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Functional polymorphisms in receptors for IgG (FcγR), affecting IgG binding, were first described in 1983, when a clear non-responder population was identified in the mitogenic effect on T cells of mouse IgG1 anti-CD3 antibodies (1). In the following years, our knowledge of structural diversity and heterogeneity in FcγR increased substantially, and clinical consequences of this variety became apparent (2,3). With the development of single and multiple Fc receptor deficient mice in the past decade, our view on Fc receptor interactions in the inflammatory response changed markedly (4, Chapter 1). More recent findings force us to revise our view on the involvement of FcγR polymorphisms in this response as well (Chapter 9).

New insights in FcγR heterogeneity and function contribute considerably to our understanding of the pathophysiology of various autoimmune and infectious diseases (2-4). In particular for immune complex-mediated diseases, such as systemic lupus erythematosus (SLE), recent advances in our knowledge have important consequences (Chapter 1). This thesis addresses the effect of genetic diversity and differential FcγR-mediated responses on systemic autoimmune disease, in particular with regard to clearance of immune complexes and the development of inflammation.

**Heterogeneity of FcγR in autoimmune disease**

Genetically-determined variation with functional consequences has been described for FcγRIIa, FcγRIIIa and FcγRIIIb, including the arginine (R) to histidine (H) change at amino acid position 131 for FcγRIIa, the valine (V) to phenylalanine (F) change at amino acid position 158 for FcγRIIIa, and the four amino acid changes termed neutrophil antigen polymorphism (NA1 and NA2) for FcγRIIIb (5-7). The effect of this variation is most pronounced for FcγRIIa, as the FcγRIIa-H131 allele was shown to be crucial for firm binding of IgG2-containing complexes (5,8), phagocytosis of IgG2-opsonized bacteria (7,9,10), and the mitogenic effect on T cells of human IgG2 anti-CD3 antibodies (11), which requires firm binding to FcγRIIa-H131 for adequate cross-linking of CD3. Although several association studies have demonstrated marked skewing towards low-binding alleles (FcγRIIa-R131, FcγRIIIa-F158 and FcγRIIIb-NA2) in patients with autoimmune diseases such as SLE, discrepancies between different studies exist (2,3). Inconsistencies may arise from variation in population sizes, clinical criteria used to define disease, as well as ethnic differences between populations (12).

In Chapter 2, we determined the influence of all 3 functional FcγR polymorphisms on susceptibility to SLE in a large and strictly Caucasian population, using well-defined and universally acknowledged criteria for disease. In this study, we found a strong trend toward skewing of FcγRIIa, with an enrichment of the homozygous low-binding genotype in patients compared with controls. This association was confirmed in a partly overlapping study population, but shown to be independent of single nucleotide polymorphisms in the interleukin-10 gene promoter (Chapter 4). Both FcγRIIa and IL-10 are located on chromosome 1, and have been indicated as potential susceptibility loci for SLE in genome scans (13,14). In contrast to previous observations (2,3), we did not find a correlation between low-binding alleles of
FcγRIIa, nor of FcγRIIIa, and the development of lupus nephritis. Due to discrepancies between different studies, a thorough meta-analysis is warranted to determine the exact contribution of different FcγR polymorphisms to disease susceptibility and clinical manifestations in SLE.

We also determined the influence of all 3 FcγR polymorphisms on susceptibility to Wegener's granulomatosis (WG), a systemic vasculitis with chronic nasal carriage of Staphylococcus aureus as an important risk factor for disease relapses (Chapter 3). Interestingly, patients with WG were more prone to relapse in the first five years after diagnosis when homozygous for the low-binding alleles of both FcγRIIa and FcγRIIIa. As these polymorphic variants are associated with decreased Fc receptor mediated clearance (7,9,10), this finding may be relevant for the chronic nasal carriage of S aureus in patients, and the subsequent risk to develop a disease relapse (15). Furthermore, this study was the first to demonstrate combinations of particular FcγR alleles to contribute to disease susceptibility. Although such allelic combinations could be of relevance in SLE as well, we were unable to demonstrate this (Chapter 2).

Studies in mice have recently shown an important role for FcγRIIb in the regulation of autoimmunity (16-18). This receptor is unique in its ability to transmit inhibitory signals, and is generally believed to act as an inflammation inhibiting receptor (Chapter 1). In Chapter 5, we screened the FcγRIIB gene for allelic variation, and determined whether specific alleles were associated with SLE in a Japanese population. For the first time, a single nucleotide polymorphism in FcγRIIB was detected in humans, resulting in an isoleucine to threonine change at amino acid position 232 within the transmembrane region of the molecule. Moreover, we found that the homozygous FcγRIIb-T/T232 genotype was significantly enriched in patients with SLE, compared with controls. Although functional differences between these two alleles remain to be determined, a recent study on deletion mutants of FcγRIIb revealed the transmembrane region of this molecule to contribute to its function (19). A confirmation of this polymorphism in Caucasian populations, as well as its contribution to disease susceptibility, is now eagerly awaited.

Clearance of immune complexes
As FcγR polymorphisms mediate binding and phagocytosis of immune complexes (5-10), they may also influence clearance of circulating immune complexes in vivo. Clearance of immune complexes is normally regulated efficiently by the mononuclear phagocyte system (MPS). In this system, erythrocytes bind immune complexes via complement receptor 1, providing a transport mechanism to mononuclear phagocytes located in liver and spleen. The complexes are subsequently removed from the erythrocytes, and internalized by fixed tissue phagocytes. The importance of FcγR in this process has been demonstrated clearly by studies of FcγR blockade in primates (20) and mice (21). In Chapter 2, we were able to show that the R-H polymorphism of FcγRIIa affects clearance of immune complexes in humans as well, as demonstrated by an extended half-life in blood of IgG-coated erythrocytes in subjects homozygous for the low-binding allele of FcγRIIa. This finding could explain the observed skewing
Summarizing discussion

towards this genotype in patients with SLE (Chapter 2 and 4), as reduced clearance of immune complexes may -indeed- influence the development of this disease.

To examine the role of FcγR in MPS function in more detail, we studied the processing of labeled immune complexes in mice deficient for different FcγR (FcγRI, FcγRII or FcγRIII). As described in Chapter 6, the liver was found to be the primary organ of immune complex uptake in mice. Clearance of immune complexes to the liver was substantially reduced when FcγRII was not present, whereas the absence of FcγRI or FcγRIII did not influence liver uptake. FcγRII in mice is analogous to FcγRIIb in humans, and is an inflammation inhibiting receptor. Indeed, in vivo production of the pro-inflammatory cytokine IL-6 in response to immune complex clearance was significantly reduced in the absence of FcγRI and FcγRIII. These findings allude to an important role for FcγRII in MPS function, as this receptor is efficient in immune complex clearance without improper production of pro-inflammatory cytokines. In view of this, we propose FcγRII to be the primary receptor for non-inflammatory IC clearance by the MPS in mice. It now remains a matter of debate whether MPS regulation in humans is predominantly at the level of activating receptors such as FcγRIIa, or the inhibitory receptor FcγRIIb.

**Inflammation in autoimmune disease**

When handling of immune complexes by the MPS is impaired, subsequent tissue deposition of complexes may result in inflammation and tissue damage. IgG subclasses differ in their potential to induce an inflammatory response, as they differentially interact with FcγR and complement. In Chapter 7, we investigated the influence of different autoantibody subclasses on the development of renal and extra-renal relapses in patients with SLE. Autoantibodies closely associated with renal involvement in SLE are commonly directed against dsDNA and nucleosomes (22). Indeed, in the majority of patients, a renal relapse is preceded by a significant rise in anti-dsDNA IgG (23). In our study, we found that IgG2 and IgG3 antibodies to nucleohistone were present more often in plasma of patients with a renal relapse compared to patients with an extra-renal relapse. Moreover, a significant rise in IgG2 anti-nucleohistone preceded a renal relapse in 78% of patients, compared to 18% of patients with an extra-renal relapse. For antibodies to dsDNA, only a significant rise in IgG1 could be detected prior to a renal relapse, although IgG2 antibodies were overrepresented in these patients. These data implicate a role for FcγR polymorphisms in the development of lupus nephritis, although we could not demonstrate such an involvement in our association study (Chapter 2).

The results of this study prompted us to investigate renal FcγR expression in more detail. Although FcγR have never been detected in frozen tissue sections of renal biopsy specimens from healthy donors (24,25), in vitro studies have shown expression of FcγRI and FcγRIIa to be induced on cultured human mesangial cells upon stimulation with LPS and/or IFNγ (26,27). In Chapter 8, we therefore examined whether FcγR expression could be detected in frozen tissue sections of renal biopsy specimens from SLE patients. We were not able to demonstrate mesangial expression
of any class of FcγR, although infiltrating leukocytes, presumably monocytes, were found positive in the majority of patients. These findings are in accordance with reconstitution studies in FcγR deficient mice, confirming FcγR-mediated activation by immune complexes deposited in kidney to be triggered by bone-marrow derived, circulating effector cells, rather than resident (mesangial) cells (4).

A challenging new concept in our understanding of inflammation is the influence of differential FcγR-mediated responses, which has been elegantly shown in panels of Fc receptor deficient animal models (4, Chapter 1). In this respect, FcγR polymorphisms may also modulate the severity of an inflammatory response. In Chapter 9, we investigated effector functions of neutrophils and monocytes upon activation with human IgG subclasses in donors expressing low-binding or high-binding FcγR alleles, in particular with regard to FcγRIIa. In contrast to our expectations, we did not find differences in cellular activation by IgG1 or IgG2 between different donors, despite an apparent distinction in firm IgG2 binding. This finding is in favour of a new mechanism for IgG-mediated leukocyte activation, in which a low-affinity interaction between IgG and FcγR appears to be sufficient to generate an inflammatory response, independent of IgG subclass and FcγR genotype of the host.

The implications of these findings for the inflammatory response in a disease such as SLE are profound. When clearance of immune complexes is impaired in the presence of low-binding FcγR alleles, our results suggest that the host is still capable of mounting adequate effector cell responses. Persistence of immune complexes, as is the case in SLE, may thus lead to excessive inflammatory responses, that can eventually be detrimental to the host. Conversely, in inflammatory processes that do not involve clearance of immune complexes, a correlation between IgG-mediated activation and FcγR polymorphisms will probably not be observed. This may also explain why we detect skewed distribution of FcγR alleles in susceptibility to SLE, but not in renal involvement, as an inflammatory response will be triggered independent of the FcγR genotype of the host once immune complexes are deposited (Chapter 2).

Concluding remarks
Whereas this thesis primarily focused on the effect of genetic diversity and differential FcγR-mediated responses on systemic autoimmune disease, it should be noted that other factors also contribute to clearance of immune complexes and development of inflammation. An important role for the complement system in processing of immune complexes has been supported by human studies showing abnormal immune complex clearance in complement-deficient and hypocomplementaemic patients, including patients with SLE (28,29). A critical role for complement has been demonstrated in the clearance of apoptotic cells as well (30), an important aspect of SLE pathophysiology. Indeed, it is intriguing that in more than 90 percent of patients with a Clq deficiency, this defect alone is sufficient to develop an SLE-like syndrome. However, it remains a matter of debate whether complement activation is crucial to the development of inflammation and tissue damage (31-33). An important role for activating FcγR has been suggested in this respect, using various animal models for human diseases (4,
Chapter 1). Furthermore, pathologic antibodies in SLE are often of the IgG2 subclass, a subclass that does not activate complement efficiently, which is in support of a dominant role for FcγR in inflammation (34, 35, Chapter 7). In view of these findings, it is likely to assume that complex diseases such as SLE develop and aggravate in the presence of multiple abnormalities. Future research should clarify the exact role of each factor in the development and progression of autoimmune disease, including the contribution of individual FcγR.

References