4.1.10. Modeling of epitope residues on the GPI crystal structure.

As the crystal structure co-ordinates of GPI for humans and rabbits were already available and considering the fact that the mice GPI protein is highly homologous to rabbit GPI, the murine GPI epitopes identified by mass spectrometry were modeled on the crystal of rabbit GPI homodimer, PDB entry: 1HOX (Lee, Chang et al. 2001), utilising the structure visualizing software RASMOL. The GPI protein monomer overall folding is divided into two globular domains (designated as large and small domains) and an "arm-like" C-terminal tail. The active site is situated in a cleft between the large and small domains of the monomer and is formed by the association of the two monomer subunits (Lee, Chang et al. 2001; Arsenieva, Hardre et al. 2002).

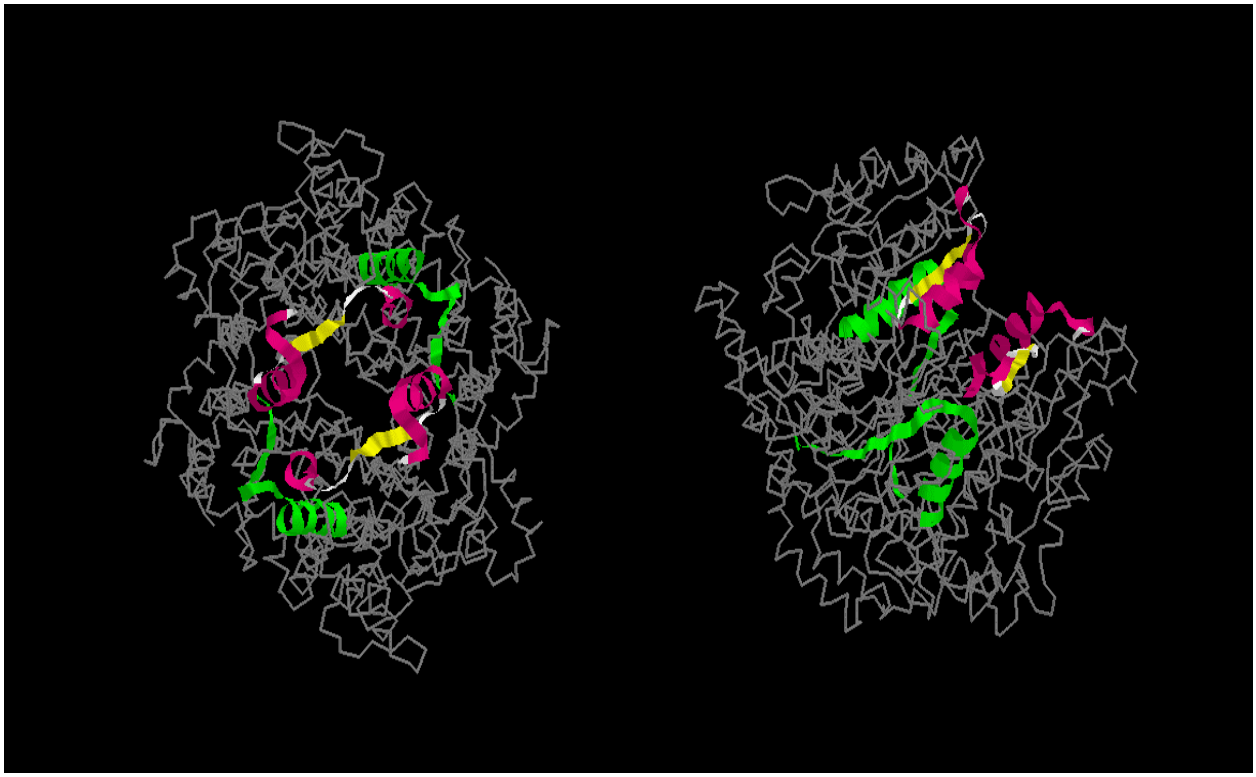


Figure 25. Modeling of murine GPI epitopes identified by mass spectrometric on rabbit GPI crystal structure. *Pink/Yellow-GPI 170-202 (11H3 & 1E3); Green-GPI 470-495 (46H9).*

It was observed that the two epitopes identified, GPI peptide residues 170-202 (ALKPYSKGGPRVWFVSNIDGTHIAKTLASLSPE) and the GPI peptide residues 470-495 (GNRPTNSIVFTKLTPFILGALIAMYE) were located on the surface of the GPI homodimer protein and hence would be accessible for binding to the antibodies. Interestingly the epitope regions of 1E3 and 46H9 were found to be at the interface of GPI homodimer units (figure 25).

4.2 Role of Innate immunity mediators in KBN Rheumatoid Arthritis mice model.

4.2.1. KBN Sera transfer induced RA in laboratory mice strains- BALB/C mice are the most susceptible strain.

KBN sera transfer induced arthritis was carried out in the laboratory mice strains BALB/c, C57BL/6, and KRN transgenic. Mice were injected with 100μl K/BxN sera or control sera from C57BL/6 mice at day 0 and 10. The mice were monitored for induction and severity of arthritis at regular intervals.

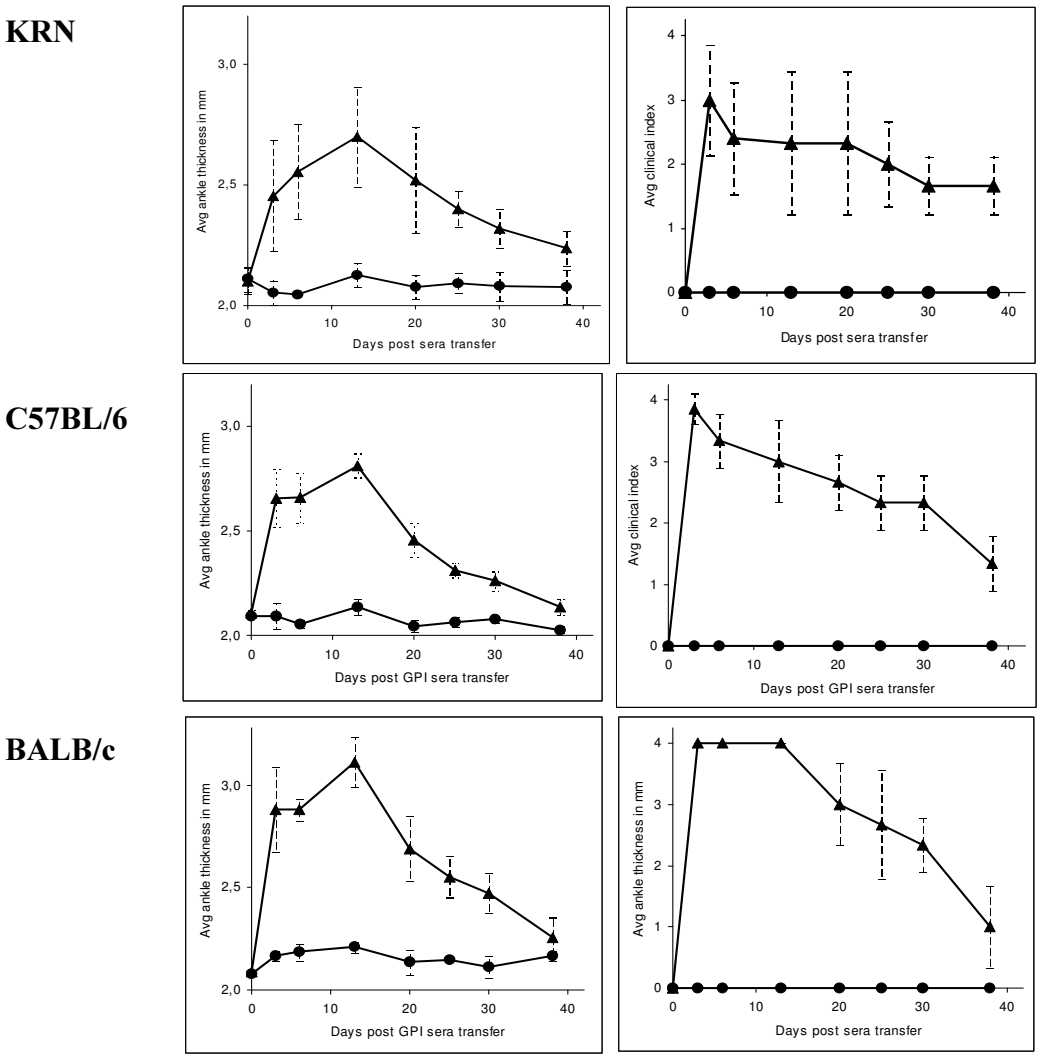


Figure 26. K/BxN sera transfer in laboratory mice strains. The mice strains KRN, C57BL/6 and BALB/c were injected *i.p* with 100 μl K/BxN serum or control C57BL/6 serum on days 0 and day 10. The mice were assessed for average ankle thickness by calliper measurement and clinical index(I) score on days 0, 3, 6, 13, 20, 25, 30 and 38. K/BxN serum transferred mice (◆)control sera transferred mice (■). Data are expressed as mean ± SEM; n=7-8 for experimental mice groups and n=3-4 for control mice groups.

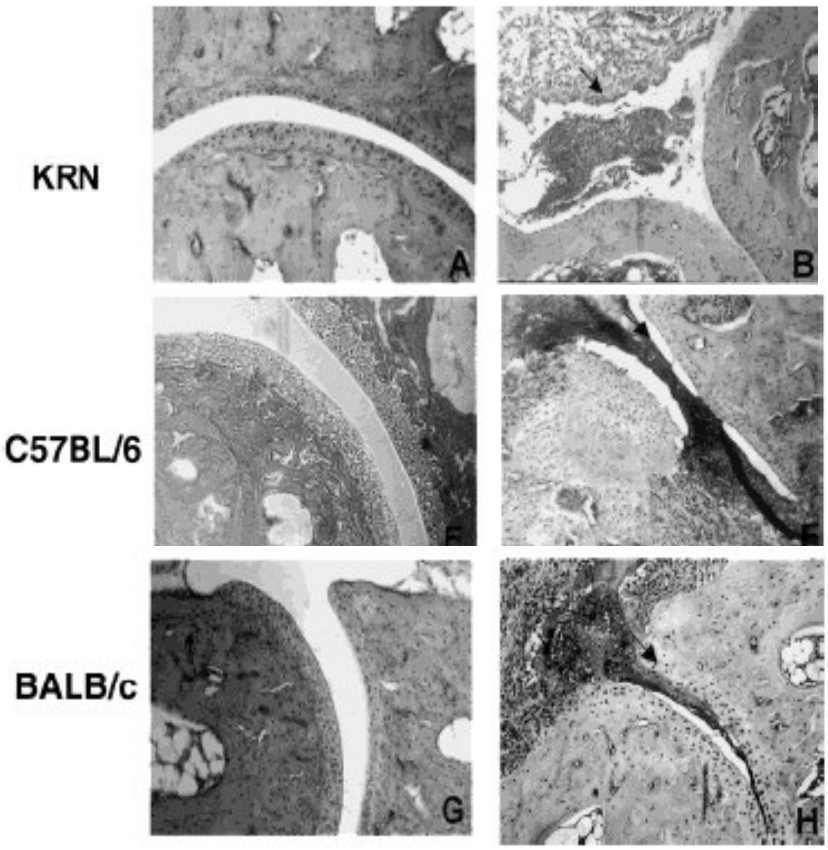


Figure. 27. Histology of ankle joints of K/BxN serum induced RA in KRN, C57BL/6 and BALB/c mice. A, E, and G are sections of ankle joints before K/BxN serum injection (day 0). B, F, and H are section of mice (day13) after serum transfer. All mice joints show active synovitis (arrow) and infiltration into the joints.

The mouse strains C57BL/6, BALB/c, as well as KRN mice developed arthritis within 36-72 hours after sera transfer. Generally, we observed that BALB/c mice were the most affected with maximum clinical index of 4, developing reddening and inflammation of knee joints in less than 36 hours after transfer, whereas the same symptoms appeared with some delay in the C57BL/6, KRN strains. 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 The joint sections were monitored for signs of synovial inflammation, hyperplasia, and cellular infiltration. All the affected mice show signs of pannus growth with cellular infiltration into synovia and joint cavity. Based on a reproducible high susceptibility of BALB/c strain it was decided to use this strain to do inhibition and therapy studies.

4.2.2. Role of complement activation and complement receptors in K/BxN sera induced RA.

4.2.2.1 Complement *C4*$^{-/-}$ mice defective in classical complement and lectin complement pathway are susceptible to KBN sera induced arthritis.

C4$^{-/-}$ mice are defective in classical and lectin complement activation pathways. To assess the contribution of these complement pathways in KBN sera induced RA, *C4*$^{-/-}$ Mice were injected i.p with K/BxN sera or C57BL/6 sera, at day 0 and 10. The mice were monitored for induction and severity of arthritis at regular intervals by measurement of ankle thickness, clinical index and ankle joint histology.

***C4*$^{-/-}$**

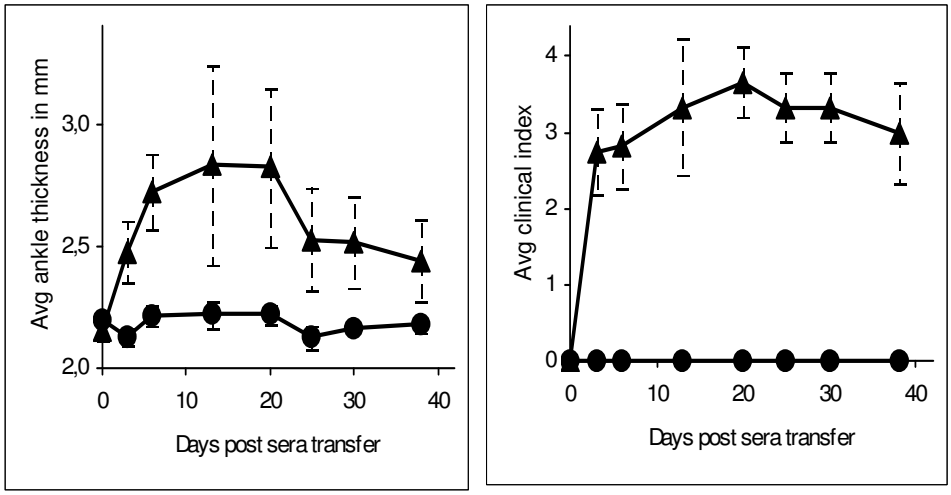
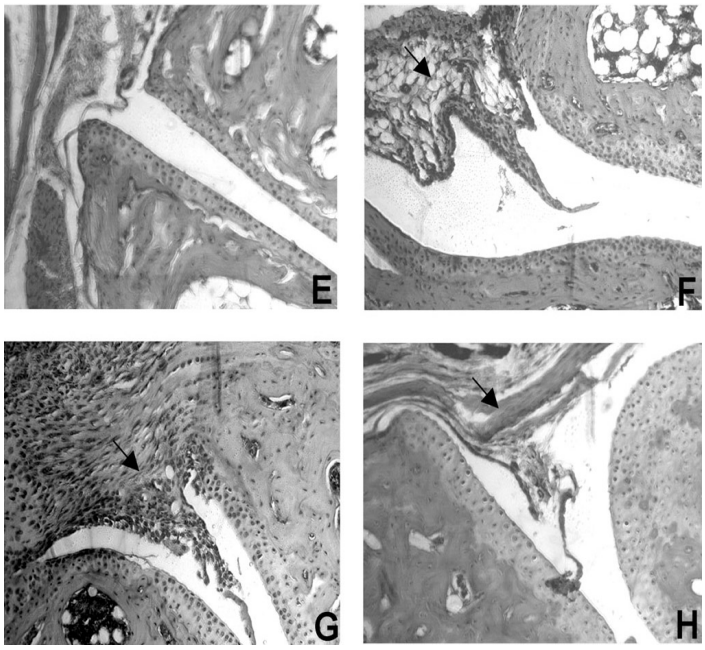


Figure 28. C4*$^{-/-}$ *mice are susceptible to K/BxN induced RA. *C4*$^{-/-}$ mice was injected i.p. with 100 *µl* equivalent of pooled K/BxN serum or with 100 *µl* C57BL/6 serum on days 0 and day 10. The mice were assessed for average ankle thickness by calliper measurement and a clinical index score 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.



CR2 $^{-/-}$

Figure 29. Histology of C4 $^{-/-}$ ***ankle joints***. Mice were injected with K/BxN serum or with control serum on days 0 and 10. Mice were sacrificed on days 0 (E), 3 (F), 10 (G) and 38 (H). Ankle joints were stained with H and E. Arrows indicate synovial hyperplasia and invasion. (E) Shows an ankle joint of a mouse before serum transfer. (F and G) show active pannus growth with infiltration into joint space. (D) Shows an ankle joint after recovery with residual fibrotic synovial tissue.

C4 $^{-/-}$ mice developed arthritis within 36-72 hours after sera transfer. Histological examination of the *C4* $^{-/-}$ mice ankle joints depicted in Figure 29 (E-H) shows an active initiation of synovial hyperplasia by day 3 (Figure 29, F) and strong synovitis by day 10 (Figure 29, G). Sections from day 38 (Figure 29, H) show residual fibrotic tissue lacking any morphological sign of infiltrating inflammatory cells.

4.2.2.2 Mice lacking complement C3 or C5 are resistant to KBN sera induced RA.- Alternative complement pathway has a key role.

NOD mice have a genetic defect in the complement C5 locus and lack C5. C5 is the central component of all the complement activation pathways and hence these mice lack apart from the classical and lectin pathways (like the *C4-/-* mice*)* also the alternative complement pathway. K/BxN sera transfer experiments were done in NOD mice to look at the role of C5 and complement activation.

NOD

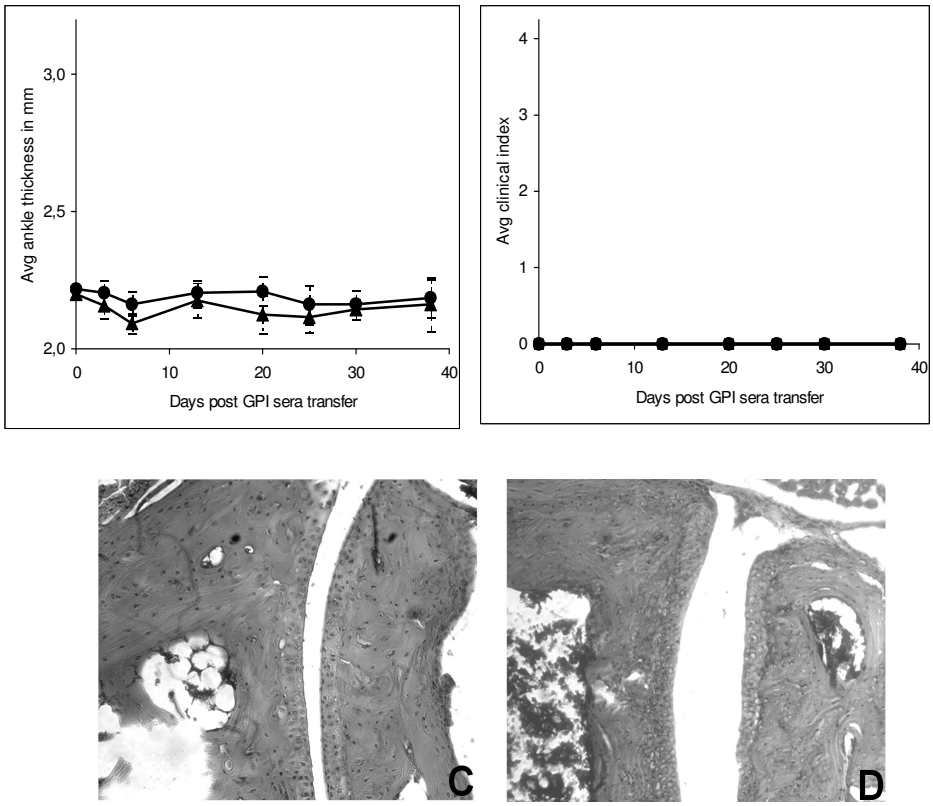


Figure 30. Complement C5-/- NOD mice are resistant to K/BxN sera induced RA. NOD mice were injected *i.p* with 100 *µl* K/BxN serum or C57BL/6 serum on days 0 and day 10. The mice were assessed for ankle thickness by clinical index(I) score on days 0, 3, 6, 13, 20, 25, 30 and 38. K/BxN serum transferred mice(◆). control sera transferred mice (■). Data are expressed as mean ± SEM; n=7-8 for experimental mice groups and n=3-4 for control mice groups. Histology of ankle joints of K/BxN serum induced arthritis of NOD mice. C is section of ankle joints before K/BxN serum injection (day 0). D is section of mice (day13) after serum transfer, which show no signs of inflammation in ankle joints seen.

The C5 deficient NOD mice were found to be completely resistant K/BxN sera induced RA with all the treated mice showing no signs of increase in ankle thickness and clinical index score (figure 30). The absence of any signs of inflammation in ankle joint histology at day 0 and after K/BxN sera transfer (day 13) of the mice also confirmed this (figure 30).

Complement C3 plays a central role in the activation and regulation of the alternative as well as classical pathway of complement. Cobra Venom Factor (CVF) is a structural and functional analog of C3 can form a C3 convertase by binding factor B, several orders of magnitude more stable than the C3 containing enzyme not subject to regulation by other complement proteins. Hence injection of CVF depletes all the complement components including C3. To study the role of complements K/BxN sera were injected into CVF treated BALB/c mice to study role of complement proteins.

CVF Treatment

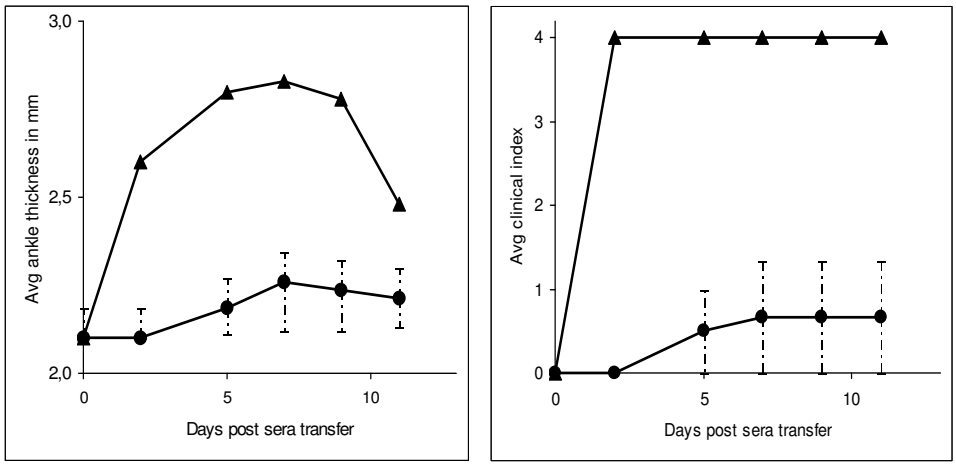


Figure 31. C3 depleted mice are resistant to K/BxN sera induced RA. BALB/c mice were depleted of complement C3 by a single *i.p* injection of 500 U CVF/Kg body weight and 100 *µl* K/BxN serum or as control with 100 *µl* C57BL/6 serum on days 0. The mice were assessed for average ankle thickness by clinical index score(I) on day 0, 3, 5, 7,.9, 11. (▲)KBN sera and no CVF,(●) KBN sera and CVF. Data are expressed as mean ± SEM; n=3-4 for experimental mice groups and n=1 for control mice groups.

CVF treated mice had a drastic reduction in ankle thickness when compared to untreated mice. Also the CVF treated mice had a maximum clinical index around 1 while all the untreated mice has a clinical index of 4. (figure 31).