General Introduction

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*The type I interferon system in multiple sclerosis: towards a personalized medicine approach for interferon-β therapy.*

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*Relevance of the type i interferon signature in Multiple sclerosis towards a personalized medicine approach for interferon-beta therapy.*
Discov med. 2013 Jan;15(80):51-60. Review

Verweij CL, Vosslamber S.
*New insight in the mechanism of action of rituximab: The interferon signature towards personalized medicine.*
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THE HETEROGENEOUS NATURE OF AUTOIMMUNITY

Clinical and molecular heterogeneity is an important issue in autoimmune diseases. Besides the wide variety of autoimmune disorders, they are all manifested by a clinical spectrum ranging from mild to severe disease, with differences in disease progression and variable involvement of secondary organ systems. Most likely, this heterogeneity has its origin in the multifactorial nature of autoimmune diseases, e.g. specific combinations of environmental and genetic risk factors are likely to influence not only susceptibility but also the disease severity and prognosis. The heterogeneity of autoimmune diseases is further reflected by inter-individual variation in responsiveness to treatment modalities, within the many treatment options with proven efficacy that are available, still a notable percentage of patients do not respond satisfactory to treatment modalities. Currently, the criteria for subtyping of patients, e.g. to select those patients who will benefit from a specific treatment option, are lacking because our understanding of the molecular and physiological complexity of autoimmune diseases is incomplete. Better insight into the processes underlying disease heterogeneity will lead to better diagnosis, prognosis and optimized ‘patient-tailored’ treatment strategies. Molecular profiling studies of blood cells and/or affected tissues of patients with autoimmune diseases begin to reveal some of the key pathways that seem to underlie disease heterogeneity. Observational profiling studies in two common autoimmune diseases, i.e. multiple sclerosis (MS) and rheumatoid arthritis (RA) already reveal the heterogeneous nature of both diseases. This thesis aims to unravel the heterogeneity in MS and RA with respect to clinical outcome of respectively IFNβ and rituximab treatment. Subsequently, we searched for genetic variation in the components of the observed molecular pathways that related to responsiveness to IFNβ treatment in MS. We also validated and showed the clinical value of the identified molecular biomarker for rituximab responsiveness in RA. Since our and other profiling studies revealed that differential activity of the type I IFN pathway is characteristic for heterogeneity in autoimmune diseases, we also studied the role of type I IFN activity in relation to pathogenic and clinical characteristics of autoimmune diseases. Ultimately this knowledge will lead to better subclassification of patients and, among others, personalized treatment approaches, thereby enabling patient tailored treatment.
AUTOIMMUNE DISEASES

MULTIPLE SCLEROSIS: PATHOPHYSIOLOGY

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system that affects more than 2 million people worldwide. The disease usually starts between 20 and 40 years of age, and occurs more often in women. MS is a multifactorial disease in origin that involves both genetic and environmental factors. Known environmental factors involve geographical latitude of residence, gender and sexual hormones, pathogen exposure, smoking and vitamin D levels. The genetic contribution is polygenic, i.e. multiple gene variants are involved. Known susceptibility genes are the MHC class II gene (HLA-DRB1*1501 allele), and interleukin-2 (IL-2) and IL-7 genes, although their contribution to disease development is weak. The disease can be divided in four clinical forms of which Relapsing-Remitting (RRMS) is the most frequent clinical form (~80%), besides Secondary Progressive (SPMS), Primary Progressive (PPMS), and Progressive Relapsing (PRMS). Each clinical form has a heterogeneous nature, which is reflected by the variability in clinical presentation, which can range from a mild to a severe demyelinating disease. The RR subtype is characterized by episodes of acute inflammation and demyelination in the brain resulting in clinical disabilities/symptoms that can develop over hours to several days, followed by remyelination and complete or partial recovery. Progressive MS, which can be PPMS or following a phase of RRMS, which is then called SPMS, is marked by the absence of acute relapses and follows a gradual scale of accumulating disabilities.

Brain tissue of MS patients show typical lesions, or sclerotic plaques. Accumulation of inflammatory cells, gliosis and axonal injury are hallmarks of these lesions. The inflammatory cells present in the lesions attack the myelin sheet that surrounds the axons, resulting in demyelination, which ultimately leads to neurological dysfunction and disabilities.

The genetic background provides an intrinsic susceptibility level, which provides a basis for environmental factors to derail the immune system. It is believed that the environmental factors in the background of the susceptibility MHC class II alleles and immune regulators such as IL-2 and IL-7 leads to activation of myelin-specific T cells in MS patients.

Evidence for autoimmunity is based on the presence of these autoreactive myelin-specific T cells and antibodies within MS plaques. Whether these reactive T cells are primarily directed against self-peptides of e.g. myelin basic protein (MBP) or a result of cross-reactivity with myelin after activation by microbial antigens remains unknown. In the CNS, the autoreactive T-cells initiate a prolonged inflammatory response resulting in local production and secretion of pro-inflammatory cytokines including IFN-gamma, TNF-alpha, IL-1beta, and IL-6. The cytokines are thought to induce the expression of adhesion molecules on endothelial cells and thereby contribute to extravasation of immune cells through the blood brain barrier (BBB). The immune cell activity ultimately results in the
breakdown of the myelin sheet of axons and oligodendrocytes. Next to autoreactive T cells, B cells and plasma cells play a role in MS pathology. The production of autoantibodies directed against oligodendrocytes and myelin is well known, but also the influence on B-cell proliferation and role of susceptibility agents (e.g. vitamin D and the Epstein-Barr virus) suggest additional roles for B cells in MS. Although there is a lot of evidence that MS is an autoimmune disease, the are indications to state that MS is primarily a neurodegenerative disease that results in an autoimmune reaction. It is however for sure that both, immune reactions as well as neurodegeneration, are important processes in MS. However, the incomplete understanding of the underlying cause of MS makes it is difficult to effectively treat patients.

MULTIPLE SCLEROSIS: TREATMENT
Treatment options can be either used to treat acute relapses as well as aimed at reducing symptoms and slowing down the progression of the disease. Acute relapses that significantly impact the abilities of a patient are generally treated with a short, 3-5 day treatment with high dose intravenous steroids such as methylprednisolone or dexamethasone. The main goal of these treatment options is to shorten and decrease the impact of the relapses, however, there is no evidence available that steroids impact the long-term course of the disease. Disease progression can be treated by a number of agents with a long-term immunosuppressive effects such as mitoxantrone, cyclophosphamide, methotrexate and cladribin. Although the effects of these agents are substantial, the mechanism of action in autoimmunity in relatively poorly understood. Interferon-β (IFNβ) is an immunomodulatory agent that is currently most broadly used in RRMS and will be described in more detail further in this introduction. Glatiramer-acetate (GA) is another approved therapy for RR-MS, with similar or slightly lower efficacy than IFNβ at high doses. A number of activities for GA were shown, including polyclonal T cell stimulation, Th2 activation and cross-reactivity with myelin peptides, shift of the antibody response toward IgG4, interference with DC differentiation, and induction of brain-derived neurotrophic factors. The most important effect of GA is most likely the relative skewing toward Th2 reactivity. Other promising therapeutic strategies include, among others, humanized monoclonal antibodies against VLA-4 (natalizumab), which blocks blood-brain barrier migration of T cells and their activation and reduces brain inflammation, and Fingolimod (FTY720) which is an agonist of four out of the five known G protein coupled receptors (GPCRs) for S1P and blocks lymphocyte egression from lymph nodes via downregulation and degradation of the S1P receptor on lymphocytes.
RAHMATOID ARTHRITIS: PATHOPHYSIOLOGY

Rheumatoid Arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation of the joints that may cause permanent cartilage and bone destruction. The prevalence is about 1% worldwide and the disease is more prominent in women than in men. Because of its chronic nature, and the prospect of permanent damage to cartilage and bone, the disease has a major impact on the patients’ well-being and costs to society.

The pathogenesis of RA involves chronic inflammation of the synovium, a delicate one-to-two cell layer thick membrane that lines the surfaces of the joint. The rheumatoid synovium is infiltrated with inflammatory cells and the synovial fibroblasts (synoviocytes) become hyperproliferative, due to the upregulation of oncogenes and inhibition of apoptosis. Subsequent secretion of inflammatory mediators, such as cytokines and chemokines, results in expansion of the synovial membrane that leads to the so called “pannus” tissue. The pro-inflammatory mediators such as tumor necrosis factor-α (TNFα), interleukin (IL)-1, IL-6, IL-17 and granulocyte-macrophage colony stimulating factor (GM-CSF) have been demonstrated to have pathogenic roles in the disease. The pannus protrudes into the joint cavity and destroys adjacent articular cartilage due to the action of matrix metalloproteinases, reactive oxygen species and other toxic molecules. Bone erosions are the consequence of subsequent activation of osteoclasts. In addition tendon sheats are affected, which results in tendon weakness and rupture. RA is considered an autoimmune disease since clinical and laboratory studies reveal an immune mediated attack against self-antigens. The loss of immunological tolerance against IgG-Fc and citrullinilated proteins, represented by the presence of rheumatoid factor (RF) and anti-citrullinilated protein antibodies (ACPA), reflects the first sign of autoimmunity in RA. These autoantibodies are present in approximately 70-90% of the RA patients. Evidence from several studies demonstrated that these autoantibodies precede the appearance of clinical symptoms by many years. However the etiology of RA is still not well understood. It has become clear that a specific combination of genetic and environmental features ignite autoimmunity and inflammatory processes that drive development of arthritis. Genetic studies revealed a role for the MHC class HLA-DRB1 01 and 04 variants (so called shared epitope alleles) in RA providing evidence for a contribution of MHC class II dependent T cell activation in the disease process. Until now approximately 40 genetic variants from large genome-wide association studies have been identified, most of which have a function in the adaptive immune system. Several environmental risk factors have been associated with RA. Among these are pathogen exposure, smoking, mineral oil, silica dust and specific diets. Currently evidence is presented that suggests a role for Porphyromonas gingivalis, the bacterium that causes periodontitis. Porphyromonas gingivalis is more prevalent in RA and is the only bacterium that expresses the enzyme peptidylarginine deaminase known to convert arginine to citrulline. An environmental risk factor that has been repeatedly connected with RA is smoking. It was demonstrated
that the risk of disease was considerably increased for SE-allele HLA-DR and ACPA positive individuals in a dose dependent manner. Together with evidence that smoking induced protein citrunillation in the lungs, a model has been proposed suggesting that smoking may break tolerance to citrunillated proteins in a susceptible background of SE allele HLA-DR positivity that initiates the development of RA.

**RHEUMATOID ARTHRITIS: TREATMENT**

Classical drugs for the treatment of RA are the so-called disease modifying anti-rheumatic drugs (DMARDS), such as gold, penicillamine, hydroxychloroquine, sulphasalazine and methotrexate. These drugs retard joint damage but lose their benefit with time. New pathogenic insights led to the development of so-called targeted therapies making use of biologicals. The introduction of the biologicals, in particular TNF antagonists, marked a dramatic improvement in patient outlook. Currently 5 TNF inhibitors (etanercept, infliximab, adalumimab, certolizumab pegol, golimumab) are registered for the treatment of RA. Following the successful introduction of TNF antagonists in the early 1990s additional targeted approaches with biologicals aimed at B-cell depletion (rituximab), which will be discussed in greater detail in a later session of this introduction, an IL-1 inhibitor (anakinra), T cell co-stimulation blockade (abatacept) and IL-6 receptor blockade (tociluzimab) have entered the clinic. These drugs control disease activity and retard joint damage.

**PHARMACOGENOMICS**

Clinical experience revealed that both (RR)MS and RA are heterogeneous diseases that are difficult to treat. The existence of heterogeneity is thought to represent different pathogenic processes underlying the disease. Thus, disease mechanisms may vary among patients, and perhaps in different phases of disease. As a consequence, response to treatment varies between patients. A subset of patients may clinically respond to a given treatment, whereas others may only benefit from another treatment.

The term pharmacogenomics emerged in the late 1990s and is associated with the application of genomics in drug development. Pharmacogenomics is defined as: “The investigation of variations of DNA and RNA characteristics as related to drug response”. This applies to genetics and transcriptomics studies. Transcriptomics is the study of the transcriptome i.e. the complete set of RNA transcripts produced by the genome in organisms, tissues or cells. The transcriptome represents the activation state of the genome. Since by definition, nearly every aspect of a disease phenotype should be represented in the pattern of genes that are expressed, transcriptomics is a powerful tool to study molecular mechanisms underlying disease heterogeneity and can be used to identify pre-existing and
drug-induced transcripts that would predict or explain the clinical response to the drug. Analysis of longitudinal patterns of gene expression after treatment will provide an in-depth understanding of the pharmacodynamic consequences of a certain therapy and may provide insight in the mechanism of action. For both MS and RA, the blood compartment is a suitable compartment to study these molecular profiles in relation to treatment response and outcome since immune cells migrate to and from the affected tissues.

MICROARRAY TECHNOLOGY
Transcriptomics is mainly based on DNA microarray technology which can be used to study mRNA levels of all the genes of the genome simultaneously. Application of this technology on blood samples from patients with autoimmune diseases allows an hypothesis-free survey to identify comprehensively the fraction of genes that are specific for a disease subtype or phase. This molecular signature typically represents the contributions and interactions of specific factors and distinct cells that are associated with disease characteristics and subtypes and thus defines the samples unique biology. Studying expression profiles of whole blood or (subsets of) blood cells has already shown to be very relevant in the field of (auto)immunity. Comparison of healthy versus diseased expression profiles can provide general (global) insights in underlying disease mechanism, whereas comparing expression profiles between patients with different clinical phenotypes such as disease stage, progression of the disease or response to treatment can help us to understand heterogeneity within a disease.

DNA microarrays consist of a solid phase to which thousands of gene-specific oligonucleotides are spotted or synthesized on a predefined location. Thus each spot on the microarray contains one type of single stranded oligonucleotides that is specific for one distinct gene. Currently, the most popular array systems are the Affymetrix GeneChip and Illumina BeadChip. Affymetrix GeneChips consist of a solid phase on which the oligonucleotides are synthesized. Illumina BeadChips consist of a collection of microscopic polystyrene beads, coated with gene-specific probes. After hybridization of the solid phase bound oligonucleotides with fluorescent labeled mRNA from an experimental sample the fluorescent intensities for each spot (=specific gene transcript) are determined and quantitated. The data are corrected for unspecific binding by background subtraction and spot intensities or intensity-ratios are obtained, also known as the raw data. Before data can be used for analysis purposes, the raw data need to be normalized, e.g. corrected for inter- and intra-array differences (figure 1). Due to the complexity of the microarray technology, several important aspects need to be considered when conducting microarray experiments and analyzing data. In particular sample collection, processing and preparation need to be performed according to quality controlled and preferably standardized procedures. Moreover, the number of transcripts that are measured on an array is much higher than
the number of samples included in the study and therefore there is a high change of ‘false positives’ of which one should be aware of and account for in the analysis. Hence subsequent interpretation and analysis of large amounts of data requires special algorithms that e.g. visualize the data, correct for multiple testing, compare expression levels of groups of related genes and translate gene expression data in biological relevant information (biological pathway or process). The hierarchical clustering algorithm\textsuperscript{52} is very useful to identify and visualize co-expressed genes in large datasets from different experimental samples obtained with microarray experiments. Significant Analysis of Microarray (SAM)\textsuperscript{53} is an algorithm for finding important differences in the expression levels of single genes or groups of genes between two or more subgroups of samples or patients. Additionally, pathway-level analysis such as Gene Set Enrichment Analysis (GSEA)\textsuperscript{54}, PANTHER\textsuperscript{55} and Ingenuity\textsuperscript{56} can provide insight in the functional pathways or biological processes that are represented by the differentially expressed genes between subgroups of samples or patients. Prediction algorithms such as Predictive Analysis of Microarrays (PAM)\textsuperscript{57} or Random Forest (RF)\textsuperscript{58} can be used to select (sets of) genes that associate with, or predict, for example clinical outcome (Figure 1). In general, sufficiently powered studies, good laboratory proficiency for sample collection and processing, and data acquisition needs to be ensured and appropriate and properly used data analyses practices are required. Finally, technical and biological validation of obtained results using independent technologies such as qPCR or histochemistry is necessary.

**TRANSCRIPTOMICS IN MS**

For Multiple Sclerosis, microarray experiments were performed using either brain lesion samples, PBMCs, T cells or whole blood to study the molecular complexity in MS compared to healthy controls. Although many of these studies used arrays with a limited amount of
genes and included relatively small numbers of patients and controls, they already provided important information relevant to disease pathogenesis.

Comparison of different types of affected postmortem brain tissue identified several genes and pathways important for the pathogenesis in MS, such as osteopontin (OPN) in brain plaques from MS patients and increased transcription of genes encoding inflammatory cytokines like interleukin (IL)-6, IL-17 and interferon-γ, interferon regulatory factor-2 and tumor necrosis factor alpha receptor-2 in MS lesions. Furthermore, differential expression of inflammatory cytokines and genes such as granulocyte colony-stimulating factor and immunoglobulin Fc receptor common γ chain was observed in acute inflammatory versus silent non-inflammatory lesions. Comparison of active and inactive plaques revealed that chronic active and chronic inactive lesions differ in gene expression profiles for immune- and inflammation related genes, apoptosis related and stress-induced genes. Overall, these studies show an increased inflamed status of the affected region of the postmortem brain of MS patients, including gene signatures related to immune activity, apoptosis, adhesion molecules and regulation of MMP pathways, reflecting ongoing inflammation as an important phenomenon in MS pathogenesis.

Although the brain is the primary site of disease in MS, limitations to access this compartment for biopsies prevented transcriptome studies in affected brains of living MS patients. Instead, the peripheral blood is an easily accessible source to study. Since immune cells and inflammatory mediators migrate between the brain and blood compartment, the cellular composition and activation/differentiation status of the peripheral blood cells reflects the processes involved in MS pathogenesis. Initial studies that have been performed using blood cells of MS patients identified 34 out of 4000 gene transcripts expressed by peripheral blood mononuclear cells (PBMCs) that discriminated patients in stable remission from healthy controls. Twelve of these genes have inflammatory and/or immunological functions, including P protein, LCK, cAMP responsive element modulator, IL-7 receptor, matrix metalloproteinase-19, M130 antigen, and peptidyl-prolyl isomerase. Other studies identified differential expression of heat shock protein-70, together with the group of histones and kinase-2 of the CDC28 complex and low levels of the inhibitor of metalloproteinase-1 (TIMP1). Differential expression of genes related to T-cell activation and expansion, inflammation and apoptosis was also observed. Further evaluation for disease activity-associated genes (acute relapse versus remission) in the same cohort revealed over-expressed genes, that play a regulatory role in epitope spreading and in macrophage recruitment to the inflammatory injury during relapse. Apoptosis-related genes were significantly down regulated during relapse. Altogether, from these first microarray studies in MS, a general picture of overexpression of inflammation and apoptosis related pathways related to the pathogenesis of MS clearly emerges. These explorative studies revealed important aspects of disease pathogenesis in the affected brain tissue and
peripheral blood compartment. However, these studies focus on the group average and the issue of inter-individual heterogeneity, which was shown to be an important feature of the disease, remained to be determined.

Given the heterogeneous nature of MS, comparisons made between e.g. healthy controls and MS patients as a whole group might rule out relevant inter-individual differences within the MS population. The first study that also focused on inter-individual differences between MS patients at the molecular level showed that many genes are differentially expressed between MS patients. Thereto, whole blood expression profiles of healthy controls and patients with clinically well-defined RRMS were compared. As was shown in other studies, these analyses revealed several biological networks represented by clusters of genes that were differentially expressed between healthy controls and RRMS patients. Most prominent was an upregulation of IFN responsive genes and a downregulation of genes involved in protein translation and degradation in MS patients. Importantly, besides being a significant phenomenon for the MS patients as a group, it was also observed that the activity of IFN responsive genes varied significantly between MS patients. This so-called “IFN signature” was part of a gene program that reflected immune defense activation, which involved besides the IFN-response genes, also genes involved in Toll-like receptor (TLR) signaling and chemokine/interleukin signaling. The IFN signature consist of genes that are activated upon a biological response to IFN. It was demonstrated that the IFN response genes belonged to the type I IFN pathway, indicating that the IFN gene signature has a type I IFN origin. This study not only revealed new pathways affected by MS, but also provides a basis for uncovering novel mechanisms underlying disease heterogeneity.

TRANSCRIPTOMICS IN RA
Synovitis is the hallmark of rheumatoid arthritis and gene expression analysis was initially aimed to provide insight in the molecular features and biological pathways at play in the affected synovium. Not only comparison between RA and healthy tissue were made, but especially comparisons between affected synovial tissue from patients with RA and osteoarthritis (OA), a joint disease with limited joint inflammation, were made.

The first study on gene expression profiling in rheumatoid synovium highlighted the increased expression of genes involved in chronic inflammation such as immunoglobulins and HLA-DR in RA synovium when compared with healthy control synovium. Comparative analysis of synovial tissue specimen from RA and osteoarthritis (OA) patients revealed marked differences between OA and RA tissues. In particular genes involved in the adaptive immunity (B and T cell regulation) were upregulated in RA tissues, this finding confirmed histological findings of an increased infiltration of T cells and B cells in the rheumatoid synovium compared to OA. In addition, a number of non-immune genes were found to be differentially expressed between the RA and OA synovium, which were involved
in diverse biological processes such as extracellular matrix biology, transcription and cell cycle regulation, receptor/signaling, protease biology, adhesion and apoptosis. Functionally, the majority of the identified pathways are involved in the regulation of inflammation, proliferation, cell survival, and angiogenesis.

A large-scale gene expression profiling study in synovial tissue from patients with erosive RA revealed considerable heterogeneity among patients. RA tissue characterized by histological inflammation showed relative high expression of genes involved in inflammation and adaptive immune response, including genes related to type I IFN activity whereas RA tissues characterized by a low inflammation showed a relative low expression of immune-related genes and higher expression of genes involved in tissue remodeling activity, which is associated with fibroblast differentiation.70,71 Also Huber and colleagues noted the broad intra-group inter-individual expression variances in RA for genes representing different pathways (such as Toll-like receptor signaling pathway, T-cell receptor signaling pathway, Fc epsilon receptor I signaling pathway, adherence junction, classical TGF-β sub-pathway and the anti-apoptotic sub-complex.76 Accordingly, Lindberg and colleagues showed that synovial biopsies had gene expression signatures that were unique for each patient.77

Although the synovium in RA is the primary site of disease, many studies addressed the question, whether disease specific features were also reflected in whole blood or peripheral blood cells of RA patients. Initial studies revealed that the expression of immune related genes (e.g. glutaminyl cyclase, IL1RA, the calcium-binding proteins S100A8, S100A12 and Grb2-associated binding protein (GAB2)) were differentially expressed between RA patients and healthy controls.78,79 Additional studies on peripheral blood cells, including analyses on whole blood cell samples (Paxgene) confirmed and further extended the molecular differences between healthy controls and RA patients.80-82 Additional genes that Teixeira and colleagues identified include Ly96/MD2, NFAT5, thioredoxin, CAP/LL37, ORM1, ORM2, SLC11A1, PGLyRP1 and Factor V.

Interestingly, in line with the observations in MS, an increased expression of the type I IFN response genes was characteristic for approximately half of the RA patients. Van der Pouw Kraan and colleagues observed that a prominent cluster of IFN-response genes was significantly upregulated in a subset of patients with RA.80 These findings have now been replicated in several other studies using independent cohorts.83-85 Moreover, measurements of IFN-bioactivity in RA serum confirmed the presence of type I IFN-bioactivity in RA serum.86

These observations are consistent with the heterogeneous nature of RA and indicates that this type I IFN pathway is systemically activated in a subset of patients with RA.
INTERFERONS

The activation of the type I IFN system in a subset of MS and RA patients reflects to a large extend the inter-individual heterogeneity in these diseases and is one of the most striking observations in the above described genomic studies. One could wonder why IFNs are associated with autoimmune disease as type I IFNs are mainly involved in viral defence, suggesting they have additional important regulatory functions.87

TYPE I INTERFERONS

The type I IFNs comprise 16 subtypes, IFNβ, IFN, IFNκ, IFNω, and 12 subtypes of IFNα, that share sequence homology ranging from 20-60%. IFNβ and the subtypes of IFNα are the most relevant subtypes of type I interferon. They signal through a receptor complex that is composed of IFNAR1 and IFNAR2 present on almost all nucleated cells.88 Binding of a type I IFN to IFNAR2 results in dimerization of IFNAR1 and IFNAR2 which are both required for activation of Janus kinases (JAKs), thereby initiating a complex cascade of intracellular secondary messengers that emerge in two divergent pathways.89 One pathway leads to activation of the transcription factor IFN-stimulated gene factor 3 (ISGF3), a complex of phosphorylated Signal Transducer and Activator of Transcription 2 (STAT2) with STAT1 and IFN regulatory factor 9 (IRF-9; p48) that binds to the IFN-stimulated response element (ISRE) present in multiple genes.90,91 The other pathway involves STAT2/1 and STAT2/3 heterodimers and STAT1 homodimer (IFN-α-activated factor, AAF), which bind to the IFN gamma-activated sequence (GAS) response element.91-94 Ultimately, the IFN-induced activation of ISRE and GAS enhancer elements switch on a wide variety of genes (Figure 2).95 Although IFNα and IFNβ show overlap in their activities, they differ in their potency against different viruses as well as their ability to activate cells of the immune system. The exact mechanisms behind these differential physiological effects enabled through a common receptor are not elucidated yet, however, it has been shown that functional differences between the IFNs result in different receptor binding features and ligand-induced conformational changes in the IFNAR which may lead to differential signaling.96

REGULATION OF TYPE I INTERFERON

Many cells of the immune system, like monocytes, macrophages and neutrophils, are able to bind and phagocytize pathogens using receptors that recognize pathogen-associated molecular patterns (PAMPs) such as LPS, RNA and DNA on microbial structures leading to type I IFN production. These pattern recognition receptors (PRRs) include Toll-like receptors (TLR), retinoid acid-inducible gene I (RIG-1)-like receptors (RLRs) and nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs). TLRs are transmembrane proteins that have a key role in the activation of the innate immune response and are crucial in the activation of IFN type I genes.97
General Introduction

Figure 2. Regulation of Interferon and downstream effects.

This scheme presents an overview of the components involved in the regulation of type I IFNs as well as the effect of type I IFNs on receptor binding, signal transduction, gene program and successive biological effects. ISRE: IFN-stimulated response element; GAS: IFN-γ-activated sequence.

The human TLR family consists of 10 functional members (TLR1-TLR10). They can be classified based on subcellular distribution: TLR3, TLR7, TLR8 and TLR9 are typically located in intracellular compartments such as the endosomes, whereas TLR1, TLR2, TLR4, TLR5 and TLR6 are generally expressed on the cell surface. The cell surface receptors recognize conserved microbial patterns, such as lipopolysaccharide (LPS), whereas the intracellular receptors mainly detect nucleic acids. To tailor the innate response to different types of pathogens the TLRs use different but overlapping pathways, such as the MyD88 and the IRF3/7 pathways resulting in the activation of NF-kB and ISGF3 signaling, respectively.98 Bacterial pathogens that activate the innate response via TLR2 and TLR4 generate a dominant NF-kB pathway, whereas viruses trigger the innate immune system through TLR3, TLR7 and TLR9 that are more involved in activation of type I IFN production via the IRF3/7 pathway.99 After ligand binding to these TLRs, participation of Toll/IL-1 receptor (TIR) domain-containing adapters is needed to initialize signaling events. The adapters involved in
IFN-α/β induction are TIR-containing adapter-inducing IFN (TRIF) and TRIF-related adapter molecule (TRAM) for TLR4, TRIF for TLR3, and myeloid differentiation factor 88 (MyD88) for TLR7, TLR8, and TLR9.100 Next to TLRs, RIG-I (retinoic acid–inducible gene I) and MDA5 (melanoma differentiation–associated gene 5, also called Helicard) also mediate induction of type I IFNs. Their ligands are viral cytosolic RNA. They bind through caspase recruitment domains (CARD) to mitochondrial IPS-1, initiating a signaling pathway that leads to IRF3, IRF7, NFκB and AP1 activation and IFNα/β expression. Via a positive feedback loop, IFNα/β expression can result in upregulation of RIG1 and MDA5. The TLR pathways are essential for production of type I IFNs by pDCs, whereas RIG-1/MDA5 pathways appear to be essential for IFN production by fibroblasts, cDCs and macrophages.101

ROLE OF GENETIC VARIANTS

Interferon Regulatory Factor 5 (IRF5) is a transcription factor which is much more restricted compared to IRF3 or IRF7. It functions as a central mediator of Toll-like receptor signaling and is involved in the production of type I IFN, apoptosis, cell-cycle regulation, cell adhesion and pro-inflammatory reactions.102, 103 Moreover, expression of IRF5 is induced after activation of the IFN type I receptor, which is indicative for a key role of IRF5 in the induction of antiviral and inflammatory responses.103 Genetic studies revealed a important contribution for variants of IRF5 in the susceptibility of autoimmune disease.104

INTERFERONS IN AUTOIMMUNITY

The observed type I IFN signature in a subset of patients with MS and RA seems to reflect the activation status of the type I IFN system, although the potential trigger(s) and involved routes responsible for this signature in these diseases remain, so far, unknown. Apart from RA and MS, other autoimmune diseases such as SLE,105 Systemic Sclerosis106 and Idiopathic Inflammatory Myopathies107 are characterized by an increased type I IFN activity in a subset of patients, suggesting a common role for IFNs in autoimmune pathogenesis. In SLE however, the pathogenic mechanism behind IFNα production and subsequent upregulation of the type I IFN signature has been described extensively. Essential in this process is the ability of endogenous stimuli such as nucleic acids as part of immune complexes to stimulate the innate immune system via TLRs (and RIG1).101

In SLE, immune complexes (ICs), consisting of autoantibodies in combination with DNA or RNA-containing autoantigens cause ongoing IFNα production. Such interferogenic ICs are internalized via the FcγRIIa expressed on pDCs, reach the endosome, and stimulate Toll-like receptor (TLR)-7 or -9, which subsequently leads to IFN-α gene transcription. Comparable to this, chromatin, snRNPs or related immune complexes can also be taken up by B cells expressing antigen receptors (BCR) after binding of either the antigenic moieties of the macromolecule (BCR specific for DNA/histone or Sm/RNP) or the Fc moiety of the
autoantibody (BCR with rheumatoid factor activity). Again, transportation into endosomal compartments follows where DNA interacts with TLR9 and RNA with TLR7/8. Whether comparable mechanisms are at play in RA, MS or other autoimmune diseases needs to be investigated.

**UNMET MEDICAL NEEDS IN MS AND RA**

Clinical and molecular heterogeneity are important issues in MS and RA. Not only in understanding the mechanisms underlying disease pathology but also in efficient treatment of (the symptoms of) the diseases. As mentioned earlier, the improved understanding of involved pathogenic pathways led to the development of multiple new (targeted) treatment options in both diseases. Unfortunately, a high proportion up to 50% of the patients do not or poorly respond to these drugs. However, treatment in MS and RA still fully relies on trial-and-error approach. In the worst-case scenario, patients can be treated for longer than a year without beneficial outcome. Given the serious side effects and considerable costs for therapy, the challenge is to predict the response outcome before the start of treatment instead of the currently followed “trial and error” approach. It is therefore extremely important to understand the nature of the heterogeneous response outcome to treatment. As shown in previous sections, transcriptomic studies in MS and RA revealed marked inter-individual molecular heterogeneity. Better understanding of this molecular complexity with respect to treatment outcome, is key for identification of predictive markers and will ultimately enable us to set criteria to select patients who will or will not benefit from a certain treatment before the start of therapy.

In this thesis, we studied this relationship between treatment and response transcriptomics (pharmacogenomics) in order to find predictive markers for treatment outcome as well as better understand pharmacological pathways underlying treatment with IFNβ in MS and rituximab in RA.

**INTERFERON-β TREATMENT IN MS**

IFNs were the first biological agents to show clinical efficacy in the most prominent (RR) form of MS. Currently, a prolonged course of IFNβ treatment is still the best available therapy for RRMS. There are three preparations available for therapy: recombinant IFNβ1b (Betaseron®/Betaferon®), produced in Escherichia coli (Chiron, Emeryville CA, and Schering AG, Berlin, Germany) and two recombinant IFNβ1a, derived from Chinese hamster ovary (CHO) cells (Avonex®, Biogen Inc., Cambridge, MA, and Rebif®, Ares-Serono S.A., Geneva, Switzerland). IFNβ reduces clinical relapses, has an ameliorating effect on brain disease activity, and possibly slows down progression of disability. Unfortunately, IFN-based therapy
is associated with adverse reactions such as flu-like symptoms. Moreover, the response to IFNβ is often only partial, i.e. disease activity is not fully suppressed.\textsuperscript{108}

Despite the ameliorative effects of IFNβ its precise mechanism of action is not defined. The anti-inflammatory activities of IFNβ are believed to dominate in the context of RRMS. These activities involve anti-proliferative and pro-apoptotic effects via a variety of molecular changes, including increases in both cyclin kinase inhibitors and several pro-apoptotic molecules (Fas/FasL, p53, Bax, Bak) as well as activation of pro-caspases 8 and 3.\textsuperscript{109} Their anti-inflammatory effects also include the reduction of IL-1 production, an outcome that could contribute to its efficacy in the treatment of inflammatory diseases.\textsuperscript{110} It was demonstrated that monocytes from RRMS patients under IFNβ treatment produced substantially less IL-1β than monocytes derived from healthy donors. Evidence exists that IFNβ induced STAT1 activation repressed the activity of the NLRP1 and NLRP3 inflammasomes, thereby suppressing caspase-1-dependent IL-1β maturation. In addition, type I IFNs induce IL-10 that may signal via STAT3 to reduce the abundance of pro-IL-1α and pro-IL-1β.\textsuperscript{111} IFNβ also induces IL-27, which inhibits the pathogenic Th17 cells by impairing the ability of DC to promote IL-17 production by CD4(+) T cells, suggesting an anti-inflammatory effect of IFNβ via the induction of IL-27.\textsuperscript{112} Moreover, IFN-beta treatment was shown to inhibit TLR9 agonist-specific secretion of chemokines (CCL3, CCL4 and CCL5), which act as ligands for CCR5-positive Th1 cells.\textsuperscript{113} IFNβ also inhibits dendritic cell migration through STAT-1-mediated transcriptional suppression of CCR7 and matrix metalloproteinase 9.\textsuperscript{114} Inhibition of TNF by IFNβ might also contribute to amelioration of disease activity.\textsuperscript{115} These anti-inflammatory properties not only may explain the effectiveness of type I IFN in the treatment of inflammatory diseases such as RRMS, but also the observed “weakening” of the immune system after viral infection.

According to results from clinical experience, IFNβ treatment reduces relapse rates by about 30%, decreases the formation of inflammatory lesions in the CNS and extends remission periods.\textsuperscript{116} Moreover, clinical experience indicates that there are IFN ‘responders’ as well as ‘non responders’. A high proportion of about 50% of the patients do not or only poorly respond to IFNβ treatment however clear criteria for such classification are still lacking.\textsuperscript{117}

Part of the unresponsiveness to IFNβ can be explained by immunogenicity, i.e. the development of neutralizing antibodies (NAb). However, since not all patients develop NAb, and, if induced after at least six months of treatment, NAb can disappear again over time,\textsuperscript{118-121} other mechanisms are likely to be involved that explain (complete to partial) unresponsiveness. Hence, other mechanisms that result in insensitivity or resistance to the effects of IFNs are likely to underlie differential responsiveness.
PHARMACODYNAMICS OF IFNβ TREATMENT IN MS

Large scale molecular profiling technologies were already widely applied to identify pharmacodynamic changes for IFNβ in healthy controls and MS. This provided a framework to better understand the biological changes and pharmacological markers. IFNβ therapy in MS induced a broad spectrum of genes with potential immune regulatory effects. Most of the groups that studied the in-vivo response of IFNβ focused on the analysis of peripheral blood mononuclear cells, rather than on whole blood. Hilpert and colleagues reported on the expression kinetics and biological response genes regulated in healthy volunteers after a single dose administration of IFNβ1b. Most of the differentially regulated genes (total 227) peaked at 6-12 hrs. Overall a vast majority of IFNβ responsive genes were upregulated. Changes in mRNA of IFN-stimulated genes occurs as early as 1-2 hrs after treatment. In most cases the magnitude of the response peaks between 4 till 24 hrs and falls to baseline levels between 48 and 144 hrs after administration of IFNβ treatment naïve patients. The analysis revealed that mRNA levels of typical response genes such as RSAD2, GCH1, OASL, MxA and MxB, were still increased after 48 hrs and returned to baseline at 96 hrs. The dynamic expression kinetics of RSAD2, GCH1, OASL, MxA and MxB renders them interesting candidates as IFNβ biological response markers, peaking between 1 and 3 days post injection. Equivalent doses of IFNβ1a and IFNβ1b induced similar profiles of a selected set of genes and proteins in healthy controls.

The upregulated IFNβ-responsive genes fall into several biological classes, involving antiviral and immune-regulation, cell survival, metal ion homeostasis, cell cycle control and transcription regulation. IFNβ also strongly induced the chemokines CCL8, CCL2, CXCL10 and CXCL11. These chemokines generally recruit leukocytes to inflammatory lesions. A single dose of IFNβ1b induced expression of inflammatory Th1 cytokines but the regulated genes do not support a simple Th1 to Th2 shift. Other genes relevant to MS pathogenesis include IL1RN, which neutralizes inflammatory responses, genes that augment (STAT1, STAT2, IRF7) or inhibit (SOCS1) IFNβ-signaling, and BAFF (B cell activating factor of TNF family). Downregulated genes included integrin-αX (CD11c) and intracellular adhesion molecule 3 (ICAM3), which mediate cell adhesion and chemotaxis. Phenotypic analysis of blood cells revealed a transient increase in neutrophil counts and a decrease in the lymphocyte counts, which was reversed at 24 hrs. The increase of monocytes and the decrease of eosinophils were more sustained with maximum effects at 24 hrs, which lasted till 72 hrs. Expression of a group of NK marker genes decreased within the PBMC population but only transiently after the start of treatment. Accordingly, Hartrich and colleagues reported a transient decline of CD56 NK cells from 4-48h following a single dose of IFNβ. With long-term treatment NK cells were also reported to decline. This suggest an effect of IFNβ on NK cell activation and/or trafficking. Accordingly, Weinstock-Guttman and colleagues reported that peak levels of β2-microglobulin protein levels in plasma were co-ordinately regulated with immunological
processes related to NK cell activation. Kantor and colleagues applied a systems biology approach and reported a striking increase in peripheral monocytes, both in absolute counts and relative to total leukocytes, shortly after injection. There was no change for known T and B cell markers. Since the gene expression differences they observed, by far exceed the shifts in the blood cell composition it is concluded that most gene expression differences detected were due to genuine direct effects of IFNβ on gene transcription rather than resulting from altered blood cell counts. Equivalent doses of IFNβ1a and IFNβ1b induced similar profiles of a selected set of genes and proteins in healthy controls.

Altogether, these studies show the involvement of general immune related processes in the working mechanism of IFNβ treatment in MS. However, although the pharmacological studies provide a rich source for the selection of new and better biomarkers to improve the pharmacological monitoring of IFNβ therapy these studies again only provided insight into the IFNβ responsiveness in terms of a group average and the issue of inter-individual heterogeneity was not explicitly considered.

B-CELL DEPLETING TREATMENT IN RA

Accumulated evidence demonstrated the role of B-cells as essential players of the disturbed immune system in patients with RA. Especially their role as producers of RF and ACPA is supposed to play a crucial role in disease pathogenesis in RA. This was based on evidence demonstrating a role for surface expressed RF to bind immune complexes on B cells which serve a role as efficient antigen presenting cells that could lead to a break in T-cell tolerance against autoantigens. In addition, an arthritogenic role for ACPA in experimental models of arthritis was demonstrated. Subsequent findings also provided evidence for an autoantibody-independent function for B cells in disease pathogenesis related to their role in antigen presentation, lymphoneogenesis and cytokine release. In 2001, the clinical outcome of B-cell depletion in RA was first investigated. Thereto rituximab was used, a chimeric-human monoclonal antibody directed against the B cell marker CD20, that was first approved in 1998 for CD20+non-Hodgkin's lymphoma. CD20 is a 35-37 kD tetra-spanning integral membrane phosphoprotein that is highly expressed by naïve, mature and memory B cells, but not by precursor B cells and antibody-producing plasma cells. Although CD20 is expressed mostly on B cells at various stages of development, a small number of T cells and natural killer (NK) cells also express low levels of CD20. The CD20 target is stably expressed on the cell surface and when bound it is not shed, internalized or downregulated.

Significant clinical improvement was demonstrated in RA patients refractory to disease-modifying anti-rheumatic drugs (DMARD) who were treated with rituximab in combination with cyclophosphamide and high-dose corticosteroids. Subsequent results from randomised clinical trials showed that rituximab was highly effective in controlling disease activity in RA after a single treatment with two 1000 mg infusions during 14 days, resulting
in regulatory approval for patients refractory to tumor necrosis factor (TNF) inhibitors. Treatment is successively repeated after 6 months when many patients relapse.\textsuperscript{153,154}

Rituximab successfully depletes CD20 expressing B-cells by a mixture of apoptosis, antibody-dependent cell-mediated cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).\textsuperscript{155} As anticipated rituximab directly depletes the intermediate B-cell stages and doesn’t affect pre-B cells and long-lived plasma cells\textsuperscript{154,156}. Rituximab also directly depletes a small population NK cells of functionally active CD20+ T cells.\textsuperscript{157,158} CD20+ B-cell depletion (mean decrease of 97%) in RA is essentially complete at 1 month after the start of a single treatment and sustains for several months.\textsuperscript{150,157,159,160} Peripheral B-cells repopulate to almost baseline levels between 6-10 months after treatment.\textsuperscript{161,162} Repopulation starts with the appearance of CD5+CD38\textsuperscript{high} naive B cells, followed by an increase in immature CD19/IgD/CD38\textsuperscript{high}/CD10\textsuperscript{low}/CD24\textsuperscript{high} B cells.\textsuperscript{157} Memory B cells can stay low for more than 2 years. The rate of B cell repopulation correlated with serum levels of the B cell-chemokine CXCL13.\textsuperscript{163} B-cell-activating factor (BAFF) (=B Lymphocyte Stimulator (BLyS)) levels in the serum increased in patients with RA after rituximab-induced B cell depletion.\textsuperscript{160} Levels of visfatin, also known as pre-B cell colony-enhancing factor (PBEF), decreased and correlated with total B cell number after treatment.\textsuperscript{164} Serum titres of RF and ACPA significantly decreased at 24 and 36 weeks respectively, whereas total Ig levels and antibody titres against recall antigens and total immunoglobulin were not affected. This finding may suggest that rituximab selectively affects short-lived autoantibody-secreting plasma cells.\textsuperscript{161,162,165}

The observation that this treatment led to long-lasting depletion of peripheral blood B cells initially raised concerns with respect to the long-term effects regarding safety. As it stands, the safety of rituximab is comparable to that of other biologic DMARDs. Adverse events included mild to moderate infusion reactions in only 2% of the rituximab-treated patients. In addition, few cases of fulminant hepatitis B reactivation and rare cases of progressive multifocal leukoencephalopathy (PML) have been reported. A recent meta-analysis did not reveal a statistically significant increased risk of serious infection for rituximab.\textsuperscript{166} The relatively low rate of infections in B-cell depleted patients may be explained by the fact that, despite a dramatically impaired humoral activity, the cellular immunity was preserved.\textsuperscript{167}

Although circulating B cells are effectively depleted in nearly all treated patients, a substantial percentage of patients do not benefit from rituximab treatment. In order to effectively utilize rituximab and prevent unnecessary costs, risk for adverse effects and delays in effective treatment it is required to restrict treatment to only those patients who will benefit. Thereto biomarkers to stratify patients in responders and non-responders prior to the start of treatment, and assess the time for retreatment are badly needed.

Since rituximab equally depletes circulating B cells in responders as well as non-responders and many patients experience a relapse after 6 months when the B cell number is still low, the mechanism by which the clinical response is achieved is not entirely clear. Based on
these findings it is speculated that more subtle B cell-related processes and/or indirect effects contribute to clinical benefit. Consequently, a detailed insight in the mechanism of action associated with clinical response may likely provide insight in the mechanism of action and identify biomarkers of response.

Several studies have been published that proposed pathophysiological differences between responders and non-responders that could provide a basis for the identification of biomarkers to predict response to rituximab. Since the synovial tissue is the primary site of inflammation in RA, efforts have been made to look for associations between synovial markers and clinical response. No baseline characteristics were identified that could predict clinical response to treatment. Histochemical analyses revealed that the impact of rituximab on synovial B cells at 8 weeks is less than that in the periphery. The incomplete synovial depletion suggests that B cell infiltration in the synovial tissue is less vulnerable to the effects of rituximab which could be the consequence of micro environmental effector circumstances for B cell depletion (ADCC, CDC, and apoptosis). Patients with higher levels of clinical response had more consistent depletion of synovial B cells. The association between clinical benefit and decreased free light chains in the synovium is supportive with this observation. At week 16 an overall significant decrease in the number of synovial macrophages was observed. Although, at this stage the decrease in synovial B cells was not significantly different between responders and non-responders, changes in synovial macrophages and plasma cells were significantly associated with clinical response outcome, indicative for secondary effects of B cell depletion. The change in plasma cell numbers correlated with a decrease in ACPA at week 16. In another study, Teng et al. found that baseline differences in circulating ACPA (IgM) along with high infiltration of CD79a+ B cells were involved in incomplete response to rituximab. These results are somewhat surprising with respect to other observations that suggest a beneficial effect in disease with a B cell phenotype. Van de Veerdonk et al. reported decreased retinoic acid-related orphan receptor -t and interleukin-22 and the number of Th17 positive cells which correlated with better clinical outcome.

Overall, the studies suggest that synovial CD20+ B cell depletion is necessary but insufficient for therapeutic benefit. Instead, effects resulting in, e.g., a disturbed balance between B cell subsets and/or indirect effects affecting synovial plasma cells, macrophages, and/or Th-17 cells appear to play a role.

At the systemic level, several investigators found a decline in RF in patients who respond to rituximab. However, clinical efficacy does not depend on complete removal of these autoantibodies, questioning the pathophysiological role of this observation. Pooled data from 10 European registries (CARRERA) demonstrated that seropositive patients achieved significantly greater reductions in DAS28 at 6 months than seronegative patients. Fluorescence activated cell sorter (FACS) methodology revealed that neither B cell depletion
nor B cell repopulation correlated with clinical response. Preliminary findings using highly sensitive FACS technology suggested variation in peripheral B cell depletion and proposed that the failure for complete B cell depletion at 6 months was associated with a poor response. Evidence exists that a good response is associated with decreased IgD+/CD27+ memory B cells and delayed repopulation in the circulation. Others reported that clinical benefit was associated with depletion of CD19+/CD27+ memory B cells in both the peripheral blood and bone marrow compartment. Repopulation of IgD+/CD27+ memory B cells goes along with delayed acquisition of somatic hypermutations. In addition, long-term depletion of IgD-/CD27+ class-switched memory B cells was reported to be associated with a good response outcome. These findings suggest that control of adaptive immune processes involving germinal center-derived mature B cells is essential for successful RTX treatment. Another study revealed that patients at relapse had significantly reduced expression of BAFF receptor on naive and memory B cells. Whereas no association between clinical outcome and total numbers of T cells has been observed, rituximab decreased cytokine production representative of helper T cells (Th1-, Th2-, and Th17-type responses). Serum levels of monocyte chemo-attractant protein-1 and epidermal growth factor were found to be significantly higher after treatment in non-responders. In-vitro studies with monocyte-derived macrophages cultured before and after rituximab treatment revealed that changes in macrophage function, reflected by an increased BAFF/BLyS, interleukin-10, and CD86 mRNA expression accompanied by a reduction in TNF release in the cell supernatant, were associated with clinical response. These results suggest a change in macrophage function during rituximab therapy towards more mature macrophages with a less pro-inflammatory phenotype in responders. Other potential predictors of clinical outcome of rituximab treatment are serum BAFF/BLyS levels, Fc-RIII and interleukin-6 genotype, blood cell transcripts, and Epstein-Barr virus genome in bone marrow.

Although the above findings may contribute to our insight in the mechanism of action related to efficacy further research is required to understand the pharmacodynamics changes and molecular characteristics that are associated with the clinical outcome of rituximab treatment.

**THESIS OUTLINE**

Better understanding of molecular mechanisms underlying non-response to IFNβ treatment in MS and RTX treatment in RA will provide the basis for more efficient, patient tailored treatment strategies. In this thesis, we used transcriptomic profiling to achieve this goal. We applied large scale DNA microarray technology for a hypothesis generating survey to identify comprehensively the fraction of genes that are differentially expressed between responders...
and non-responders and could serve as a clinically relevant biomarker. Moreover, we aimed to specifically focus on the clinical relevance of differential activity of the type I IFN system in RA and MS patients and the contribution of genetic factors. Furthermore, we studied the association of the type I IFN system and autoantibody specificities in another autoimmune disorder, i.e. IIM.

In chapter 1.1, we studied gene expression profiles of MS patients before they started with IFNβ treatment in order to identify molecular markers to predict treatment outcome and to better understand (non)-responsiveness to treatment.

In chapter 1.2 we investigated whether genetic variation in central components of the type I IFN pathway is associated with the observed differential activity of the type I IFN system in MS patients and response to IFNβ therapy. We studied Single Nucleotide Polymorphisms (SNPs) in Interferon Regulatory Factor 5, which is a key factor of the type I IFN activation pathway. We compared IRF5 genotypes with transcriptional activity of the type I IFN system and clinical response parameters of IFNβ treated MS patients. In chapter 2.1 we studied pharmacogenomics in RA patients that were treated with rituximab, a B cell depleting antibody. We searched for genes and processes involved in response outcome and aimed to understand the biological mechanism underlying treatment (non)-responsiveness.

In chapter 2.2 we further tested the association of baseline type I IFN activity and clinical response to rituximab treatment of RA patients and we evaluated the diagnostic value of type I IFN response genes for prediction of non RESPONSIVENESS to rituximab treatment using ROC curve analysis.

In chapter 3.1 we studied genetic variation in IRF5 in relation to carotid intimal medial thickness in RA patients in order to better understand variability in the IFN system in relation to cardio vascular diseases under inflammatory conditions such as RA.

In chapter 3.2 we aimed to associate type I IFN related gene expression profiles to autoantibody profiles in patients with idiopathic inflammatory myopathies. We studied the link between presence of antibodies directed against RNA-binding protein complexes and the activation status of the type I IFN pathway in order to better understand the possible role of the type I IFN pathway in the pathology of these disorders.
REFERENCES


36. Stolt P; Kallberg H; Lundberg I; Sjogren B; Klareskog L, Alfredsson L. Silica exposure is associated with increased risk of developing rheumatoid arthritis: results from the Swedish EIRA study. Ann Rheum Dis, 2005 64, 582-6.


43. HELIOVAARA M; Aho K; AROMAA A; Knekt P; REUNANEN A. Smoking and risk of rheumatoid arthritis. J Rheumatol, 1993 20, 1830-5.


45. UHLIG T; HAGEN KB, KVieten TK. Current tobacco smoking, formal education, and the risk of rheumatoid arthritis. J Rheumatol, 1999 26, 47-54.


56. www.Ingenuity.com


77. Lindberg J, af Klint E, Ulfgren AK, et al., Variability in synovial inflammation in rheumatoid arthritis investigated by microarray technology. *Arthritis Res Ther* 2006a, 8:R47


98. Moynagh PN. TLR signalling and activation of IRFs: revisiting old friends from the NF-kB pathway. Trends Immunol 2005; 26:469-476


100. Roberto Baccala, Dwight H. Kono, Argyrios N. Theofilopoulos Interferons as pathogenic effectors in autoimmunity. Immunological Reviews, Volume 204, Issue 1, pages 9–26, April 2005


123. Satoh J, Nanri Y, Tabunoki H, Yamamura T. Microarray analysis identifies a set of CXCR3 and CCR2 ligand chemokines as early IFNβ1-responsive genes in peripheral blood lymphocytes in vitro: an implication for IFNβ-related adverse effects in multiple sclerosis. BMC Neuro 2006; 6:18
125. Satoh J, Nanri Y, Tabunoki H, Yamamura T. Microarray analysis identifies a set of CXCR3 and CCR2 ligand chemokines as early IFNβ1-responsive genes in peripheral blood lymphocytes in vitro: an implication for IFNβ-related adverse effects in multiple sclerosis. BMC Neuro 2006; 6:18


147. Hultin LE, Hausner MA, Hultin PM, Giorgi JV. CD20 (pan-B cell) antigen is expressed at a low level on a subpopulation of human T lymphocytes. Cytometry 14:196-204, 1993


PART 1
Molecular Markers for IFN-β Therapy in Multiple Sclerosis