Genetic variants contribute to differences in response and toxicity to drugs used in autoimmune diseases: Rheumatoid arthritis and systemic lupus erythematosus

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Chapter 1

General introduction and outline of the thesis

RHEUMATOID ARTHRITIS AND OTHERS AUTOIMMUNE DISEASES

Rheumatoid arthritis (RA) is an autoimmune disease associated with progressive disability and systemic complications. RA is characterized by synovial inflammation and hyperplasia, autoantibody production (rheumatoid factor and anti-citrullinated protein antibody ACPA), cartilage and bone destruction, and systemic features, including cardiovascular, pulmonary, psychological and skeletal disorders (1). The etiology of this inflammatory disease remains unclear due to complexity of interacting factors including both genetic and environmental determinants. The long-established association with *HLA-DRB1* locus has been confirmed in patients who are positive for rheumatoid factor or ACPA (2). Smoking (3), infectious agents and female gender have been recognized as risk factors associated with RA (1). Moreover, gene-gene and gene-environment interactions increase the risk for RA. The environment-gene interactions promote loss of tolerance to self-proteins that contain a citrulline residue, which is generated by post-translational modification and detected in T-cell and B-cell compartments. Why the systemic loss of tolerance is linked to a localized onset of inflammation in the joint is still unclear (1).

Disease-modifying anti-rheumatic drugs (DMARDs), including methotrexate (MTX) in RA have the capacity of reducing or preventing damage to the joints and preserving their integrity and function by modulating the immune response. However, the results of treatment with these drugs in patients diagnosed with RA are variable and unpredictable.

The anti-inflammatory mechanism of action of MTX is explained by its ability to inhibit cellular proliferation by reducing purine and pyrimidine synthesis, particularly in the cells most pertinent to synovial inflammation, such as T lymphocytes (4). MTX is well established as a competitive inhibitor of dihydrofolate reductase, and thereby prevents the regeneration from dihydrofolate of tetrahydrofolate, which is essential for the generation of folate cofactors required for de novo purine and pyrimidine synthesis (5).

The understanding of how MTX is effective at low doses in inflammatory diseases also provides some insights into how its well-known toxicities arise. The toxic effects have been suggested to result from a depletion of hepatic folate stores and the accumulation of MTX polyglutamates in the liver (6). When no complete response is obtained with MTX, other DMARDs can be used in sequential or combined therapy, or to add a biologic agent or with targeted therapy.

The introduction of biologic agents has notably altered the treatment of RA; these agents not only reduce symptoms and signs of the disease, but also delay its radiologic progression (7).

At present, five TNF inhibitors are available for the treatment of RA, three of which are full-length monoclonal antibodies: infliximab, adalimumab and golimumab. The fourth agent, etanercept, is a fusion protein of two TNFR2 receptor extracellular domains and the Fc fragment of human immunoglobulin 1 (IgG1). Certolizumab is a humanized Fab fragment conjugated to polyethylene glycol (PEG) without IgG1 region (8).

Biologic agents exert their pharmacological effects through their variable portion (designed to block the target molecule) and their constant portion (the Fc fragment of IgG1), which specifically binds the human FcG receptors (FcGRs) (9-12). FcGRs are expressed on the surface of most immune cells. Engagement of FcGRs by TNF antagonists could affect a number of cellular functions, including phagocytosis, antibody-dependent cellular cytotoxicity (ADCC), induction of apoptosis, cytokine release and macrophage-mediated clearance of immune-complexes (12, 13).

However, these treatments are substantially more expensive than traditional DMARDs and, unfortunately not efficacious in all patients (14). Some studies point out that between 25% and 30% of subjects with RA do not respond to anti-TNF treatment (15). There are other biologic agents used in the treatment of RA: anti-IL-6 receptor (tocilizumab, sarilumab), anti-IL-1 receptor (anakinra), anti-CD20 (rituximab) and anti-CD80/86 (abatacept). Similarly, targeted synthetic DMARDs, as baricitinib and tofacitinib, were developed to interfere with a specific molecule, Janus kinases (JAKs), based on advances in molecular and structural biology. They interfere with JAKs— intracellular signal transduction molecules that translate the effects of some cytokines to cellular responses (16).

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease of unknown cause that can affect virtually any organ of the body. Immunologic abnormalities, especially the production of a number of antinuclear antibodies (ANA), are a prominent feature of the disease. Patients present with variable clinical features ranging from mild joint and skin involvement to life-threatening renal, hematologic, or central nervous system involvement (17). The clinical heterogeneity of SLE and the lack of pathognomonic features or tests complicate the diagnostics (18).

The choice of therapy for SLE is highly individualized and depends on the predominant symptoms, organ involvement, response to previous therapy, and disease severity. A general approach to treating the predominant symptomatology include immunosuppressive agents: hydroxychloroquine or chloroquine, glucocorticoids, mycophenolate, cyclophosphamide and other agents targeting B-cell pathways as belimumab (19, 20) and rituximab (21). Belimumab has been approved by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) for use in SLE patients, whereas use of rituximab is considered off-label use.

Rituximab is a chimeric monoclonal immunoglobulin G1 antibody against the CD20 protein of B-lymphocytes promoting B cell depletion (22, 23). It has become a crucial therapy against systemic autoimmune diseases, since an aberrant B cell regulation is among the common pathogenic mechanisms of these diseases (24). FDA and EMA have approved the use of rituximab in Non-Hodgkin's lymphoma, chronic lymphocytic leukemia, and RA in combination with MTX in adult patients with moderately to severely active RA who have an inadequate response to one or more tumor necrosis factor anti-TNF, Wegener's granulomatosis, and microscopic polyangiitis in adult patients in combination with glucocorticoids. Recent studies in other systemic autoimmune diseases show the importance of this therapy in refractory patients (25-30).

PHARMACOGENETICS OF DRUGS USED IN RA AND SLE

Pharmacogenetics is defined as the study of variability in drug responses attributed to genetic factors (31, 32). It has become one of the leading and potentially most actionable areas of the personalized medicine paradigm, as evidenced by the increased availability of clinical pharmacogenetic testing (33).

The consequences of treatment with DMARDs in patients diagnosed with RA or SLE are variable and largely unpredictable. A possible cause that explains the interindividual differences in both efficacy and adverse events can be the genetic variations in genes encoding drug metabolizing enzymes or drug transporters (34-43).

The treatment with biologic agents is substantially more expensive than use of traditional DMARDs and, moreover, are not effective and safe for everyone (14). Early identification of subjects who respond to these drugs may be helpful when establishing a (cost)effective and safe treatment with these drugs (44).

OUTLINE OF THIS THESIS

The primary objective of this thesis is to investigate the role of pharmacogenetics in predicting drug response in treatments for the autoimmune diseases: RA and SLE. For this reason, this thesis is divided in two parts: pharmacogenetics related with drugs used in RA and pharmacogenetics of rituximab used in SLE and other autoimmune diseases.

Part 1: Pharmacogenetics of drugs used in RA

MTX is the most common DMARD used in RA. However, its use is hampered by frequent adverse drug events among which gastrointestinal toxicity is most frequent. Hepatotoxicity is a relatively rare but serious adverse event related to the use of MTX and is largely unpredictable. In **chapter 2** an overview is presented of the previously performed studies concerning pharmacogenetic predictive biomarkers for MTX-induced hepatotoxicity.

Treatment with anti-TNF agents results in a reduction of disease activity in most RA patients. However, a substantial part of patients does not respond to this therapy for unknown reasons. It would be highly beneficial to be able to predict whether or not an individual patient responds to treatment. **Chapters 4** and **5** describe the investigations on the role of different candidate SNPs related to the efficacy of the treatment with different anti-TNFs in RA. In addition, in **chapter 3** a replication study is presented based on 4 polymorphisms that were found associated with anti-TNF response in RA in a previously published genome-wide association study.

Part 2: Pharmacogenetics of rituximab used in SLE and other autoimmune diseases

In **chapters 6–8** the role of different genetic variants related to the pharmacodynamics of the drug or of the diseases are evaluated to study the contribution to differences in the response to rituximab in patients with SLE and other systemic autoimmune diseases.

In **chapter 6**, the possible involvement of the *-174 IL-6* polymorphism in the clinical response to rituximab in different systemic autoimmune diseases is assessed. In **chapter 7**, the aim is to investigate the possible involvement of the *FCGR3A-158F/V* polymorphism in the clinical response to rituximab in Spanish patients with different systemic autoimmune diseases. In **chapter 8**, the role of G/T polymorphism at the *IL2–IL21* region in the rituximab response in a cohort of SLE patient and different autoimmune disorders is analyzed.

Chapter 9 provides a summary of this thesis, **chapter 10** the Dutch summary (Nederlandse samenvatting), and **chapter 11** the general discussion and future perspective of this thesis.

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