smoking, with an odds ratio (OR) of 2.35 (61). Smoking has been shown to increase the production of antibodies targeting citrullinated proteins (ACPA) in RA patients, particularly in current smokers (62). The effect of smoking in RA is dose-dependent and long-term, as demonstrated by the high OR observed decades after cessation in heavy smokers (63). Additionally, periodontitis has been suggested to be associated with RA (64), likely due to microbial synergy and dysbiosis. Other possible risk factors such as obesity and dietary factors are still under debate.

1.3.3 Therapeutic interventions of RA

Over the last twenty years, the prognosis for RA patients has shown significant improvement due to early diagnosis, reliable clinical activity assessment, efficient use of immunosuppressants such as methotrexate, and the availability of biological reagents in medical treatment. The current focus of biological disease-modifying antirheumatic drugs is on targeting antigen presentation events (e.g., CD80 and CD86 inhibitors), B cells (e.g., anti-CD20 antibody), and the actions of proinflammatory cytokines (e.g., TNF inhibitors, IL-6R inhibitors, and JAK inhibitors). However, their use still poses challenges such as insufficient response and adverse effects. In addition, symptomatic agents such as pain mediators or non-steroidal anti-inflammatory drugs can be used to improve signs and symptoms, while glucocorticoids provide rapid anti-inflammatory activity but should be used for a limited period to avoid toxicity.

As the diagnostic and therapeutic approaches for RA continue to evolve, it is becoming evident that a more nuanced understanding of the underlying biological mechanisms is needed. Our findings in Study II and III have uncovered the involvement of dysregulated T cell tolerance and plasma cell formation by ROS deficiency in the development of the disease, which provides potential targets for future treatments.

1.3.4 Murine models of RA

Murine models have greatly advanced our understanding of the mechanisms underlying autoimmune arthritis and are valuable tools for validating therapeutic targets. These models generally mirror many features of the human disease, such as lymphocytic autoreactivity, autoantibody- and complement-mediated effects, accumulation and proinflammatory functions of neutrophils, macrophage infiltration, and cartilage or bone injuries.

The most commonly used induced model is the collagen-induced arthritis (CIA) model, where rats or mice develop an erosive polyarthritis after immunization with COL2, one of the dominant collagen forms of articular cartilage, along with collagen type IX, type XI, and cartilage oligomeric matrix protein. The CIA mouse model is typically induced in strains carrying certain MHCII alleles such as H-2^q and H-2^r (65), and usually results in severe

arthritis with extensive cartilage and bone damage. Besides clinical phenotypes, CIA shares many similarities in pathogenetic mechanisms with human RA, including MHCII restriction, breach of tolerance, and generation of autoantibodies against self collagen, making it the gold standard model for RA. Chronic inflammation in CIA depends on the use of oily adjuvant, which itself may also induce similar inflammatory arthritis in rodents, leading to the development of adjuvant-induced arthritis models. Examples of such models include Complete Freund's Adjuvant-induced arthritis and pristane-induced arthritis, where the adjuvant is intradermally injected (66,67). In contrast to human RA, which is limited to bone erosion, both CIA and adjuvant-induced models exhibit pronounced bone apposition at the joint margins, possibly due to less involvement of TNF in these models (68).

The derivative of CIA, collagen antibody-induced arthritis model, is a passive immunization model established by intravenous administration of arthritogenic antibodies (69). Its clinical and histological characteristics, such as synovitis, formation of pannus, cartilage degradation, and bone remodelling, are similar to those of CIA and mimic human RA. It is characterized by macrophage and neutrophil activation via ICs, complements, and Fc receptors, demonstrating a role of humoral immunity in the development of arthritis. However, it is independent of T or B cell responses and therefore does not recapitulate the complexity of the human disease. Despite this limitation, it is widely used because of rapid disease onset and high prevalence. Another transfer model, the K/BxN serum-transfer arthritis model, relies on pathogenic antibodies derived from K/BxN mice that spontaneously develop severe inflammatory arthritis (70). This model involves antibodies targeting the ubiquitous self-antigen GPI as well as innate components, including neutrophils, macrophages, proinflammatory cytokines, and complement factors. Both transfer models are useful for studying immune responses and potential therapeutics especially in genetically modified mice on arthritis-unsusceptible backgrounds. The latter model inspired the establishment of the GPI-induced arthritis model, in which mice are immunized with either human GPI protein or the $hGPI_{325-339}$ peptide (71). This model is characterized by H-2^q-restricted Th1 and Th17 cells and B cells that cross-react with the mouse GPI protein.

In addition, proteoglycan-induced arthritis, antigen-induced arthritis, and spontaneous mouse models (e.g., K/BxN, SKG, and human TNF transgenic mice) are also commonly used, each mimicking different aspects of human RA, including the involvement of ICs, the presence of rheumatoid factors, Th1 or Th17 responses, and defective T cell negative selection. It is worth noting that none of the arthritis models entirely recapitulate human RA, and there are limitations to testing and drawing conclusions about the success of new therapeutic approaches due to differences between the human and murine immune systems. However, a variety of newly established humanized models are gradually overcoming this issue (72). Despite these limitations, murine models are still useful for dissecting the processes involved in disease development and providing valuable information regarding the pathogenesis of RA.

1.4 REDOX SIGNALING

1.4.1 Reactive oxygen species

Reactive oxygen species (ROS) refer to a group of reactive molecules derived from molecular oxygen, including radicals such as superoxide and hydroxyl, as well as non-radicals such as hydrogen peroxide. ROS play a crucial role in regulating normal cellular functions, including cell differentiation, proliferation, and migration, and are strictly controlled through antioxidant enzymes and various negative feedback mechanisms in physiological conditions. They can be produced in a living organism on the plasma membrane, in the cytosol, in peroxisomes, in lysosomes, or on the membrane of mitochondria and endoplasmic reticulum, by a variety of systems, including electron transport chains and

ROS-generating enzymes, such as oxoglutarate dehydrogenase complex, NADPH oxidase (NOX), and xanthine oxidase (73).

Mitochondrial respiratory chain is the primary cellular source of ROS. Although the major biological function of mitochondria is ATP synthesis via electron transfer, superoxide and hydrogen peroxide are generated as by-products during the process (74). When ROS accumulate, they may target mitochondrial membranes, proteins, and mitochondrial DNA, resulting in the inhibition of energy transduction and altered redox signalling (75). However, these ROS also act as second messengers in many signalling pathways, such as the NF-κB pathway (76).

In contrast, NOX is the only family of enzyme complexes with the sole function of generating ROS (73). These complexes transfer electrons from NADPH to reduce molecular oxygen to superoxide radicals. So far, seven members have been discovered in the family, including NOX1, NOX2, NOX3, NOX4, NOX5, DUOX1, and DUOX2. They vary in their regulation pathways, cellular localization, and biological functions.

1.4.2 NADPH oxidase 2

NOX2 is the first identified isoform of NOX family (77). Its structures and biochemical features have been extensively studied, providing insights into the other isoforms. NOX2 is present in endothelial and phagocytic cells, and it is critical for antimicrobial defence. Patients with mutations in NOX2 develop chronic granulomatous disease (CGD), which is characterized by recurrent and life-threatening bacterial and fungal infections.

The NOX2 complex comprises two transmembrane proteins, cytochrome b subunit alpha (CYBA/ $p22^{phox}$) and beta (CYBB/$gp91^{phox}$), as well as cytosolic components NCF1/$p47^{phox}$, NCF2/$p67^{phox}$, NCF4/$p40^{phox}$, and a small GTP binding protein Rac (Figure 2). The CYBA and CYBB heterodimer serves as the core catalytic enzyme, with CYBA providing stabilization and a docking site for NCF1, while CYBB contains all the elements necessary for electron transfer. NCF2 is proposed to promote enzymatic activity, which relies on its translocation to and interaction with CYBB, assisted by NCF1 and Rac (78). NCF4 may act as a membrane anchor for the NOX2 complex via its N-terminal phox homology (PX) domain, although its exact function remains unclear.

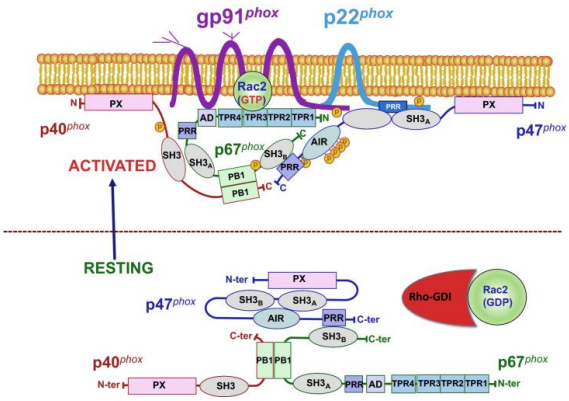

Figure 2. Assembly of the NOX2 complex.
PX, phox homology;
SH3, Src homology 3;
PRR, proline-rich region;
AIR, auto-inhibitory region;
PB1, Phox and Bem1;
AD, activation domain;
TPR, tetratricopeptide-rich region;
Rho-GDI, Rho GDP-dissociation inhibitor.
Adapted from Belambri, Sahra A., et al. 2018 (79). *CC-BY.*

In a resting cell, the components of the NOX2 complex are distributed between the cytosol and the plasma or phagosome membrane. GDP-bound Rac is sequestered by Rho GDP-

dissociation inhibitor (79). NCF2 forms a complex with NCF1 through one of the Src homology 3 (SH3) domains of NCF2 and the proline-rich domain of the NCF1 C-terminal tail. Meanwhile, NCF4 is bound to and stabilized by NCF2 via their Phox and Bem1 domains. NCF1 is kept in an inactive state by the interaction between its $SH3_A$-$SH3_B$ tandem and the auto-inhibitory region (AIR). Similar autoinhibited confirmation is present in NCF4 (80).

Upon stimulation, NCF1 is phosphorylated on several serine residues in the AIR, which inhibits the SH3-AIR interaction and induces the binding of the SH3 tandem to the proline-rich domain of CYBA (79). The conformational change also releases the NCF1 PX domain and promotes its binding to the phospholipids on the membranes. NCF4 undergoes a similar conformational switching process upon phosphorylation, but exhibits different lipid-binding properties, which will be described in detail later. NCF1 and NCF4 thereby bring their linked protein NCF2 in proximity to CYBB, where NCF2 enhances the catalytic activity via its activation domain. The interaction between CYBB and NCF2 is further enhanced by GTP-bound Rac disconnected from Rho GDP-dissociation inhibitor (78).

As previously mentioned, NCF1 and NCF4 exhibit different phospholipid-binding specificities. While the NCF1 PX domain binds to phosphatidic acid, phosphatidylserine, and phosphatidylinositol 3,4-bisphosphate, which are present in various biological membranes, the NCF4 PX domain prefers phosphatidylinositol 3-phosphate (PtdIns3P), which accumulates significantly in phagosomal membranes (81,82). NCF4 is therefore crucial for NOX2 assembly on the membranes of endosomes and phagosomes, and subsequently, for the production of intracellular ROS (83). In Study III, we focus on the regulatory function of NCF4-dependent intracellular ROS specifically in relation to the development of CIA.

1.4.3 ROS-related pathologies

Maintaining homeostasis of ROS is critical for preventing oxidative damage while promoting proper pathogen resistance, cellular signal transduction, and metabolism. This balance relies on the interplay between ROS production and their clearance through various scavenging mechanisms involving both enzymatic and non-enzymatic antioxidants, such as superoxide dismutase, catalase, glutathione-ascorbate cycle, glutathione peroxidase cycle, α-tocopherol, carotenoids, flavonoids, and proline (84).

However, excessive ROS production can result from genetic mutations in ROS-producing enzymes or pathological conditions such as persistent infections, ischaemia-reperfusion, and vascular inflammation (85). These conditions may induce cell death and tissue injuries in vital organs, leading to organ failure if not managed. Moreover, excessive ROS levels can cause oxidative damage to macromolecules, which is associated with carcinogenesis, metastasis, and reproductive issues, as well as promote inflammation and insulin resistance by affecting cellular signalling. Conversely, physiological levels of ROS play important roles in immune responses, wound repair, and cell development. In general, imbalanced ROS levels may contribute to inflammatory diseases and infections, cardiovascular and metabolic diseases, cancers, neurological disorders, and autoimmune diseases (86,87). The following text will focus on CGD and autoimmune diseases, the two main ROS-related pathological conditions resulting from immune dysregulation.

1.4.3.1 Chronic granulomatous disease

CGD was firstly reported in four cases of children in 1950 who presented with life-threatening bacterial or fungal infections and tissue granuloma formation (88). Later, CGD was recognized as a rare primary immunodeficiency disease caused by defects in genes encoding the subunits of the NOX2 complex (77) and EROS, a chaperone that assists in CYBB-CYBA heterodimer expression (89).

Since ROS generated by NOX2 are critical for microbial killing and contribute to neutrophil extracellular traps (NETs) formation, most CGD patients initially present with infections (90). ROS production is the main factor that mediates the effect of genotype on the age of onset, disease severity, and survival (91). Germline null mutations in *CYBB* lead to absent ROS production in all types of phagocytes and result in X-linked CGD. *NCF1* mutations, most commonly the c.75_76del, are associated with decreased levels of ROS bursts (92) and are responsible for two-thirds of autosomal recessive CGD (93). Patients with autosomal recessive CGD tend to develop less severe infections and present with later onset than those with X-linked CGD, due to residual production of ROS (90,91). Besides *NCF1* mutations, genetic defects in *CYBA* and *NCF2* are also associated with autosomal recessive CGD but with lower mutant allelic frequency (93). Defective NCF4, on the other hand, is rare and only leads to a mild atypical form of CGD, manifested as hyperinflammation and peripheral infections (94).

Given the involvement of NOX2 in programmed cell death (95) and suppression of proinflammatory responses such as the NF-κB pathway (96), CGD patients who have defects in NOX2 activation usually exhibit chronic inflammatory conditions, such as granulomatous inflammation in the lungs and gastrointestinal and genitourinary tracts, as well as hemophagocytic lymphohistiocytosis (93). In addition, a range of diverse autoimmune conditions have been observed in CGD patients (97), which will be discussed in the next section.

1.4.3.2 Autoimmune diseases

As a result of the imbalance between ROS generation and antioxidant systems, excessive ROS may cause DNA, proteins, and lipid oxidation, leading to the formation of neoantigens relevant to the development of autoimmune responses in SLE, RA, and Type 1 diabetes (98). Under these autoinflammatory conditions, ROS also trigger tissue injury indirectly by activating neutrophils, NETs, and NETosis (99). In SLE, patients' neutrophils produce ROS more rapidly and at higher concentrations than those of healthy individuals (100,101). Likewise, RA patients exhibit a remarkable increase in ROS formation, accompanied by lipid peroxidation, protein oxidation, DNA damage, and decreased antioxidant defence capacity (102). Antioxidant supplements such as vitamin C for RA (103) and melatonin (104) for lupus have been studied in preclinical or clinical settings, indicating a promising therapeutic option for both diseases.

However, recent studies have challenged the traditional view that ROS play an exclusively proinflammatory role. For example, besides infections, CGD patients also commonly suffer from non-infectious inflammatory bowel disease (105), as well as other inflammatory and autoimmune manifestations such as antiphospholipid syndrome, juvenile idiopathic arthritis, IgA nephropathy, cutaneous lupus erythematosus, and autoimmune pulmonary disease (97), indicating a link between autoimmunity and impaired NOX2 function. To date, numerous murine models (86,106,107) and human cohorts (108–113) have shown an association between ROS deficiency due to genetic variants in NOX2 subunits, including NCF1, NCF2, NCF4, and RAC2, and multiple autoimmune diseases. In the following section, the discussion will mainly focus on the role of *NCF1* and *NCF4* variants in lupus and arthritis.

1.4.4 NOX2 deficiency in autoimmune diseases

1.4.4.1 Defects in the NCF1 gene

Several genome-wide association studies have identified the genomic regions encoding some of the NOX2 subunits as susceptible loci for autoimmune diseases (114,115). However, in most of these loci, the causal polymorphisms have not been discovered. *Ncf1* was the first

major gene identified within the quantitative-trait loci for arthritis through positional cloning in rat models (116). The disease allele was surprisingly associated with lower production of NOX2-derived ROS, suggesting a regulatory role of ROS in autoinflammatory conditions. It was first found in rats that a naturally occurring polymorphism of *Ncf1* regulates the severity of pristane-induced arthritis (116). Later, the finding was confirmed using a common mouse strain with a spontaneous point mutation at the -2 position of exon 8 of the *Ncf1* gene, denoted $Ncf1^{m1j}$, which leads to a truncated protein and thereby deficiency in NOX2-derived ROS production (117,118). $Ncf1^{m1j}$ mice also develop exacerbated PIL (119), myelin oligodendrocyte glycoprotein$_{1\text{-}125}$-induced chronic experimental autoimmune encephalomyelitis (120), and mannan-induced psoriatic arthritis (121), highlighting the causal link between low ROS production due to dysfunctional NOX2 complex and the development of autoimmunity.

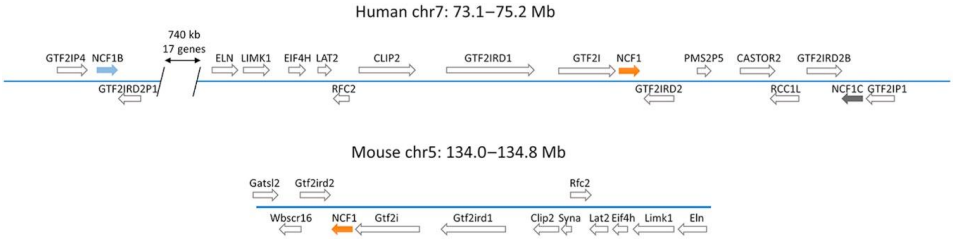

Figure 3. NCF1/Ncf1 genetic organization in humans and mice. Adapted from Urbonaviciute, Vilma, et al. 2019 (122).

The human *NCF1* gene is much more complex compared to its orthologs in rodents (Figure 3). The mouse *Ncf1* gene, located on chromosome 5, shows high conservation throughout the region, albeit in a reverse direction. In contrast, the human *NCF1* gene is accompanied by two pseudogenes, *NCF1B* and *NCF1C*, on chromosome 7. These pseudogenes are highly similar to the functional *NCF1* gene, except for a 2-bp GT deletion (ΔGT) in exon 2 (123). The surrounding region of *NCF1* in the human genome contains multiple large duplications, known as low-copy repeats (LCRs), and several small inversions, duplications, and deletions (124). The *NCF1* genes are located in separate LCRs, with the pseudogenes in between. Due to the high similarity among LCRs, unequal crossover events between the *NCF1* gene and the pseudogenes are prone to occur, leading to the formation of fusion genes that consist of segments from both the *NCF1* gene and *NCF1B* or *NCF1C*. This crossover has been observed in NCF1-deficient CGD patients who carry a non-functional ΔGT *NCF1* gene because exon 2 of the functional *NCF1* gene has been substituted with the reciprocal sequence of the pseudogenes (125). These patients have two copies of ΔGT *NCF1* genes instead of normal *NCF1* genes on two chromosomes, resulting in absent or extremely low phagocyte NOX2 activity (92). The opposite outcome of this crossover event has also been found, where the pseudogenes gain the GTGT sequence in exon 2 to become functional *NCF1* genes, known as *NCF1*-pseudo fusion genes (126). Although it remains unclear whether these fusion genes have full functionality, a cohort study has shown that low ROS production due to an *NCF1* polymorphism can be restored by having a copy of the *NCF1*-pseudo fusion gene (108). In addition to pseudogenes and fusion genes, the *NCF1* gene itself exists in various numbers of copies. Studies have shown that increased copy numbers of functional *NCF1* can protect against RA and SLE, while decreased copy numbers predispose to the diseases (109,110). Due to the complexity of the *NCF1* gene and its surrounding region, it is generally excluded from genome-wide association studies and full sequencing analysis.

With the help of nested PCR and pyrosequencing strategy, three single-nucleotide polymorphisms in the functional *NCF1* gene were identified in a Swedish RA cohort (110). Among them, the missense variant p.Arg90His (rs201802880), showed significantly lower ROS production and resembled the *Ncf1* polymorphism discovered in rats. Although no association between the p.Arg90His variant and RA was found in the Swedish RA cohort, a study of a Korean RA cohort reported that it predisposes individuals to RA with an OR of 1.65 (109,110). In a Swedish SLE cohort, the p.Arg90His variant was found to be more frequent in patients (OR=3.0) and was also associated with a younger age at lupus diagnosis (108). The p.Arg90His variant's association with SLE was confirmed in a separate study of Asians (OR=3.47), European Americans (OR=2.61), and African Americans (OR=2.02) (109). Compared to other genetic risk variants identified by genome-wide association studies or candidate gene studies with a relatively low risk and OR usually <2 (Figure 1) (28,30,31), the p.Arg90His variant has the strongest known genetic association with SLE. In addition to RA and SLE, the p.Arg90His variant was also associated with another autoimmune disease, primary Sjögren's syndrome, with OR=2.45 in Chinese and OR=2.35 in European Americans (109).

The NCF1 protein is highly conserved across species, comprising 390 amino acids and weighing 47kDa. It consists of an PX domain, two SH3 domains ($SH3_A$ and $SH3_B$), a C-terminal AIR, and a proline-rich region (Figure 2). In rats, the natural polymorphism substitutes Threonine with Methionine at position 153 located in the hinge region between the PX and $SH3_A$ domain of the NCF1 protein, affecting the interaction of NCF1 with phosphatidylinositol 3,4-bisphosphate (127), but not the expression of NCF1 (116). The spontaneous mutation in *Ncf1*^{m1j} mice leads to a lack of 8 residues in the NCF1 $SH3_B$ domain, which may affect the assembly of NCF1 with CYBA (128,129). The human p.Arg90His variant, on the other hand, causes an amino acid shift from Arginine to Histidine at position 90 in the PX domain, affecting the binding of NCF1 to phospholipids (130). In general, the identified polymorphisms or mutations in both human and rodents impair or fail the translocation of NCF1 to the membrane, leading to lower NOX2-derived ROS production and an association with autoimmunity (116,122,129,131).

1.4.4.2 Defects in the NCF4 gene

NCF4 is the most recently discovered protein among the NOX2 subunits (132). It has a molecular weight of 40kDa, 339 amino acids, and consists of a PX domain, an SH3 domain, and a Phox and Bem1 domain. Compared to NCF1, NCF4 is more conserved across humans, mice, and rats. Although less studied, NCF4 has been associated with autosomal recessive CGD (94), Crohn's disease (133), and RA (112,113). More than 200 variants of *NCF4* have been identified, including intron variants, splice-site variants, missense variants, in-frame deletion, frameshift deletion, and nonsense variants. These variants may lead to undetectable or truncated protein, resulting in no or low NOX2-derived ROS production (94,134).

The first single-nucleotide polymorphism of *NCF4* be associated with RA is rs729749, located in the middle of intron 4 of the *NCF4* gene (112). However, its genetic effect remains unclear, as no evidence of influence is shown on any transcription factor binding site or known regulatory element. Another intronic single-nucleotide polymorphism, rs4821544, was previously identified in Crohn's disease and later found to be associated with RA in a Chinese cohort (113,135).

In the absence of NCF4, the expression of NCF1 and NCF2 is decreased by more than 50% (83,136). Neutrophils from *Ncf4*$^{-/-}$ mice exhibit substantially decreased intracellular and extracellular ROS responses, leading to severe, CGD-like defects in bacterial killing (136). Moreover, the knockout enhances the susceptibility to CIA without affecting COL2-specific T cell responses, antigen presentation of COL2, or the delayed-type hypersensitivity reaction

(83). The development of CIA promoted by *Ncf4*$^{-/-}$ is accompanied by dramatic activation of innate inflammatory pathways, including delayed neutrophil apoptosis and increased secretion of proinflammatory cytokines.

Ncf4^{R58A}, a single point mutation in the PX domain of NCF4, was initially induced in mice to study the role of PtdIns3P-NCF4 binding in phagosomal NOX2 activity (137). In contrast to *Ncf4*$^{-/-}$, this mutation does not affect the expression of NCF1 or NCF2. It significantly decreases intracellular but not extracellular ROS production in neutrophils in response to various stimuli (83,137). Similarly, *Ncf4*^{R58A} mice are deficient in bacteria killing and susceptible to CIA, yet the diseases are much milder in these mice than in *Ncf4*$^{-/-}$ mice (83,136,137). In addition, with relatively restricted deficiency in ROS production both quantitatively and spatially, *Ncf4*^{R58A} mice exhibit less innate immune activation and susceptibility to a T cell-independent mannan-induced psoriatic arthritis-like disease model than *Ncf4*$^{-/-}$ mice and *Ncf1*^{m1j} mice (83).

1.4.4.3 NOX2-derived ROS in SLE

ROS deficiency has been suggested to contribute to the development of SLE by affecting efferocytosis and NETs formation, which are the two main sources of proinflammatory autoantigens. Efferocytosis, a process of dead cell clearance by phagocytes, is insufficient or delayed in both mice and humans with low NOX2-derived ROS production, and in those with lupus (138–140). This leads to the accumulation of cell debris in ROS-deficient phagocytes, which promotes the production of inflammatory mediators and the development of autoreactive memory to apoptotic-cell-derived self-antigens (139,141).

The role of NETs in SLE is still under debate. Although NETs contain lupus autoantigens such as histones and DNA (142) and have been suggested to promote the disease (143,144), NOX2-derived-ROS-dependent NETs may also resolve inflammation by degrading proinflammatory mediators with NET serine proteases (145,146), and ameliorate lupus (119).

Apoptotic-cell- and NETs-derived DAMPs are sensed by pattern recognition receptors on the surface of APCs. Under ROS-deficient conditions, these APCs become overactivated, leading to enhanced T cell responses and a break of tolerance in the adaptive immune system (138,139,147). Autoreactive B cells secrete autoantibodies that bind to nuclear components to form ICs, which are internalized by various innate immune cells and stimulate them upon ligation of different receptors. The resulting secretion of proinflammatory cytokines further promotes adaptive immune responses, leading to more autoantibody production and IC formation (122). This vicious cycle ultimately results in chronic autoimmunity and organ injuries. Although the exact mechanisms remain unclear, ROS deficiency has been proposed to contribute to lupus development by altering the aforementioned processes (86,130,139). In Study I, we investigated the role of NCF1-dependent ROS in protecting against lupus and the potential redox-sensitive signalling pathways involved. In Study III, we discussed the role of NCF4-dependent ROS in lupus-associated autoantibody production.

1.4.4.4 NOX2-derived ROS in RA

In genetically susceptible individuals, autoimmunity may already be present before the clinical onset of RA, as evidenced by the presence of circulating polyclonal autoantibodies, including rheumatoid factor, ACPA, and anti-carbamylated protein antibodies (52,148). These antibodies may arise from an immune response to neoantigens that are continuously generated in the body due to inflammation or respiratory bursts. In sera, joint tissues, and synovial fluids from RA patients, autoantibodies are usually detectable against ROS-modified biomacromolecules (e.g., COL2, C1q, and IgG) or antigens formed through other post-translational modification (149). Cysteine is the key amino acid in most redox reactions, and

the functional groups of cysteine residues, the thiol groups, have been found decreased in RA patients (150). Within an oxidative environment, thiol groups tend to form disulfide bonds, which may influence the selective display of MHCII-restricted T cell epitopes during antigen processing (151). In the GPI-induced arthritis model, the major immunogenic peptide carries cysteines and is reported to be more efficiently processed under NOX2-derived-ROS-deficient conditions, leading to enhanced antigen-specific T cell responses and exacerbated arthritis (152).

Except for putative autoantigens that are presented directly by B cells, most antigens are internalized by macrophages or DCs, proteolytically processed, and subsequently presented on the surface of these cells by MHCII molecules or through the process of cross-presentation via MHCI molecules. Both MHCII- and MHCI-restricted antigen presentation have been suggested to play a role in autoreactive T cell priming, leading to later activation of synovial migratory memory T cell and prolonging joint inflammation (153). NOX2 deficiency has been shown to promote phagocytosis and proteolysis of antigens in APCs, possibly by modulating phagosomal pH or the redox microenvironment, which leads to overactivation of T cells (130,139,154).

In a healthy individual, $CD4^+$ T cell activation, differentiation, and proliferation in response to antigen presentation and local cytokines can be inhibited by regulatory T cells during resolution of inflammation. However, Treg-mediated suppression is defective in RA patients, leading to expansion of proinflammatory T cell populations, such as Th1 and Th17 (155). These T cells secrete cytokines to recruit and activate other immune cells, in which multiple signaling pathways, such as hematopoiesis, apoptosis, and innate immune responses, have been suggested to be regulated by NOX2-derived ROS (83,156).

The source of ROS with the most significant impact on T cell regulation, whether from intracellular NOX2, mitochondria, or bystander cells such as APCs, remains a topic of debate. Nevertheless, numerous studies have documented the regulatory effects of ROS on T cell function. Some redox-sensitive proteins, such as protein tyrosine phosphatases, that play crucial roles in TCR (as well as BCR) signaling, are genetically associated with arthritis (157,158). Insufficient NOX2-derived ROS production has been shown to increase the number of reduced thiol groups on T cell surface, lowering the threshold for T cell reactivity and proliferation, as well as driving T cell-dependent autoimmune responses (118,159). The deficiency also impairs the induction and suppressive function of peripheral regulatory T cells, which can be rescued by ROS from macrophages (147,160).

In addition to peripheral T cell malfunction, abnormalities in T cell selection have also been suggested as contributing factors to the development of RA. For example, a mutation in *Zap70* in the SKG strain, which spontaneously develops chronic autoimmune arthritis, alters TCR signaling, changing the thresholds of T cells to thymic selection and subsequently maintaining the autoreactive T cell clones (161). Moreover, post-translational modifications of autoantigens that affect T cell epitopes, such as glycosylated $COL2_{259\text{-}273}$, present only in peripheral tissues but not thymus, are correlated with a high frequency of autoreactive T cells and autoantibodies, as well as susceptibility to RA (162,163). Despite increasing knowledge of the cellular and molecular mechanisms of central tolerance, our understanding of how it is maintained remains largely unknown. However, considering that changes in T cell surface redox levels occur during T cell interaction with APCs (159), it is conceivable that ROS may be especially important during thymic selection, where TCR-MHCII interaction is critical. In Study II, we found that selection and activation of autoreactive T cells are regulated by NOX2-derived ROS in an autoreactive-T-cell-driven arthritis model.

Similar to T cells, B cell disturbances in central and peripheral tolerance, activation, and proliferation also contribute to RA pathogenesis, by promoting autoreactive B cell

accumulation, ectopic germinal center formation, support for T cells, proinflammatory cytokine secretion, and antibody production in the inflamed synovium. Increased levels of autoantibodies have been observed in CGD patients and rodents carrying defective NOX2, accompanied by altered IgG subtype distribution (86,118,164,165). These could potentially result from overactivation of innate immune cells and T cells due to ROS deficiency, while direct effects of NOX2-derived ROS on B cells remain unclear (83,147). Study III focuses on investigating the regulatory role of intrinsic ROS in B cells against autoimmune arthritis.

2 RESEARCH AIMS

For several decades, ROS has been considered as a pathogenic factor in autoimmune diseases. However, in the early 21st century, a polymorphic *Ncf1* allele in rats was found to cause lower oxygen bursts and result in severe arthritis, challenging the dominant paradigm. Since then, genetic defects in *Ncf1* have been associated with multiple autoimmune models in rodents. Studies on human cohorts have also revealed a strong association between *NCF1* variants and autoimmune diseases like SLE and RA. Similar observations have been reported on other subunits in NOX2 complex, such as NCF4. Together, these findings suggest a regulatory property of NOX2-derived ROS in autoimmunity, yet the underlying mechanism remains incompletely understood. The overall aim of this study was to investigate the mechanisms linking deficits in NOX2 subunits NCF1 and NCF4 to the development of autoimmune diseases, specifically lupus and arthritis.

Research objectives were as follows:

Study I: Investigate the regulatory mechanisms of NCF1-dependent ROS in lupus;

Study II: Investigate the role of NCF1 in T cell tolerance and the development of arthritis driven by autoreactive T cells;

Study III: Investigate NCF4 regulation on B cell functions in autoimmune diseases.

3 MATERIALS AND METHODS

3.1 MOUSE MODELS OF LUPUS

Pristane-induced lupus. Lupus is induced in mice by intraperitoneal injection of pristane. Balb/c mice develop a lupus-like disease, characterized by IC glomerulonephritis, mild erosive arthritis, and circulating lupus-associated antibodies several months after pristane injection (166). Mice with C57 black backgrounds, such as B10 and B6, develop a lower level of autoimmunity than Balb/c, accompanied by pulmonary vasculitis and pulmonary hemorrhage. Some pathways in pristane-injected mice closely resemble those in patients with SLE, such as overproduction of type I IFNs. PIL is the most widely used induced model of lupus, serving as an example of how an environmental factor can trigger a lupus-like disease in genetically unsusceptible strains.

***Yaa*-accelerated spontaneous lupus.** In study I, we established a new mouse strain carrying both the *Ncf1*^{m1j} mutation and the Y-linked autoimmune accelerating locus (*Yaa*). *Yaa* is a translocated region from the X chromosome onto the Y chromosome of the BXSB strain, responsible for the spontaneous development of lupus-like autoimmune abnormalities, which have been confirmed to be driven by the duplicated *Tlr7* gene in the locus and the resultant overexpression of TLR7 (167). The *Yaa* locus has been bred onto other lupus-prone backgrounds, where it has been shown to promote disease progression.

3.2 MOUSE MODELS OF ARTHRITIS

Collagen-induced arthritis. Mice immunized intradermally with COL2 in Complete Freund's Adjuvant develop severe and progressive joint lesions, which are characterized by synovitis, pannus formation, and damage to cartilage and bone. The model is dependent on MHCII, T cells, and B cells, and demonstrates autoimmune responses driven by COL2-reactive T cells (168) and the development of arthritogenic autoantibodies (169). Autoreactive T-cell clones in both *DRB1**04-positive RA patients and H-2^{q} CIA mice recognize the same immunodominant peptide COL2$_{259\text{-}273}$, predominantly in its glycosylated form (163). The mouse peptide differs from that in other species, including bovine, rat, and human, by only one amino acid at position 266, with Aspartic acid in the mouse self-peptide and Glutamic acid in the heterologous peptide.

Glucose-6-phosphate isomerase-induced arthritis. Serum from K/BxN transgenic mice which spontaneously develop severe inflammatory arthritis transfer the disease to a wide range of mouse strains, due to autoantibodies against GPI (70). Intradermal immunization with human GPI protein or the derived hGPI$_{325\text{-}339}$ peptide in Complete Freund's Adjuvant induces self-limiting arthritis in mice with certain MHCII haplotypes (71). This model is T and B cell-dependent and driven by GPI autoreactive lymphocytes.

Collagen antibody-induced arthritis. Mice receiving a combination of monoclonal anti-COL2 antibodies intravenously develop a rapidly-onset and short-term arthritis that is independent of T or B cells. It is instead caused by neutrophil and macrophage infiltration, which is activated by aggregated antibodies on the cartilage through Fc receptors and complement pathways (170).

3.3 ETHICAL CONSIDERATIONS

The research included in the thesis involves animal experimentation, which raises ethical concerns. However, due to the complex and systemic nature of SLE and RA, *in vivo* models are currently necessary to gain a deeper understanding of these diseases. We justify our use of animals by showing that the potential benefits to humans overweigh the harm caused to

animals. The research provides insights into the pathogenesis of lupus and arthritis, which may lead to the development of more effective treatment strategies and ultimately save lives or increase the quality of life. We strictly adhere to the Three Rs principle – Replacement, Reduction, and Refinement, to minimize animal suffering and sacrifice. While Replacement is not currently possible, we make every effort to improve animal welfare. For example, we carefully monitor the animals' health and use humane endpoints to determine when euthanasia is necessary. We also design our experiments to minimize the number of mice used. Our trackable electronic animal medical record system at KI (tickatlab.ki.se) ensures full control of each mouse's conditions, further reducing suffering and waste.

4 RESULTS AND DISCUSSION

4.1 STUDY I: NCF1-dependent production of ROS protects against lupus by regulating plasmacytoid dendritic cell development and functions.

Lupus has been linked to deficient NCF1-dependent ROS production in both humans and mouse models (86,108,109). Several mechanisms have been proposed to explain this association, including impaired formation of NETs (119), insufficient efferocytosis of apoptotic cells by macrophages due to reduced endosomal acidification (139,140), and overactivation of TLR signalling caused by acidic pH in pDC endosomes (130). In this study, we systemically screened and identified pDCs as the major immune cell type responsible for lupus exacerbation due to NCF1 deficiency, using a set of cell-specific Cre-deleter, the human p.Arg90His variant knockin, and transgenic mouse strains. We demonstrated that restoring NCF1-dependent ROS specifically in pDCs can protect against PIL and *Yaa*-accelerated spontaneous lupus by limiting AKT/mTOR-dependent pDC generation, CCR2-mediated tissue accumulation, and type I IFN responses.

Overactivation of the type IFN system is a hallmark of SLE, and pDCs, the primary producers of IFNα, have long been considered the main contributors to the pathogenesis and development of lupus (171). However, the mechanisms behind the increased number of pDCs in lupus tissues and their hyperactivity are still not fully understood (21,172,173). This study showed that NCF1-dependent intrinsic ROS regulate pDC development and accumulation in multiple organs, correlating with a local type I IFN signature. PDCs are known to produce IFNα upon stimulation by nucleic-acid-containing materials, which are phagocytosed via Fc receptors and sensed by endosomal pattern recognition receptors. Several receptors and downstream factors involved in this process have been associated with lupus, including FcγRIIa (174), TLR7 (175), TLR9 (176), STING (177), and ISGs such as IRF and STAT1 (178), among which TLR7/9 and STAT1 have been proven to be regulated by NCF1-derived ROS (86,130). Our study added STING and JAK1, one of the upstream signals of STAT1, to the list of redox-regulated proteins and showed that hyperactivation of the STING/IFNα/JAK1/STAT1 cascade in ROS-deficient pDCs contributes to disease exacerbation.

Targeting pDCs has been considered a potential therapeutic strategy against lupus. Ablation of pDCs with anti-BDCA2 antibody and downregulation of type I IFN signalling with JAK inhibitors were both tested in clinical trials of SLE, where pDC depletion but not JAK inhibitors successfully passed phase II efficacy trials (179–182). Our study demonstrated that injection of NCF1-defecient pDCs promoted PIL, and oral administration of baricitinib, a JAK inhibitor, ameliorated spontaneous lupus. These findings suggest that the aforementioned strategies may be more effective in subgroups of patients with insufficient ROS production. The discovery of the protective role of pDC-derived ROS in lupus exacerbated by NCF1 deficiency and the underlying mechanisms open up new possibilities for the treatment of SLE, at least in patients carrying relevant genetic variants.

4.2 STUDY II: Two major genes associated with autoimmune arthritis, *Ncf1* and *Fcgr2b*, additively protect mice by strengthening T cell tolerance.

Several adaptive T cell transfer experiments have confirmed the regulatory role of NCF1-dependent ROS in T cell activity in rodent models of arthritis, such as pristane-induced arthritis and CIA (159,183,184). However, classical CIA is not a model for autologous T cell response, since the COL2-reactive T cells elicited by immunization with heterogenous COL2 do not recognize endogenous COL2 peptides (185). To investigate the role of ROS in T cell autoreactivity and tolerance, a mouse strain expressing transgenic COL2 was established

(186). The *Col2*^{266E} mutation in the transgene makes the immunodominant peptide COL2$_{259-273}$ identical to the corresponding peptide in rat COL2, thus protecting the mice from CIA. By inducing the *Ncf1*^{m1j} mutation, T cell tolerance is broken, resulting in the development of chronic autoimmune arthritis (187). The shortcoming of this strain lies in the presence of endogenous COL2, leading to the formation of heterotrimeric molecules, which confounds the interpretation of the results. In this study, we established a knockin strain that completely replaced *Col2*^{D266} with *Col2*^{266E} in the immunodominant peptide. The BQ.*Col2*^{266E} mice exhibited more pronounced protection against CIA under ROS-deficient conditions when compared to the transgenic mice. To confirm that *Col2*^{266E} does not affect T cell recognition of the peptide COL2$_{259-273}$, we established another strain carrying both the *Col2*^{266E} and the *Col2*^{264R} mutation, which changes the major TCR recognition site by mutating Lysine at position 264 (188). The BQ.*Col2*^{264R} mice elicited a strong non-self T cell response and developed severe arthritis.

NCF1-dependent ROS have been proposed to regulate T cell activity by oxidizing antigenic peptides (152), modulating APC responses (147), and directly affecting redox-sensitive T cell signaling pathways (159,160). However, the regulation of thymic selection of T cells by ROS has not been fully investigated. In this study, we showed that the *Ncf1*^{m1j} mutation decreased the expression of AIRE in medullary thymic epithelial cells (mTECs) and B cells. As a result, potentially autoreactive T cells may escape negative selection and enter the periphery.

One of the upstream activators of NOX2 is the Fc receptor family, among which FCGR2B is considered a critical inhibitory factor which plays a protective role against autoimmune diseases (189,190). Deficiency in *Fcgr2b* has been reported to increase the susceptibility and severity of CIA by dysregulating DCs and B cells, resulting in increased T cell responses and autoantibody production (191). In this study, we showed that FCGR2B deficiency increases COL2-specific autoreactive T cells and leads to the development of CIA in BQ.*Col2*^{266E} mice. Interestingly, we observed that mice with deficiencies in both NCF1 and FCGR2B developed more severe disease than those with either single defect, indicating that these two genes regulate tolerance through different mechanisms.

In conclusion, our study establishes a new autologous model for RA, which enables tracking of the autoreactive MHCII-restricted T cell response to COL2. Using this model, we demonstrated the critical roles of NCF1 and FCGR2B in regulating T cell tolerance, enhancing our understanding of their regulatory functions in the context of RA.

4.3 STUDY III: NCF4-dependent intracellular reactive oxygen species regulate plasma cell formation.

NCF4 is generally considered to be less critical than NCF1 for the function of the NOX2 complex, and its deficiency usually leads to milder symptoms of CGD and lower proinflammatory responses (83,134). As a result, NCF4 has not received as much research attention. Despite this, several *NCF4* defects have been linked to RA, suggesting a unique role of NCF4 in the development of autoimmunity (112,113).

To investigate the distinct role of NCF4, we analyzed the pattern of ROS production in cells carrying the *Ncf4*^{R58A} mutation, which has been shown to cause severe CIA without affecting the function of NCF1 or NCF2 (83). Consistently, we observed no alteration in the expression of NCF1, NCF2, or NCF4 due to the mutation. Moreover, we found that NCF4-deficient neutrophils and B cells exhibited decreased intracellular ROS production, while extracellular ROS production remained unaffected. This contrasts with the *Ncf1*^{m1j} mutation, which leads to both intracellular and extracellular ROS deficiencies. Therefore, the *Ncf4*^{R58A} mutation offers a useful tool for studying the regulatory effect of NCF4-dependent intracellular ROS

specifically. We also found that the severity of CIA was dose-dependent, with one copy of the mutation resulting in milder disease and less impact on ROS production.

In a previous study on *Ncf1*^{m1j} mice, enhanced antigen presentation and autoreactive T cell activation were observed. In contrast, *Ncf4*^{58A} mice, which had normal extracellular ROS production, did not show similar effects. One possible explanation for this difference is that the TCR signaling cascade may be regulated by extracellular ROS secreted by APCs into the immunological synapse (157). Although the *Ncf4*^{58A} mutation did not affect T cells, it did promote the formation of antibody-secreting cells (ASCs), resulting in higher levels of autoreactive COL2-specific antibodies in the immunized mice. These results suggest that NCF4-dependent ROS primarily modulate arthritis by regulating the activation and differentiation of B cells.

The NOX2 complex is activated in B cells upon antigen binding during two possible occasions: extrafollicular activation and germinal center reaction. In the CIA mice, we observed increased plasma cells two weeks after immunization, indicating that these plasma cells were more likely to have been formed via a germinal center response. In contrast, extrafollicular responses, which produce a relatively small number of short-lived plasmablasts within 4-6 days post immunization, are less likely to be responsible (192,193). The sustained higher levels of isotype-switched anti-COL2 antibodies and larger numbers of ASCs in the synovium of *Ncf4*^{58A} mice in the chronic phase of CIA also suggest a regulatory effect of NCF4-dependent ROS in the germinal center reaction, although only a minor difference was observed in germinal center formation.

The anti-COL2 antibodies target a series of highly conserved epitopes in the triple-helical part of COL2 in rodents and primates (194). Autoantibodies produced in response to certain conformational COL2 epitopes, such as $COL2_{359\text{-}369}$, have been proven to be arthritogenic in CIA and RA (195). Interestingly, autoreactive anti-$COL2_{359\text{-}369}$ B cells are not deleted or anergized in healthy mice; instead, they are positively selected during development, indicating a regulatory function (196). However, during B cell activation and differentiation, dysregulated pathways may cause these regulatory B cells to transition into pathogenic ASCs, which can be a process sensitive to redox regulation (197). Additionally, we have found that the number of ASCs producing anti-nucleosome and anti-dsDNA antibodies was also higher in *Ncf4*^{58A} mice at the early stage of PIL, suggesting that ROS regulation is not limited to the formation of cartilage-specific ASCs.

In addition to increased formation, the migration of plasma cells to inflamed joints also contributes to the severity and chronicity of arthritis. In RA, alterations in the expression of chemokine receptors on B cells and plasma cells have been noted (198), including CXCR3 and CXCR4, which drive ASCs to inflammatory sites and bone marrow, respectively (199,200). Our study found that the expression pattern of CXCR3 and CXCR4 on B cells and ASCs was modulated intrinsically by NCF4-dependent intracellular ROS, possibly through the BCR and/or TLR4 pathway.

In summary, our findings highlight the regulatory role of intrinsically produced NCF4-dependent ROS by B cells in plasma cell formation and migration. These results advance our understanding of the contribution of ROS to autoimmune responses and the production of autoantibodies.

5 CONCLUSIONS

At a physiological level, NOX2-derived ROS act as second messengers, contributing to multiple aspects of the complex immunoregulatory network. Deficiency in ROS production due to genetic variants in the subunits of the NOX2 complex promotes the development of autoimmune diseases by dysregulating innate and adaptive immune cells. Specifically, Study I describes the regulatory role of intrinsic NCF1-dependent ROS in pDC development and functions, as well as their protective effects against lupus. Study II addresses the importance of functional NCF1 in T cell tolerance, providing protection against arthritis. Study III focuses on the regulation of NCF4-dependent intracellular ROS in plasma cell formation in the context of both arthritis and lupus. Together, these studies demonstrate the regulatory roles of NOX2-derived ROS in a series of autoimmune events. Further insights into these processes may provide additional opportunities for therapeutic intervention.

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7 REFERENCES

1. Takeuchi O, Akira S. Pattern Recognition Receptors and Inflammation. Cell. 2010 Mar;140(6):805–20.
2. Nagamine K, Peterson P, Scott HS, Kudoh J, Minoshima S, Heino M, et al. Positional cloning of the APECED gene. Nat Genet. 1997 Dec;17(4):393–8.
3. Gambineri E, Torgerson TR, Ochs HD. Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis: Curr Opin Rheumatol. 2003 Jul;15(4):430–5.
4. Wang L, Wang FS, Gershwin ME. Human autoimmune diseases: a comprehensive update. J Intern Med. 2015 Oct;278(4):369–95.
5. Aslani S, Rezaei R, Jamshidi A, Mahmoudi M. Genetic and epigenetic etiology of autoimmune diseases: lessons from twin studies. Rheumatol Res. 2018 Apr 1;3(2):45–57.
6. Ruff WE, Greiling TM, Kriegel MA. Host–microbiota interactions in immune-mediated diseases. Nat Rev Microbiol. 2020 Sep;18(9):521–38.
7. Theofilopoulos AN, Kono DH, Baccala R. The multiple pathways to autoimmunity. Nat Immunol. 2017 Jul;18(7):716–24.
8. Barber MRW, Drenkard C, Falasinnu T, Hoi A, Mak A, Kow NY, et al. Global epidemiology of systemic lupus erythematosus. Nat Rev Rheumatol. 2021 Sep;17(9):515–32.
9. Scherlinger M, Mertz P, Sagez F, Meyer A, Felten R, Chatelus E, et al. Worldwide trends in all-cause mortality of auto-immune systemic diseases between 2001 and 2014. Autoimmun Rev. 2020 Jun;19(6):102531.
10. Carter EE, Barr SG, Clarke AE. The global burden of SLE: prevalence, health disparities and socioeconomic impact. Nat Rev Rheumatol. 2016 Oct;12(10):605–20.
11. Yen EY, Singh RR. Brief Report: Lupus-An Unrecognized Leading Cause of Death in Young Females: A Population-Based Study Using Nationwide Death Certificates, 2000-2015. Arthritis Rheumatol. 2018 Aug;70(8):1251–5.
12. Cojocaru M, Cojocaru IM, Silosi I, Vrabie CD. Manifestations of systemic lupus erythematosus. Maedica. 2011 Oct;6(4):330–6.
13. Zoma A. Musculoskeletal involvement in systemic lupus erythematosus. Lupus. 2004 Nov;13(11):851–3.
14. Sawada T, Fujimori D, Yamamoto Y. Systemic lupus erythematosus and immunodeficiency. Immunol Med. 2019 Jan 2;42(1):1–9.
15. Frazzei G, van Vollenhoven RF, de Jong BA, Siegelaar SE, van Schaardenburg D. Preclinical Autoimmune Disease: a Comparison of Rheumatoid Arthritis, Systemic Lupus Erythematosus, Multiple Sclerosis and Type 1 Diabetes. Front Immunol. 2022 Jun 30;13:899372.
16. Lu R, Munroe ME, Guthridge JM, Bean KM, Fife DA, Chen H, et al. Dysregulation of innate and adaptive serum mediators precedes systemic lupus erythematosus classification and improves prognostic accuracy of autoantibodies. J Autoimmun. 2016 Nov;74:182–93.
17. Fanouriakis A, Tziolos N, Bertsias G, Boumpas DT. Update on the diagnosis and management of systemic lupus erythematosus. Ann Rheum Dis. 2021 Jan;80(1):14–25.
18. Tsokos GC. Systemic Lupus Erythematosus. N Engl J Med. 2011 Dec;365(22):2110–21.
19. Schwartz MM. The Pathology of Lupus Nephritis. Semin Nephrol. 2007 Jan;27(1):22–34.
20. Hoffman R. T cells in the pathogenesis of systemic lupus erythematosus. Clin Immunol. 2004 Oct;113(1):4–13.

21. Jacobi AM, Zhang J, Mackay M, Aranow C, Diamond B. Phenotypic Characterization of Autoreactive B Cells—Checkpoints of B Cell Tolerance in Patients with Systemic Lupus Erythematosus. PLoS One. 2009 Jun 2;4(6):e5776.
22. Moulton VR, Suarez-Fueyo A, Meidan E, Li H, Mizui M, Tsokos GC. Pathogenesis of Human Systemic Lupus Erythematosus: A Cellular Perspective. Trends Mol Med. 2017 Jul;23(7):615–35.
23. Ma K, Du W, Wang X, Yuan S, Cai X, Liu D, et al. Multiple Functions of B Cells in the Pathogenesis of Systemic Lupus Erythematosus. Int J Mol Sci. 2019 Nov 29;20(23):6021.
24. Rönnblom L, Alm GV. A Pivotal Role for the Natural Interferon α–producing Cells (Plasmacytoid Dendritic Cells) in the Pathogenesis of Lupus. J Exp Med. 2001 Dec 17;194(12):F59–64.
25. Kuo CF, Grainge MJ, Valdes AM, See LC, Luo SF, Yu KH, et al. Familial Aggregation of Systemic Lupus Erythematosus and Coaggregation of Autoimmune Diseases in Affected Families. JAMA Intern Med. 2015 Sep 1;175(9):1518.
26. Generali E, Ceribelli A, Stazi MA, Selmi C. Lessons learned from twins in autoimmune and chronic inflammatory diseases. J Autoimmun. 2017 Sep;83:51–61.
27. Lo MS. Monogenic Lupus. Curr Rheumatol Rep. 2016 Dec;18(12):71.
28. Teruel M, Alarcón-Riquelme ME. The genetic basis of systemic lupus erythematosus: What are the risk factors and what have we learned. J Autoimmun. 2016 Nov;74:161–75.
29. Kwon, Chun, Kim, Mak. Update on the Genetics of Systemic Lupus Erythematosus: Genome-Wide Association Studies and Beyond. Cells. 2019 Sep 30;8(10):1180.
30. Bentham J, Morris DL, Cunninghame Graham DS, Pinder CL, Tombleson P, Behrens TW, et al. Genetic association analyses implicate aberrant regulation of innate and adaptive immunity genes in the pathogenesis of systemic lupus erythematosus. Nat Genet. 2015 Dec;47(12):1457–64.
31. Zhong J, Olsson LM, Urbonaviciute V, Yang M, Bäckdahl L, Holmdahl R. Association of NOX2 subunits genetic variants with autoimmune diseases. Free Radic Biol Med. 2018 Sep;125:72–80.
32. Skopelja-Gardner S, An J, Tai J, Tanaka L, Sun X, Hermanson P, et al. The early local and systemic Type I interferon responses to ultraviolet B light exposure are cGAS dependent. Sci Rep. 2020 May 13;10(1):7908.
33. Vaglio A, Grayson PC, Fenaroli P, Gianfreda D, Boccaletti V, Ghiggeri GM, et al. Drug-induced lupus: Traditional and new concepts. Autoimmun Rev. 2018 Sep;17(9):912–8.
34. Gustafsson JT, Gunnarsson I, Källberg H, Pettersson S, Zickert A, Vikerfors A, et al. Cigarette smoking, antiphospholipid antibodies and vascular events in Systemic Lupus Erythematosus. Ann Rheum Dis. 2015 Aug;74(8):1537–43.
35. Barbhaiya M, Tedeschi SK, Lu B, Malspeis S, Kreps D, Sparks JA, et al. Cigarette smoking and the risk of systemic lupus erythematosus, overall and by anti-double stranded DNA antibody subtype, in the Nurses' Health Study cohorts. Ann Rheum Dis. 2018 Feb;77(2):196–202.
36. Fanouriakis A, Kostopoulou M, Alunno A, Aringer M, Bajema I, Boletis JN, et al. 2019 update of the EULAR recommendations for the management of systemic lupus erythematosus. Ann Rheum Dis. 2019 Jun;78(6):736–45.
37. Manzi S, Sánchez-Guerrero J, Merrill JT, Furie R, Gladman D, Navarra SV, et al. Effects of belimumab, a B lymphocyte stimulator-specific inhibitor, on disease activity across multiple organ domains in patients with systemic lupus erythematosus: combined results from two phase III trials. Ann Rheum Dis. 2012 Nov;71(11):1833–8.

38. Gross JA, Johnston J, Mudri S, Enselman R, Dillon SR, Madden K, et al. TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. Nature. 2000 Apr;404(6781):995–9.
39. Zagury D, Le Buanec H, Mathian A, Larcier P, Burnett R, Amoura Z, et al. IFNα kinoid vaccine-induced neutralizing antibodies prevent clinical manifestations in a lupus flare murine model. Proc Natl Acad Sci U S A. 2009 Mar 31;106(13):5294–9.
40. Haas KM, Watanabe R, Matsushita T, Nakashima H, Ishiura N, Okochi H, et al. Protective and Pathogenic Roles for B Cells during Systemic Autoimmunity in NZB/W F1 Mice. J Immunol. 2010 May 1;184(9):4789–800.
41. Mihara M, Tan I, Chuzhin Y, Reddy B, Budhai L, Holzer A, et al. CTLA4Ig inhibits T cell–dependent B-cell maturation in murine systemic lupus erythematosus. J Clin Invest. 2000 Jul 1;106(1):91–101.
42. Rowland SL, Riggs JM, Gilfillan S, Bugatti M, Vermi W, Kolbeck R, et al. Early, transient depletion of plasmacytoid dendritic cells ameliorates autoimmunity in a lupus model. J Exp Med. 2014 Sep 22;211(10):1977–91.
43. Reeves WH, Lee PY, Weinstein JS, Satoh M, Lu L. Induction of autoimmunity by pristane and other naturally occurring hydrocarbons. Trends Immunol. 2009 Sep;30(9):455–64.
44. Satoh M, Kumar A, Kanwar YS, Reeves WH. Anti-nuclear antibody production and immune-complex glomerulonephritis in BALB/c mice treated with pristane. Proc Natl Acad Sci U S A. 1995 Nov 21;92(24):10934–8.
45. Lee PY, Kumagai Y, Li Y, Takeuchi O, Yoshida H, Weinstein J, et al. TLR7-dependent and FcγR-independent production of type I interferon in experimental mouse lupus. J Exp Med. 2008 Dec 22;205(13):2995–3006.
46. Yokogawa M, Takaishi M, Nakajima K, Kamijima R, Fujimoto C, Kataoka S, et al. Epicutaneous Application of Toll-like Receptor 7 Agonists Leads to Systemic Autoimmunity in Wild-Type Mice: A New Model of Systemic Lupus Erythematosus: Cutaneous TLR-7 Activation Leads to SLE in Wild-Type Mice. Arthritis Rheumatol. 2014 Mar;66(3):694–706.
47. Morris SC, Cohen PL, Eisenberg RA. Experimental induction of systemic lupus erythematosus by recognition of foreign Ia. Clin Immunol Immunopathol. 1990 Nov;57(2):263–73.
48. Minichiello E, Semerano L, Boissier MC. Time trends in the incidence, prevalence, and severity of rheumatoid arthritis: A systematic literature review. Joint Bone Spine. 2016 Dec;83(6):625–30.
49. Markusse IM, Akdemir G, Dirven L, Goekoop-Ruiterman YPM, van Groenendael JHLM, Han KH, et al. Long-Term Outcomes of Patients With Recent-Onset Rheumatoid Arthritis After 10 Years of Tight Controlled Treatment: A Randomized Trial. Ann Intern Med. 2016 Apr 19;164(8):523.
50. Matcham F, Scott IC, Rayner L, Hotopf M, Kingsley GH, Norton S, et al. The impact of rheumatoid arthritis on quality-of-life assessed using the SF-36: A systematic review and meta-analysis. Semin Arthritis Rheum. 2014 Oct;44(2):123–30.
51. Cojocaru M, Cojocaru IM, Silosi I, Vrabie CD, Tanasescu R. Extra-articular Manifestations in Rheumatoid Arthritis. Maedica. 2010 Dec;5(4):286–91.
52. Ge C, Holmdahl R. The structure, specificity and function of anti-citrullinated protein antibodies. Nat Rev Rheumatol. 2019 Aug;15(8):503–8.
53. Salmon M, Scheel-Toellner D, Huissoon AP, Pilling D, Shamsadeen N, Hyde H, et al. Inhibition of T cell apoptosis in the rheumatoid synovium. J Clin Invest. 1997 Feb 1;99(3):439–46.
54. Liu Y, Aryee MJ, Padyukov L, Fallin MD, Hesselberg E, Runarsson A, et al. Epigenome-wide association data implicate DNA methylation as an intermediary of genetic risk in rheumatoid arthritis. Nat Biotechnol. 2013 Feb;31(2):142–7.

55. Crowson CS, Matteson EL, Myasoedova E, Michet CJ, Ernste FC, Warrington KJ, et al. The lifetime risk of adult-onset rheumatoid arthritis and other inflammatory autoimmune rheumatic diseases. Arthritis Rheum. 2011 Mar;63(3):633–9.
56. Padyukov L. Genetics of rheumatoid arthritis. Semin Immunopathol. 2022 Jan;44(1):47–62.
57. Petrovská N, Prajzlerová K, Vencovský J, Šenolt L, Filková M. The pre-clinical phase of rheumatoid arthritis: From risk factors to prevention of arthritis. Autoimmun Rev. 2021 May;20(5):102797.
58. van der Helm-van Mil AHM, Verpoort KN, Breedveld FC, Huizinga TWJ, Toes REM, de Vries RRP. The HLA–DRB1 shared epitope alleles are primarily a risk factor for anti–cyclic citrullinated peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis. Arthritis Rheum. 2006 Apr;54(4):1117–21.
59. Terao C, Brynedal B, Chen Z, Jiang X, Westerlind H, Hansson M, et al. Distinct HLA Associations with Rheumatoid Arthritis Subsets Defined by Serological Subphenotype. Am J Hum Genet. 2019 Sep;105(3):616–24.
60. Vignal C, Bansal AT, Balding DJ, Binks MH, Dickson MC, Montgomery DS, et al. Genetic association of the major histocompatibility complex with rheumatoid arthritis implicates two non-DRB1 loci. Arthritis Rheum. 2009 Jan;60(1):53–62.
61. Sugiyama D, Nishimura K, Tamaki K, Tsuji G, Nakazawa T, Morinobu A, et al. Impact of smoking as a risk factor for developing rheumatoid arthritis: a meta-analysis of observational studies. Ann Rheum Dis. 2010 Jan;69(01):70–81.
62. Bellatin MF, Han M, Fallena M, Fan L, Xia D, Olsen N, et al. Production of Autoantibodies against Citrullinated Antigens/Peptides by Human B Cells. J Immunol. 2012 Apr 1;188(7):3542–50.
63. Liu X, Tedeschi SK, Barbhaiya M, Leatherwood CL, Speyer CB, Lu B, et al. Impact and Timing of Smoking Cessation on Reducing Risk of Rheumatoid Arthritis Among Women in the Nurses' Health Studies. Arthritis Care Res. 2019 Jul;71(7):914–24.
64. Bello-Gualtero JM, Lafaurie GI, Hoyos LX, Castillo DM, De-Avila J, Munevar JC, et al. Periodontal Disease in Individuals With a Genetic Risk of Developing Arthritis and Early Rheumatoid Arthritis: A Cross-Sectional Study. J Periodontol. 2016 Apr;87(4):346–56.
65. Wooley PH, Luthra HS, Griffiths MM, Stuart JM, Huse A, David CS. Type II collagen-induced arthritis in mice. IV. Variations in immunogenetic regulation provide evidence for multiple arthritogenic epitopes on the collagen molecule. J Immunol. 1985 Oct 1;135(4):2443–51.
66. Vingsbo C, Sahlstrand P, Brun JG, Jonsson R, Saxne T, Holmdahl R. Pristane-induced arthritis in rats: a new model for rheumatoid arthritis with a chronic disease course influenced by both major histocompatibility complex and non-major histocompatibility complex genes. Am J Pathol. 1996 Nov;149(5):1675–83.
67. Gauldie SD, McQueen DS, Clarke CJ, Chessell IP. A robust model of adjuvant-induced chronic unilateral arthritis in two mouse strains. J Neurosci Methods. 2004 Oct;139(2):281–91.
68. Diarra D, Stolina M, Polzer K, Zwerina J, Ominsky MS, Dwyer D, et al. Dickkopf-1 is a master regulator of joint remodeling. Nat Med. 2007 Feb;13(2):156–63.
69. Nandakumar K, Holmdahl R. Antibody-induced arthritis: disease mechanisms and genes involved at the effector phase of arthritis. Arthritis Res Ther. 2006;8(6):223.
70. Monach PA, Mathis D, Benoist C. The K/BxN Arthritis Model. Curr Protoc Immunol. 2008 May;81(1):15–22.
71. Pizzolla A, Laulund F, Wing K, Holmdahl R. A new model of arthritis induced by a glucose-6-phosphate isomerase peptide: immunological requirements and peptide characterisation. Ann Rheum Dis. 2012 Feb;71(Suppl 1):A83.2-A83.

72. Schinnerling K, Rosas C, Soto L, Thomas R, Aguillón JC. Humanized Mouse Models of Rheumatoid Arthritis for Studies on Immunopathogenesis and Preclinical Testing of Cell-Based Therapies. Front Immunol. 2019 Feb 19;10:203.
73. Di Meo S, Reed TT, Venditti P, Victor VM. Role of ROS and RNS Sources in Physiological and Pathological Conditions. Oxid Med Cell Longev. 2016;2016:1–44.
74. Han D, Williams E, Cadenas E. Mitochondrial respiratory chain-dependent generation of superoxide anion and its release into the intermembrane space. Biochem J. 2001 Jan 15;353(2):411–6.
75. Ballinger SW, Patterson C, Yan CN, Doan R, Burow DL, Young CG, et al. Hydrogen Peroxide– and Peroxynitrite-Induced Mitochondrial DNA Damage and Dysfunction in Vascular Endothelial and Smooth Muscle Cells. Circ Res. 2000 May 12;86(9):960–6.
76. Mohan N, Meltz ML. Induction of Nuclear Factor κB after Low-Dose Ionizing Radiation Involves a Reactive Oxygen Intermediate Signaling Pathway. Radiat Res. 1994 Oct;140(1):97.
77. Royer-Pokora B, Kunkel LM, Monaco AP, Goff SC, Newburger PE, Baehner RL, et al. Cloning the gene for an inherited human disorder—chronic granulomatous disease—on the basis of its chromosomal location. Nature. 1986 Jul;322(6074):32–8.
78. Pick E. Role of the Rho GTPase Rac in the activation of the phagocyte NADPH oxidase: Outsourcing a key task. Small GTPases. 2014 Jan;5(1):e27952.
79. Belambri SA, Rolas L, Raad H, Hurtado-Nedelec M, Dang PMC, El-Benna J. NADPH oxidase activation in neutrophils: Role of the phosphorylation of its subunits. Eur J Clin Invest. 2018 Nov;48:e12951.
80. Honbou K, Minakami R, Yuzawa S, Takeya R, Suzuki NN, Kamakura S, et al. Full-length p40phox structure suggests a basis for regulation mechanism of its membrane binding. EMBO J. 2007 Feb 21;26(4):1176–86.
81. Song ZM, Bouchab L, Hudik E, Le Bars R, Nüsse O, Dupré-Crochet S. Phosphoinositol 3-phosphate acts as a timer for reactive oxygen species production in the phagosome. J Leukoc Biol. 2017 May 1;101(5):1155–68.
82. Stahelin RV, Burian A, Bruzik KS, Murray D, Cho W. Membrane binding mechanisms of the PX domains of NADPH oxidase p40phox and p47phox. J Biol Chem. 2003 Apr 18;278(16):14469–79.
83. Winter S, Hultqvist Hopkins M, Laulund F, Holmdahl R. A Reduction in Intracellular Reactive Oxygen Species Due to a Mutation in NCF4 Promotes Autoimmune Arthritis in Mice. Antioxid Redox Signal. 2016 20;25(18):983–96.
84. Das K, Roychoudhury A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Front Environ Sci. 2014 Dec 2;2:53.
85. Yang S, Lian G. ROS and diseases: role in metabolism and energy supply. Mol Cell Biochem. 2020 Apr;467(1–2):1–12.
86. Kelkka T, Kienhöfer D, Hoffmann M, Linja M, Wing K, Sareila O, et al. Reactive oxygen species deficiency induces autoimmunity with type 1 interferon signature. Antioxid Redox Signal. 2014 Dec 1;21(16):2231–45.
87. Vermot A, Petit-Härtlein I, Smith SME, Fieschi F. NADPH Oxidases (NOX): An Overview from Discovery, Molecular Mechanisms to Physiology and Pathology. Antioxidants. 2021 Jun 1;10(6):890.
88. Bridges RA. A Fatal Granulomatous Disease of Childhood: The Clinical, Pathological, and Laboratory Features of a New Syndrome. AMA J Dis Child. 1959 Apr 1;97(4):387.
89. Thomas DC, Charbonnier LM, Schejtman A, Aldhekri H, Coomber EL, Dufficy ER, et al. EROS/CYBC1 mutations: Decreased NADPH oxidase function and chronic granulomatous disease. J Allergy Clin Immunol. 2019 Feb;143(2):782-785.e1.

90. Marciano BE, Spalding C, Fitzgerald A, Mann D, Brown T, Osgood S, et al. Common Severe Infections in Chronic Granulomatous Disease. Clin Infect Dis. 2015 Apr 15;60(8):1176–83.
91. Kuhns DB, Alvord WG, Heller T, Feld JJ, Pike KM, Marciano BE, et al. Residual NADPH Oxidase and Survival in Chronic Granulomatous Disease. N Engl J Med. 2010 Dec 30;363(27):2600–10.
92. Kuhns DB, Hsu AP, Sun D, Lau K, Fink D, Griffith P, et al. NCF1 (p47phox)–deficient chronic granulomatous disease: comprehensive genetic and flow cytometric analysis. Blood Adv. 2019 Jan 22;3(2):136–47.
93. Yu HH, Yang YH, Chiang BL. Chronic Granulomatous Disease: a Comprehensive Review. Clin Rev Allergy Immunol. 2021 Oct;61(2):101–13.
94. van de Geer A, Nieto-Patlán A, Kuhns DB, Tool AT, Arias AA, Bouaziz M, et al. Inherited p40phox deficiency differs from classic chronic granulomatous disease. J Clin Invest. 2018 Aug 31;128(9):3957–75.
95. Sanmun D, Witasp E, Jitkaew S, Tyurina YY, Kagan VE, Åhlin A, et al. Involvement of a functional NADPH oxidase in neutrophils and macrophages during programmed cell clearance: implications for chronic granulomatous disease. Am J Physiol-Cell Physiol. 2009 Sep;297(3):C621–31.
96. Trevelin SC, Dos Santos CX, Ferreira RG, de Sá Lima L, Silva RL, Scavone C, et al. Apocynin and Nox2 regulate NF-κB by modifying thioredoxin-1 redox-state. Sci Rep. 2016 Oct 4;6(1):34581.
97. De Ravin SS, Naumann N, Cowen EW, Friend J, Hilligoss D, Marquesen M, et al. Chronic granulomatous disease as a risk factor for autoimmune disease. J Allergy Clin Immunol. 2008 Dec;122(6):1097–103.
98. Ryan BJ, Nissim A, Winyard PG. Oxidative post-translational modifications and their involvement in the pathogenesis of autoimmune diseases. Redox Biol. 2014;2:715–24.
99. Lood C, Blanco LP, Purmalek MM, Carmona-Rivera C, De Ravin SS, Smith CK, et al. Neutrophil extracellular traps enriched in oxidized mitochondrial DNA are interferogenic and contribute to lupus-like disease. Nat Med. 2016 Feb;22(2):146–53.
100. Elloumi N, Ben Mansour R, Marzouk S, Mseddi M, Fakhfakh R, Gargouri B, et al. Differential reactive oxygen species production of neutrophils and their oxidative damage in patients with active and inactive systemic lupus erythematosus. Immunol Lett. 2017 Apr;184:1–6.
101. Niwa Y, Sakane T, Shingu M, Miyachi Y. Role of stimulated neutrophils from patients with systemic lupus erythematosus in tissue injury, with special reference to serum factors and increased active oxygen species generated by neutrophils. Inflammation. 1985 Jun;9(2):163–72.
102. Mateen S, Moin S, Khan AQ, Zafar A, Fatima N. Increased Reactive Oxygen Species Formation and Oxidative Stress in Rheumatoid Arthritis. PLoS One. 2016 Apr 4;11(4):e0152925.
103. Jaswal S, Mehta HC, Sood AK, Kaur J. Antioxidant status in rheumatoid arthritis and role of antioxidant therapy. Clin Chim Acta. 2003 Dec;338(1–2):123–9.
104. Dos Santos M, Favero G, Bonomini F, Stacchiotti A, Rodella LF, Veronese FV, et al. Oral supplementation of melatonin protects against lupus nephritis renal injury in a pristane-induced lupus mouse model. Life Sci. 2018 Jan;193:242–51.
105. Marciano BE, Rosenzweig SD, Kleiner DE, Anderson VL, Darnell DN, Anaya-O'Brien S, et al. Gastrointestinal involvement in chronic granulomatous disease. Pediatrics. 2004 Aug;114(2):462–8.
106. Hultqvist M, Sareila O, Vilhardt F, Norin U, Olsson LM, Olofsson P, et al. Positioning of a polymorphic quantitative trait nucleotide in the Ncf1 gene controlling oxidative burst response and arthritis severity in rats. Antioxid Redox Signal. 2011 Jun 15;14(12):2373–83.

107. Zhong J, Li Q, Holmdahl R. Natural Loss-of-Function Mutations in Qa2 and NCF1 Cause the Spread of Mannan-Induced Psoriasis. J Invest Dermatol. 2021 Jul;141(7):1765-1771.e4.
108. Olsson LM, Johansson AC, Gullstrand B, Jonsen A, Saevarsdottir S, Ronnblom L, et al. A single nucleotide polymorphism in the NCF1 gene leading to reduced oxidative burst is associated with systemic lupus erythematosus. Ann Rheum Dis. 2017;76(9):1607–13.
109. Zhao J, Ma J, Deng Y, Kelly JA, Kim K, Bang SY, et al. A missense variant in NCF1 is associated with susceptibility to multiple autoimmune diseases. Nat Genet. 2017;49(3):433–7.
110. Olsson LM, Nerstedt A, Lindqvist AK, Johansson SC, Medstrand P, Olofsson P, et al. Copy number variation of the gene NCF1 is associated with rheumatoid arthritis. Antioxid Redox Signal. 2012;16(1):71–8.
111. Jacob CO, Eisenstein M, Dinauer MC, Ming W, Liu Q, John S, et al. Lupus-associated causal mutation in neutrophil cytosolic factor 2 (NCF2) brings unique insights to the structure and function of NADPH oxidase. Proc Natl Acad Sci U S A. 2012 Jan 10;109(2):359–60.
112. Olsson LM, Lindqvist AK, Källberg H, Padyukov L, Burkhardt H, Alfredsson L, et al. A case-control study of rheumatoid arthritis identifies an associated single nucleotide polymorphism in the NCF4 gene, supporting a role for the NADPH-oxidase complex in autoimmunity. Arthritis Res Ther. 2007;9(5):R98.
113. Zhang TP, Li R, Huang Q, Pan HF, Ye DQ, Li XM. Association of NCF2, NCF4, and CYBA Gene Polymorphisms with Rheumatoid Arthritis in a Chinese Population. J Immunol Res. 2020 Oct 20;2020:1–11.
114. Cunninghame Graham DS, Morris DL, Bhangale TR, Criswell LA, Syvänen AC, Rönnblom L, et al. Association of NCF2, IKZF1, IRF8, IFIH1, and TYK2 with systemic lupus erythematosus. PLoS Genet. 2011 Oct;7(10):e1002341.
115. Rioux JD, Xavier RJ, Taylor KD, Silverberg MS, Goyette P, Huett A, et al. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nat Genet. 2007 May;39(5):596–604.
116. Olofsson P, Holmberg J, Tordsson J, Lu S, Akerstrom B, Holmdahl R. Positional identification of Ncf1 as a gene that regulates arthritis severity in rats. Nat Genet. 2003;33(1):25–32.
117. Huang CK, Zhan L, Hannigan MO, Ai Y, Leto TL. P47phox-deficient NADPH oxidase defect in neutrophils of diabetic mouse strains, C57BL/6J-m db/db and db/+. J Leukoc Biol. 2000 Feb;67(2):210–5.
118. Hultqvist M, Olofsson P, Holmberg J, Backstrom BT, Tordsson J, Holmdahl R. Enhanced autoimmunity, arthritis, and encephalomyelitis in mice with a reduced oxidative burst due to a mutation in the Ncf1 gene. Proc Natl Acad Sci U S A. 2004;101(34):12646–51.
119. Kienhöfer D, Hahn J, Stoof J, Csepregi JZ, Reinwald C, Urbonaviciute V, et al. Experimental lupus is aggravated in mouse strains with impaired induction of neutrophil extracellular traps. JCI Insight. 2017 May 18;2(10):e92920.
120. Zhong J, Yau ACY, Holmdahl R. Independent and inter-dependent immunoregulatory effects of NCF1 and NOS2 in experimental autoimmune encephalomyelitis. J Neuroinflammation. 2020 Dec;17(1):113.
121. Zhong J, Scholz T, Yau ACY, Guerard S, Hüffmeier U, Burkhardt H, et al. Mannan-induced Nos2 in macrophages enhances IL-17–driven psoriatic arthritis by innate lymphocytes. Sci Adv. 2018 May 4;4(5):eaas9864.
122. Urbonaviciute V, Luo H, Sjöwall C, Bengtsson A, Holmdahl R. Low Production of Reactive Oxygen Species Drives Systemic Lupus Erythematosus. Trends Mol Med. 2019 Oct;25(10):826–35.

123. Görlach A, Lee PL, Roesler J, Hopkins PJ, Christensen B, Green ED, et al. A p47-phox pseudogene carries the most common mutation causing p47-phox- deficient chronic granulomatous disease. J Clin Invest. 1997 Oct 15;100(8):1907–18.
124. Cuscó I, Corominas R, Bayés M, Flores R, Rivera-Brugués N, Campuzano V, et al. Copy number variation at the 7q11.23 segmental duplications is a susceptibility factor for the Williams-Beuren syndrome deletion. Genome Res. 2008 May;18(5):683–94.
125. Roesler J, Curnutte JT, Rae J, Barrett D, Patino P, Chanock SJ, et al. Recombination events between the p47-phoxgene and its highly homologous pseudogenes are the main cause of autosomal recessive chronic granulomatous disease. Blood. 2000 Mar 15;95(6):2150–6.
126. Heyworth PG, Noack D, Cross AR. Identification of a novel NCF-1 (p47-phox) pseudogene not containing the signature GT deletion: significance for A47 degrees chronic granulomatous disease carrier detection. Blood. 2002 Sep 1;100(5):1845–51.
127. Shen K, Sergeant S, Hantgan RR, McPhail LC, Horita DA. Mutations in the PX–SH3A Linker of p47phox Decouple PI(3,4)P2 Binding from NADPH Oxidase Activation. Biochemistry. 2008 Aug 1;47(34):8855–65.
128. Groemping Y, Lapouge K, Smerdon SJ, Rittinger K. Molecular Basis of Phosphorylation-Induced Activation of the NADPH Oxidase. Cell. 2003 May;113(3):343–55.
129. Sareila O, Jaakkola N, Olofsson P, Kelkka T, Holmdahl R. Identification of a region in p47phox/NCF1 crucial for phagocytic NADPH oxidase (NOX2) activation. J Leukoc Biol. 2013 Mar 1;93(3):427–35.
130. Meng Y, Ma J, Yao C, Ye Z, Ding H, Liu C, et al. The NCF1 variant p.R90H aggravates autoimmunity by facilitating the activation of plasmacytoid dendritic cells. J Clin Invest. 2022 Aug 15;132(16):e153619.
131. Li XJ, Marchal CC, Stull ND, Stahelin RV, Dinauer MC. p47phox Phox homology domain regulates plasma membrane but not phagosome neutrophil NADPH oxidase activation. J Biol Chem. 2010 Nov 5;285(45):35169–79.
132. Wientjes FB, Hsuan JJ, Totty NF, Segal AW. p40phox, a third cytosolic component of the activation complex of the NADPH oxidase to contain src homology 3 domains. Biochem J. 1993 Dec 15;296(3):557–61.
133. Hong M, Ye BD, Yang SK, Jung S, Lee HS, Kim BM, et al. Immunochip Meta-Analysis of Inflammatory Bowel Disease Identifies Three Novel Loci and Four Novel Associations in Previously Reported Loci. J Crohns Colitis. 2018 May 25;12(6):730–41.
134. Matute JD, Arias AA, Wright NAM, Wrobel I, Waterhouse CCM, Li XJ, et al. A new genetic subgroup of chronic granulomatous disease with autosomal recessive mutations in p40phox and selective defects in neutrophil NADPH oxidase activity. Blood. 2009 Oct 8;114(15):3309–15.
135. Roberts RL, Hollis-Moffatt JE, Gearry RB, Kennedy MA, Barclay ML, Merriman TR. Confirmation of association of IRGM and NCF4 with ileal Crohn's disease in a population-based cohort. Genes Immun. 2008 Sep;9(6):561–5.
136. Ellson CD, Davidson K, Ferguson GJ, O'Connor R, Stephens LR, Hawkins PT. Neutrophils from p40phox−/− mice exhibit severe defects in NADPH oxidase regulation and oxidant-dependent bacterial killing. J Exp Med. 2006 Aug 7;203(8):1927–37.
137. Ellson C, Davidson K, Anderson K, Stephens LR, Hawkins PT. PtdIns3P binding to the PX domain of p40phox is a physiological signal in NADPH oxidase activation. EMBO J. 2006 Oct 4;25(19):4468–78.
138. Bagaitkar J, Huang J, Zeng MY, Pech NK, Monlish DA, Perez-Zapata LJ, et al. NADPH oxidase activation regulates apoptotic neutrophil clearance by murine macrophages. Blood. 2018 May 24;131(21):2367–78.

139. Geng L, Zhao J, Deng Y, Molano I, Xu X, Xu L, et al. Human SLE variant NCF1-R90H promotes kidney damage and murine lupus through enhanced Tfh2 responses induced by defective efferocytosis of macrophages. Ann Rheum Dis. 2021 Sep 23;81(2):255–67.
140. Hahn J, Euler M, Kilgus E, Kienhöfer D, Stoof J, Knopf J, et al. NOX2 mediates quiescent handling of dead cell remnants in phagocytes. Redox Biol. 2019 Sep;26:101279.
141. Duhlin A, Chen Y, Wermeling F, Sedimbi SK, Lindh E, Shinde R, et al. Selective Memory to Apoptotic Cell–Derived Self-Antigens with Implications for Systemic Lupus Erythematosus Development. J Immunol. 2016 Oct 1;197(7):2618–26.
142. Schnabel A, Csernok E, Isenberg DA, Mrowka C, Gross WL. Antineutrophil cytoplasmic antibodies in systemic lupus erythematosus. Arthritis Rheum. 1995 May;38(5):633–7.
143. Leffler J, Martin M, Gullstrand B, Tyden H, Lood C, Truedsson L, et al. Neutrophil Extracellular Traps That Are Not Degraded in Systemic Lupus Erythematosus Activate Complement Exacerbating the Disease. J Immunol. 2012 Apr 1;188(7):3522–31.
144. Hakkim A, Furnrohr BG, Amann K, Laube B, Abed UA, Brinkmann V, et al. Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. Proc Natl Acad Sci U S A. 2010 May 25;107(21):9813–8.
145. Remijsen Q, Berghe TV, Wirawan E, Asselbergh B, Parthoens E, De Rycke R, et al. Neutrophil extracellular trap cell death requires both autophagy and superoxide generation. Cell Res. 2011 Feb;21(2):290–304.
146. Schauer C, Janko C, Munoz LE, Zhao Y, Kienhöfer D, Frey B, et al. Aggregated neutrophil extracellular traps limit inflammation by degrading cytokines and chemokines. Nat Med. 2014 May;20(5):511–7.
147. Gelderman KA, Hultqvist M, Pizzolla A, Zhao M, Nandakumar KS, Mattsson R, et al. Macrophages suppress T cell responses and arthritis development in mice by producing reactive oxygen species. J Clin Invest. 2007;117(10):3020–8.
148. Shi J, van de Stadt LA, Levarht EWN, Huizinga TWJ, Hamann D, van Schaardenburg D, et al. Anti-carbamylated protein (anti-CarP) antibodies precede the onset of rheumatoid arthritis. Ann Rheum Dis. 2014 Apr;73(4):780–3.
149. Smallwood MJ, Nissim A, Knight AR, Whiteman M, Haigh R, Winyard PG. Oxidative stress in autoimmune rheumatic diseases. Free Radic Biol Med. 2018 Sep;125:3–14.
150. Pullaiah P, Suchitra MM, Siddhartha Kumar B. Protein carbonyls and protein thiols in rheumatoid arthritis. Int J Res Med Sci. 2018 Apr 25;6(5):1738.
151. Li P, Haque MA, Blum JS. Role of Disulfide Bonds in Regulating Antigen Processing and Epitope Selection. J Immunol. 2002 Sep 1;169(5):2444–50.
152. Yang M, Haase C, Viljanen J, Xu B, Ge C, Kihlberg J, et al. Cutting Edge: Processing of Oxidized Peptides in Macrophages Regulates T Cell Activation and Development of Autoimmune Arthritis. J Immunol. 2017 Dec 15;199(12):3937–42.
153. Benson RA, Patakas A, Conigliaro P, Rush CM, Garside P, McInnes IB, et al. Identifying the Cells Breaching Self-Tolerance in Autoimmunity. J Immunol. 2010 Jun 1;184(11):6378–85.
154. Rybicka JM, Balce DR, Khan MF, Krohn RM, Yates RM. NADPH oxidase activity controls phagosomal proteolysis in macrophages through modulation of the lumenal redox environment of phagosomes. Proc Natl Acad Sci U S A. 2010 Jun 8;107(23):10496–501.
155. Aravena O, Pesce B, Soto L, Orrego N, Sabugo F, Wurmann P, et al. Anti-TNF therapy in patients with rheumatoid arthritis decreases Th1 and Th17 cell populations and expands IFN-γ-producing NK cell and regulatory T cell subsets. Immunobiology. 2011 Dec;216(12):1256–63.

156. Sareila O, Hagert C, Rantakari P, Poutanen M, Holmdahl R. Direct Comparison of a Natural Loss-Of-Function Single Nucleotide Polymorphism with a Targeted Deletion in the Ncf1 Gene Reveals Different Phenotypes. PLoS One. 2015;10(11):e0141974.
157. Holmdahl R, Sareila O, Pizzolla A, Winter S, Hagert C, Jaakkola N, et al. Hydrogen Peroxide As an Immunological Transmitter Regulating Autoreactive T Cells. Antioxid Redox Signal. 2013 Apr 20;18(12):1463–74.
158. James J, Chen Y, Hernandez CM, Forster F, Dagnell M, Cheng Q, et al. Redox regulation of PTPN22 affects the severity of T-cell-dependent autoimmune inflammation. eLife. 2022 May 19;11:e74549.
159. Gelderman KA, Hultqvist M, Holmberg J, Olofsson P, Holmdahl R. T cell surface redox levels determine T cell reactivity and arthritis susceptibility. Proc Natl Acad Sci U S A. 2006 Aug 22;103(34):12831–6.
160. Kraaij MD, Savage NDL, van der Kooij SW, Koekkoek K, Wang J, van den Berg JM, et al. Induction of regulatory T cells by macrophages is dependent on production of reactive oxygen species. Proc Natl Acad Sci U S A. 2010 Oct 12;107(41):17686–91.
161. Sakaguchi N, Takahashi T, Hata H, Nomura T, Tagami T, Yamazaki S, et al. Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice. Nature. 2003 Nov;426(6965):454–60.
162. Raposo B, Merky P, Lundqvist C, Yamada H, Urbonaviciute V, Niaudet C, et al. T cells specific for post-translational modifications escape intrathymic tolerance induction. Nat Commun. 2018 Dec;9(1):353.
163. Snir O, Bäcklund J, Boström J, Andersson I, Kihlberg J, Buckner JH, et al. Multifunctional T cell reactivity with native and glycosylated type II collagen in rheumatoid arthritis. Arthritis Rheum. 2012 Aug;64(8):2482–8.
164. Rintisch C, Förster M, Holmdahl R. Detection of arthritis-susceptibility loci, including Ncf1, and variable effects of the major histocompatibility complex region depending on genetic background in rats. Arthritis Rheum. 2009 Feb;60(2):419–27.
165. Cachat J, Deffert C, Alessandrini M, Roux-Lombard P, Le Gouellec A, Stasia MJ, et al. Altered Humoral Immune Responses and IgG Subtypes in NOX2-Deficient Mice and Patients: A Key Role for NOX2 in Antigen-Presenting Cells. Front Immunol. 2018 Jul 11;9:1555.
166. Richard ML, Gilkeson G. Mouse models of lupus: what they tell us and what they don't. Lupus Sci Med. 2018 Jan;5(1):e000199.
167. Merino R, Fossati L, Izui S. The lupus-prone BXSB strain: the Yaa gene model of systemic lupus erythematosus. Springer Semin Immunopathol. 1992 Oct;14(2):141–57.
168. Corthay A, Johansson Å, Vestberg M, Holmdahl R. Collagen-induced arthritis development requires αβ T cells but not γδ T cells: studies with T cell-deficient (TCR mutant) mice. Int Immunol. 1999 Jul;11(7):1065–73.
169. Nandakumar KS, Bäcklund J, Vestberg M, Holmdahl R. Collagen type II (CII)-specific antibodies induce arthritis in the absence of T or B cells but the arthritis progression is enhanced by CII-reactive T cells. Arthritis Res Ther. 2004;6(6):R544-550.
170. Nandakumar KS, Holmdahl R. Collagen Antibody Induced Arthritis. In: Cope AP, editor. Arthritis Research. Totowa, NJ: Humana Press; 2007. p. 215–23.
171. Bengtsson AA, Sturfelt G, Truedsson L, Blomberg J, Alm G, Vallin H, et al. Activation of type I interferon system in systemic lupus erythematosus correlates with disease activity but not with antiretroviral antibodies. Lupus. 2000 Nov;9(9):664–71.
172. Zhou Z, Ma J, Xiao C, Han X, Qiu R, Wang Y, et al. Phenotypic and functional alterations of pDCs in lupus-prone mice. Sci Rep. 2016 Feb 16;6(1):20373.
173. Blomberg S, Eloranta ML, Cederblad B, Nordlind K, Alm GL, Rönnblom L. Presence of cutaneous interferon-a producing cells in patients with systemic lupus erythematosus. Lupus. 2001 Jul;10(7):484–90.

174. Båve U, Magnusson M, Eloranta ML, Perers A, Alm GV, Rönnblom L. FcγRIIa Is Expressed on Natural IFN-α-Producing Cells (Plasmacytoid Dendritic Cells) and Is Required for the IFN-α Production Induced by Apoptotic Cells Combined with Lupus IgG. J Immunol. 2003 Sep 15;171(6):3296–302.
175. Savarese E, Steinberg C, Pawar RD, Reindl W, Akira S, Anders HJ, et al. Requirement of toll-like receptor 7 for pristane-induced production of autoantibodies and development of murine lupus nephritis. Arthritis Rheum. 2008 Apr;58(4):1107–15.
176. Santiago-Raber ML, Dunand-Sauthier I, Wu T, Li QZ, Uematsu S, Akira S, et al. Critical role of TLR7 in the acceleration of systemic lupus erythematosus in TLR9-deficient mice. J Autoimmun. 2010 Jun;34(4):339–48.
177. Murayama G, Chiba A, Kuga T, Makiyama A, Yamaji K, Tamura N, et al. Inhibition of mTOR suppresses IFNα production and the STING pathway in monocytes from systemic lupus erythematosus patients. Rheumatology. 2020 Oct 1;59(10):2992–3002.
178. Baechler EC, Batliwalla FM, Karypis G, Gaffney PM, Ortmann WA, Espe KJ, et al. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proc Natl Acad Sci U S A. 2003 Mar 4;100(5):2610–5.
179. Furie RA, van Vollenhoven RF, Kalunian K, Navarra S, Romero-Diaz J, Werth VP, et al. Trial of Anti-BDCA2 Antibody Litifilimab for Systemic Lupus Erythematosus. N Engl J Med. 2022 Sep 8;387(10):894–904.
180. Hasni SA, Gupta S, Davis M, Poncio E, Temesgen-Oyelakin Y, Carlucci PM, et al. Phase 1 double-blind randomized safety trial of the Janus kinase inhibitor tofacitinib in systemic lupus erythematosus. Nat Commun. 2021 Jun 7;12(1):3391.
181. Wallace DJ, Furie RA, Tanaka Y, Kalunian KC, Mosca M, Petri MA, et al. Baricitinib for systemic lupus erythematosus: a double-blind, randomised, placebo-controlled, phase 2 trial. The Lancet. 2018 Jul;392(10143):222–31.
182. Werth VP, Fleischmann R, Robern M, Touma Z, Tiamiyu I, Gurtovaya O, et al. Filgotinib or lanraplenib in moderate to severe cutaneous lupus erythematosus: a phase 2, randomized, double-blind, placebo-controlled study. Rheumatology. 2022 May 30;61(6):2413–23.
183. Batsalova T, Dzhambazov B, Klaczkowska D, Holmdahl R. Mice Producing Less Reactive Oxygen Species Are Relatively Resistant to Collagen Glycopeptide Vaccination against Arthritis. J Immunol. 2010 Sep 1;185(5):2701–9.
184. Hultqvist M, Olofsson P, Gelderman KA, Holmberg J, Holmdahl R. A New Arthritis Therapy with Oxidative Burst Inducers. PLoS Med. 2006 Sep 12;3(9):e348.
185. Michaëlsson E, Andersson M, Holmdahl R, Engström Å. Identification of an immunodominant type-II collagen peptide recognized by T cells in H-2q mice: self tolerance at the level of determinant selection. Eur J Immunol. 1992 Jul;22(7):1819–25.
186. Malmstrom V, Michaelsson E, Burkhardt H, Mattsson R, Vuorio E, Holmdahl R. Systemic versus cartilage-specific expression of a type II collagen-specific T-cell epitope determines the level of tolerance and susceptibility to arthritis. Proc Natl Acad Sci U S A. 1996 Apr 30;93(9):4480–5.
187. Hultqvist M, Bäcklund J, Bauer K, Gelderman KA, Holmdahl R. Lack of reactive oxygen species breaks T cell tolerance to collagen type II and allows development of arthritis in mice. J Immunol Baltim Md 1950. 2007 Aug 1;179(3):1431–7.
188. Batsalova T, Dzhambazov B, Merky P, Bäcklund A, Bäcklund J. Breaking T cell tolerance against self-type II collagen in HLA-DR4 transgenic mice and development of autoimmune arthritis. Arthritis Rheum. 2010;1911–20.
189. Radstake TRDJ, Franke B, Wenink MH, Nabbe KCAM, Coenen MJH, Welsing P, et al. The functional variant of the inhibitory Fcγ receptor IIb (CD32B) is associated with the rate of radiologic joint damage and dendritic cell function in rheumatoid arthritis. Arthritis Rheum. 2006 Dec;54(12):3828–37.

190. Willcocks LC, Carr EJ, Niederer HA, Rayner TF, Williams TN, Yang W, et al. A defunctioning polymorphism in FCGR2B is associated with protection against malaria but susceptibility to systemic lupus erythematosus. Proc Natl Acad Sci U S A. 2010 Apr 27;107(17):7881–5.
191. Li F, Smith P, Ravetch JV. Inhibitory Fcγ Receptor Is Required for the Maintenance of Tolerance through Distinct Mechanisms. J Immunol. 2014 Apr 1;192(7):3021–8.
192. Smith KGC, Hewitson TD, Nossal GJV, Tarlinton DM. The phenotype and fate of the antibody-forming cells of the splenic foci. Eur J Immunol. 1996 Feb;26(2):444–8.
193. Di Niro R, Lee SJ, Vander Heiden JA, Elsner RA, Trivedi N, Bannock JM, et al. Salmonella Infection Drives Promiscuous B Cell Activation Followed by Extrafollicular Affinity Maturation. Immunity. 2015 Jul;43(1):120–31.
194. Lindh I, Snir O, Lönnblom E, Uysal H, Andersson I, Nandakumar K, et al. Type II collagen antibody response is enriched in the synovial fluid of rheumatoid joints and directed to the same major epitopes as in collagen induced arthritis in primates and mice. Arthritis Res Ther. 2014;16(4):R143.
195. Burkhardt H, Koller T, Engström Å, Nandakumar KS, Turnay J, Kraetsch HG, et al. Epitope-specific recognition of type II collagen by rheumatoid arthritis antibodies is shared with recognition by antibodies that are arthritogenic in collagen-induced arthritis in the mouse: Shared B Cell Epitopes in CIA and RA. Arthritis Rheum. 2002 Sep;46(9):2339–48.
196. Cao D, Khmaladze I, Jia H, Bajtner E, Nandakumar KS, Blom T, et al. Pathogenic Autoreactive B Cells Are Not Negatively Selected toward Matrix Protein Collagen II. J Immunol. 2011 Nov 1;187(9):4451–8.
197. Bertolotti M, Farinelli G, Galli M, Aiuti A, Sitia R. AQP8 transports NOX2-generated H2O2 across the plasma membrane to promote signaling in B cells. J Leukoc Biol. 2016 Nov 1;100(5):1071–9.
198. Henneken M, Dörner T, Burmester GR, Berek C. Differential expression of chemokine receptors on peripheral blood B cells from patients with rheumatoid arthritis and systemic lupus erythematosus. Arthritis Res Ther. 2005;7(5):R1001-1013.
199. Hauser AE, Debes GF, Arce S, Cassese G, Hamann A, Radbruch A, et al. Chemotactic Responsiveness Toward Ligands for CXCR3 and CXCR4 Is Regulated on Plasma Blasts During the Time Course of a Memory Immune Response. J Immunol. 2002 Aug 1;169(3):1277–82.
200. Hargreaves DC, Hyman PL, Lu TT, Ngo VN, Bidgol A, Suzuki G, et al. A Coordinated Change in Chemokine Responsiveness Guides Plasma Cell Movements. J Exp Med. 2001 Jul 2;194(1):45–56.