Beyersdorf et al. www.jem.org/cgi/doi/10.1084/jem.20051060

MATERIALS AND METHODS

Histopathology and immunohistochemistry. For immunohistochemistry, 10-μm cryostat sections were stained with mAb B115-1 (dilution 1:500; Hy-Cult, via Sanbio) to identify T cells or with mAb ED1 (Sigma-Aldrich) to identify macrophages. We used the ABC detection system (DakoCytomation) with 3,3-diaminobenzidine as peroxidase substrate. Endogenous peroxidase activity was suppressed by incubating the sections for 10 min in 3% H_2O_2 before adding the ABC reagents. Finally, sections were counterstained with Mayer's hemalaun, dehydrated, and mounted in Vitro-clueR.

Apoptotic T cells were identified by TUNEL reaction as described previously (1) using a cell death detection kit (Roche), and nitro blue tetrazolium/5-bromo-4-chloro-3-indolylphosphate (NBT/BCIP) as chromogenic substrate. For simultaneous T cell immunohistochemistry, we applied the immunocytochemical protocol described before, except that an alkaline phosphatase-based detection method was used with New-fuchsin (DakoCytomation) as chromogenic substrate. Sections were mounted in aqueous mounting media Aquatex (Merck). For the myelin staining, Luxol Fast blue (LFB), and axonal Bielschowski silver impregnation we used standard procedures described by Linker et al. (2).

Analysis of inflammatory infiltrates and of T cell apoptosis was performed by an observer who was masked to the respective treatment analyzing 20–24 visual fields (3.2–3.84 mm^2) at 250 magnification of three to four spinal cord sections per animal. Apoptosis was assessed by morphological criteria (3) or labeling by TUNEL reaction (1).

Statistical analysis. Two-tailed Student's *t* tests were performed as indicated.

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