The Effect of Systemic Depletion of Natural Killer Cells in an EAE Mouse Model of Multiple Sclerosis Examined by Magnetic Resonance Imaging and Bioluminescence Imaging

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Introduction

The natural killer (NK) cells of the innate immune system can profoundly impact the development of adaptive immune responses against foreign invaders, as well as self-antigens [1]. Inflammatory and autoimmune responses in anatomical locations such as the central nervous system (CNS) differ substantially from those found in peripheral organs [2]. In experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis characterized by cellular infiltration, demyelination and axonal transaction, we find that systemic depletion of NK cells using a monoclonal antibody leads to exacerbated CNS inflammation and neurological deficits [3, 4]. For this study a combination of in vivo MRI and bioluminescence imaging was used to examine lesion development and measure reactive oxygen species (ROS) in EAE mice given the antibody anti-NK1.1.

Methods

C57BL/B6 mice (n=12) were injected subcutaneously in the hind flank with 200 µg of MOG35-55 peptide in complete Freund’s adjuvant (CFA) (Difco, Detroit, MI, USA) containing 500 µg of non-viable, desiccated Mycobacterium tuberculosis. On the day of and 2 days after immunization, the mice were inoculated with 200 ng of pertussis toxin (List Biologic, Campbell, CA, USA) intraperitoneally. For depletion of NK1.1+ cells in vivo, 100 ug anti-NK1.1 mAb or normal control mouse immunoglobulin (IgG) was injected intraperitoneally (i.p.) into each mouse (n=6 each group) at day - 2 post immunization. Every five days thereafter, 50 ug anti-NK1.1 mAb were injected i.p. until the termination of experiments.

In vivo MRI was performed on a 7 Tesla small-animal scanner (Bruker BioSpin, Billerica, MA). Coronal fat-suppressed T2-weighted images were acquired over the entire brain of each animal (RARE; TE1 14.5 ms, TE2 65.5 ms, TR 4500 ms, 0.5 mm slice thickness, Matrix 256x256, FOV 2.8cm, eight averages, 40 coronal slices, scan time 28 minutes, and 20 axial slices, scan time 28 minutes). For imaging of ROS generation in brain, bioluminescence images in live mice were captured with a 1 min acquisition time using a cooled IVIS imaging system (Xenogen IVIS-200, Alameda, CA) 18 hours after injection of 27 mg/kg DHE (Molecular Probes, Eugene, OR).

Results

T2 weighted images show focal lesions located around the ventricles of EAE mice from both groups (Figure 1). The mice given Anti-NK1.1 produced more pronounced focal lesions than animals given IgG. Optical imaging revealed a significantly larger ROS signal in the Anti-NK1.1 mice than the IgG controls (Figure 2A). Measurement of ROS signal showed significantly larger signal in the Anti-NK1.1 mice than in control mice (Figure 2b, 3.86±0.79 p/s/cm² Anti-NK1.1 vs. 1.73±0.36 p/s/cm² IgG, *P*=0.0092). In these models, the intensity of CNS inflammation visualized by MRI and quantified by in vivo bioluminescence imaging highly correlates with clinical and histological pictures of EAE.

Conclusion

These studies suggest an organ-specific activity of NK cells on the magnitude of CNS inflammation. Neuroimaging with both bioluminescence and MRI revealed CNS pathology that is highly pertinent to the role of NK cells in this pathology.

References

1. Abbas & Lichtman, Cell Mol Imun, 2003
2. Andersson et al., Neuroscience, 1992
3. Alvord et al, Prog Clin Biol Res, 1984

Figure 1 Example of image of periventrical lesions in EAE mice given A) IgG and B) Anti-NK1.1 antibody. Lesions are indicated by arrows.

Figure 2. A) Example of optical florescence images of mice given IgG and Anti-NK1.1 antibody. B) Significant difference in ROS signal was found between Anti-NK1.1 and control (3.86±0.79 Anti-NK1.1 vs. 1.73±0.36 IgG, *P*=0.0092).