# Effect of Interleukin 17 Receptor Deficiency on the Development of Experimental Lyme Borreliosis

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## Abstract

Lyme disease is caused by infection with the spirochete, *Borrelia burgdorferi*, and is the most common vector-borne disease in the United States. Individuals who are not treated with antibiotics near the time of infection will typically develop secondary disease manifestations such as arthritis and carditis. Despite much effort, the immunological mechanisms driving the development of disease are not clearly defined. Interleukin 17 (IL-17) is a cytokine that is a significant contributor to the inflammatory response through recruitment of macrophages and neutrophils to the site of infection. It has been found in the synovial fluid of Lyme disease patients and in the serum of *Borrelia*-infected mice, and has been suggested to play an important role in the development of Lyme arthritis. To directly test the requirement for IL-17 activity in Lyme arthritis, we infected arthritis-susceptible C3H mice deficient in the common A chain of the IL-17 receptor and followed the development of disease. Severity of arthritis and carditis were determined by histology on days 21 and 35 post-infection and levels of spirochete DNA in tissues were measured using real-time PCR. Production of cytokines was measured directly from tissue homogenates. These studies indicate that IL-17 signaling is not essential for the development of disease in the murine model of Lyme borreliosis.

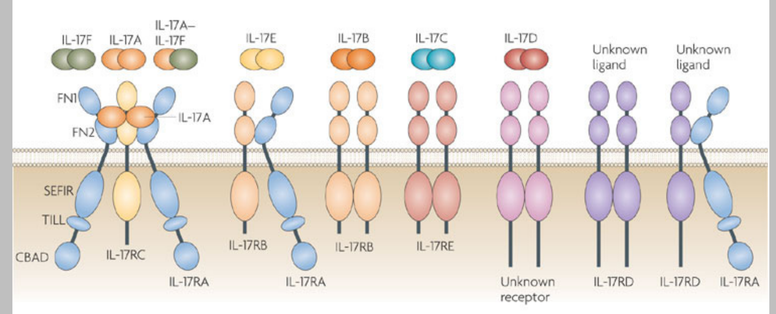
## Methods

**Infection**: Stocks of virulent, N40 strain of *Borrelia burgdorferi* were grown to log phase in 7.5 mls of Barbour, Stoenner, Kelly II (BSK) medium with 6% rabbit serum. Spirochetes were counted and diluted in sterile BSK medium to $1x10^6$ bacteria/ml. Wild type (WT) and IL-17RA$^{-/-}$ C3H/HeJ mice were anesthetized, and inoculated in both hind feet with 50 uL of bacteria. Five WT control mice and five IL-17RA$^{-/-}$ mice were sacrificed at 21 and 35 days post infection.

**Protein Isolation:** Hearts and knees were harvested from each mouse. The tissues were pulverized and placed in a mixture of HBSS and PIC. Samples were sonicated, centrifuged, and then filtered through a 45µm filter. Then, a BCA assay was run to quantify the amount of protein obtained. This was used for comparison of results of ELISAs ran on these samples to measure the cytokines KC, IL-12, IL-17, and IL-6.

**Determination of *Bb* loads:** DNA was extracted from one ear and the right ankle of each mouse using Trizol. Following extraction, samples were analyzed for *Bb* loads using RealTime PCR.

## IL-17 Receptor Signaling Background




Gaffen, S., 2009

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## Joint Swelling Curve

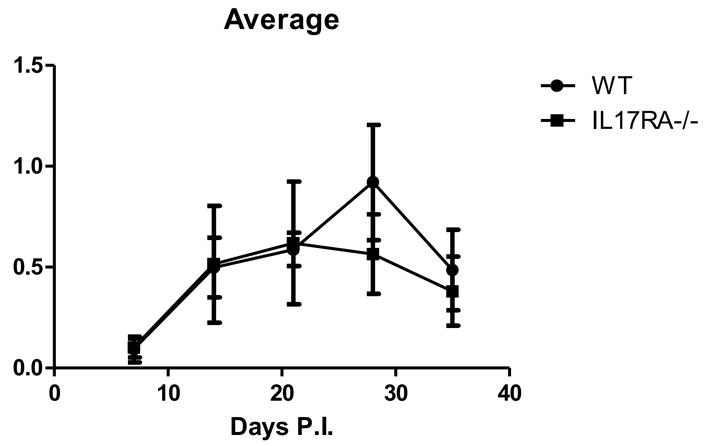


**Figure 1**: The swelling curve between the IL-17RA$^{-/-}$ mice and WT mice is very similar. This data may indicate that the IL-17RA$^{-/-}$ mice begin resolving at an earlier time point than the WT controls.

## *Borrelia* Loads

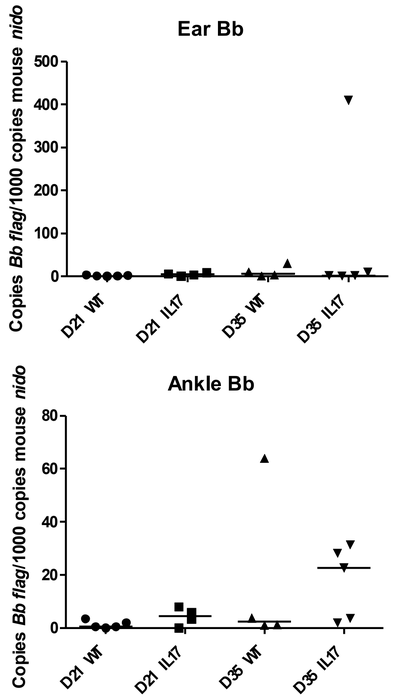


**Figure 2:** *Borrelia* loads in the ear of IL-17RA$^{-/-}$ mice are similar to that of the WT control mice. By day 35 in the joint, *Borrelia* loads trend higher in the IL-17RA$^{-/-}$ mice. This may indicate reduced *Borrelia* clearance.

## Histological Analysis

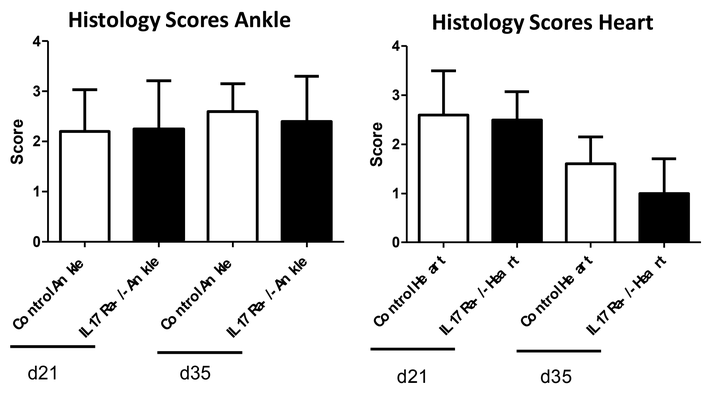


**Figure 4:** Histological analysis reveals no difference in disease severity between IL-17RA$^{-/-}$ and WT mice.

## Cytokine Analysis of IL-17RA$^{-/-}$ mice

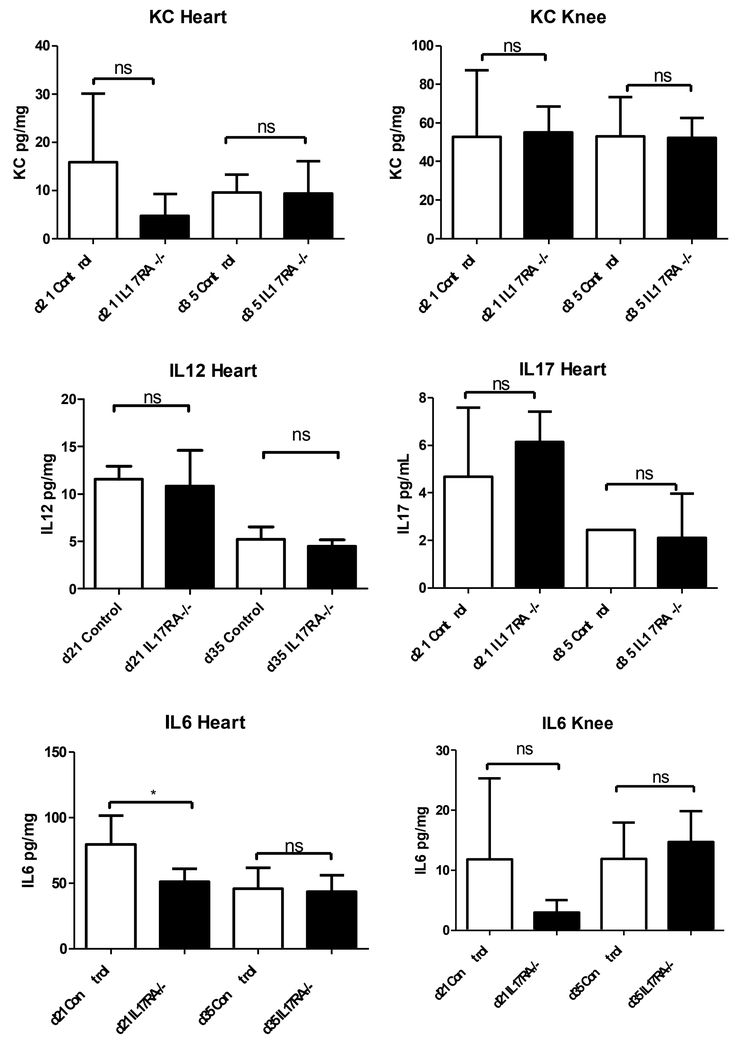


**Figure 3:** IL-17RA$^{-/-}$ mice have significantly lower IL-6 levels at the peak of the infection. Surprisingly, KC levels in both heart and ankle are not significantly different.

## Conclusions

- IL-17RA$^{-/-}$ mice show no difference in inflammatory cell influx upon infection with *Borrelia burgdorferi*.
- It appears that IL-17RA$^{-/-}$ mice may resolve arthritis earlier, but the experiment needs to be repeated for verification.
- *Borrelia* clearance may be slightly hindered in IL-17RA$^{-/-}$ mice.
- Overall, IL-17 receptor signaling plays no apparent role in the development of Lyme arthritis.

## Acknowledgements

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