Immune regulation by receptors for IgG

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Chapter 8

Discussion

DISCUSSION

This thesis describes the role of FcγR family members in mouse models of the autoimmune diseases arthritis and SLE. In addition, it presents the generation and basic analysis of newly generated advanced genetically modified mouse models, which provide new opportunities for a more detailed analysis of the function of the inhibiting FcγRIIB.

New insights into the role of FcγRs in arthritis

In the past years, the analysis of various murine models has provided valuable information about the role of FcγRs in arthritis (Chapter 2). In this thesis these findings have been extended by using mice that are selectively inactivated for one or more FcγRs.
In contrast to other IC-induced autoimmune models, such as antibody (Ab)-induced trombocytopenia, nephrotoxic nephritis, hamolitic aneamia, the role of FcγRIV in arthritis has not yet been addressed (1-3). Destructive arthritis that develops in FcγRI/II/III KO mice but not in FcRγ KO mice (deficient for all activating FcγR; FcγRI, FcγRIII, FcγRIV) provide for the first time strong indication that FcγRIV alone can be sufficient to drive the downstream antibody effector pathways resulting in arthritis pathology (Chapter 3). Since this could be demonstrated in both the active collagen-induced arthritis (CIA) and the passive K/BxN serum-induced arthritis, a role for another FcRγ-chain-associated receptor is unlikely, but cannot fully be excluded. To confirm these observations more directly, FcγRIV KO C57Bl/6 mice should be tested as soon as they become available in passive arthritis models, such as anti-bCII-mediated arthritis and K/BxN serum-induced arthritis. Since FcγRIV binds IgG2a and IgG2b isotypes of IgG, it is expected that passive arthritis induced by anti-bCII Abs of these isotypes, but not by IgG1, will be attenuated in FcγRIV KO mice. To confirm the results obtained in C57Bl/6 background and to complete the analysis of FcγR KO mice on DBA/1 background, it is be desirable to cross FcγRI KO and FcγRIV KO mice onto the widely used arthritis-sensitive DBA/1 background and to study the course of active and passive arthritis. Alternatively CIA can be induced in C57Bl/6 mice deficient for one or more activating FcγR by using a protocol suitable for arthritis induction in C57Bl/6 mice (4). Moreover, mice with full deletion of the FcγRs locus on Chr1 (FcγRIII/ IV/ II), combined with FcγRI KO, but with a functional FcRγ-chain, which will become available in the near future, should be tested in CIA. If these quadruple KO mice, deficient for all FcγR, are completely resistant to CIA, it can be concluded that there is an indispensable FcγR-mediated phase in arthritis. This is of particular interest, since it is reported that FcRγ KO mice deficient for all activating FcγR develop bone destruction in the absence of inflammation in antigen-induced arthritis (AIA) (5) and in the K/BxN model after repeated injections of K/BxN serum (6).
Studies on different murine arthritis models have shown that the role of the individual FcγR family members in arthritis is often redundant and under specific conditions their function is interchangeable (7). Our observation that in FcγRI/II KO and FcγRII/III KO mice CIA develops with the same incidence suggests that FcγRI and FcγRIII can fully compensate for each other (Chapter 3). An earlier study showed that FcγRIII deficient DBA/1 mice are almost fully protected against CIA (8). This can be explained by the different background, the presence of the inhibiting FcγRIIB or the much shorter observation period compared to our

study. FcγRIII mainly regulates the inflammatory response in the early phase, whereas FcγRI is more prominently involved in the induction of chrondrocyte death and matrix erosion via activation of matrix methalloproteinases (MMPs) in the late phase of the disease (7;9). Cartilage erosion is more pronounced in T cell-dependent arthritis models, indicating that T cell-derived cytokines might play an important role in that pathogenic process. In the IC-induced arthritis (ICA) model, severe cartilage erosion is enhanced by local over-expression of the pro-inflammatory Th1 cytokine IFNγ (10). In FcγRI KO mice, chrondrocyte death remained low, even after IFNγ over-expression, suggesting a crucial role for FcγRI in this process. However, MMP-mediated cartilage destruction was enhanced by IFNγ in arthritic knee joints of FcγRI KO mice indicating that FcγRIII can compensate for the lack of FcγRI (10). In Chapter 4 we show, using FcγRIII KO mice, that in ICA the requirement for FcγRIII in the induction of joint inflammation and chondrocyte death can be bypassed by local over-expression of IFNγ. Whereas in these experiments upregulation of FcγRI was induced artificially by IFNγ-expressing adenovirus vectors most likely similar upregulation occurs physiologically during CIA and possibly also in RA. Importantly, IFNγ also increases expression of FcγRIV (1), which may synergize with FcγRI in mediating induction of chondrocyte death, even though FcγRI has been shown to be crucially required for this process (10). Clearly, these experiments show that IFNγ has the potency to shift the balance in the involvement of the different FcγR family members during arthritis through differential regulation of their expression. It is highly relevant to identify the cytokines and their sources which are responsible for this action *in vivo*. The recently identified Th17 cells seem to play a more important role in autoimmune pathology than Th1 cells (11). IL-17, secreted by Th17 cells, is a more potent cytokine in inducing autoimmune inflammation than IFNγ (12). Therefore it is highly relevant to evaluate the effects of IL-17 on FcγR expression. In arthritis the inflamed joint contains many cytokines, both pro- and anti-inflammatory, which may have opposing effects on FcγR expression levels. The susceptibility of local effector cells for IC-induced triggers will be determined by the net cytokine signals they receive.

The role of FcγRIV in arthritis as deduced from studies in FcγRI/II/III KO mice might be overestimated, since these mice, due to the absence of FcγRIIB, exhibit higher antibody titres, defective IC clearance and lack of control by an inhibiting receptor, while FcγRIV is the solely available FcγR. On the other hand, FcγRIIB deficiency might mimic the conditions in patients in which cytokine profiles induce a shift in the ratio between the inhibiting and activating receptors in favor of the activating ones. We have shown in Chapter 5 that in FcγRI/III KO mice FcγRIIB can act as a potent endocytic receptor and in that way can reduce joint inflammation by IC-clearance during the development of arthritis. In the AIA model FcγRI/III KO mice hardly develop disease, whereas FcγRI/II/III KO mice show increased joint inflammation compared to WT controls but without severe cartilage destruction. Joint macrophages exhibited an activated phenotype in FcγRI/II/III KO but not in FcγRI/III KO mice. In the light of the discovery of FcγRIV two years after the publication of this paper, this finding can also be explained by uncontrolled FcγRIV function in FcγRI/II/III KO mice compared to FcγRI/III KO mice, in which FcγRIIB is still present. The observations that FcγRIV expressing effector cells (neutrophils, macrophages) are present in high numbers in the inflamed joint and that the dominant isotype of anti-mBSA Abs is IgG2a, which binds strongly to FcγRIV, make it plausible that FcγRIV is involved. With the AIA model no severe

cartilage destruction was found in FcγRI/II/III KO mice (Chapter 4), in contrast with the finding in the CIA model, in which the FcγRI/II/III KO mice exhibited fully destructive arthritis (Chapter 3). One possible explanation is that the AIA experiments are terminated after seven days whereas our CIA experiments were terminated after more than 120 days. Differential contribution of FcγRIV to the disease process as a consequence of differences in regulatory cytokine profiles between the two arhritis models could be another explanation. We found indications that FcγRIV contributes to joint inflammation in the passive K/BxN model (duration 2 weeks) but whether the induced mild inflammation resulted in cartilage destruction was not determined (Chapter 3).

The dominant function of FcγRIIB under physiological conditions is most likely inhibition of FcγR-mediated activation rather than IC clearance. To prove that FcγRIIB contributes *in vivo* to IC clearance in the presence of functional activating FcγRs is experimentally challenging. To dissect the inhibitory role of FcγRIIB from its role in uptake of soluble IC *in vivo*, the B cell-specific b1-isoform of FcγRIIB that is incapable of mediating endocytosis should be expressed specifically in macrophages in the absence of the endogenous b2-isoform of FcγRIIB. Alternatively, FcγRIII KO mice may be used in a passive model, taking advantage of the strong difference in affinity of FcγRI and FcγRIIB for the different IgG subclasses. FcγRIII KO macrophages should take up IgG2a-IC via FcγRI and IgG1-ICs via FcγRIIB.

The definition of the role of FcγRs in arthritis has emerged from *in vivo* studies performed in a large variety of arthritis models, each with their characteristic similarities and differences when compared to RA, in different mouse strains enabling the analysis of different aspects of the disease process (Chapter 2). CIA is the model of choice from all mouse models described in this thesis. The development of CIA after immunization with collagen type II from another species is based on cross-reactivity of the induced antibodies with mouse collagen that is present in the joints. The disease has a T cell-dependent phase and its induction does not rely on intra-articular injection. In addition, CIA has a self-perpetuating, chronic nature, which mirrors RA. Intriguingly, despite the considerable differences between the various arthritis models the role of FcγRIII seems to be dominant (6;8;13-16). An obvious explanation is that from all activating FcγRs, FcγRIII is most widely expressed and has the highest basal expression level. FcγRIII is the only activating FcγR expressed on mouse mast cells, which are reported to play a substantial role in arthritis (17). In contrast, our studies on CIA in the FcγRIII KO model showed that although FcγRIII deficiency resulted in a delay in the onset of the disease in the chronic phase, 70-130 days after the immunization with collagen, the role of FcγRIII can be taken over by FcγRI and/or FcγRIV suggesting that the dominance of FcγRIII is restricted only to the initial phase of the chronic disease (Chapter 3).

From the many studies published so far it is still unclear which FcγR family member (FcγRI, III or IV) on which effector cell type (e.g. macrophages, neutrophils, mast cells etc.) is involved in the observed activation of downstream effector mechanisms, like secretion of the effector cytokines IL-1β and TNFα and secretion and activation of MMPs in the different stages of arthritis. For a detailed understanding of the complex role of the individual FcγR family members in the different stages of disease, inducible cell type-specific FcγR KO mice could be very helpful. Identification of well-defined steps, such as secretion of a certain cytokine after triggering of one FcγR family member on a particular cell type in the context of the complex pathological processes of arthritis will provide better understanding of the

disease.

The role of FcγRIIB in autoimmunity redefined

The contribution of FcγRIIB to the development of autoimmunity has been debated extensively in the last decade (18;18-22). It has been suggested that linked 129-derived sequences may contribute to the strong autoimmune phenotype of FcγRIIB$_{129}^{-/-}$ mouse (23). In Chapter 6 of this thesis, for the first time, we provide direct evidence that FcγRIIB on its own plays a limited role in the development of spontaneous autoimmunity in C57Bl/6 mice. FcγRIIB$_{B6}^{-/-}$ mice, generated on full C57Bl/6 background, exhibited a milder (non-lethal) autoimmune phenotype compared to FcγRIIB$_{129}^{-/-}$ mice. However, as we showed, FcγRIIB$_{B6}^{-/-}$ mice still lack both a negative control of FcγR-mediated antibody effector pathways and a negative feedback loop on the antibody production of B cells. Importantly, FcγRIIB$_{B6}^{-/-}$ mice are susceptible to induced autoimmune disease, although the disease develops with lower incidence than in FcγRIIB$_{129}^{-/-}$ mice. In conclusion, our results show that FcγRIIB is indeed a negative regulator of B cells and antibody-mediated effector cell functions. The role of FcγRIIB in the development of spontaneous autoimmunity (SLE) is more restricted as originally postulated on the basis of the phenotype of FcγRIIB$_{129}^{-/-}$ mice, whereas its role in induced autoimmunity (CIA) is supporting the hypothesis that FcγRIIB represents a late checkpoint in B cell tolerance. Impaired FcγRIIB function may have the potential to amplify the development of lupus, when combined with other loci associated with autoimmunity, such as the Sle1-region, as demonstrated by the severe lupus phenotype of FcγRIIB$_{129}^{-/-}$ mice and the mild autoimmunity of Sle1-congenic C57Bl/6 mice. These data redefine the intrinsic role of FcγRIIB in autoimmunity.

However, several aspects of the phenotype of the FcγRIIB$_{129}^{-/-}$ mouse are not well understood. FcγRIIB$_{129}^{-/-}$ mice as well as in C57Bl/6 mice congenic for the129-derived SLE locus develop spelenomegaly and the composition of their splenocytes is altered (24;25). In contrast FcγRIIB$_{B6}^{-/-}$ mice do not develop splenomegaly and preliminary analysis by flow cytometry showed a normal splenocyte composition, however this analysis needs to be extended to more specific subtypes of cells. From our observations so far it is unclear why FcγRIIB$_{B6}^{-/-}$ mice do not die prematurely as FcγRIIB$_{129}^{-/-}$ do. Analysis of the kidneys of aged mice showed increased inflammation in FcγRIIB$_{B6}^{-/-}$ mice compared to WT controls and histology scores of FcγRIIB$_{B6}^{-/-}$ mice were almost comparable to the histology scores of FcγRIIB$_{129}^{-/-}$ mice. However, at the age the kidneys were collected the majority of the FcγRIIB$_{129}^{-/-}$ mice had died, most likely due to kidney failure, and the kidneys of these death mice have not been included in the histological analysis. In contrast, within the first year of life mortality was not increased in FcγRIIB$_{B6}^{-/-}$ mice compared to controls. From these data it is impossible to conclude whether there is a difference in kidney inflammation between the two strains. To address the cause of death more precisely, moribund animals, showing increased proteinuria as a sign of kidney failure, should be sacrificed and analyzed. It is possible that (a) certain autoantibodie(s), most likely specific for (a) kidney constituent(s), make(s) the difference between FcγRIIB$_{B6}^{-/-}$ and FcγRIIB$_{129}^{-/-}$ mice. This is supported by the results of our analysis of the autoantibody titres of the two strains which suggest that FcγRIIB$_{B6}^{-/-}$ mice have a more restricted repertoire compared to FcγRIIB$_{129}^{-/-}$ mice. Therefore it would be interesting to test

for the presence of a wider range of autoAbs in FcγRIIB$_{129}^{-/-}$ mice. However, it cannot be excluded that there is hardly any qualitative difference in lupus-like disease between the FcγRIIB$_{B6}^{-/-}$ and FcγRIIB$_{129}^{-/-}$ mice. The difference between the two strains at the age of 12 months (87 and 17 % survival respectively) can also be explained by a substantial delay in the development of autoimmune disease in FcγRIIB$_{B6}^{-/-}$ mice. Therefore we should extend the observation period with 2-6 months.

The nature of the Sle1-associated gene(s)/allele(s) that in combination with the C57Bl/6 background causes autoimmunity is subject to intense investigations. The contradictory results on the interaction of FcγRIIB with the MRL/lpr background using the FcγRIIB$_{129}^{-/-}$ mice (26;27) suggest that complex interactions of 129-derived genes with each other and/or with the C57Bl/6 genome play a dominant role in determining whether autoimmunity develops. Since penetrance of autoimmunity greatly depends on the health status of the mice, it is not unlikely that some of the genes/alleles located in the Sle1 locus influence responses to infections or alter composition of the gut flora. It is known that environmental triggers have major influence on the development of autoimmunity. It is likely that these Sle1-associated genes act in a B or T cell-intrinsic manner. A good candidate is the SLAM/CD2 region that carries several genes that are expressed in B cells with a significant different expression level in the 129 haplotype compared to the C57Bl/6 haplotype (20). Within the SLAM/CD2 region the allelic variant of Ly108 present in the NZW-type autoimmune prone Sle1b sublocus, which shows a strong shift in the ratio between two splice isoforms compared to the Ly108 gene in C57Bl/6 mice resulting in a dramatic change in a variety of B cell responses upon triggering the B cell receptor, is a promising candidate lupus susceptibility gene (28). The identification of susceptible genes or combination of genes in the Sle1 region might be hampered by the strong genetic and perhaps functional linkage between these susceptibility genes. An approach would be to generate KO mice for one or more candidate genes using 129-derived ES cells and to analyze these mice for the development of autoimmunity.

Using the 56R transgenic model it has been suggested that FcγRIIB acts at a peripheral checkpoint either by inhibiting the secretion of IgG autoAb by autoreactive B cells (29) or by preventing B cells to enter into GC reaction (30). To exclude a contribution of the linked 129-derived Sle1 locus in these processes these studies should now be repeated using the FcγRIIB$_{B6}^{-/-}$ mice presented here. Especially, since it has been suggested that unidentified genes present in the C57Bl/6 background decrease the efficacy of receptor editing (29).

Recently we have shown that in FcγRIIB$_{129}^{-/-}$ mice the induction of oral and nasal tolerance is impaired (31). Our preliminary results with the FcγRIIB$_{B6}^{-/-}$ mice confirmed the phenotype of the FcγRIIB$_{129}^{-/-}$ mice suggesting that the 129-derived Sle1-region is not involved in nasal and oral tolerance induction in FcγRIIB$_{129}^{-/-}$ mice (J.M.Ramirez, unpublished results).

The precise role of FcγRIIB in IVIG-mediated inhibition of inflammation is somewhat controversial. In mouse models of ITP, rheumatoid arthritis, and nephrotoxic nephritis, IVIG administration blocked autoAb-mediated inflammation. This protective effect was abolished in FcγRIIB KO mice (32-34). Other studies have found a dominant role for FcγRIII on DCs and identified a downstream, indirect role for FcγRIIB (35). The role of FcγRIIB could be clarified by re-analyzing (conditional) FcγRIIB$_{B6}^{-/-}$ mice in the above mentioned models.

In addition to redefining the role of FcγRIIB in autoimmunity, altogether these data demonstrate also the pitfall of using KO mice generated with 129-derived ES cells and subsequently

backcrossed on C57Bl/6 background to define the function of a gene. After 20 generations backcrossing about 10 cM 129-derived sequences linked to the KO allele are still present in the KO offspring. This underscores the importance of the generation of KO mice directly in C57Bl/6 background using C57Bl/6-derived ES cells.

B cell-specific inhibition by FcγRIIB

There is circumstantial evidence that FcγRIIB on B cells plays a direct role in autoimmunity. Transfer of FcγRIIB$_{129}^{-/-}$ bone marrow into WT recipients causes humoral autoimmunity (18). However, in this experiment, the effect of FcγRIIB-deficiency is not dissected from the contribution of the 129-derived linked Sle1 locus. Moreover, the authors do not show data on kidney function and mortality, therefore it is not clear whether the severity of autoimmune disease in the adoptively transferred mice is comparable to the severity of autoimmune disease in FcγRIIB$_{129}^{-/-}$ mice. In a different study, using autoimmune-prone mice without or with reduced FcγRIIB expression, complementation of FcγRIIB on 30% of all B cells was sufficient to reduce humoral autoimmunity (19). Moreover, in a transgenic model, in MRL/lpr mice, FcγRIIB over-expression on B cells, but not on macrophages, is able to delay development of autoimmune disease (36). Unfortunately, none of these studies address the intrinsic cell-type-specific contribution of FcγRIIB on C57Bl/6 background in maintaining tolerance in the absence of any genomic region derived from other strains associated with autoimmunity.
To address the B cell-specific role of FcγRIIB in autoimmunity, the novel inducible B cell-specific FcγRIIB mice presented in Chapter 7 should be monitored for the development of autoimmunity with age. Since FcγRIIB$_{B6}^{-/-}$ mice show only a mild autoimmune phenotype the expected autoimmunity in the conditional KO mice may even be milder, therefore large numbers of mice are required. Backcrossing these mice on an autoimmune background (such as Yaa or MRL/lpr) would increase disease incidence and/or disease severity and, depending on the cell type-specific effect of the other loci, would result in synergy. For instance, Yaa contributes to autoimmunity in a B cell-intrinsic manner (37;38). Analysis of Yaa mice in combination with cell type-specific FcγRIIB deletion could confirm these findings, although the synergy is between FcγRIIB deficiency and Yaa in the development of autoimmunity is mild.
Our preliminary studies using B cell-specific inducible FcγRIIB$_{B6}^{-/-}$ mice in the CIA model show that the presence of only 30% FcγRIIB-deficient B cells can already confer to high IgG anti-bCII titres, comparable to what is seen in FcγRIIB$_{B6}^{-/-}$ mice. However, in contrast to what was observed in FcγRIIB$_{B6}^{-/-}$ mice these high IgG anti-bCII titres are not sufficient to induce joint inflammation in these mice with a myeloid-cell compartment expressing normal FcγRIIB levels (PB, JSV, unpublished results). It would be interesting to determine the minimum number of B cells lacking FcγRIIB that is needed to break tolerance. In addition, it would be important to assess, whether the B cells which secrete pathogenic IgG anti-bCII Abs all belong to the FcγRII deficient B cell compartment. FcγRIIB KO B cells may be sorted after immunization and transferred into WT or myeloid cell-specific FcγRIIB$_{B6}^{-/-}$ mice to delineate the distinct steps in CIA pathology.
To gain better insight into how FcγRIIB influences B cell ontogeny and selection in an

antigen-specific system, these novel inducible B-cell specific FcγRIIB mice may be crossed onto some well established model systems, such as the HEL (39) or the 56R BCR transgenic mouse strains (40).
Since FcγRIIB is expressed throughout almost the entire B cell development from pro-B cells to plasma cells, several B cell stages may be affected by FcγRIIB-mediated regulation. It has been suggested that FcγRIIB is a distal regulator of autoimmunity and controls development of IgG+ Ab-secreting B cells, whereas IgM+ B cells are not affected (29). Using the novel, inducible B cell-specific FcγRIIB$_{B6}^{-/-}$ mice, presented in Chapter 7, several important questions can now be answered. First, it should determined, whether in healthy mice FcγRIIB-deficient B cells live longer/shorter, express different surface activation markers or migrate preferentially to certain anatomical locations when compared to FcγRIIB sufficient B cells. Next, the same parameters should be studied after immunization with a foreign (OVA) or with a self-antigens (bCII) using different immunization routes (oral, subcutaneous) with IFA or CFA.

FcγRIIB-mediated inhibition of myeloid effector cells

It has been shown that FcγRIIB on myeloid cells plays an important role in controlling antibody mediated effector pathways involved in chronic inflammation, hypersensitivity, protection against bacterial infections and septic shock (36;41). In order to provide a direct confirmation of these observations we have tried to generate myeloid cell-specific FcγRIIB$_{B6}^{-/-}$ mice by crossing FcγRIIB$_{B6}^{fl/fl}$ mice to LysM-Cre mice (42). However, in these mice only about 40% of the macrophages was FcγRIIB deficient while the remaining 60% showed only decreased expression of this receptor (Figure 1). The most likely explanation for the partial deletion is that during B cell development the lyzozymeM promoter becomes active after FcγRIIB expression is initiated, while the turnover of surface expression of FcγRIIB is low. Nevertheless, the elevated phagocytic activity we observed in BMDMs from myeloid cell-specific FcγRIIB KO mice indicates that this strain can be useful for *in vivo* studies (PB, JSV, unpublished results).
There are several Cre transgenic mouse strains available that provide myeloid cell-specific deletion (hCD68-, F4/80-Cre), but unfortunately none these strains provide effective deletion specifically in all macrophages (43;44). This restriction probably reflects the highly heterogeneous nature of macrophages in the different tissues as well as their terminally differentiated state.
FcγRIIB plays a major regulatory role on mast cells by not only regulating FcγRIII but also FcεRI-mediated antibody effector pathways in these cells (45). Recently, two Cre transgenic mouse strains have been described that provide recombination of floxed genes exclusively in mast cells (Mcpt5-Cre (46) and Chm:Cre, using the baboon alpha-chymase promoter (47). Mast cell-specific FcγRIIB KO mice should not only be tested in IgE-and IgG1-mediated passive anaphylaxis experiments but also in arthritis models given the possible role of mast cells in disease initiation (17).
Neutrophils can neither be cultured *ex vivo* due to their short life-time, nor can they be transferred into recipient mice. Therefore it would especially interesting to analyze the neutrophil-specific role of FcγRIIB *in vivo*. Unfortunately, at the moment there are no

neutrophil-specific Cre mice available. It has been shown that the human FcγRIIIB promoter recapitulates neutrophil-specific expression, therefore Cre mice using this promoter should provide neutrophil-specific deletion (48).

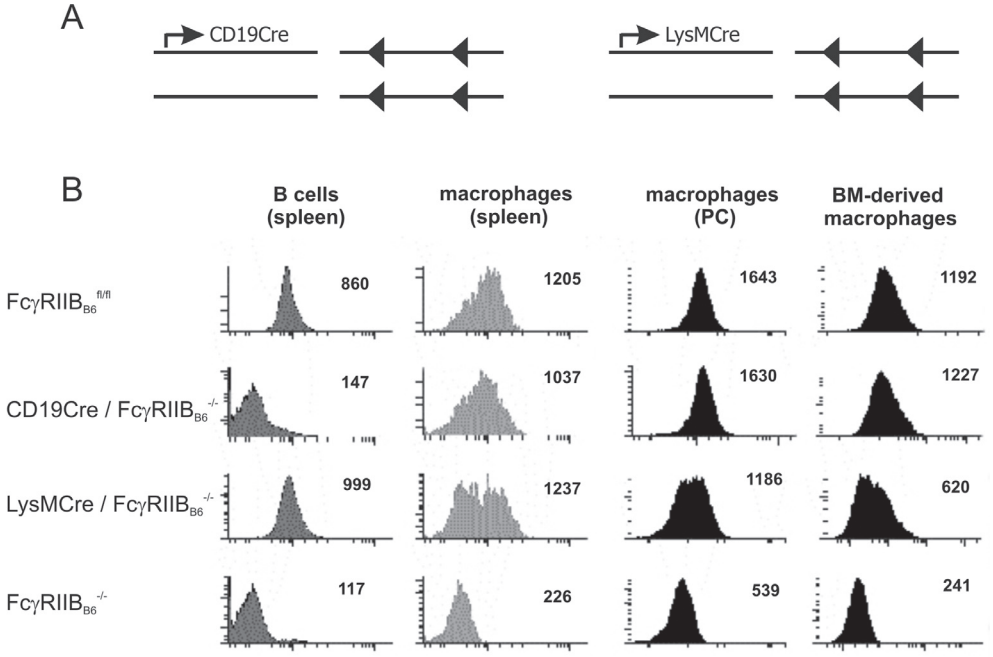

Figure 1.
A: Schematic representation of conditional FcγRIIB KO mice
B: Cytofluorimetric analysis of FcγRIIB expression in conditional FcγRIIB KO mice using the K9.361 mAb. Splenic B cells: CD19+, splenic macrophages CD11b+Gr1-, peritoneal cavity macrophages; CD11b+, bone marrow-derived macrophages CD11b+.

FcγRIIB on DCs inhibits activating FcγRs and therefore DC activation by ICs, which keeps DCs in an immature state contributing to the maintenance of immune tolerance (49). In addition, ICs taken up by FcγRIIB are recycled to the cell surface and presented to B cells as intact conformational antigens, inducing potent T cell independent (TI) immune responses (50). The role of FcγRIIB on DCs may be addressed in detail by DC-specific deletion of FcγRIIB using CD11c-Cre mice (51). The disadvantage of this approach is that CD11c promoter may also be active in certain macrophage populations as suggested by results with CD11c-DTR mice (52). Our hypothesis that the role of FcγRIIB in nasal and oral tolerance induction depends exclusively on the expression of this receptor on DCs (31) can be tested with these mice.

In conclusion, by crossing $FcγRIIB_{B6}^{fl/fl}$ mice with different cell type-specific Cre mice and analyzing the phenotype of the conditional KO offspring the complex role of FcγRIIB in immune regulation and autoimmunity can be defined in great detail.

The impact of new technologies in modifying the mouse genome on FcγR research

Conditional mutagenesis is a powerful tool to study the specific function of a molecule on a defined cell-type. Projects are now ongoing to delete all mouse genes (~25k) conditionally (53). As a result of coordinated effort by large-scale international consortia, the NIH Knockout Mouse Project (KOMP), the European Conditional Mouse Mutant Program (EUCOMM) and the Canadian NorCOMM program as well as different companies (eg. Regeneron, Lexicon, Artemis), it is expected that within a few years ES cells with any floxed gene can be ordered. Through intelligent vector design these large scale KO strategies allow conditional gene inactivation and simultaneous follow up of gene expression. With the availability of F0 generation mice fully derived from gene-targeted ES cells the time-scale of generating conditional KO mice is greatly reduced (54). These new developments will make conditional KO mice widely and easily available, allowing basic scientists to focus on the scientific analysis of these mice. However, specific models (among others the FcγRII/III/IV triple KO mentioned in this thesis) which cannot be generated by intercrossing the single FcγR KOs would still have to be generated by individual researchers.

Despite these technical advances one remaining bottleneck is the generation of effective, cell type-specific Cre transgenic mice. Therefore the generation and extensive characterization of novel constitutive and inducible cell type-specific Cre mice is a prerequisite for a successful application of conditional KO mouse models (55). This is also aided by new technologies, such as lentiviral transgenesis, or the generation of a well-characterized genomic locus with a build-in docking site, allowing easy, rapid delivery of transgenic constructs in the mouse genome with reproducible expression pattern by recombinase mediated cassette exchange.

How FcγR research will benefit from these developments? Within the next few years in addition to the conditional FcγRIIB KO presented here, conditional KOs on C57Bl/6 background from all other FcγR family members will become available enabling the analysis of the cell-type specific role of these receptors in the absence of the effects of linked 129-derived loci like the Sle1 locus. The possibility to introduce large genomic regions in the mouse genome enables the generation of transgenic mice expressing human FcγRs. Such mice will be valuable tools to test therapeutic mAbs. The biggest hurdle in this strategy is the correct expression pattern. Even when this is all solved, such models would still have limitations, since complement and FcRn would be of mouse origin.

Introducing subtle mutations through oligo targeting will allow easier generation of knockin mice (56). This will enable the generation of *in vivo* models with relevant disease-associated SNPs found in human FcγRs, such as the FcγRIIB Ile232Thr, which is associated with SLE (57;58).

There is growing awareness that the genetic background of KO mice and epistasis between linked genes, the KO allele and the background may have great influence on the phenotype of the KO mouse (as demonstrated in Chapter 6 of this thesis). Therefore there is now a general consensus that genetically modified mice for (immunological) research have to be of C57Bl/6 origin (see EUCOMM program). However, one should keep in mind that C57Bl/6 mice exhibit a clear Th1 bias. Due to the complex genomic interactions one should always keep in mind the limitations of studying one single inbred strain.

Concluding remarks

In this thesis the *in vivo* role of murine FcγRs in autoimmune diseases is analyzed with special focus on arthritis and lupus. The results with murine arthritis models extend our knowledge on FcγR function during arthritis. By generating and analyzing a novel conditional knockout mouse model in C57Bl/6 background we were able to redefine the role of FcγRIIB in autoimmunity. Since FcγRIIB is under consideration as a target for therapeutic intervention it is important to understand its function in great detail. The spatial and temporal conditional FcγRIIB KO mouse models permit a detailed analysis of this receptor in both protective immunity and immuno-pathology, which will support the design of new therapeutic interventions.

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