Validation of Piroxicam-Accelerated Colitis in the Interleukin-10 Knock out Mouse - a Preclinical Model Mimicking Human Inflammatory Bowel Disease

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Predictive validity and immune cell involvement in the pathogenesis of piroxicam-accelerated colitis in interleukin-10 knock out mice.

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Introduction

- Piroxicam-accelerated colitis (PAC) in interleukin-10 knock out (IL-10 k.o.) mice combines a dysregulated immune response against the gut microbiota with a decreased mucosal integrity (Berg *et al.* Gastroent, 2002; Holgersen *et al.* JCC, 2013)

- The PAC IL-10 k.o. mouse is an useful *in vivo* model of inflammatory bowel disease (IBD). However, the predictive validity and pathogenic mechanisms of the model have not been thoroughly investigated.

- The aim of this study was:

1. To qualify the PAC IL-10 k.o. model by examining the efficacy of IBD reference drugs on colonic inflammation.
2. To elucidate the pathophysiologic role of IBD-relevant immune cells in the PAC IL-10 k.o. model by depletion of specific immune cell subsets.

Methods

- C57BL/6 IL-10 k.o. mice received piroxicam in the chow, throughout the study.

- Mice were treated prophylactically with anti-IL-12/23p40 monoclonal antibodies (mAb), anti-TNFα mAb, cyclosporine A (CsA) or oral prednisolone. n = 8-12 mice per group.

- $CD4^+$ cells, $CD8^+$ cells and macrophages were depleted prophylactically by treatment with anti-CD4 mAb, anti-CD8 mAb and clodronate-encapsulted liposomes, respectively. T cell receptor co-stimulation was blocked by CTLA4-Ig (Orencia). n = 10-14 mice per group.

- Histological analysis, cytokine profiling ELISAs and calprotectin immunohistochemistry were performed on colon tissue from studies showing treatment effect in the PAC IL-10 k.o. model.

Figure 1. Anti-IL-12/23p40 mAb, anti-TNFα mAb and cyclosporine A (CsA) treatment prevented weight loss and attenuated colonic pathology of PAC IL-10 k.o. mice. W: L = weight: length. *p<0.05,**p<0.01,***p<0.001.

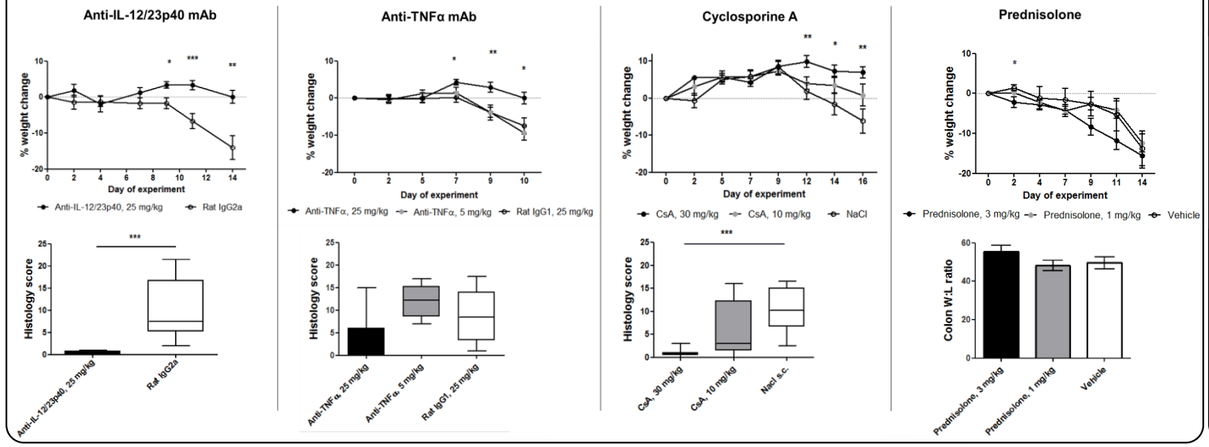


Figure 2. Depletion of $CD8^+$ cells tended to increase mortality, whereas depletion of $CD4^+$ cells or treatment with CTLA4-Ig (10 mg/kg) had no effect on disease progression. Depletion of macrophages induced body weight loss; nevertheless it was associated with significantly reduced colonic pathology. *p<0.05,**p<0.01,***p<0.001.

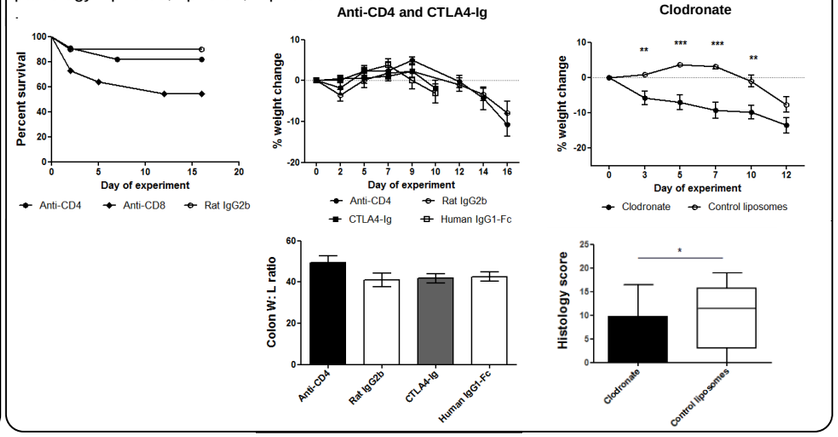


Figure 3. Colon cytokine profile of PAC IL-10 k.o. mice treated with the specified drugs. *p<0.05,**p<0.01,***p<0.001.

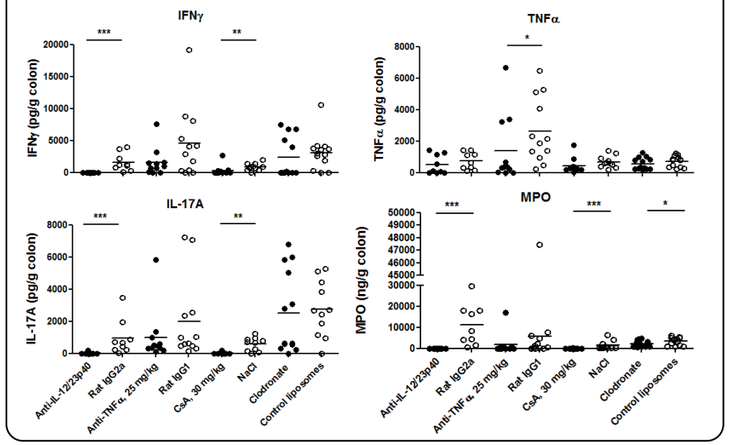
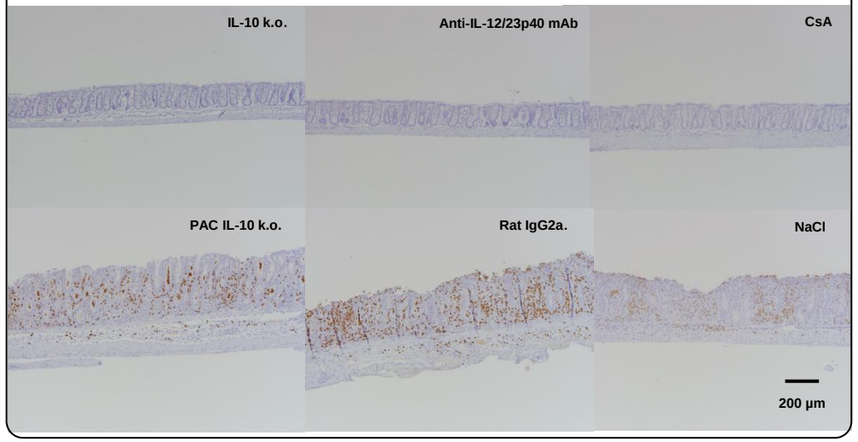


Figure 4. The colonic calprotectin density was correlated with disease activity of PAC IL-10 k.o. mice. Anti-IL-12/23p40 mAb and CsA treatment significantly decreased the level of calprotectin in the colon.



Conclusions

- Reference drugs with known efficacy in severe IBD were efficacious in the PAC IL-10 k.o. model.

- The ameliorative drugs reduced the colonic levels of IFNγ, IL-17A, MPO and calprotectin, which indicates that these cytokines, and/or the cells that secrete them, play an important role in disease development.

- $CD8^+$ cells seem to protect against disease in the PAC IL-10 k.o. model. In contrast, our data indicate that macrophages are a main driver of the colitis, whereas $CD4^+$ cells are not.

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