

## Visualization of Antigen-specific T Cell in Living Arthritic Mice

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Fig. 1

It has been shown that CD4<sup>+</sup> T cells play a crucial role in the pathogenesis of several autoimmune diseases such as rheumatoid arthritis. In fact, CD4<sup>+</sup> T cells can be found in target organs in both human and mouse models of autoimmunity; thus, autoantigen-specific CD4<sup>+</sup> T cells have tissue-specific homing properties<sup>1</sup>. However, homing kinetics of such cells in vivo has not been clearly understood. To directly examine whether type II collagen (CII)-specific T cells home to the site of inflammation, we generated T cell hybridomas derived from CII-specific T-cell receptor (TCR)-transgenic mice. We transduced a CII-specific T cell with a gene encoding a green fluorescent protein (GFP)-luciferase fusion protein (termed CII-GFP-Luc), and tested the patterns of cell trafficking with whole-body bioluminescence imaging of the labeled cells in living animals<sup>2,3</sup>. We injected either CII-GFP-Luc or myelin basic protein (MBP)-specific T cells that carry GFP-luciferase genes (termed MBP-GFP Luc) into mice that had severe arthritis and obtained serial images of the mice. Three days after the cell transfer, photons emitted from the cells were detected in arthritic joints from all mice tested. Interestingly, MBP-GFP-Luc initially homed to inflamed joints as efficiently as did the CII-GFP-Luc cells. However, the trafficking of MBP-GFP-Luc cells to the inflamed joints was transient, whereas the CII-GFP-Luc cells were detected in the arthritic joints for more than 7 days following the injection. These results indicate that both CII- and MBP-specific T cells efficiently trafficked into the inflamed joints, but that TCR specificity seemed to be required for retention of T cells in inflamed joints<sup>4,5</sup>.

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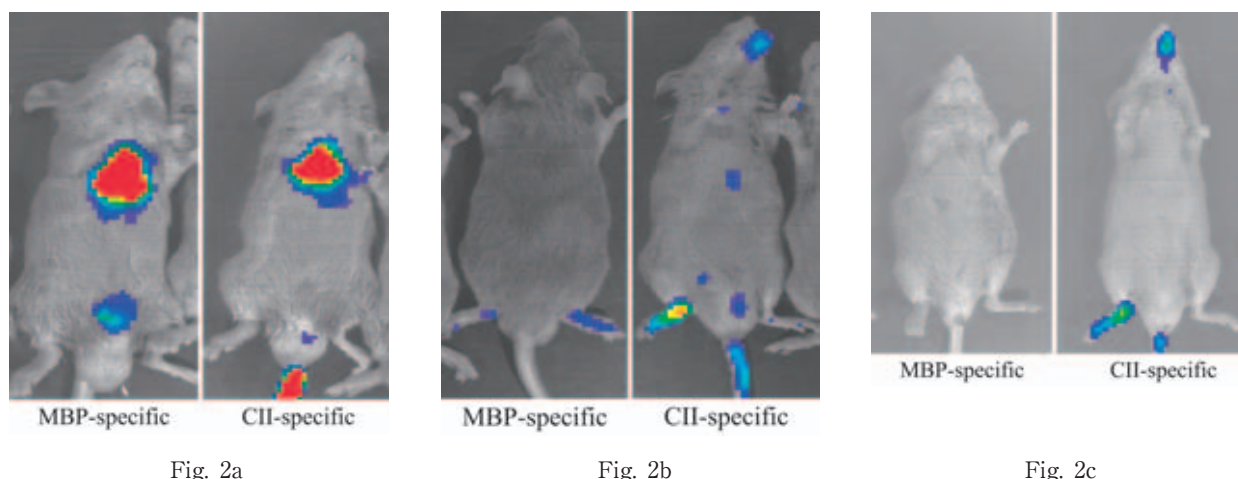


Fig. 2a

Fig. 2b

Fig. 2c

### Fig. 1 Basic method of Imaging

Before imaging, mice were anesthetized with avertin. An aqueous solution of the substrate luciferin was injected into the peritoneal cavity 5 min before imaging. The animals were then placed in the light-tight chamber of a low light imaging system equipped with a cooled charge-coupled device camera and a Navitar f 0.95 lens (Navitar Inc., Rochester, NY, USA), and a gray-scale body-surface reference image was obtained under weak illumination. Photons emitted from luciferase within the animal and then transmitted through the tissue were collected using the IVIS imaging system (Xenogen Corp., Hopkinton, MA, USA) with 5 min integration times. A pseudocolor image representing light intensity (blue least intense and red most intense) was generated using LivingImage Software (Xenogen Corp.) as an overlay on the IGOR image analysis package (WaveMetrics, Inc., Portland, OR, USA). Gray-scale reference images and pseudocolor images were superimposed by the LivingImage software, and annotations were added.

### Fig. 2 Homing and retention of CII-specific T cell hybridomas in sites of inflammation

After intravenous adoptive transfer, CD4<sup>+</sup> T cell hybridomas retrovirally transduced to express the enzyme luciferase (pGC-GFP-Luc vector construct) can be visualized in vivo in real time using bioluminescence imaging. On day 1, injected T cell hybridomas specific for either CII or MBP first travel to pulmonary circulation (**Fig. 2a**) and subsequently home to inflamed paws (**Fig. 2b**). Some bioluminescent activity is also seen from paravasally injected cells at the tail vein injection site. Interestingly, the trafficking of MBP-GFP-Luc cells to the inflamed joints was transient, whereas the CII-GFP-Luc cells were detected in the arthritic joints on day 5 (**Fig. 2c**) and for more than 7 days following the injection (data not shown).

## References

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