The Role of Plasmacytoid Dendritic Cells and Natural Killer Cells in Systemic Lupus Erythematosus

NIKLAS HAGBERG
Abstract

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by autoantibody production, which can eventually lead to immune complex (IC)-mediated organ damage. Due to the stimulation of plasmacytoid dendritic cells (pDC) by nucleic acid-containing ICs (DNA- or RNA-IC), patients with SLE have an ongoing interferon (IFN)-α production. IFN-α induces a general activation of the immune system that may initiate or propagate an autoimmune process if not properly regulated. Previous studies have shown that natural killer (NK) cells potently enhance the IFN-α production by pDCs.

In study I, the mechanisms behind the NK cell-mediated increased IFN-α production by RNA-IC-stimulated pDCs were investigated. ICs triggered CD56<sup>dim</sup> NK cells via FcγRIIIA to the secretion of cytokines (e.g. MIP-1β) that promoted IFN-α production. Additionally, an LFA-1-dependent cell-cell interaction between pDCs and NK cells strongly contributed to the increased production of IFN-α. In study II, the RNA-IC-induced regulation of surface molecules on pDCs and NK cells was investigated. The expression of CD319 and CD229, which are two SLAM family receptors genetically associated with SLE, was induced on pDCs and NK cells by RNA-IC. IFN-α-producing pDCs displayed an increased expression of CD319 and CD229, whereas pDCs from patients with SLE had a decreased expression of CD319. In study III, we serendipitously identified an SLE patient harboring autoantibodies to the NK cell receptor CD94/NKG2A. In study IV, sera from 203 patients with SLE were analyzed for autoantibodies to the CD94/NKG2A, CD94/NKG2C and NKG2D receptors. Seven patients harbored anti-CD94/NKG2A autoantibodies, and two of these patient’s autoantibodies also reacted with CD94/NKG2C. Anti-CD94/NKG2A and anti-CD94/NKG2C autoantibodies both interfered with the HLA-E-mediated regulation of NK cell cytotoxicity, and facilitated the elimination of target cells expressing these receptors. Furthermore, these autoantibodies were found in a group of severely diseased SLE patients and their titers closely followed disease activity.

In conclusion, this thesis provides insights to molecular mechanisms whereby NK cells regulate the IFN-α production, it further links the SLAM receptors to SLE, and it describes novel autoantibodies to receptors regulating NK cell cytotoxicity. Together these findings strengthen the assumption that NK cells are involved in the pathogenesis of SLE.

Keywords: Systemic lupus erythematosus, plasmacytoid dendritic cells, natural killer cells, type I interferon, immune complex, SLAM receptors, autoantibodies, CD94/NKG2A, CD94/NKG2C

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List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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Related Papers


### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ACR</td>
<td>American College of Rheumatology</td>
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<tr>
<td>ADCC</td>
<td>Antibody-dependent cellular cytotoxicity</td>
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<td>ANA</td>
<td>Anti-nuclear antibody</td>
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<td>BAFF</td>
<td>B cell activating factor</td>
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<td>BCR</td>
<td>B cell receptor</td>
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<tr>
<td>BDCA</td>
<td>Blood dendritic cell antigen</td>
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<tr>
<td>BTK</td>
<td>Bruton’s tyrosine kinase</td>
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<tr>
<td>CRACC</td>
<td>CD2-like receptor activating cytotoxic cells</td>
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<tr>
<td>DC</td>
<td>Dendritic cell</td>
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<tr>
<td>dsDNA</td>
<td>Double-stranded DNA</td>
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<tr>
<td>EAT-2</td>
<td>Ewing’s sarcoma-activated transcript 2</td>
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<tr>
<td>GAS</td>
<td>Interferon gamma-activated site</td>
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<tr>
<td>HSV</td>
<td>Herpes simplex virus</td>
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<tr>
<td>IC</td>
<td>Immune complex</td>
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<tr>
<td>IFN</td>
<td>Interferon</td>
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<td>IFNAR</td>
<td>Interferon alpha receptor</td>
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<td>IFNGR</td>
<td>Interferon gamma receptor</td>
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<tr>
<td>IFNLR</td>
<td>Interferon lambda receptor</td>
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<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>ILT</td>
<td>Immunoglobulin-like transcript</td>
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<tr>
<td>IRAK</td>
<td>Interleukin-1 receptor-associated kinase</td>
</tr>
<tr>
<td>IRF</td>
<td>Interferon regulatory factor</td>
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<tr>
<td>ISGF</td>
<td>Interferon-stimulated gene factor</td>
</tr>
<tr>
<td>ISRE</td>
<td>Interferon-stimulated response element</td>
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<tr>
<td>ITAM</td>
<td>Immunoreceptor tyrosine-based activation motif</td>
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<tr>
<td>ITIM</td>
<td>Immunoreceptor tyrosine-based inhibitory motif</td>
</tr>
<tr>
<td>JAK</td>
<td>Janus kinase</td>
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<tr>
<td>KIR</td>
<td>Killer cell immunoglobulin-like receptor</td>
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<tr>
<td>LAIR</td>
<td>Leukocyte-associated immunoglobulin-like receptor</td>
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<tr>
<td>LAMP</td>
<td>Lysosomal-associated membrane protein</td>
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<tr>
<td>LFA</td>
<td>Lymphocyte function-associated antigen</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>MAPK</td>
<td>Mitogen-activated protein kinase</td>
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<td>MHC</td>
<td>Major histocompatibility complex</td>
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<td>MIP</td>
<td>Macrophage inflammatory protein</td>
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<tr>
<td>MS</td>
<td>Multiple sclerosis</td>
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<tr>
<td>MyD88</td>
<td>Myeloid differentiation factor 88</td>
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<tr>
<td>NET</td>
<td>Neutrophil extracellular trap</td>
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<tr>
<td>NFκB</td>
<td>Nuclear factor-κB</td>
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<td>NIPC</td>
<td>Natural interferon producing cell</td>
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<td>NK</td>
<td>Natural killer</td>
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<tr>
<td>NZBxW</td>
<td>New Zealand Black/White</td>
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<tr>
<td>ODN</td>
<td>Oligodeoxynucleotide</td>
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<tr>
<td>PAMP</td>
<td>Pathogen-associated molecular pattern</td>
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<tr>
<td>PBMC</td>
<td>Peripheral blood mononuclear cell</td>
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<tr>
<td>pDC</td>
<td>Plasmacytoid dendritic cell</td>
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<tr>
<td>pSS</td>
<td>Primary Sjögren’s syndrome</td>
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<tr>
<td>RA</td>
<td>Rheumatoid arthritis</td>
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<tr>
<td>RNA-IC</td>
<td>RNA-containing immune complex</td>
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<tr>
<td>SAP</td>
<td>Signaling lymphocyte activating molecule-associated protein</td>
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<tr>
<td>SLAM</td>
<td>Signaling lymphocyte activating molecule</td>
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<td>SLE</td>
<td>Systemic lupus erythematosus</td>
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<td>SLEDAI</td>
<td>SLE disease activity index</td>
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<tr>
<td>SLICC</td>
<td>Systemic Lupus International Collaborating Clinics</td>
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<tr>
<td>snRNP</td>
<td>Small nuclear ribonucleoprotein</td>
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<tr>
<td>STAT</td>
<td>Signal transducer and activator of transcription</td>
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<tr>
<td>TCR</td>
<td>T cell receptor</td>
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<tr>
<td>TGF</td>
<td>Transforming growth factor</td>
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<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>Th</td>
<td>T helper cell</td>
</tr>
<tr>
<td>TRAF</td>
<td>TNF receptor-associated factor</td>
</tr>
<tr>
<td>TRAIL</td>
<td>TNF-related apoptosis-inducing ligand</td>
</tr>
<tr>
<td>Treg</td>
<td>T regulatory cell</td>
</tr>
<tr>
<td>TYK</td>
<td>Tyrosine kinase</td>
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<td>UV</td>
<td>Ultraviolet</td>
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Introduction

The immune system consists of a complex network of cells, which protects the organism against disease. In order to accomplish this, the immune system must recognize a wide array of pathogens, including viruses, bacteria and parasites. The immune system can be divided into the innate and the adaptive immune system. The innate immune system confers a broad non-specific protection against infectious disease by recognizing conserved structures of pathogens, such as pathogen-associated molecular patterns (PAMPs), or the absence of major histocompatibility complex (MHC) molecules, typically induced by some viral infections. In contrast, the adaptive immune system display antigen-specificity and is characterized by an immunological memory, which yields a faster and stronger immune response the next time it encounters the pathogen.

An important property of the immune system is to distinguish “self” from “non-self”, as failure to do so may result in an immune response to autoantigens, and finally an autoimmune disease. The autoimmune disorders can broadly be divided into organ specific diseases, for example diabetes mellitus type I, which affects the insulin-secreting pancreatic β cells, and systemic autoimmune diseases, such as systemic lupus erythematosus (SLE) and primary Sjögren’s (pSS) syndrome. In this thesis I have investigated the role of two innate immune cell types, plasmacytoid dendritic cells (pDC) and natural killer (NK) cells, in the pathogenesis of SLE.

Systemic lupus erythematosus

Clinical presentation and epidemiology

SLE, or lupus, is a chronic inflammatory disease with a heterogeneous clinical picture. The disease is characterized by autoantibodies to nuclear antigens and immune complex (IC) deposition in tissues that can trigger inflammatory damage in multiple organs, including muscle and joints, brain and peripheral nervous system, lungs, heart, kidneys, and skin. The severity of the disease can be quite variable, ranging from only mild skin or joint involvement to a severe life-threatening disease. There is no standard test to diagnose lupus. Instead, the diagnosis of lupus is based on a combination of clinical manifestations (e.g. involvement ≥2 organ system), laboratory find-
ings (e.g. presence of typical autoantibodies), and the exclusion of an alternative diagnosis explaining the symptoms. For research purposes, SLE is classified according to 11 different criteria from the American College of Rheumatology (ACR), where four of the criteria should be fulfilled in a patient to be diagnosed with lupus (Table 1) (1, 2). In order to improve the clinical relevance, a revised classification was proposed by the Systemic Lupus International Collaborating Clinics (SLICC) group in 2012 (3). This classification is based on 11 clinical and 6 immunologic criteria. In agreement with the ACR classification, 4 criteria should be met, but according to the SLICC rule, a patient must fulfill at least one clinical and one immunologic criterion to be diagnosed with lupus. Another important difference is that, regardless of whether 4 criteria are met, a biopsy-proven lupus nephritis together with anti-nuclear or anti-double-stranded DNA (dsDNA) antibodies, is sufficient for the classification of SLE. Because the studies in this thesis were initiated before 2012, all patients in these investigations were classified using the ACR criteria.

Table 1. The 1997 update of the 1982 ACR Classification Criteria for SLE

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Definition</th>
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<tr>
<td>Malar rash</td>
<td>A rash on the cheeks and nose, often in the shape of a butterfly</td>
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<tr>
<td>Discoid rash</td>
<td>A rash that appears as red, raised, disk-shaped patches</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>Skin rash after exposure to sun</td>
</tr>
<tr>
<td>Oral ulcers</td>
<td>Sores in the mouth</td>
</tr>
<tr>
<td>Arthritis</td>
<td>Pain and swelling of two or more joints</td>
</tr>
<tr>
<td>Serositis</td>
<td>Pleuritis or pericarditis</td>
</tr>
<tr>
<td>Kidney disorder</td>
<td>Persistent protein or cellular casts in the urine</td>
</tr>
<tr>
<td>Neurologic disorder</td>
<td>Seizures or psychosis, in the absence of other causes</td>
</tr>
<tr>
<td>Hematological disorder</td>
<td>Anemia, leukopenia, lymphopenia or thrombocytopenia</td>
</tr>
<tr>
<td>Immunologic disorder</td>
<td>Positive test for anti-dsDNA, anti-Sm or anti-Phospholipid-antibodies</td>
</tr>
<tr>
<td>Antinuclear antibodies</td>
<td>Positive antinuclear-antibody test</td>
</tr>
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</table>


The incidence and prevalence of SLE varies between different ethnicities. In the Northern European population approximately 5 out of 100,000 individual are diagnosed with lupus each year and the prevalence is estimated to be 68/100,000 (5). In the Afro-American population these figures are considerably higher (6). The disease predominantly affects women during their child bearing age, and a female/male ratio of 9:1 is observed among patients with SLE (6, 7). The mortality in patients with SLE has decreased significantly over the past decades, with a 5-year survival rate of less than 50% in
1955 (8) to a 10-year survival rate of ~80-90% today (9, 10). Nevertheless, patients with SLE still have a 2-5-fold increased mortality risk (11), which is mainly due to infections and cardiovascular diseases (12). The reason behind the increased survival can be manifold, but one important factor is the use of corticosteroids and other immunosuppressant drugs.

During the disease course, patients may have periods of disease exacerbation (flares) and periods with fewer symptoms (remission). There are several indices to evaluate the activity of the disease. The most widely used index is the SLE disease activity index (SLEDAI), which is a global index based on 24 weighted clinical and laboratory variables (13, 14). A disadvantage of the SLEDAI score is its inability to detect partial improvements, and therefore more sensitive composite indices, such as the SLEDAI-2000 Responder Index 50 (SRI-50), have recently been developed and are now used in clinical trials (15). To measure the long term impact of SLE the SLICC/ACR damage index, which assess the accumulated irreversible organ damage, can be used (16).

Etiology

Although the specific cause of SLE is unknown, multiple genetic, environmental, and epigenetic factors have been shown to contribute to the disease.

**Genetic factors**

The concordance rate of SLE in monozygotic twins is 25-40%, whereas for dizygotic twins it is only 2-5% (17). Thus, emphasizing a strong genetic component in SLE. The majority of the known genetic risk factors only confers a modest increased risk for SLE and is commonly found in the general population. To date, risk variants in more than 40 genes have been robustly associated to SLE (18). Most of the risk loci reside in immune related pathways (**Figure 1**) and similar to many other autoimmune diseases, gene variants in the MHC region display the strongest association to SLE (19, 20). Due to the tremendous advances in technologies for genetic analysis, the number of identified genetic risk variants has increased rapidly. However, in most cases, the causal variants and their functional effect on the disease remain to be determined.

In addition to the common risk gene variants, there are some rare, but highly penetrant mutations described. For instance, deficiencies in genes for classical complement components are an extremely strong genetic risk factor and >90% of individuals with a complete C1q-deficiency develop SLE (21). Other monogenic deficiencies that results in lupus like symptoms are mutations in the gene encoding the DNA degrading nuclease three prime repair exonuclease 1 (TREX1) (22, 23) or the tartrate resistant acid phosphatase 5 (TRAP) (24).
Environmental factors

Several environmental factors can contribute to the onset as well as the triggering of flares in SLE. One of the most prominent environmental factor is ultraviolet (UV)-light (i.e. sun exposure), which can trigger a systemic flare. The effect of UV-light is probably mediated through the induction of apoptosis in keratinocytes and the release of nuclear antigens which, as will be described below, can trigger an activation of the type I interferon (IFN) system (25).

Viral infections are common in SLE and have been suggested to trigger the disease. For instance, autoantibodies to certain Epstein Barr virus (EBV)-derived proteins cross-reacts with the lupus antigen Ro/SSA, and injection of this protein in mice induce lupus like symptoms (26). Besides molecular mimicry, bystander activation and epitope-spreading could also be potential mechanisms by which viral infections contribute to the disease (27). Other viruses that have been suggested to be linked to SLE are Parvovirus B19 and cytomegalovirus (CMV) (28).

Some case-control studies have identified smoking as a risk factor for SLE (29, 30), whereas other large population based studies have not been able to find such associations (31, 32). Moreover, certain dietary agents (e.g. alfalfa-sprouts) may exacerbate the disease (33).

Another important environmental factor is drugs. Drug-induced lupus was first described in 1945 following treatment with Sulphadiazine (34) and
to date more than 80 drugs are considered to induce a reversible lupus disease (35). The mechanisms behind drug-induced lupus are in many cases not clear, but some of the drugs that most frequently induce lupus (e.g. hydralazine, procainamide, and 5-azacytidine) are DNA de-methylating agents which alter the epigenetics (36).

**Epigenetic factors**
Epigenetics comprises the heritable changes in gene expression that is not caused by a change in DNA sequence (37). The most common forms of epigenetic modifications are DNA methylation and histone modifications. In contrast to germline mutations, epigenetic modifications differ between cell types and are reversible.

An important epigenetic alteration described in SLE is the hypomethylation of CD4+ T cells (38), which results in an increased expression of genes contributing to the formation of autoreactive T cells and an increased immunoglobulin (Ig)G production by B cells (39-42). Recent data suggest that microRNA may be involved in the altered methylation status of lupus T cells (43, 44).

**Sex**
The reason behind the strong predominance of lupus in females is not completely understood, but the X chromosome and sex hormones may be involved.

Several risk genes are located on the X chromosome and it is believed that an incomplete inactivation of the X chromosome could be one factor explaining the increased prevalence of SLE among women. In support of an X chromosome dosage effect, males who carry an extra X chromosome (XXY, Klinefelter’s syndrome) have a 14-fold increased risk of developing SLE compared to men carrying the normal XY chromosomes (45).

Sex hormones are potent modulator of the immune system, and their contribution to SLE has been suggested by the fact that the disease tends to worsen during pregnancy and to remit after menopause (46). The influence of sex hormones is also supported by data from murine models. In the lupus prone New Zealand Black/White (NZBxW F1) strain, female mice have a more severe lupus-like disease, but administration of estrogen to male mice exacerbates the disease to comparable levels as those seen in female mice (47).

To conclude, there are a large number of genetic, environmental, and epigenetic factors which acts in concert to trigger SLE.
Pathogenesis

Several different immune cells are involved in the disease process of SLE, and a central characteristic of the disease is the lack of tolerance and the production of autoantibodies.

Autoantibodies

The most prominent feature of SLE is an activation of B cells with production of autoantibodies to nuclear antigens (ANA). These autoantibodies typically recognize nucleic acids or nucleic acid-associated proteins, and such autoantibodies are found several years before the clinical onset of the disease (48). The presence of ANA is one of the diagnostic criteria for SLE, but ANAs are also detected in a wide range of rheumatic and infectious diseases, and even in healthy individuals. The ANAs can be divided into two groups, those that react with DNA- or DNA-containing autoantigens, and those that react with RNA-containing autoantigens.

Anti-dsDNA were the first autoantibodies described in SLE and such autoantibodies are detected in the majority of patients at some time of the disease (49). There are several observations suggesting a pathogenic role of anti-dsDNA autoantibodies. Passive transfer of human or murine anti-dsDNA antibodies to mice or rats results in a lupus-like glomerulonephritis (50, 51). Furthermore, the titers of anti-dsDNA autoantibodies are associated to disease activity (52) and the presence of nephritis (53) in human lupus.

Autoantibodies to RNA-binding proteins are enriched in circulating ICs from patients with SLE (SLE-IC) (54) and their presence is associated with an increased activation of the type I IFN system (55). Examples of autoantibodies that bind to RNA-binding proteins are: anti-Sm and anti-RNP antibodies that recognize small nuclear ribonucleoprotein (snRNP) complexes, anti-Ro/SSA, anti-La/SSB, and anti-tRNA-synthetase. In two of the studies presented in this thesis, the U1snRNP complex has been used as a model autoantigen. The properties of this complex are described in the “Materials and methods” section.

More than 100 different autoantibodies have been identified in patients with SLE (56). Most of these autoantibodies are only found in a minority of patients and are not specific for SLE. Besides the ANAs, autoantibodies targeting cytokines and plasma proteins (e.g. IFN-α (57, 58), B cell activating factor (BAFF) (59), C1q (60)) and receptors (e.g. CD4 (61), CD45 (62), TCR/CD3 (63), and FcγRs (64)) are described. Thus, it is possible that mechanisms involving a direct interference with cellular functions have a pathogenic effect.

Apoptosis

Apoptosis is the process of programmed cell death that naturally occurs to maintain the homeostasis and eliminate damaged cells (65). During apopto-
sis, nuclear proteins and nucleic acids are modified and clustered in blebs at the surface of the apoptotic cell. These apoptotic blebs contain known SLE autoantigens, such as chromatin, U1-RNP, Ro/SSA, and La/SSB (66). Normally, the apoptotic cells are rapidly phagocytized by macrophages and dendritic cells (DC), but in patients with SLE this process is disturbed. Due to an increased apoptosis (67, 68) and an impaired clearance of apoptotic material (69, 70), SLE patients display an increased load of apoptotic material. Accordingly, autoantibodies from SLE patients can form ICs with apoptotic debris that can deposit in tissues and cause inflammation or, as will be described below, induce IFN-α.

**Dysregulation of immune cells**

**B cells**

Given their role as antibody producers, a lot of research in SLE has been devoted to B cells. Patients with SLE have an increased proportion of circulating, antibody-secreting, plasma cells that correlates to disease activity (71). Central tolerance is a process where developing B and T cells that are autoreactive are depleted. Due to defective mechanisms in check-points for central B cell tolerance, SLE patients have an increased number of B cells that express an autoreactive B cell receptor (BCR) (72, 73). Together with the observed hyper-responsiveness to B cell receptor (BCR) stimulation in lupus patients (74), these factors govern the formation of autoantibodies. In addition, B cells also have functions that are independent of antibody secretion. These functions include antigen-presentation, costimulation of T cells, and the production of pro-inflammatory cytokines (e.g. interleukin (IL)-6, tumor necrosis factor (TNF)-α, and lymphotoxin-α) and immunosuppressive cytokines (e.g. transforming growth factor (TGF)-β and IL-10) (75). The contribution of antibody-independent mechanisms to lupus is suggested by data from murine models, where reconstitution of B cell-depleted lupus-prone mice (MRL/lpr) with B cells without the ability to secrete Ig, still leads to the development of a lupus-like disease. However, in comparison to mice reconstituted with IgG-secreting B cells, these mice develop a milder disease (76).

**T cells**

T cells have a central role in the cell-mediated immunity and several alterations in T cells from patients with SLE have been observed. For instance, T cells from lupus patients display an increased T cell receptor (TCR) signaling (77, 78). This increased sensitivity can be attributed to the pre-clustering of lipid rafts that overexpress adhesion and costimulatory molecules (79), as well as the “rewiring” of the TCR of SLE T cells. The latter term refers to the substitution of the TCR signaling TCRζ chain with the common γ chain of the immunoglobulin receptors (FeRγ) (80). T cells from
patients with SLE also display an impaired function of regulatory T cells (Treg) (81), which normally function to suppress the immune response of other cells. Furthermore, patients with SLE display a decreased activation-induced cell death of T cells (82). This process is essential in terminating the immune response after clearance of pathogens. Both the impaired function of Treg cells and the decreased activation-induced cell death could possibly be ascribed to a defective IL-2 production (83, 84). Together with the increased sensitivity of TCR stimulation, the compromised activation-induced cell death likely contributes to the persistence of activated T cells seen in SLE. In addition, SLE patients have an increased frequency of interleukin IL-17 producing T cells (85, 86), which infiltrate inflamed kidneys, skin, and lungs of patients with SLE (86, 87).

Innate immune cells
Alterations in the innate immune system of patients with SLE include the increased spontaneous apoptosis of monocytes and neutrophils (88), the impaired clearance of apoptotic cells by macrophages (69, 70), and the differentiation of monocytes into antigen-presenting DCs (89).

Several alterations are also found in pDCs and NK cells and they will be discussed in a separate section of the thesis.

Cytokines
Cytokines are small soluble proteins that activate or regulate the function of cells. Given their role in the initiation, propagation, and resolution of an immune response, it is clear that a dysregulation in the cytokine production or signaling can contribute to the autoimmune disease process.

The concentration of numerous cytokines are increased, or decreased in serum from patients with SLE. In addition to IL-2 and IL-17, which were mentioned above, these include: TNF-α (90), IFN-γ (91), BAFF (92), IL-6 (93), IL-10 (94), IL-12 (95), IL-18 (96), IL-21 (97), and IFN-α (98, 99).

TNF-α is a central upstream inducer of the inflammatory response, which is mainly produced by activated monocytes and macrophages (100). TNF-α inhibitors are successfully used to treat RA, but despite initial improvement of inflammatory symptoms in lupus patients, the occurrence of severe adverse events (101) have led to the termination of clinical trials with these drugs to treat SLE.

IFN-γ is produced by NK cells and T cells. This cytokine activates monocytes and stimulate cell-mediated immune responses, which promote tissue injury (102). Administration of IFN-γ to lupus prone mice (NZB/W F1) accelerates the disease (103), whereas deletion of the IFN-γ receptor prevents autoantibody production and glomerulonephritis in this model (104). However, the evidence for a role of IFN-γ in human lupus is not well documented.

BAFF is a cytokine produced by myeloid cells that act exclusively on B cells. The properties of BAFF include the induction of isotype switching,
the differentiation of B cells into plasma cells, and the survival of
B cells (105-107). In clinical trials, the anti-BAFF mAb belimumab was
shown to reduce the number of naïve and activated B cells and plasma cells,
without affecting the numbers of memory B cells, or T cells. Belimumab
treated patients also displayed a reduction of total IgG and autoantibody
titers, and a normalization of complement levels (108). Accordingly, as the
first new drug in more than 50 years, belimumab was recently approved for
the treatment of SLE.

IL-6 is a cytokine that affects both B cells and T cells. In B cells, an in-
creased survival, differentiation, and antibody-production is observed after
IL-6 stimulation (109). Concerning T cells, IL-6 promotes the differentia-
tion of T helper (Th)17 cells and suppresses the differentiation of Treg cells
(110). Inhibition of IL-6 ameliorates lupus symptoms and reduces the titers
of anti-dsDNA in the NZB/W model (111). A mAb against the IL-6 receptor
(toziluzumab) is approved for treating RA and clinical trials in SLE are on-
going (112).

Gene variants in IL-10 are associ ated to SLE (113), but the functional
consequence of these variants is not known. IL-10 potently suppress antigen
presenting cells through inhibiting the upregulation of MHC class II mole-
cules and costimulatory molecules. On the other hand, IL-10 also has pro-
inflammatory effects, such as costimulation, activation and survival of B
cells as well as activation of NK cells (114). Despite promising results in an
open-labeled pilot study of patients with SLE (115), no clinical trials with
mAbs targeting IL-10 are currently ongoing.

IL-12 and IL-18 are cytokines, which strongly activates NK cells and
T cells to IFN-γ production. In a murine model of lupus (MRL/lpr), inhibi-
tion of IL-18 resulted in a decreased mortality and reduced renal damage
(116). In patients with lupus nephritis, the expression of IL-18 is increased in
the glomeruli and IL-18 is proposed to be involved in the observed recruit-
ment of pDCs to the glomeruli of these patients (117). IL-18 is also abun-
dantly expressed in lesional skin from lupus patients (118).

IL-21 is one of the most recently described cytokines. It is produced by
T cells and promotes B cell proliferation and differentiation (119) as well as
Th17 differentiation (120). Gene variants of IL-21 are associated to human
SLE (121) and in experimental lupus models, IL-21 receptor knock-out mice
have a reduced disease severity (122).

IFN-α was the first cytokine which was shown to be increased in patients
with SLE (98). The increased concentration of IFN-α correlates to disease
activity (123) and patients with SLE have an increased expression of
type I IFN induced genes (termed a type I IFN signature) in peripheral blood
cells (124-126) and affected tissues (127). Several of the risk gene variants
identified in recent genome-wide association studies, resides in genes that
belong to the type I IFN system (128). Furthermore, a direct causative role
for IFN-α in the pathogenesis of SLE is supported by the fact that patients treated with IFN-α occasionally develop SLE (129-131).

Together these observations strongly suggest that the type I IFN system is central in the pathogenesis of SLE.

The interferons

The IFNs were first described in 1957 when Isaacs and Lindenmann discovered that incubation of tissue cultures with heat-inactivated viruses induced the release of soluble factor that interfered with viral replication (132).

By definition all IFNs have antiviral properties, but based on their structural features and receptor usage, the IFNs can be subgrouped into three different types:

- type I: IFN-α, IFN-β, IFN-ω, IFN-κ, IFN-ε
- type II: IFN-γ
- type III: IFN-λ

Of the type I IFNs, IFN-α is encoded by 13 highly homologous genes (IFNA1, 2, 4, 5, 6, 7, 8, 10, 13, 14, 16, 17, and 21). In contrast, IFN-β, IFN-ω, IFN-κ, and IFN-ε are encoded by one single gene each, and these genes display considerably lower homology to IFN-α (133).

The type II and type III IFNs display no, or very low, homology with the type I IFNs. The type II IFNs are encoded by one gene (IFNG), whereas the type III IFNs are encoded by 3 separate genes (IL-28A, IL-28B and IL-29). The different types of IFNs signal through three distinct receptors, the IFN-α receptor (IFNAR), the IFN-γ receptor (IFNGR) and the IFN-λ receptor (IFNLR). In this thesis, I have studied the regulation of IFN-α production by pDCs and therefore, the next section is focused on the type I IFN system.

The type I interferon system

**The interferon-α receptor**

All type I IFN subtypes signal through the ubiquitously expressed IFNAR. The IFNAR is a heterodimeric receptor, composed of the two subunits IFNAR1 and IFNAR2 (134). The canonical signaling pathway downstream of the IFNAR is mediated through two Janus kinases, tyrosine kinase 2 (TYK2) and janus kinase 1 (JAK1), which activates signal transducer and activator of transcription (STAT)1 and STAT2. Following phosphorylation, STAT1/STAT2-heterodimers translocate to the nucleus, where they together with interferon regulatory factor-9 (IRF9) form the transcription factor com-
plex interferon-stimulated gene factor 3 (ISGF3). This complex binds to IFN-stimulated response elements (ISRE) and initiates the transcription of >2000 type I IFN regulated genes (135, 136) (Figure 2). Similar to the IFNAR, the IFNLR also signals through the ISGF3 complex (137). However, due to the restrictive expression pattern of the IFNLR (138, 139), only 150 of the >2000 genes induced by IFN-α, are induced by IFN-λ (136).

IFNAR stimulation also results in the phosphorylation of STAT1 by JAK1 and JAK2. In this case, STAT1-homodimers translocate to the nucleus and bind to IFN-γ-activated sites (GAS) (135) (Figure 2). This signaling pathway is the same pathway as used by the IFNGR, and consequently, approximately 700 of the >2000 genes induced by IFN-γ are shared by IFN-α (136).

Figure 2. Signaling pathways of the interferon-α receptor.

**Immunological properties of the type I interferons**

The type I IFNs are important in protecting the organism from viruses. The direct antiviral effects of IFN-α are mediated through inhibition of viral replication and induction of apoptosis. IFN-α also have indirect antiviral properties, through its induction of antiviral proteins (e.g. MxA) (140) and the general activation of the immune system. In terms of immune activating effects, IFN-α promotes the differentiation of monocytes to antigen-presenting cells (89) and induce the expression of costimulatory molecules on DCs (133). The increased antigen-presenting and costimulatory ability of these cells leads to an increased activation of T cells. Concerning T cells, IFN-α promotes the polarization of Th cells to Th1 cells, suppress the differentiation into Treg cells and directly activates cytotoxic T cells (141). Furthermore,
IFN-α activates B cells to differentiate into antibody producing plasma cells (142) and enhance the cytotoxicity of NK cells (143) (Figure 3). This broad activation of the immune system is of course beneficial in terms of clearing pathogens. However, if the activation, or resolution, of the IFN-α driven immune response is not properly regulated, these effects may lead to an autoimmune process.

Although most cell types can produce small amounts of IFN-α, the principal IFN-α producer is pDC.

**Figure 3.** Immune activating effects of IFN-α. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Rheumatology, Rönnblom et al., copyright 2010 (144).

**The plasmacytoid dendritic cell**

The pDC was originally described as the natural IFN producing cell (NIPC) because of its extraordinary ability to secrete high levels of IFN-α (3-10 pg/cell) (145, 146). Due to the plasma cell-like morphology of unstimulated NIPC and their ability to adopt a mature dendritic cell morphology following activation with CD40 ligand and IL-3 (147), the NIPC was later termed the “plasmacytoid dendritic cell”. In human peripheral blood, <1% of the mononuclear cells are pDCs and in freshly isolated PBMCs they can be identified by the expression of blood dendritic cell antigen (BDCA)-2 (CD302) or BDCA-4 (CD303) (148). Following culturing of pDCs *ex vivo*, surface expression of BDCA-2 is down-regulated on pDCs. In contrast, the expression
of BDCA-4 is not down-regulated, but since the expression of BDCA-4 is induced on monocytes and conventional DCs (148) this surface marker cannot be used alone to identify pDCs in cultured cells. Other means to identify pDCs are by their phenotype as CD4⁻CD11c⁻Lineage⁻ or CD123ʰighHLA-DR⁻Lineage⁻ cells (149).

The secretion of IFN-α and other pro-inflammatory cytokines (e.g. TNF-α and IL-6) by pDCs is induced through stimulation of the endosomal toll-like receptor (TLR)7 and TLR9 that recognize RNA and DNA, respectively. Activation of TLR7 or TLR9 initiate signaling via the adaptor molecule myeloid differentiation factor 88 (MyD88), which associates with a signal complex comprised of Bruton’s tyrosine kinase (BTK), TNF receptor-associated factor 6 (TRAF6), IL1R-associated kinase (IRAK)1 and IRAK4 (150-152). The subsequent phosphorylation and nuclear translocation of IRF7 eventually results in the expression of IFN-α (153, 154) (Figure 4). Additionally, translocation of nuclear factor-κB (NFκB) induces the expression of pro-inflammatory cytokines, whereas nuclear translocation of mitogen-activated protein kinases (MAPK) induces the expression of costimulatory molecules (155).

Following activation, pDCs lose their ability to produce IFN-α and instead acquire a mature dendritic cell phenotype (156) with an increased capacity to present antigens and activate T cells (157). Activated pDCs have also been proposed to exert cytolytic killing of tumor cells (158).

Although pDCs are perhaps best known for their immune stimulating functions following activation, recent findings suggest that immature pDCs have a tolerogenic role. In terms of central tolerance, pDCs transport and present antigens to CD4⁺ T cells in the thymus, which cause a clonal deletion of autoreactive T cells (159). In the periphery, immature pDCs induce the differentiation of Treg cells that suppress autoreactive T cells (160, 161).
Figure 4. Signaling pathways involved in the activation of pDCs. The picture is modified from Dubois’ Lupus Erythematosus and Related syndromes 8th edition.

The type I interferon system in SLE

Normally, the type I IFNs are produced in response to viruses. In order to avoid an excessive activation of the immune system, it is important that the production of type I IFNs is stringently regulated and limited in time. However, as described above, many patients with SLE have a continuous activation of the type I IFN system, which can be seen as an increased concentration of IFN-α in serum and/or an IFN signature in peripheral blood (124-126) or affected tissues (127). Supporting an important role for pDC-produced IFN-α in the continuous activation of the type I IFN system in SLE, murine lupus models of IFNAR gene-knockouts (162), or knock-outs that results in an absence of pDCs or the inability of pDCs to produce type I IFN in response to endosomal TLR-stimulation (163), ameliorates the lupus symptoms. These findings have led to a great interest in understanding the role of IFN-α in the etiopathogenesis of SLE and clinical trials with therapies neutralizing IFN-α or the IFNAR are currently ongoing (164-167).
Endogenous IFN-α inducer

A key finding to understand the mechanisms behind the activated type I IFN system, was that sera from patients with SLE could induce IFN-α production by peripheral blood mononuclear cells (PBMCs) cultured in vitro (168, 169). This observation suggested that patients with SLE had an endogenous IFN-α inducer, responsible for the continuous activation of the type I IFN system.

The proposed endogenous IFN-α inducer was later shown to consist of ICs formed by autoantibodies against nucleic acids or nucleic acid-containing proteins (170). Such ICs can be created in vitro by SLE-IgG and apoptotic or necrotic material (171, 172), or SLE-IgG and purified nucleic acid containing autoantigens, such as small nuclear ribonucleoproteins (snRNPs) (173, 174). Due to the defect clearance of apoptotic cells in SLE (175), the availability of autoantigens with the potential to create interferon-inducing (interferogenic) ICs is increased. Once formed, the interferogenic ICs are internalized in pDC through the low-affinity FcγRIIA-receptor (CD32A) (176, 177) and transferred to the endosomes. The subsequent stimulation of TLR7 or TLR9 by the nucleic acid-part induces the secretion of IFN-α as described above.

Neutrophils undergoing a specific type of cell death, NETosis, release nuclear DNA in the form of neutrophil extracellular traps (NETs). These associate with antimicrobial peptides, such as LL37, and represent another type of endogenous IFN-α inducer which triggers pDCs (178, 179). In addition to pDC-derived IFN-α, chromatin-stimulated neutrophils have recently been proposed to be another source of IFN-α production in lupus patients (180).

The plasmacytoid dendritic cell

The frequency of pDCs in peripheral blood of patients with SLE is markedly decreased (168). This is probably due to migration of activated pDCs to inflamed tissues such as skin (181, 182), kidney (117), or lymph nodes (183). The remaining pDCs in circulation from patients with SLE are functionally competent and produce normal levels of IFN-α/cell following stimulation with herpes simplex virus (HSV) (168).

pDCs from patients with SLE also have an increased ability to stimulate T cell proliferation and a reduced capacity to induce Treg cells (184).

A type I IFN driven etiopathogenic model of SLE

By combining the environmental factors, the effects of type I IFN, and the endogenous type I IFNs inducers, described so far in this thesis, an etiopathogenic disease model of SLE can be generated (Figure 5). In this model, it can also be envisioned that several of the genetic risk variants, described in Figure 1, participate.
Figure 5. An etiopathogenic model of SLE. Viral infections induce IFN-α production by pDC and release of autoantigens from dying cells. IFN-α activates the immune system as described in the text. In individuals susceptible to develop lupus, autoantibodies targeting nucleic-acid containing autoantigens are produced. Together with autoantigens from dying cells, ICs that induce IFN-α production by pDCs and stimulate autoantibody production are formed. In this way a self-perpetuating vicious circle is created. The picture is reprinted from Rönnblom L., Ups J Med Sci 2011 (185).

Regulation of IFN-α production

The IFN-α production by pDCs is regulated by a complex network of cytokines and receptors. IFN-α itself is a strong positive modulator of the IFN-α expression (170, 186). This effect is known as priming and the mechanism involves the increased expression of IRF-7 (187). An IFN-α promoting effect has also been shown for the cytokines GM-CSF and IL-3 (170, 188). In contrast, IL-10 and TNF-α are cytokines which inhibit the IFN-α production (186).
With regard to surface receptors on pDCs, BDCA-2 (189), immunoglobulin-like transcript 7 (ILT-7) (190), FcRγIIA (176, 191), FcεRIA (192), NKp44 (193), and leukocyte-associated immunoglobulin-like receptor 1 (LAIR-1) (194) negatively modulates the IFN-α production by pDCs. These receptors all have in common that they signal through immunoreceptor tyrosine-based activation motifs (ITAMs). In contrast, CD300a which signals via immunoreceptor tyrosine-based inhibitory motifs (ITIMs) is one of the few, if not the only, receptor described which mediate an increased IFN-α production when cross-linked on human pDCs (195). Another negative modulator of the IFN-α production is the complement system. The mechanisms are not clear, but both a direct effect on pDCs (196) through binding of C1q to LAIR-1 (197) and a preferential binding of C1q-containing ICs to monocytes over pDCs (198) have been suggested.

In terms of interactions with other cells, NK cells promote the IFN-α production by pDCs stimulated with the TLR9 agonist oligodeoxynucleotide (ODN)2216 (199), or RNA-containing IC (RNA-IC) (200). In the case of RNA-IC stimulation, monocytes potentely inhibit the help from natural killer (NK) cells through secretion of reactive oxygen species, TNF-α and prostaglandin E2 (200). Notably, the suppressive effect of monocytes is significantly decreased in monocytes from patients with SLE. In addition to NK cells, both B cells (201) and platelets (202) have been shown to increase the IFN-α production by IC-stimulated pDCs.

Thus, as summarized in Figure 6, the IFN-α production is regulated by an intricate network of cytokines and receptors.

![Figure 6](image.png)

*Figure 6. Cytokines and receptors which inhibit (red) or enhance (green) the IFN-α production by TLR7- or TLR9-stimulated pDCs. The picture is modified from Bao et al., Protein Cell 2013 (203).*
Natural killer cells

NK cells were first recognized for their ability to detect and kill cells that do not express MHC class I molecules, a phenomenon referred to as “missing self” (204). NK cells are therefore important in the defense against virally infected (205) or transformed cells (206), but they also contribute to the homeostasis of the immune system by killing activated immune cells (207-209). The direct effector functions of NK cells are mediated through the secretion of lysosomes containing the lytic granules perforin and granzymes (210), or the engagement of the death-receptor ligands TNF-related apoptosis-inducing ligand (TRAIL) and Fas ligand (FasL) (211, 212). NK cells can also kill antibody coated cells through a process termed antibody-dependent cellular cytotoxicity (ADCC).

The activation of NK cells results in the secretion of cytokines and chemokines (e.g. TNF-α, IFN-γ, and macrophage inflammatory protein (MIP)-1β) which affects the survival of pathogens both directly, and indirectly, through enhancing and shaping the innate and adaptive immune response (213, 214).

Although NK cells belong to the innate immune cells, recent studies have shown that NK cells can acquire characteristics of an immune memory, such as an expansion of pathogen-specific cells, an extended lifespan, self-renewal, and the ability to mount an enhanced secondary immune response to specific antigens (215-217).

In humans, NK cells are usually defined as CD3−CD56+ cells and they represent approximately 5-20% of the peripheral blood lymphocytes (218). Two major subpopulations of NK cells exist, the CD56dimCD16+ and the CD56brightCD16− NK cells. The CD56dimCD16+ NK cell constitutes roughly 90% of the NK cells in peripheral blood and spleen. These cells express perforin, are cytotoxic, and produce IFN-γ after interactions with tumor cells in vitro. In lymph nodes and tonsils, the majority of NK cells are CD56brightCD16− NK cells. These cells lack perforin and are activated by IL-12, IL-15, and IL-18 to produce cytokines and chemokines (218, 219).

Unlike B cells and T cells, NK cells do not express receptors that undergo somatic gene rearrangements to generate receptor diversity and specificity. Instead, NK cell functions rely on an array of germ-line encoded activating and inhibitory receptors (220). The activating receptors sense the expression of ligands typically induced on stressed, virally infected, or malignant cells. Some of the activating and coactivating receptors are summarized in Table 2, and include the low affinity Fc receptor for IgG (FcγRIIIA/CD16), the natural cytotoxicity receptors (NCRs, NKP30, NKP44, and NKP46), the SLAM family receptors (2B4, CRACC, LY-9, and NTB-A), and other receptors, such as NKG2D and DNAM-1.

To avoid an inappropriate response to healthy cells, NK cells are equipped with inhibitory receptors monitoring the expression of MHC class I molecules, as well as receptors that recognize non-MHC ligands. The former
group consists of the inhibitory killer cell immunoglobulin-receptors (iKIRs), the CD94/NKG2A receptor, and ILT-2, whereas the latter group is comprised of KLRG1 and LAIR-1 (Table 2). All inhibitory receptors signals through ITIM containing motifs and these signals are dominant over the activating signal. Thus, the NK cell response is the function of a complex hierarchical integration of signals (221).

Table 2. Activating, coactivating and inhibitory receptors expressed by resting human NK cells and their ligands

<table>
<thead>
<tr>
<th>Activating/Coactivating</th>
<th>Inhibitory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor</td>
<td>Ligand</td>
</tr>
<tr>
<td>FcγRIIIA (CD16)</td>
<td>IgG</td>
</tr>
<tr>
<td>NKp30 (CD337)</td>
<td>B7-H6, BAT-3</td>
</tr>
<tr>
<td>NKp44 (CD336)</td>
<td>Viral hemagglutinin</td>
</tr>
<tr>
<td>NKp46 (CD335)</td>
<td>Viral hemagglutinin</td>
</tr>
<tr>
<td>KIR (CD158)</td>
<td>HLA-Class I</td>
</tr>
<tr>
<td>NKG2C (CD94/CD159c)</td>
<td>HLA-E</td>
</tr>
<tr>
<td>NKG2D (CD314)</td>
<td>ULBP1-4, MICA/B</td>
</tr>
<tr>
<td>DNAM-1 (CD226)</td>
<td>CD112, PVR (CD155)</td>
</tr>
<tr>
<td>2B4 (CD244)</td>
<td>CD48</td>
</tr>
<tr>
<td>CRACC (CD319)</td>
<td>CRACC (CD319)</td>
</tr>
<tr>
<td>LY-9 (CD229)</td>
<td>LY-9 (CD229)</td>
</tr>
<tr>
<td>NTB-A (CD352)</td>
<td>NTB-A (CD352)</td>
</tr>
<tr>
<td>LFA-1 (CD11/CD18)</td>
<td>ICAM1-5</td>
</tr>
</tbody>
</table>

Modified from Bryceson Y.T et al., J Innate Immun, 2011 (222)

Since the activating and inhibitory receptors are expressed stochastically, subsets of NK cells will not express any inhibitory receptors which recognize self-MHC. Such cells would theoretically be autoreactive. However, tolerance against self is maintained in these cells by the process of NK cell education where recognition of self-MHC class I by inhibitory receptors on NK cells are necessary for NK cells to be fully activated (223-225).

NK cells have been suggested to participate in the pathogenesis of several autoimmune diseases, including multiple sclerosis (MS), rheumatoid arthritis (RA), and SLE (226). Although their exact functions are still unclear, both a disease promoting and preventing function has been proposed. With regard to disease promoting properties, NK cell can drive Th1 polarization through secretion of IFN-γ (227), activate DCs (228), costimulate T cells (229, 230), and directly kill “self”-cells. On the other hand, production of IL-10 (231), TGF-β (232), and the killing of DCs (233) and activated T cells (234) are functions which may counteract the autoimmune disease process.
Natural killer cells in SLE

Several alterations of NK cells in SLE have been described. Patients with SLE have reduced numbers of NK cells in peripheral blood (235-237) and the numerical deficit correlates to disease activity and the presence of nephritis (236, 238, 239). In addition, circulating NK cells from patients with SLE display a reduced cytotoxicity (235, 237, 239). Whether the low numbers of NK cells in circulation reflects the trafficking of NK cells to inflamed tissues is not known, but studies in patients with RA (240, 241), psoriasis (242), pSS (243), and diabetes mellitus type 1 (244) have shown an increased number of infiltrating NK cells, or subsets of NK cells, in affected tissues. In experimental murine models of MS and RA, a reduced cytotoxicity of NK cells has been proposed to lead to a defect in killing of activated T cells that may contribute to the pathogenesis of these diseases (245, 246). Whether this also applies to SLE has not been studied. Moreover, peripheral blood SLE-NK cells are activated as determined by expression of the early activation marker CD69 (237) and the proportion of CD56bright NK cells is increased in SLE. The latter observation might be explained by the fact that IFN-α, at least in vitro, increases the proportion of CD56bright NK cells (247).

Based on the findings that the production of IFN-γ by cytokine-activated SLE-NK cells correlates to serum levels of IFN-α (237), a link between the increased IFN-α production and the altered NK cell function seen in SLE has been suggested.

NK cells are also linked to SLE by genetic associations to certain KIR genotypes (248, 249) and polymorphisms in the FcγRIIIA (250, 251). However, in this context, it is important to bear in mind that subsets of T cells also express KIR molecules (252) and FcγRIIIA (253, 254).

Thus, there are a lot of data suggesting a role of NK cells in SLE, but their involvement and the exact function in the pathogenesis of SLE remains to be established.
Present investigations

Aims of the thesis

The general aim of this thesis was to investigate the role of pDCs and NK cells in SLE. The specific aims of each study were:

- **Study I** - To clarify the mechanisms behind the capacity of NK cells to enhance the IFN-\(\alpha\) production by pDCs stimulated with RNA-IC.

- **Study II** - To identify surface molecules involved in the interaction between pDCs and NK cells and investigate their potential role in regulating the production of IFN-\(\alpha\).

- **Study III** - To characterize a novel anti-NKG2A autoantibody serendipitously found in serum from a patient with an active and severe SLE disease.

- **Study IV** – To investigate the occurrence of autoantibodies to the CD94/NKG2A, CD94/NKG2C, and NKG2D receptors in SLE and to characterize such autoantibodies.
Materials and methods

Several methods have been used in these studies, and they are described in detail in the manuscripts. In this section some of the central methods are briefly described and discussed.

Samples

PBMCs and serum were collected from patients with SLE and pSS visiting the Rheumatology Unit (Uppsala University Hospital), or healthy blood donors (Department of Transfusion Medicine, Uppsala University Hospital). All SLE and pSS patients fulfilled the ACR criteria for SLE (1, 2) and the American European consensus criteria for pSS (255), respectively.

Cell isolation and in vitro stimulation

PBMCs were prepared from healthy donor buffy coats using Ficoll-Hypaque density gradient centrifugation. pDCs and NK cells were isolated from PBMCs using a magnetic bead-based separation technology (MACS, Miltenyi Biotec) (256). In this method, cells are labelled with biotinylated mAbs to specific surface antigens. After addition of anti-biotin mAbs conjugated to magnetic nanobeads the labeled cells can be separated from unlabeled cells in a magnetic column. Collecting the labeled cells (positive selection) is usually the fastest way to isolate a particular cell subset with high purity and yield. However, to avoid inappropriate activation or inhibition of cells bound by mAbs, cell separations in this thesis have, when possible, been performed by labeling the cells to be removed and isolating the unlabeled cells (negative selection).

To induce IFN-α, cells were stimulated with RNA-IC, HSV, or the CpG-containing ODN2216. The RNA-IC was generated in vitro by combining purified U1snRNP particles (257) and IgG from patients with SLE, purified by protein G chromatography (174). The U1snRNP complex is one of the five snRNPs that forms the spliceosome. Structurally, the U1snRNP contains seven core proteins (Sm proteins), the U1 specific U1-70K, U1-RNP A and U1-RNP C proteins, and a small nuclear RNA molecule (258).

In study I and study II, IgG from a patient with autoantibodies to SmB, SmD, U1-RNP A, U1-RNP C, ribosomal P, histone and dsDNA, was used. Although not included in the publications, the major findings in these studies were confirmed using IgG from at least two other SLE patients harboring autoantibodies to the U1snRNP complex.

HSV was prepared by propagation of the virus in WISH cells and inactivation by UV-light as previously described (172). The TLR9 agonist ODN2216 is a phosphorothioate-modified oligonucleotide belonging to the class A of ODN molecules. This class of agonists induces high expression of type I IFN by pDCs, but is only weak stimulators of B cells (259).
Detection of IFN-α

Levels of IFN-α in culture supernatants were determined with a dissociation-lanthanide fluoroimmunoassay (DELFIA), using the anti-IFN-α mAbs LT27:293 as capture antibody and europium-labelled LT27:297 as detection antibody (168). This assay measures the majority of the IFN-α subtypes, but do not recognize IFN-α2b. An advantage of this method is therefore that the effect of priming can be studied using recombinant IFN-α2b. This assay has a detection limit of 2 U/ml (~10 pg/ml). To measure IFN-α in serum samples, a modified, more sensitive assay with a detection limit of 0.5 U/ml (~2.5 pg/ml) was used. In the latter assay, the anti-IFN-α mAbs LT27:273 and LT27:293 were used as capture antibody and europium-labelled LT27:297 as detection antibody. In this assay, IFN-α2b is also recognized.

Activation of NK cells

Activation of NK cells was determined as CD107a mobilization using flow cytometry. CD107a (lysosomal-associated membrane protein-1 (LAMP-1)) is a protein lining the membrane of cytotoxic granules (260) and will therefore be exposed to the surface following NK or T cell degranulation (261, 262). Consequently, this method measures the initial event that takes place in the activation of target cell lysis by NK cells and T cells. For determination of the actual lysis of target cells, the standard chromium release assay can be used (263). These two assays show a strong correlation and the reason for using the CD107a mobilization assay in the present investigations was that this method enables the study of distinct subsets of NK cells (e.g. CD94/NKG2A+ and CD94/NKG2C+ cells).
Results and discussion

Study I

*IFN-α Production by Plasmacytoid Dendritic Cells Stimulated with RNA-containing Immune Complexes is Promoted by NK cells via MIP-1β and LFA-1.*

pDCs increase the cytotoxicity of NK cells by producing IFN-α (264, 265) and reciprocally, NK cells promote the production of IFN-α by pDCs (199, 200). Supporting that such a pDC-NK cell cross-talk may occur in vivo, colocalization of pDCs and NK cells have been shown in human inflamed tonsils (266), and lesional skin of HSV-infected patients (267) or patients with lichen planus (268). The molecular mechanisms, by which NK cells enhance the IFN-α production by pDCs, were at this time unknown. Accordingly, this study was undertaken to clarify the mechanisms behind this observation.

In this study, we showed that RNA-IC triggered NK cells via FcγRIIIA (CD16) to promote the IFN-α production by pDCs. In line with this finding, CD56<sup>dim</sup>CD16<sup>+</sup> NK cells were as efficient as total NK cells in promoting the IFN-α production by RNA-IC-stimulated pDCs, while CD56<sup>bright</sup>CD16<sup>−</sup> NK cells had no effect. However, stimulation of CD56<sup>bright</sup>CD16<sup>−</sup> NK cells by IL-12 and IL-18 rendered these cells as efficient as RNA-IC-stimulated CD56<sup>dim</sup>CD16<sup>+</sup> NK cells in increasing the IFN-α production.

Using supernatants from stimulated NK cells, we could show that the increased IFN-α production by pDC was partially mediated through soluble factors produced by NK cells after triggering of FcγRIIIA or stimulation by IL-12 and IL-18. One of these factors was identified as MIP-1β. MIP-1β is a chemokine, which signals through the CCR5 receptor. Although conflicting data exist, some studies have shown that a genetic deletion leading to a nonfunctional CCR5 receptor is protective for SLE (269, 270). In a follow-up study, the IFN-α production in RNA-IC-stimulated pDC-NK cell cocultures with cells from healthy individuals without this deletion and individuals that were heterozygous for the deletion, were analyzed. No differences in the IFN-α response was observed between these two groups (p=0.94*, Figure 7, unpublished data, Berggren et al.). In this study, only one individual were homozygous for the CCR5 deletion, thus precluding any statistical comparisons.

* Mann-Whitney U test, 77 individuals without CCR5 deletion and 27 individuals heterozygous for the CCR5 deletion
Figure 7. The influence of CCR5 genotype on IFN-α production. pDCs and NK cells from healthy donors were stimulated with RNA-IC and the concentration of IFN-α in culture supernatants was determined after 20 hours. Data show IFN-α production for individuals that lack (WT/WT), are heterozygous (WT/∆) or homozygous (∆/∆) for the 32 base-pair deletion in CCR5 as determined by the SNP rs333.

The increased IFN-α production by RNA-IC-stimulated cells was largely dependent on cell-cell contact between pDCs and NK cells, as antibody-mediated blocking of the lymphocyte function-associated antigen (LFA)-1 receptor strongly decreased the IFN-α production. In contrast to RNA-IC, the increased production of IFN-α in ODN2216- or HSV-stimulated pDC-NK cell cocultures was not affected by blocking LFA-1. This finding is in line with a previous report, which could not detect any effect of LFA-1 inhibition on the ODN2216-induced IFN-α production (199). The specificity of LFA-1 blockade for RNA-IC-stimulated cells may reflect the fact that FcγRIIIA-stimulation induces an active conformation of LFA-1 (271).

In addition to IFN-α, the secretion of several other pro-inflammatory cytokines and chemokines, associated to SLE (e.g. IFN-γ, IL-6, IL-8, and MIP-1β), were increased in RNA-IC-stimulated cocultures of pDCs and NK cells. Whether this was the consequence of the increased IFN-α production or a direct effect of the pDC-NK cell interaction is still unknown. This observation also suggests that in cocultures of pDC and NK cells, there may be other cytokines, besides MIP-1β, that have a stimulatory role in the IFN-α response.

When NK cells from patients with SLE were compared to healthy donor NK cells, we found that SLE-NK cells had a reduced capacity to enhance IFN-α production by RNA-IC-stimulated pDCs. However, addition of exogenous IL-12 and IL-18 restored their IFN-α promoting function. The exact reason for the decreased capacity of SLE-NK cells to stimulate IFN-α production remains to be determined. Hypothetically, a redistribution of subsets of NK cells from peripheral blood to inflamed tissues, tolerance induction through a continuous stimulation by ICs in vivo, or a treatment effect might be some explanations for the decreased activity of NK cells from patients with SLE.

In summary, this study revealed novel findings of the mechanisms whereby NK cells promote the IFN-α production in response to interferogenic ICs. Given the pivotal role of IFN-α in SLE, it is of great interest to elucidate the
detailed mechanisms behind the increased IFN-α production, and in particular to identify the receptors and ligands involved, as this could provide new target molecules for modulating the IFN-α production.

Study II

*Systemic Lupus Erythematosus Immune Complexes Increase the Expression of SLAM Family Members CD319 (CRACC) and CD229 (LY-9) on Plasmacytoid Dendritic Cells and CD319 on CD56dim NK Cells.*

This study aimed to identify receptors and ligands responsible for the increased IFN-α production by pDCs cultured together with NK cells. In a screen for RNA-IC-induced regulation of surface molecules, the expression of 42 molecules was analyzed in RNA-IC- or unstimulated PBMCs using flow cytometry. In this screen, we found that the expression of CD319 was increased on pDCs and CD56dim NK cells, following RNA-IC stimulation. CD319 belongs to the signaling lymphocyte activating molecule (SLAM) family. This family comprise seven receptors (Table 3), which are involved in the regulation of several different types of immune cells (272).

**Table 3. Human SLAM family receptors and their nomenclature**

<table>
<thead>
<tr>
<th>SLAMF</th>
<th>CD</th>
<th>Alternative names</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLAMF1</td>
<td>CD150</td>
<td>SLAM</td>
</tr>
<tr>
<td>SLAMF2</td>
<td>CD48</td>
<td></td>
</tr>
<tr>
<td>SLAMF3</td>
<td>CD229</td>
<td>LY-9</td>
</tr>
<tr>
<td>SLAMF4</td>
<td>CD244</td>
<td>2B4</td>
</tr>
<tr>
<td>SLAMF5</td>
<td>CD84</td>
<td></td>
</tr>
<tr>
<td>SLAMF6</td>
<td>CD352</td>
<td>NTB-A</td>
</tr>
<tr>
<td>SLAMF7</td>
<td>CD319</td>
<td>CRACC, CS1</td>
</tr>
</tbody>
</table>

In this context, it is of particular interest that the locus harboring the SLAM genes is associated to both human SLE (273-275) and to murine models of SLE (276, 277). In addition, recent data suggest that the SLAM family receptors are functionally involved in the pathogenesis of SLE (278, 279).

Except for CD48 and CD244, which interacts with each other, all the other SLAM receptors signal via homophilic interactions. Consequently, the increased expression of CD319 on both pDCs and NK cells may facilitate their interaction.

When the regulation of all SLAM family members was analyzed, we found that the expression of CD229 on pDCs was also induced by RNA-IC. To study the mechanisms behind the regulation of C319 and CD229, we used isolated pDCs and NK cells. RNA-IC-stimulation of purified pDCs did not induce CD319 or CD229, but addition of NK cells, cytokines (e.g. IL-3 or GM-CSF), or stimulation with ODN2216 restored the up-regulation to the
same levels as that seen in PBMCs. In contrast, activation of isolated NK cells by RNA-IC or heat-aggregated IgG induced a stronger increase in CD319 expression on CD56^{dim} NK cells than in PBMCs. Addition of pDCs further increased the induction of CD319 when RNA-IC was used. Thus, the expression of CD319 on pDCs and CD56^{dim} NK cells is regulated through a bi-directional crosstalk between pDCs and NK cells, involving activation of both endosomal TLRs and Fc-receptors.

On NK cells, CD319 and CD229 function as costimulatory molecules, which increase the killing of target cells expressing CD319 and CD229 (280, 281). Concerning pDCs, the expression of CD319 and CD229 was first described in 2009 (282), but the functional role of SLAM molecules on pDCs has not yet been established. In this study, we found an increased expression of CD319 and CD229 on IFN-α producing pDCs, but we could not demonstrate any effect on the RNA-IC- or ODN2216-induced IFN-α production by cross-linking CD319 or CD229. In other immune cells, CD319 functions as an activating or an inhibitory receptor. The function depends on the expression of the activating signaling adaptor molecules, SLAM-associated protein (SAP) and Ewing’s sarcoma-activated transcript 2 (EAT-2), and the inhibitory adaptor molecules, Src homology region 2 domain-containing phosphatase (SHP)-1, SHP-2, SHIP-1, and C-Src kinase (CSK) (283, 284). By Western blot we showed that pDCs lack expression of SAP and EAT-2, but express SHP-1, SHP-2, SHIP-1 and CSK. This may suggest that the function of CD319 on pDCs is to inhibit another receptor. Further studies to determine the function of SLAM molecules on pDCs are warranted, but such experiments are hampered by the fact that specific blocking antibodies to CD319 and CD229 are not commercially available. Another approach to assess the function of SLAM molecules on pDCs would be to use gene knockouts. To date, there are no methods to genetically manipulate primary pDCs, but there are three cell lines originating from the leukemic counterpart of pDCs (blastic plasmacytoid dendritic cell neoplasm) described, which may be used (285-288). Two of these cell lines produce IFN-α after stimulation with viruses or synthetic TLR7/9 agonists, but whether these cells produce IFN-α in response to interferogenic ICs has not been published.

Contrasting the in vitro data, patients with SLE had a significant decreased expression of CD319 on pDCs. The reason for this is still unknown, but plausible explanations for this include a treatment effect or the redistribution of activated pDCs from peripheral blood to inflamed tissues. Another possible explanation is that a continuous stimulation by SLE-IC may induce a negative feed-back loop resulting in a decreased expression of CD319. However, the latter explanation is contradicted by the fact that the RNA-IC-induced expression of CD319 and CD229 was equally strong in pDCs from SLE patients and controls.

35
NK cells from patients with SLE displayed a decreased expression of CD229 on CD56<sup>dim</sup> NK cells and an increased expression of CD319 on CD56<sup>bright</sup> NK cells.

In conclusion, this study further strengthens the association of SLAM family members to SLE by showing that the expression of CD319 and CD229 is strongly increased on pDCs after RNA-IC-stimulation, that they are increased on IFN-α producing pDCs and that their expression is altered on pDCs and NK cells from patients with SLE.

Study III

*Anti-NKG2A Autoantibodies in a Patient with Systemic Lupus Erythematosus.*

A serendipitous finding in *study II* suggested that a patient with SLE had autoantibodies targeting NKG2A. Together with CD94, NKG2A forms a heterodimeric inhibitory receptor, which is expressed on subsets of NK cells and CD8<sup>+</sup> T cells. The CD94/NKG2A complex binds to HLA-E that is stabilized by signal peptides derived from other HLA molecules, and in that way the CD94/NKG2A receptor monitors both the expression of classical HLA molecules and the function of antigen processing in target cells (289-291). In contrast, CD94/NKG2C is a closely related activating receptor complex that displays considerably lower affinity for HLA-E with HLA-derived signal peptides, but has some specificity for CMV-derived peptides (292-294).

The presence and specificity of anti-NKG2A autoantibodies was confirmed by detecting binding of IgG from this patient to a CD94/NKG2A-transfected murine B cell line, but not to cells transfected with CD94/NKG2C, or the structurally unrelated activating receptor NKG2D. The anti-NKG2A autoantibodies selectively blocked the binding of HLA-E tetramers to CD94/NKG2A-transfected, but not to CD94/NKG2C-transfected, cells. In line with these data, the anti-NKG2A autoantibodies blocked the NKG2A-mediated inhibition of NK cell activation by target cells expressing HLA-E, whereas NKG2C-mediated activation was unaffected.

When four longitudinally sampled sera from this patient were analyzed, we could only detect anti-NKG2A antibodies in the two sera sampled during a severe flare. Although the presence of anti-NKG2A autoantibodies correlated to increased serum levels of IFN-α, we could not find a direct effect of anti-NKG2A antibodies on the IFN-α production.

Anti-NKG2A autoantibodies, which blocked the binding of HLA-E to CD94/NKG2A-transfected cells, were only found in 1 out of 136 SLE patient sera and in none of the 60 pSS patient sera analyzed. One limitation of the assay used in the present study is that it only detects autoantibodies to NKG2A that block the binding of HLA-E. Another limitation is the fact that
the transfected cells have a much higher expression of CD94/NKG2A than primary NK cells and consequently, high titers of autoantibodies may be required to obtain a blocking effect. There is also a possibility that in vivo absorption of autoantibodies to NK cells may yield a false negative result.

In this study, we have focused on the anti-CD94/NKG2A autoantibodies effect on NK cells. However, because these autoantibodies also blocked the detection of NKG2A on T cells, it is tempting to speculate that these autoantibodies may alter T cell functions as well. The exact function of CD94/NKG2A on T cells is not known, but a role in down-regulating cytotoxicity of activated CD8⁺ T cells specific for various antigens (295-297), protecting CD8⁺ T cells from apoptosis (298), and inhibiting the induction of Treg cells (299) have been proposed. Conceivably supporting a role for C94/NKG2A⁺ T cells in SLE, we found a decreased frequency of NKG2A⁺CD8⁺ T cells and NKG2A⁺ γδ-T cells in peripheral blood of patients with SLE (p=0.0002 and p<0.0001, respectively †; unpublished data). This finding is in accordance with previously published data (300, 301).

Data from murine disease models have suggested that pharmacological targeting of NKG2A by mAbs might be an effective therapy in autoimmune diseases, such as MS and RA (245, 246). Although the in vivo effect of the anti-NKG2A autoantibody described in this study is still unknown, the presence of this autoantibody in a patient with SLE during a severe flare might raise concerns about the safety and efficacy of an anti-NKG2A-based therapy, not least in SLE.

To conclude, this is the first study describing the occurrence of autoantibodies to NKG2A. Given the presence of these autoantibodies in an SLE patient with a severe disease, the association of autoantibody titers to the disease activity and the functional effect on NK cell cytotoxicity, these autoantibodies may be relevant in a pathogenic perspective.

Study IV

Autoantibodies to the CD94/NKG2A and CD94/NKG2C Receptors in Patients with Systemic Lupus Erythematosus.

Having identified a patient with anti-NKG2A autoantibodies in Study III, we next performed a more comprehensive screening for autoantibodies to the CD94/NKG2A, CD94/NKG2C and NKG2D receptors. Initially, a method to detect binding of human Ig to the CD94/NKG2A, CD94/NKG2C and NKG2D receptors were established. In contrast to the method used in Study III, the method in the present investigation detects autoantibodies of any specificity to these receptors, rather than just HLA-E-blocking autoanti-

† Mann-Whitney U test, 19 healthy controls and 21 SLE patients
bodies. By analyzing sera from 203 patients with SLE and 90 healthy controls, we identified six additional patients with autoantibodies targeting the CD94/NKG2A receptor. Sera from two of these patients also reacted to the CD94/NKG2C receptor, whereas none of the patients had autoantibodies to the NKG2D receptor.

To determine the specificity of the autoantibodies, we studied whether they interfered with the binding of commercially available mAbs to NKG2A and NKG2C, as well as HLA-E pentamers. Serum from three of the patients interfered with binding of the anti-NKG2A mAb (clone Z199), whereas serum from the two patients with anti-CD94/NKG2C autoantibodies blocked the binding of the anti-NKG2C mAb (clone 134591). Of note, the anti-CD94/NKG2A autoantibodies from all patients, but one, inhibited the binding of HLA-E to NKG2A, whereas autoantibodies from one of the patients augmented this binding. IgG from the latter patient also stabilized the binding of HLA-E to CD94/NKG2C, whereas serum from the other patient with anti-CD94/NKG2C autoantibodies blocked the interaction between CD94/NKG2C and HLA-E. In functional studies, we could show that anti-CD94/NKG2A autoantibodies increased the degranulation of NKG2A⁺ NK cells in response to HLA-E expressing target cells and that anti-CD94/NKG2C autoantibodies decreased the degranulation of NKG2C⁺ NK cells in response to HLA-E expressing target cells. These findings suggest that SLE patients harboring anti-CD94/NKG2A autoantibodies may have an increased killing of cells, which normally should not be killed. This increased killing might lead to elevated levels of nucleic-acid containing autoantigens, which together with other autoantibodies could drive the type I IFN production. Hypothetically, given the preference of CD94/NKG2C for certain viral peptides, the decreased degranulation of CD94/NKG2C⁺ NK cells in response to HLA-E-expressing target cells may result in an increased susceptibility to certain infections. Although both patients with anti-CD94/NKG2C autoantibodies had a history of recurrent infections, we could not rule out that other factors, such as treatment, were more important for the observed infections.

When PBMCs were isolated from four of these patients, we found that two of the patients had a very low frequency of peripheral blood NK cells. Moreover, patients harboring anti-CD94/NKG2A autoantibodies that blocked the binding of the anti-NKG2A mAb (clone Z199) had a low, to no, detectable surface expression of NKG2A on both NK cells and T cells. Several explanations for the low expression of NKG2A exist. First, these patients may have genetic alterations affecting the expression of these proteins. However, no uncommon genetic variations leading to non-synonymous amino acid substitutions were found when the exons encoding NKG2A, NKG2C, or CD94 (KLRC1, KLRC2 and KLRD1) were sequenced in four of the patients. Second, autoantibodies may bind NKG2A in vivo and simply mask the detection of NKG2A. Third, all patients had autoantibodies of the
IgG3 and/or IgG1 subclasses. Since these subclasses bind complement and Fc-receptors, these autoantibodies may result in complement-mediated lysis or antibody-dependent cellular cytotoxicity (ADCC) towards CD94/NKG2A+ or CD94/NKG2C+ cells. In support of the latter hypothesis, IL-2-activated PBMCs killed CD94/NKG2A- and/or CD94/NKG2C-transfected Ba/F3 cells pre-incubated with the anti-CD94/NKG2A or anti-CD94/NKG2C autoantibodies.

A large number of autoantibodies are described in SLE (56). Some of them correlates to disease activity (e.g. anti-dsDNA antibodies (52)), or clinical manifestations (e.g. anti-phospholipid antibodies and thrombosis (302)). By analyzing longitudinally sampled sera, we found that the titers of anti-CD94/NKG2A and anti-CD94/NKG2C autoantibodies were closely associated to the disease activity. Examination of medical records showed that five of the seven patients had a SLICC/ACR damage index of ≥2 and four of them suffered from nephritis. Thus, anti-CD94/NKG2A autoantibodies were found in a subgroup of patients with a severe disease (303).

In this cross-sectional study we found anti-CD94/NKG2A autoantibodies in 3.4% of the patients in our SLE cohort. Considering the fact that these autoantibodies were preferentially detected early in the disease and in active patients and the fact that most of the patients in our cohort are well treated with a low to no disease activity, it is possible that the presence of such autoantibodies is a more common phenomenon in active SLE patients. Moreover, in this study we analyzed three of the receptors that regulate NK cell cytotoxicity. Given the multitude of such receptors, it is conceivable that the frequency of SLE patients that have autoantibodies to receptors which regulates NK cell cytotoxicity may be considerably larger. In fact, autoantibodies to KIR molecules have occasionally been described in patients with SLE (304) and preliminary data suggest that some of the patients included in the initial screening in this study have autoantibodies targeting KIR molecules.

Thus, a subset of patients with SLE produces autoantibodies to the CD94/NKG2A and CD94/NKG2C receptors. Given that these autoantibodies interfere with the HLA-E-mediated regulation of NK cell cytotoxicity and that their presence and titers correlate to disease activity, these autoantibodies may be important in the pathogenesis and clinical expression of SLE.

Concluding remarks

In this thesis, I have showed that pDCs and NK cells closely collaborate to increase the production of IFN-α and other pro-inflammatory cytokines. In comparison to HSV and the synthetic IFN-α inducer ODN2216, the cross-talk of pDCs and NK cells was most efficiently induced after stimulation with ICs mimicking those found in the circulation of patients with SLE. The potent IFN-α promoting property of SLE-IC-stimulated NK cells suggests
that the pDC–NK cell cross-talk may be involved in sustaining the continuous IFN-α production, seen in patients with SLE. In a general perspective, these mechanisms could also operate in other IFN-α driven autoimmune diseases, such as pSS, myositis and systemic sclerosis.

In addition to the increased IFN-α production, the pDC-NK cross-talk was shown to be essential in regulating the expression of the two SLAM receptors CD319 and CD229 on RNA-IC-stimulated pDCs. Because the function of these receptors on pDCs is still unknown, a potential involvement in the pathogenesis of SLE is speculative. Nevertheless, it is intriguing that receptors that are genetically associated to SLE display an altered expression on pDCs and NK cells from lupus patients, and that ICs resembling those found in lupus patients regulates these molecules selectively on pDCs and NK cells.

In the second part of the thesis, several patients with SLE who harbored functional autoantibodies to two receptors regulating the NK cell cytotoxicity, CD94/NKG2A and CD94/NKG2C, were identified. Although the exact in vivo consequence of these autoantibodies remains to be determined, the importance of a carefully regulated NK cell cytotoxicity is evident from patients with hemophagocytic lymphohistocytosis (HLH). Due to a defect perforin dependent cytotoxicity of NK cells and CD8+ T cells, these patients have an excessive activation of T cells and macrophages, which results in a severe inflammation and potentially fatal organ damage (305).

Although there have been a great improvement in the treatment of patients with SLE during the past decades, a lot of patients are still treated with drugs that exert their effect through a broad non-specific suppression of the immune system. In order to reduce adverse events, novel therapies with a more specific mechanism of action would be valuable. Despite a lot of promising results from murine experimental models of lupus, the anti-BAFF mAb belimumab is the only new drug, in more than 50 years, that has been approved for treating SLE. This could of course imply that murine models of lupus are not representative of the human disease, but it could also reflect the heterogeneity of the human disease. Understanding the molecular mechanism behind the disease may yield new therapeutic targets. In addition, such knowledge could aid in stratifying patients into subgroups, with a common pathogenic pathway affected, which may respond differently to different drugs.

Several drugs that target the type I IFN system are currently evaluated both pre-clinically and in human clinical trials. Neutralizing mAbs to IFN-α have been shown to reduce the IFN-signature, but so far no clinical effect has been proven (165, 166). Because of the antiviral effects of type I IFNs, one major concern when therapeutically targeting this system is the risk for infections. Consequently, a target that specifically blocks the SLE-IC-induced IFN-α production would be attractive. In the first study where we investigated the molecular mechanisms behind the IFN-α promoting proper-
ty of NK cells, we observed that anti-LFA-1 mAbs selectively blocked the induction of RNA-IC-induced IFN-α. Antibodies to the CD11a subunit of LFA-1 (efalizumab, Raptiva™) have previously been used in patients with psoriasis to modulate the T cell immune response (306). This treatment was later withdrawn due to the increased risk for progressive multifocal leukoencephalopathy (PML) (307). Given that patients with severe SLE already have an increased risk for developing PML (308), such a therapy for SLE cannot be considered safe. Accordingly, further studies of how the IFN-α production is regulated by SLE-IC are needed in order to find targets that selectively modulates the SLE-IC-induced IFN-α production.

With regard to CD94/NKG2A and CD94/NKG2C, future studies to clarify the potential pathogenic role of autoantibodies to these receptors may yield insights whether modulation of NK cell cytotoxicity would be beneficial in a subgroup of patients with SLE.

To conclude, although further studies are needed to determine their exact contribution to the SLE disease process, the findings in this thesis strengthen the assumption that NK cells may have an important role in the pathogenesis of SLE.
Sammanfattning på svenska

Kroppens immunförsvar består av ett stort antal olika celltyper som samspe-
lar för att bekämpa virus, bakterier och andra sjukdomsframkallande organ-
ismer. En viktig funktion för immunsystemet är att kunna skilja kroppsegna
ämnen från främmande ämnen. Sjukdomar där individens immunsystem
attackerar egna celler kallas för autoimmuna sjukdomar. I den här avhand-
lingen har jag studerat hur immunsystemet regleras vid den autoimmuna
sjukdomen systemisk lupus erythematosus (SLE).

Årligen insjuknar cirka 400 svenska individer i SLE och det uppskattas att
drygt 5000 personer i Sverige lever med sjukdomen. En viktig komponent i
sjukdomen är antikroppar mot kroppsegna ämnen (autoantikroppar) som
tillsammans med det kroppsegna ämnet (autoantigen) bildar immunkomplex.
Dessa immunkomplex kan deponeras i vävnad och orsaka inflammation som
slutligen leder till en organskada. SLE kan drabba flera av kroppens organ-
system och sjukdomens svårighet kan variera från en mild sjukdom med
lättare hud- eller led-engagemang till en allvarlig livshotande sjukdom med
inflammation och skada på t ex njurar, hjärta/kärl och/eller centrala nervsys-
stemet.

De bakomliggande mekanismerna till sjukdomen är inte fullständigt klar-
lagda men man vet att både ärfliga faktorer och miljöfaktorer bidrar till
sjukdomen. Flera observationer pekar på typ I interferonsystemets centrala
roll för utvecklingen av SLE. Redan på 1980-talet beskrevs att patienter med
SLE har en ökad koncentration av interferon (IFN)-α i serum. Senare har det
visats att behandling av vissa sjukdomar med IFN-α kan leda till lupuslik-
nande symtom och man har även påvisat ett ökat uttryck av IFN-α-
inducerade gener i celler från perifert blod eller inflammerad vävnad hos
dessa patienter. I normala fall aktiveras typ I IFN-systemet av virus eller
vissa bakterier och det producerade IFN-α bidrar till kroppens tidiga immu-
nsvar genom både dess direkt antivirus egenskaper och genom dess aktivе-
ing av andra typer av immunceller. En viktig orsak till det aktiverade IFN-
systemet vid SLE är att patienterna har kroppsegna IFN-inducerare, så kal-
lade interferogena immunkomplex. De interferogena immunkomplexen be-
står av autoantikroppar och nukleinsyror (DNA/RNA), eller nukleinsyrain-
nehållande protein. Dessa immunkomplex tas upp av plasmacytoida dendri-
tiska celler (pDC) genom Fc-receptorer och stimulerar sedan Toll-lika recep-
torer (TLR) vilket leder till IFN-α-produktion. Produktionen av IFN-α
regleras genom ett komplext nätverk av andra immunceller och det har tidi-
gare visats att naturliga mördarceller (NK-celler) kraftigt kan förstärka pDCs IFN-α-produktion. En av NK-cellernas viktigaste funktioner är att döda infekterade celler och canceromvandlade celler och NK-celler är därför en viktig del av kroppens tidiga immunsvar.

Målet med den här avhandlingen var att undersöka hur pDC och NK-celler samspelar vid SLE med ett specifikt fokus på dess effekter på typ I IFN systemet. Genom att bättre förstå dessa mekanismer skulle man kunna identifiera nya målmolekyler som skulle kunna angripas med nya läkemedel.

För att studera pDC och NK-cellers roll vid SLE har vi dels använt ett modellsystem där immunceller har isolerats från perifert blod från friska individer och sedan stimulerats med immunkomplex bestående av SLE autoantikroppar samt ett RNA-innehållande autoantigen (RNA-IC) och dels använt immunceller isolerade från SLE patienter.


I Studie II var syftet att identifiera ytterligare receptorer och ligander som är viktiga i samspelen mellan pDC och NK-celler. I en stor screening fann vi att två receptorer som tidigare visats vara genetiskt associerade till SLE, CD319 och CD229, uppreglerades kraftig på pDC och NK-celler efter stimulering med RNA-IC. De molekylära mekanismerna bakom denna reglering utreddes och vi bestämde förekomsten av signaleringsmolekyler för dessa receptorer i pDC. Vi kunde inte fastställa funktionen av CD319 och CD229 på pDC eller dess roll i samspelen mellan NK-celler, men vi fann att pDC som producerade IFN-α karaktäriserades av ett högre uttryck av dessa receptorer än de pDC som saknade IFN-α-produktion. Slutligen påvisade vi ett förändrat uttryck av båda dessa molekyler på pDC och NK-celler från patienter med SLE.

autoantikroppar ledde till ett ökat dödande av celler som hos friska individer normalt inte skulle dödas.


Sammanfattningsvis har jag i den här avhandlingen beskrivit nya molekylära mekanismer för hur NK-celler kan förstärka IFN-α-produktionen från pDC. I tillägg till den tidigare visade genetiska associationen mellan CD319 och CD229 till SLE har jag visat att uttrycket av dessa receptorer på pDC induceras av SLE-liknande immunkomplex samt kopplat ett ökat uttryck av dessa receptorer till en ökad produktion av IFN-α. Slutligen har jag beskrivit och karaktäriserat en helt ny grupp av autoantikroppar, som påverkar regleringen av NK-cellernas dödande av målceller, hos patienter med SLE. Tillsammans stärker dessa fynd uppfattningen att NK-celler är inblandade i sjukdomsprocessen vid SLE.
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