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Short communication

Antigen therapy of experimental autoimmune encephalomyelitis selectively induces apoptosis of pathogenic T cells

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Abstract

Administration of high-dose myelin antigen induces massive T cell apoptosis in experimental autoimmune encephalomyelitis (EAE) but the nature of the target cells remains elusive. Here we have used a cell line established in eGFP-transgenic Lewis rats to distinguish between pathogenic and bystander T cells in adoptive transfer EAE. Intravenous application of gpMBP strongly reduced the amount of encephalitogenic cells in spinal cord and spleen while the number of the other T cells remained constant. This could be attributed to their differential sensitivity to apoptosis. Thus, antigen therapy selectively targets pathogenic T cells and should therefore limit potential adverse effects.
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Keywords: Experimental autoimmune encephalomyelitis; Multiple sclerosis; T cell apoptosis; Antigen therapy; Transgenic rat

1. Introduction

Experimental autoimmune encephalomyelitis (EAE) is a T cell-mediated inflammatory disease of the CNS that is widely used as a model for multiple sclerosis (MS) (Gold et al., 2006). In the Lewis rat, EAE follows a monophasic disease course and can be induced by adoptive transfer of encephalitogenic T cells (AT-EAE) (Ben-Nun et al., 1981; Swanborg, 2001). The inflammatory cascade is initiated by autoreactive T lymphocytes that cross the blood brain barrier and start to secrete pro-inflammatory cytokines and chemokines. Subsequently, additional autoreactive T cells are recruited to the CNS, which then leads to an amplification of the immune response (Flügel et al., 2001). Finally, bystander T cells, macrophages and granulocytes are recruited from the recipient's lymphoid organs to the inflammatory lesion, which results in demyelination (Gold et al., 2006).

Interferon-β (IFN-β) and Glatiramer Acetate (GA) are the most commonly used drugs to treat relapsing–remitting multiple sclerosis (Murray, 2006). While IFN-β induces a rather complex immunomodulation, GA has been developed as an example of immunotherapy based on the application of a putative myelin autoantigen (Arnon and Sela, 2003). Furthermore, antigen administration in rodents was used to treat experimental autoimmune disorders of the nervous system (Critchfield et al., 1994; Weishaupt et al., 1997, 2000). In this case, intravenous injection of high-dose gpMBP results in the induction of T cell apoptosis, leading to an amelioration of the disease course. However, it remains elusive whether cell death occurs in all types of T cells or whether the effect is restricted to the pathogenic T effector

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cells. This question can be addressed using an encephalitogenic T cell line recently established from eGFP-transgenic Lewis rats (Tischner et al., 2006; van den Brandt et al., 2004). Using an AT-EAE model we now show that the pathogenic T effector cells are preferentially eliminated by apoptosis after high-dose antigen therapy, while the bystander T cells in the spinal cord and normal T cells in the secondary lymphoid organs are only marginally affected.

2. Materials and methods

2.1. Induction of EAE

AT-EAE was induced in 6-week old female Lewis rats (Charles River, Sulzfeld, Germany) by i.v. injection of 8×10^6 freshly activated $eGFP^+$ MBP-specific T cells as previously described (Tischner et al., 2006). The T cell line used in this experiment is comprised of more than 95% $CD4^+$ $TCR\beta^+$ cells and underwent five restimulation cycles with gpMBP. Around 30% of the cells are $V\beta8.2^+$ whereas 13% express the Vβ3.3 restriction element (unpublished data). Animals were inspected daily and the severity of EAE was assessed employing a 6-grade disease score according to standard protocols (Schmidt et al., 2003). On day 3 after EAE induction the rats showed first signs of disability (Tischner et al., 2006; Weishaupt et al., 2000) and were treated i.v. with 500 μg gpMBP followed by a second injection with the same amount of antigen 15 h later. Control rats received PBS at the same time points. 6 h later the animals were sacrificed, spleen and spinal cord isolated and analyzed for the abundance of $eGFP^+$ and $eGFP^-$ T cells as well as for the induction of apoptosis. All experiments were conducted according to Bavarian state regulations for animal experimentation and approved by the responsible authorities.

2.2. Histology

In situ tailing (IST) was performed on paraffin-embedded spinal cord (TUNEL staining). Tissue sections were incubated for 1 h with 50 μl of a reaction mixture containing 1 μl digoxigenin-labeled nucleotides (Dig DNA labeling mixture; Roche, Mannheim, Germany) and 12 U transferase (Promega; Heidelberg, Germany). The reaction was stopped by adding 0.5 M EDTA. Sections were then treated for 1 h with an alkaline phosphatase-labeled antibody (Roche) at a dilution of 1:600. The color reaction was visualized by alkaline phosphate histochemistry using NBT/BCIP (Roche) as chromogen. After IST, the same sections were stained for T cells using the monoclonal B115–1 antibody (Holland Biotechnology, Leiden, Netherlands) and the ABC detection system (Dako, Hamburg, Germany) with alkaline phosphatase and New fuchsin as chromogen. Serial sections from the spinal cord were analyzed in eight animals in total, followed by quantification of the number of B115-1 and $TUNEL^+$ cells per mm^3 (mean ± SEM).

2.3. Isolation of spinal cord infiltrates

Lymphocytes were isolated from the spinal cord by density centrifugation. To this end, the dissected tissue was passed through a metal mash and treated with 100 μg/ml DNase I (Roche). After centrifugation the spinal cord homogenate was resuspended in 6 ml of 30% Percoll, overlaid on a Percoll gradient containing 4 ml 45% and 2 ml 70% Percoll and spinned for 20 min (2300 rpm, 4 °C). Finally, the lymphocytes were harvested at the interfaces between the layers.

2.4. Flow cytometry

FACS analysis of lymphocyte isolates was performed as previously described (van den Brandt et al., 2005) using reagents obtained from BD Biosciences (Heidelberg, Germany): monoclonal anti-ratTCRβ antibody (clone R73), anti-ratCD4 antibody (clone Ox35), anti-active Caspase-3 antibody (clone C92–605) and AnnexinV-Cy5. Intracellular staining of active Caspase-3 was performed according to the manufacturer's instructions. To determine Caspase-3 activation in T lymphocytes in the spinal cord,

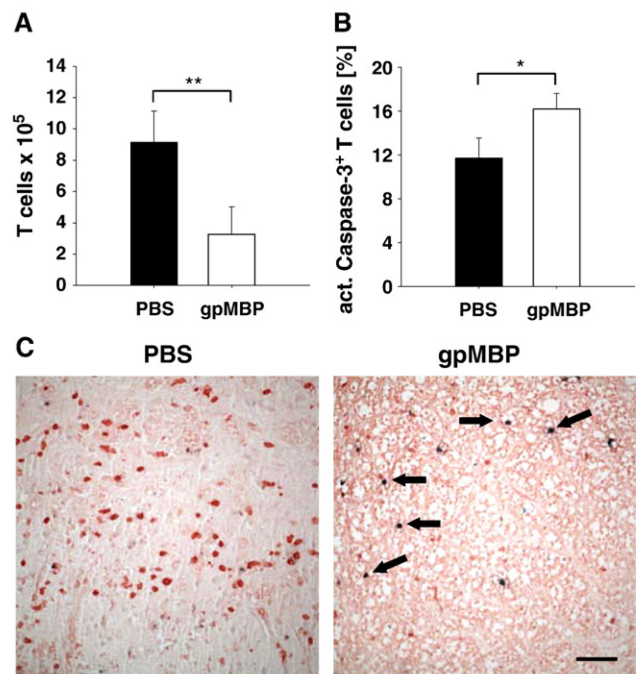

Fig. 1. T cell apoptosis after i.v. high-dose antigen therapy. Lewis rats undergoing AT-EAE were treated with gpMBP or PBS as a control. 6 h after the second injection the animals were sacrificed. (A) Absolute T cell numbers in the spinal cord were determined by flow cytometric analysis of lymphocyte isolates. Lymphocytes were gated on live cells. $^{**}P<0.01$. (B) Percentage of T cells positively staining for active Caspase-3 among all T cells in the spinal cord. $^{*}P<0.05$ (C) Histological analysis of spinal cord sections by double-labeling. Nuclei with fragmented DNA indicating apoptosis are labeled black by in situ tailing followed by anti-T-cell immunocytochemistry (red signal). Arrows indicate apoptotic T cells. Bar = 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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