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MAJOR HISTOCOMPATIBILITY COMPLEX (MHC2+) PERIVASCULAR MACROPHAGES IN THE AXOTOMIZED FACIAL MOTOR NUCLEUS ARE REGULATED BY RECEPTORS FOR INTERFERON-γ (IFNγ) AND TUMOR NECROSIS FACTOR (TNF)

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Abstract—The major histocompatibility complex (MHC) glycoproteins, MHC1 and MHC2, play a key role in the presentation of antigen and the development of the immune response. In the current study we examined the regulation of the MHC2 in the mouse brain after facial axotomy.

The normal facial motor nucleus showed very few slender and elongated MHC2+ cells. Transection of the facial nerve led to a gradual but strong upregulation in the number of MHC2+ cells, beginning at day 2 and reaching a maximum 14 days after axotomy, correlated with the induction of mRNA for tumor necrosis factor (TNF) α, interleukin (IL) 1β and interferon-γ (IFNγ) and a peak in neuronal cell death. In almost all cases, MHC2 immunoreactivity was restricted to perivascular macrophages that colocalized with vascular basement membrane laminin and macrophage IBA1-immunoreactivity, with no immunoreactivity on phagocytic microglia, astrocytes or invading T-cells. Heterologous transplantation and systemic injection of endotoxin or IFNγ did not affect this perivascular MHC2 immunoreactivity, and transgenic deletion of the IL1 receptor type I, or TNF receptor type 1, also had no effect. However, the deletion of IFNγ receptor subunit 1 caused a significant increase, and that of TNF receptor type 2 a strong reduction in the number of MHC2+ macrophages, pointing to a counter-regulatory role of IFNγ and TNFα in the immune surveillance of the injured nervous system.

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Abbreviations: DAB, diaminobenzidine; FA/PBS, formaldehyde in phosphate-buffered saline; IFNγ, interferon-γ; IFNγR1, interferon-γ receptor type 1; IL1R1, interleukin-1 receptor type 1; IL1β, interleukin-1β; IL2, interleukin-2; IL6, interleukin-6; MHC, major histocompatibility complex/class; OLV, optical luminosity value; PB, phosphate-buffered sucrose; PBS, phosphate-buffered saline; TNF, tumor necrosis factor; TNFR1, tumor necrosis factor receptor type 1; TNFR2, tumor necrosis factor receptor type 2.

Key words: perivascular macrophage, basal membrane, antigen presentation, cytokine receptors, facial axotomy.

The surveillance and effector functions of the immune system are essential in combating neural infections as well as in mediating inflammation and tissue damage in the different forms of autoimmune inflammation of the nervous system, including post-vaccination and post-infectious encephalomyelitis, Guillain Barré syndrome and multiple sclerosis (Griffin et al., 1987; Hartung, 1993; Wekerle, 2002). However, there is also evidence pointing to the involvement of the immune system extends beyond infection and autoimmune conditions, and includes neurodegenerative diseases and different forms of brain trauma. Brain injury can lead to a influx of T-lymphocytes, macrophages, granulocytes and natural killer cells to the site of CNS damage (Olsson et al., 1992; Owens et al., 1994; Hirschberg et al., 1998; Raivich et al., 1998a; Bohatschek et al., 2001a; Ransohoff, 2002), accompanied by a local induction of major histocompatibility complex cell surface glycoproteins and costimulatory factors that are prerequisite for successful antigen presentation (Streit et al., 1989a,b; Lampson, 1990; Olson et al., 2001; Bohatschek et al., 2004a,b). Recent data suggest that this trauma-induced immune response plays an important, and frequently protective role in the injured neural tissue (Serpe et al., 2003; Schwartz et al., 2003). Understanding the molecules involved in this form of immune surveillance could thus be of clinical relevance.

The T lymphocytes are known to recognize their specific antigen when associated to the class I or class II molecules of the major histocompatibility complex, abbreviated as major histocompatibility class (MHC) 1 and MHC2 (for a review see Zinkernagel and Doherty, 1997). This recognition is aided by the binding of T-cell accessory molecules CD4 and CD8, expressed by the T-helper (mainly CD4+) and the T-suppressor/cytotoxic (mainly CD8+) lymphocytes, to their respective MHC2 or MHC1 ligands (Fleury et al., 1991; Miceli and Parnes, 1991). CNS injury causes an increase in the levels of MHC1 as well as MHC2, but the increase in MHC1 appears to be more abundant, particularly in the early and mild forms of neural damage in rodents (Streit et al., 1989b; Graeber et al., 1990; Morioka et al., 1993; Raivich et al., 1993, 1999). In previous studies we explored the molecular mechanisms associated with the induction of MHC1 in the mouse facial motor nucleus after transection of the facial nerve, impli-

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cating interleukin-6 (IL6) in the early, and interleukin-1 and tumor necrosis factor (TNF) in the late microglial response (Galiano et al., 2001; Bohatschek et al., 2004a). In the current study, we turned our attention to the MHC2 system, which appears to play a key part in mediating the survival of injured facial motoneurons (Serpe et al., 2003).

The facial axotomy model is characterized by two distinct types of cellular response. In the early phase, 1–4 days after facial axotomy, the rapid molecular changes in the axotomized neurons are followed by the activation and proliferation of microglial cells and their attachment to the cell bodies of the injured but living neurons (Graeber et al., 1988; Raivich et al., 1994; Svensson et al., 1994; Kalla et al., 2001). In the second, much later phase, transection of the facial nerve leads to a loss of approximately 20–40% of the affected motoneurons (Torvik and Skjörten, 1971; Sendtner et al., 1996; Kalla et al., 2001; Serpe et al., 2003; Raivich et al., 2002), with a peak in cell death at day 14 (Möller et al., 1996; Raivich et al., 1998a; Petitto et al., 2003). This neuronal debris is taken up and phagocytosed by surrounding microglia, transforming into rounded macrophages that aggregate to form large glial nodules and interact with the invading lymphocytes (Raivich et al., 1998a; Jones et al., 2000), which could place the microglia into a strategic position to present antigen.

The initial phase is associated with a strong upregulation of IL6, transforming growth factor β1 and the receptor for the macrophage colony stimulating factor (Kiefer et al., 1993; Raivich et al., 1998b). The second phase is associated with that of interleukin-1 β (IL1β), interferon γ (IFNγ) and TNFα (Raivich et al., 1998a). Both groups of cytokines play an important, phase-specific role on the cellular and molecular changes in the adult mouse axotomized facial motor nucleus: early cytokines in the first phase, late cytokines in the second one (Rong et al., 2001; Kalla et al., 2001; Galiano at al., 2001; Raivich et al., 2002). Here, we have used the model to analyze the time course and cellular distribution of MHC2 regulation after facial axotomy, and then after finding that the peak of MHC2 activity occurs during the second phase, explored the molecular mechanisms involved using mice with a homozygous deficiency for genes encoding the receptors for the cytokines IL1, TNFα and IFNγ.

EXPERIMENTAL PROCEDURES

Animals and surgical procedures

Normal adult C57BL/6 mice were obtained from Charles River (Sulzfeld, Germany). Balb/c animals were obtained from the in-house animal facilities at the Max-Planck Institute for Neurobiology, Martinsried, Germany. The homozygous IFNγ receptor type 1 knockouts (IFNγR1−/−; Huang et al., 1993) on the 129/SvEv background (G129) and the 129/SvEv controls were purchased from B&K Universal (Grimston/Aldbrough, Hull, UK). The TNFR1 knockouts (TNFR1−/−) on C57BL/6 background (Pfeffer et al., 1993) and wild type, C57BLl/6 controls were provided by Klaus Pfeffer (Microbiology Institute, Technical University, Munich, Germany). Homozygous Aa−/− mice with the disruption of the MHC class II gene Aa (Kontgen et al., 1993) and TNFR2−/− mice (Erickson et al., 1994) on the C57BL/6 background, IL1R1−/− (Labow et al., 1997) on the B6x129 background, and the appropriate C57BL/6 and B6x129 wild type controls were provided by F. Hoffmann-La Roche Ltd, Basle, Switzerland.

The animal experiments and care protocols were approved by the Regierung von Oberbayern (AZ 211-2531-10/93 and AZ 211-2531-37/97). All experiments conformed to European Communities Council Directive of 24 November 1986 (86/609/EEC) guidelines on the ethical use of animals and were designed to minimize the number of animals used and their suffering. The surgical procedures were all performed on 2–6 month old mice under anesthesia with i.p. injection of 0.4 mg/g body weight Avertin (tribromethanol; Sigma, Deisenhofen, Germany). The right facial nerve was transected at the stylomastoid foramen and the animals were killed in ether after a survival time from 0 to 42 days. A subgroup of C57BL/6 animals also received a s.c. transplant of balb/C kidney 6 days before kill, a s.c. injection of 2.5×10^4 IFNγ or an i.p. injection of 1.0 mg of *E. coli* lipopolysaccharide (055:B5 serotype; Sigma), 2 days before kill.

For light microscopic immunohistochemistry, the mice were perfused intracardially with 100 ml phosphate-buffered saline (PBS; 10 mM Na_2HPO_4, 0.85%NaCl, pH 7.4), followed by 200 ml of 4% formaldehyde in PBS (FA/PBS). The brain stem was taken out and postfixed by a 2 h immersion in 1% FA/PBS at 4 °C on a rotator (8 r.p.m.), followed by cryoprotection overnight by rotating immersion in a phosphate-buffered sucrose solution (PB; 10 mM Na_2HPO_4, pH 7.4, 4 °C; 30% sucrose) and freezing on dry ice. The brainstem was cut in 20 μm thick sections at the level of the facial motor nucleus at −15 °C, collected on gelatin-coated slides and stored at −80 °C until use.

Light microscopic bright-field immunohistochemistry

For standard immunohistochemistry, the sections were thawed, spread in distilled water, fixed in 4% formaldehyde/PB, defatted in acetone and pre-treated with 5% goat serum (Vector, Wiesbaden, Germany) in PB, as described by Möller et al. (1996). The sections were incubated overnight at 4 °C with primary rat monoclonal antibody against mouse MHC2 (MCA09; Serotec, Wiesbaden, DE) diluted 1:50 in PB/BSA (PB/0.1% bovine serum albumin; Sigma), followed by washing with PB/BSA and incubation with a 1:100 diluted biotinylated goat-anti-rat secondary antibody (Vector). Subsequently, the sections were incubated with ABC reagent (Vector) for 1 h and the staining visualized with Co/Ni-enhanced diaminobenzidine/H_2O_2 (DAB; 0.5 g/l in PBS/0.01% H_2O_2; Sigma; $CoCl_2$ 0.25 g/l, $NiSO_4$ 0.2 g/l). After washing with distilled water, the sections were dehydrated in alcohol and xylene and mounted with Depex (BDH, Poole, UK). Omission of the primary antibody led to the disappearance of the specific staining.

Immunoelectron microscopy

For electron microscopic immunohistochemistry, slow intracardiac perfusion with 100 ml of PBS was followed by 200 ml of 4% FA in PBS. The brainstems were removed and postfixed in 1% FA/PBS for 2 h at 4 °C. Vibratome sections, 80 μm thick, were cut at the level of the facial motor nucleus and treated similarly to the bright-field immunohistochemistry protocol but with the following exceptions: the sections were floating and acetone treatment was omitted; also, preincubation with goat serum was extended to 4 h, incubation with the secondary antibody was prolonged to 8 h at 4 °C and ABC reagent was applied overnight at 4 °C. For visualization the sections were first incubated in DAB/PBS for 20 min, followed by incubation in Co/Ni supplemented DAB/PBS with H_2O_2 for 20 min. The sections were then fixed in 2% glutaraldehyde/PBS for 6 days, osmicated, dehydrated and embedded in Araldite (Fluka, Basel, Switzerland). Semithin sections were counterstained with Toluidine Blue, for light microscopy, to identify the regions for the ultrathin sections. The ultrathin 100 nm sections were counterstained with uranyl acetate and lead citrate and examined in a Zeiss EM 10 electron microscope.