# Tertiary lymphoid organs form during the long term course of murine glomerulonephritis

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**Introduction.** T helper cells (TH) such as TH1, TH17 and regulatory T cells (Tregs) as well as B cells, macrophages, dendritic cells and neutrophils infiltrate the kidney during the course of nephrotoxic serum nephritis (NTS) thereby mediating disease. This project evaluates changes in the renal leukocyte population during the long-term course of the model and focuses on appearance of tertiary lymphoid organs (TLO) formation within the kidney. TLO form in chronic diseases and have structural and functional similarities to the lymph node.

**Methods.** C57BL/6J mice were immunized and three days thereafter, the rabbit anti-GBM antiserum was injected via the tail vein. Five time points were evaluated: day 3, week 2, week 4, week 8 and week 20.

**Results.**

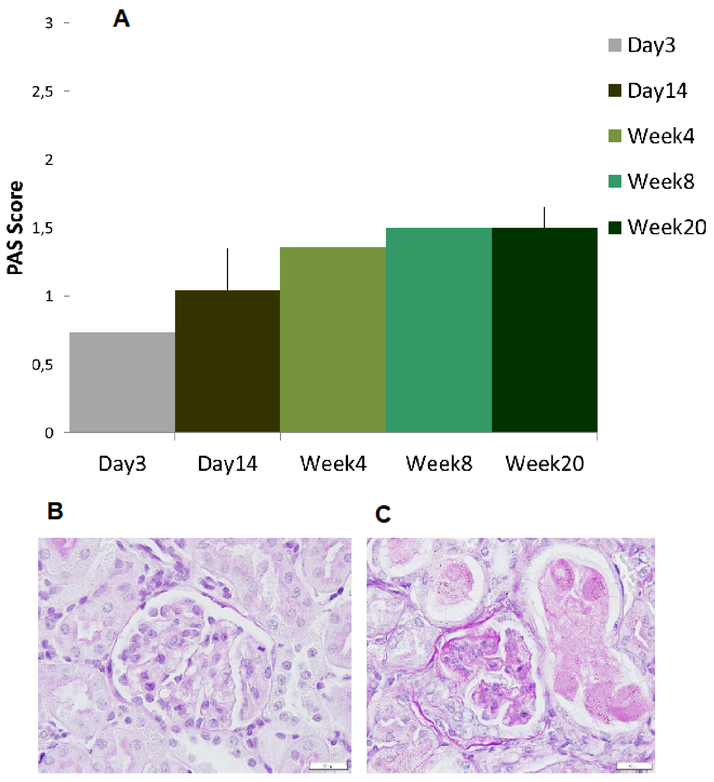



**Figure 1- Glomerular changes**. As expected, glomerulosclerosis developed progressively within the observation period (**A**). On day 3 (**B**), the glomeruli were intact. After 20 weeks (**C**), significant glomerular and tubular pathology in the form of glomeruloscerlosis and tubular inclusions could be observed.

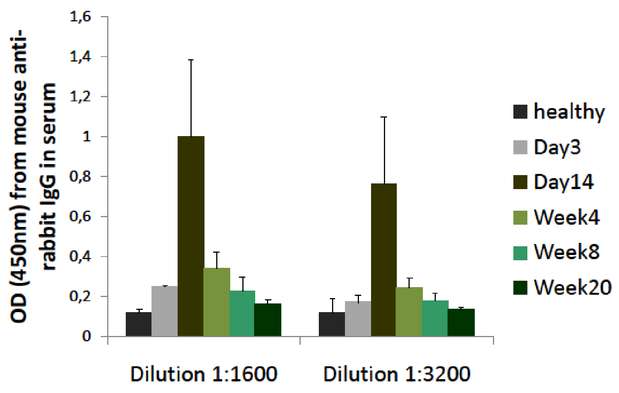



**Figure 2 – B cell response.** An ELISA for mouse anti- rabbit IgG from serum was performed. Circulating autologous IgG peaked on day 14 and reached baseline values at the end of the observation period.

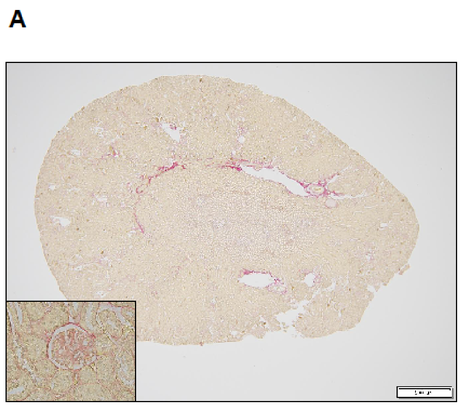



**Figure 3 – Renal Fibrosis.** The development of fibrosis was observed on day 3 (**A**) and week 20 (**B**). Fibrosis initially developed in the cortex, while all nephron segments and the renal medulla were gradually affected over the course of time.

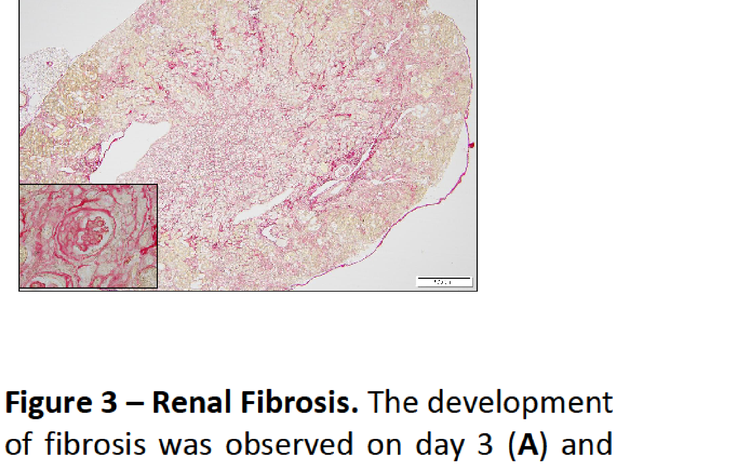
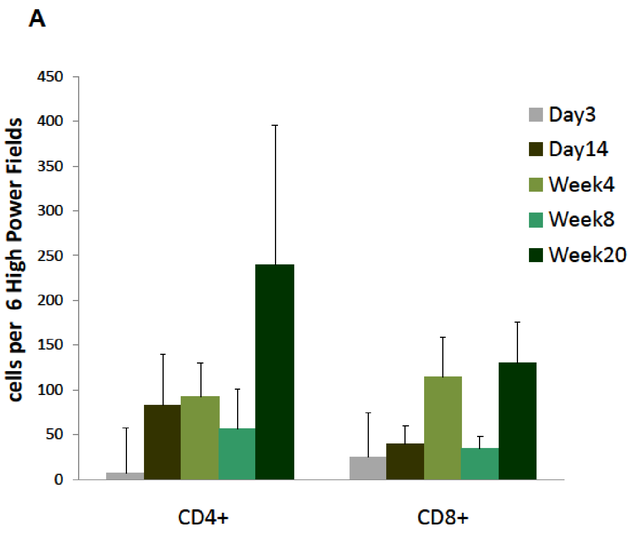



**Figure 4- Renal Leukocytes.** CD4⁺ and CD8⁺ cells were analyzed during long term-course of GN. The exact cell count of CD4⁺ and CD8⁺ cells from one kidney is shown. After 20 weeks, all cell types increased which strengthens the idea of TLO formation in the kidney.



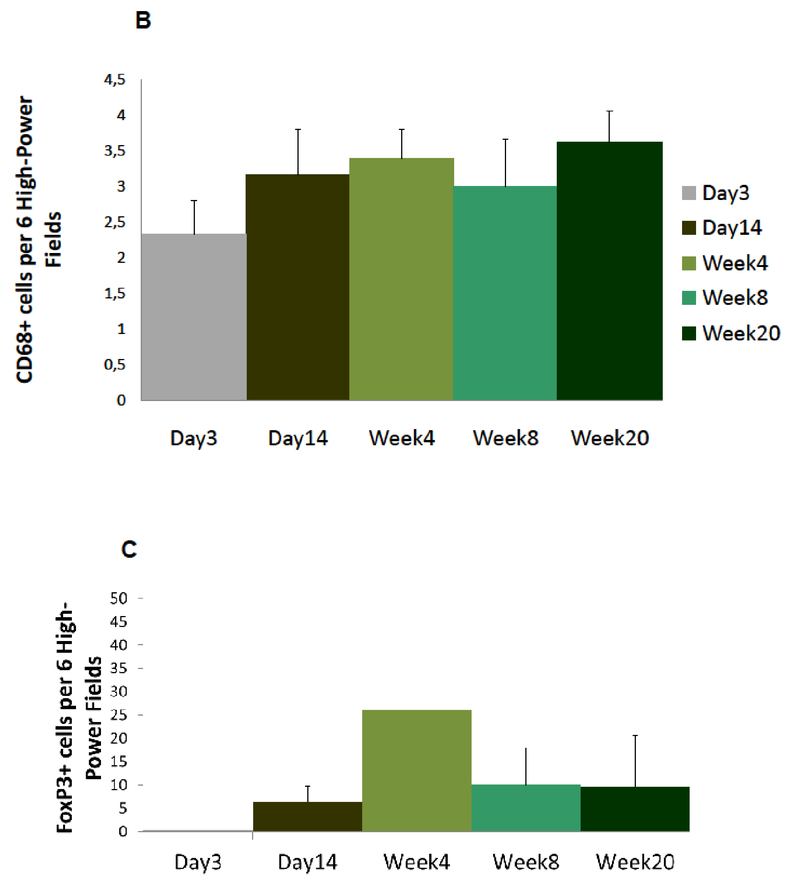




**Figure 5- Infiltrating cells.** Staining of CD4⁺, CD8⁺, CD68⁺ and FoxP3⁺ cells in the kidney were accomplished during long term-course. 6 high power fields (**A**,**C**) or 6 low power fields (**B**) were evaluated.
After 20 weeks, there was an increase of CD4⁺ and CD8⁺ cells (**A**) whereas an infiltration of CD68⁺ cells (**B**) occurred at an early timepoint and only minimal increase could be observed during later timepoints. Staining for FoxP3⁺ cells (**C**), reflecting Tregs, in the kidney showed an increase after 4 weeks compared to the other time points.

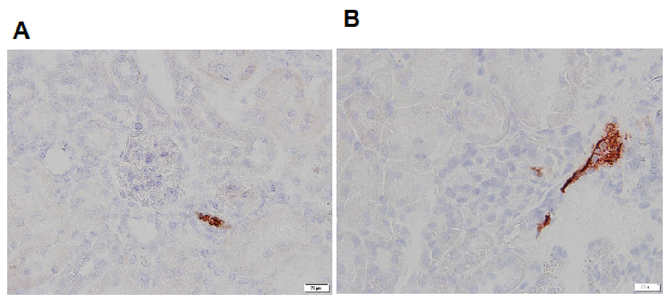



**Figure 7 – Lyve-1 Stain.** Immunohistochemical stain for evaluation of the lymphatic vascular marker Lyve-1⁺ in the kidney was performed. Positively stained areas were seen in or around the glomeruli and were found in the kidneys of healthy (**A**) and nephritic (**B**) mice to the same extent.

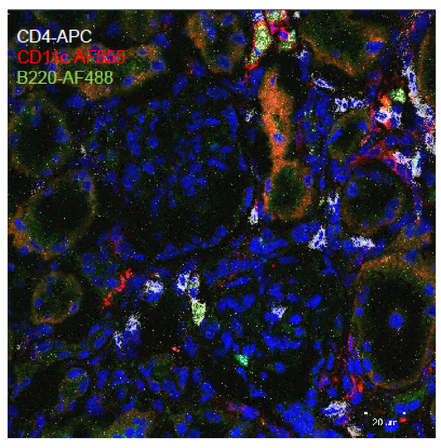



**Figure 8 – TLO formation.** An accumulation of T-, B- and dendritic cells occurred on week 20 and therefore indicates the formation of TLO in the kidney.

**Discussion.**
Similarly to published findings in lupus nephritis patients, we found evidence of TLO fomation in kidneys from mice with NTS 20 weeks after disease induction (1). The formation of TLO in our NTS model was associated with increased T effector cell, but also Treg infiltration into the kidney, suggesting involvement of TLOs in deterioration, but also resolution of glomerulonephritis.

All values indicate mean +/-SD , *p<0.05

(1)Chang,A.et al (2011) In situ B cell mediated immune responses and tubulointerstitial inflammation in human lupus nephritis. J.Immunol. 186, 1849-1860

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