4.2.2.3. No modulatory role for complement receptors CR1(CD35) and CR2(CD21) on the outcome of RA KBN sera induced mice model.

4.2.2.3.1. Cr2\(^{-}\) mice lacking complement receptors CR1 and CR2 are susceptible to RA. Complement receptors CR1 and CR2 recognize opsonized immune complexes. CR1 has an important role in immune complex clearance, inhibition of C3 and C5 convertase and as receptor for C3b/C4b while CR2 is a receptor for C3dg, involved in germinal center reaction and an activator of alternative pathway on binding iC3. To assess the contribution of the complement receptors CR1 and CR2 in the K/BxN model of sera-induced-arthritis, Cr2\(^{-}\) gene knockout mice lacking CR1 and CR2 were injected intra-peritoneally with 100 \(\mu\)l equivalent of pooled K/BxN sera or, as control, with C57BL/6 sera, at day 0 and 10. The mice were monitored for induction and severity of arthritis. The assessment of arthritis was done by calliper measurement of ankle thickness, clinical index and ankle joint histology.

Figure 32. No role of CR1 and CR2 in K/BxN sera induced RA. Cr2\(^{-}\) mice was injected i-p with 100 \(\mu\)l equivalent of pooled K/BxN serum or with 100 \(\mu\)l C57BL/6 serum on days 0 and day 10. The mice were assessed for average ankle thickness by calliper measurement and a clinical index score(I) on days 0, 3, 6, 13, 20, 25, 30 and 38. K/BxN serum transferred mice (○), control sera transferred mice (■) are shown. Data are expressed as mean ± SEM; n=7-8 for experimental mice groups and n=3-4 for control mice groups.
Results

The complement receptor 1 and 2 deficient $Cr2^{-/-}$ mice developed arthritis within 36-72 hours after sera transfer. The ankle swelling was maximal at around day 7-8 after the first sera transfer and was visible for up to 30 days after the second transfer on day 10. Mice were sacrificed at regular time intervals after sera transfer for histological examination of ankle joints. The joint sections were monitored for signs of synovial inflammation, hyperplasia, and cellular infiltration. The ankle joint sections shown in figure 2 trace the formation of the actively infiltrating synovial inflammatory tissue as RA progresses in the various mice strains. The diseased $Cr2^{-/-}$ mice ankle joints shown in Figure 33 (A-D), clearly demonstrate an active synovial hyperplasia with cellular infiltration beginning by day 3 (Figure 33, B). Synovitis peaks at day 10 (Figure 33, C). By day 38 the sick $Cr2^{-/-}$ mice had completely recovered from disease as indicated by shrinkage of synovial membrane (Figure 33, D).

$CR2^{-/-}$

Figure 33. Histology of $Cr2^{-/-}$ ankle joints. Mice were sacrificed on days 0 (A), 3 (B ), 10 (C ) and 38 (D). Ankle joints were stained with H and E. Representative sections are shown. Arrows indicate synovial hyperplasia and invasion. (A) Shows an ankle joint of a mouse before serum transfer. (B, C,)show active pannus growth with infiltration into the synovial joint space. (D) Shows an ankle joint after recovery with residual fibrotic synovial tissue.

4.2.2.3.2. Clearance of GPI-antibodies from peripheral blood and disease severity are correlated.

The ability of K/BxN serum to induce arthritis is confined to anti-GPI antibodies making up most of the Ig in the serum. $C4^{-/-}$ mice lacking complement C4 are known to have IC deposition. $Cr2^{-/-}$ mice lack the CR1 and CR2 receptors and CR1 is known to be potentially involved in IC-clearance. We wanted to study the role of antibody clearance in K/BxN sera induced RA. For this we assessed the rate of GPI antibody-clearance from sera using an
ELISA for anti-GPI-specific antibodies after K/BxN sera transfer. Mice were bled at the indicated time points tested for the presence of GPI-specific antibodies. We observed no significant difference in antibody clearance in the $C4^{−/−}$ and the $Cr2^{−/−}$ mice in comparison to C57BL/6 or KRN mice (Figure 34). However we found that BALB/c mice, the most susceptible strain to RA in our disease model, cleared GPI-specific antibodies much faster, as indicated by the rapid drop in the anti-GPI antibody titer at day 7 and its almost complete absence by day 20 in comparison to clearance at day 30 in C57BL/6, $Cr2^{−/−}$, $C4^{−/−}$ and KRN. In contrast, the completely protected NOD mice showed the lowest antibody clearance rates with a slight decrease of anti-GPI antibody titers at day 7. NOD mice needed up to day 40 to show complete clearance of anti-GPI-antibodies from the peripheral blood.

Figure 34. GPI antibody clearance in the peripheral blood of the sera transferred mice. The mice were bled on days 0, 3, 6, 13, 20, 25, 30 and 38 and screened for anti-GPI antibodies by GPI-specific ELISA. OD at 1/300 dilution of sera is shown. Results from K/BxN serum-transferred (♦) and controls (■) are shown. Data are expressed as mean ± SEM. N=2-4 for experimental mice groups; n=1-2 for control mice groups.
4.3. \( FcγRIII^{-/-} \) mice are completely resistant whereas \( FcγRIIb^{-/-} \) have exaggerated disease in KBN sera induced RA model.

In order to elucidate the role of activating \( Fcγ \) receptor \( FcγRIII \) and the inhibitory \( Fcγ \) receptor \( FcγRIIb \) in mediating disease in KBN sera induced arthritis murine model, knockout mice \( FcγRIII^{-/-} \) and \( FcγRIIb^{-/-} \) were used for inducing RA. The mice were injected with 100 \( \mu l \) equivalent of KBN sera or as control with C57Bl6 mice sera and monitored for RA by measuring ankle thickness, clinical index score and joint histology.

![Graphs showing average ankle thickness and clinical index scores over days post sera transfer for \( FcγRIII^{-/-} \) and \( FcγRIIb^{-/-} \) mice.](image)

**Figure 35.** \( FcγRIII^{-/-} \) mice are resistant and \( FcγRIIb^{-/-} \) have highly susceptible to K/BxN sera induced RA. \( FcγRIII^{-/-} \) and \( FcγRIIb^{-/-} \) mice was injected i-p with 100 \( \mu l \) equivalent of pooled K/BxN serum or with 100 \( \mu l \) C57BL/6 serum on days 0. The mice were assessed for average ankle thickness by calliper measurement and a clinical index score(I) on days 0, 3, 5, 7, 9, 11. K/BxN serum transferred mice (●), control sera transferred mice (○).
Mice were sacrificed at day 10 after sera transfer and ankle were processed and stained with H&E. Joints from control sera(right) and K/BxN sera(left) injected mice for $Fc\gamma RIII^-$ and sections of K/BxN sera injected mice for $Fc\gamma RIIb^-$ are shown. The $Fc\gamma RIII^-$ mice do not show any signs of inflammation, while the $Fc\gamma RIIb^-$ mice joints show severe inflammation of joints and cartilage damage. JC-joint cavity; Ca-cartilage; Bo-bone; Pa-pannus tissue.

The $Fc\gamma RIII$ deficient mice were found to be completely resistant to K/BxN sera induced arthritis with no signs of ankle swelling and zero clinical index. Histology of ankle joints of the K/BxN sera transferred mice showed normal synovia and no signs of inflammation. In contrast the $Fc\gamma RIIb$ deficient mice developed very severe swelling of ankles, which peaked on 5-7th day after sera transfer. The clinical index of all mice transferred with K/BxN sera was four, which again points out that the absence of inhibitory $Fc\gamma RIIb$ enhances the severity of the disease. The ankle joint histology of the affected mice clearly shows severe inflammation, with highly invasive pannus growth and degradation of cartilage and bone.

**Figure 36. Joint histology of $Fc\gamma RIII^-$ and $Fc\gamma RIIb^-$ mice.** Mice were sacrificed at day 10 after sera transfer and ankle were processed and stained with H&E. Joints from control sera(right) and K/BxN sera(left) injected mice for $Fc\gamma RIII^-$ and sections of K/BxN sera injected mice for $Fc\gamma RIIb^-$ are shown. The $Fc\gamma RIII^-$ mice do not show any signs of inflammation, while the $Fc\gamma RIIb^-$ mice joints show severe inflammation of joints and cartilage damage. JC-joint cavity; Ca-cartilage; Bo-bone; Pa-pannus tissue.