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Peripheral nerve lesion produces increased levels of major histocompatibility complex antigens in the central nervous system

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Summary

Proliferation of central nervous system (CNS) glia in response to peripheral nerve injury occurs without apparent participation of cells of the immune system. It is shown here that following transection of the rat facial nerve there is strongly elevated expression of class I, and to a lesser extent, class II antigens of the major histocompatibility complex (MHC) in the facial nucleus. It is demonstrated by double-immunofluorescence studies that the cells responsible for increased levels of MHC class I antigens are endogenous brain microglia. These findings emphasize the thought that microglia are immunocompetent cells, but, at the same time, raise the possibility for a non-immunological function of MHC antigens under conditions of neural regeneration.

Introduction

During the retrograde reaction seen in the facial nucleus after peripheral nerve section there is a massive infiltration of the region by glial cells. While conflicting views have been presented regarding the origin of proliferating glial elements, evidence from this laboratory, and others, strongly supports the idea of a proliferation of local microglia rather than an invasion of blood leucocytes (Kreutzberg, 1966; Sjöstrand, 1966; Torvik and Søreide, 1975; Schelper and Adrian, 1980; Søreide, 1981; Streit and Kreutzberg, 1987, 1988; Graeber et al., 1988). Although there is little reason to believe that the glial reaction to axotomy bears resemblance to a classical inflammatory reaction, especially since there is no introduction of an exogenous antigen, recent developments in the study of the relationship between glial cells and immune responses have changed traditional concepts profoundly in ascribing certain immune-related functions to central nervous system (CNS) glial cells (Fontana et al., 1984; Sun and Wekerle, 1986; Wekerle et al., 1986; Giulian, 1987; Suzumura et al., 1987). This has prompted us to determine in the present study whether expression of major histocompatibility complex (MHC) antigens occurs in the facial nucleus under conditions of nerve regeneration, and if so, by what cell types.
In the normal mammalian brain the expression of MHC antigens is extremely low (Schnitzer and Schachner, 1981; Lampson and Hickey, 1986; Wekerle et al., 1986; Lampson, 1987). The lack of MHC gene products together with a lack of lymphatic drainage, and the presence of the blood–brain barrier has been taken to regard the brain as an ‘immunologically privileged’ organ. This privilege is to be understood in a sense that immune responses and the concomitant appearance of MHC antigens may occur only during certain pathological states, or experimental situations, such as multiple sclerosis (Traugott et al., 1983; Traugott, 1987), experimental allergic encephalomyelitis (EAE) (Matsumoto and Fujiwara, 1986; Matsumoto et al., 1986), viral infections (Suzumura et al., 1986; Olsson et al., 1987), and following stimulation with γ-interferon in vivo and in vitro (Lampson and Fisher, 1984; Wong et al., 1984, 1985; Suzumura et al., 1987). The present report demonstrates that a lesion occurring outside of the CNS, i.e. transection of the facial nerve, results in a strong increase in MHC class I, and a minor increase in class II antigens in the facial nucleus. This adds a new perspective to the study of MHC antigen expression in that the condition leading to MHC antigen expression is one in which the integrity of the blood–brain barrier is preserved, and no direct insult to the brain takes place.

**Materials and methods**

**Surgery and tissue processing**

Young adult male Wistar rats were used for all experiments (8–10 weeks old, approx. 200 g). Under ether anesthesia the right facial nerve was cut near its exit from the stylomastoid foramen. Following survival periods of 3–7 days, the animals were sacrificed either by decapitation, or by perfusion with 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.2 under deep chloral hydrate anesthesia. The perfused brains were post-fixed in the same fixative for maximally 4 h. Either fresh-frozen or fixed-frozen sections of 20 μm thickness were prepared from the brain stem at the level of the facial nuclei, and left to dry for 1–2 h on gelatinized slides at room temperature.

**Staining methods**

Immunohistochemical staining of paraformaldehyde-fixed sections was performed with monoclonal antibodies listed in Table 1. Sections were incubated for 1–2 h at room temperature with the primary antibodies applied in optimal or serial dilutions, and the binding sites were visualized using the avidin-biotin-horseradish peroxidase method (Vectastain ABC-Kit, Vector Laboratories, Burlingame, CA, U.S.A.) with 3,3'-diaminobenzidine (DAB)-H₂O₂ as a substrate medium.

**TABLE 1**

ANTIBODIES USED FOR IMMUNOCYTOCHEMICAL STUDIES IN THE AXOTOMIZED FACIAL NUCLEUS

| Designation | Source           | Antigen                                      | Dilution     | Staining results |
|-------------|-----------------|----------------------------------------------|--------------|-----------------|
| Anti-GFAP   | Dakopatts, Denmark | Glial fibrillary acidic protein | 1:400       | + + +           |
| OX-18       | Serotec, U.K.    | Monomorphic determinant of rat MHC class I antigens | 1:2000 | + + +           |
| OX-27       | Serotec, U.K.    | Polymorphic determinant of rat MHC class I antigens | 1:100 to 1:2000 | -               |
| OX-8        | Serotec, U.K.    | Membrane glycoprotein of cytotoxic T cells | 1:100 to 1:2000 | -               |
| W3/25       | Serotec, U.K.    | Membrane glycoprotein of helper T cells | 1:100 to 1:2000 | -               |
| OX-52       | Serotec, U.K.    | Pan T cell marker                           | 1:100 to 1:2000 | -               |
| OX-3        | Serotec, U.K.    | Polymorphic determinant of rat Ia antigen | 1:100 to 1:2000 | -               |
| OX-6        | Serotec, U.K.    | Monomorphic determinant of rat Ia antigen | 1:100 to 1:406 | +               |
| OX-17       | Serotec, U.K.    | α-Chain determinant of rat Ia antigen | 1:100 to 1:2000 | -               |
Double-immunofluorescence studies were carried out on fresh-frozen tissue sections using monoclonal antibodies directed against monomorphic determinants of MHC class I antigens (OX-18) and the glial fibrillary acidic protein (GFAP), a well-defined astrocytic marker (Bignami et al., 1972), as well as the microglia-binding lectin from *Griffonia simplicifolia* (GSA I-B4). Sections were first incubated with OX-18 antibody, followed by goat anti-mouse IgG-rhodamine conjugate (Sigma, 1:60), and then with rabbit anti-GFAP followed by goat anti-rabbit IgG-fluorescein conjugate (Sigma, 1:60), or GSA I-B4-fluorescein conjugate (Sigma, 10 μg/ml in PBS + 0.1% Triton X-100 containing 0.1 mM CaCl₂, MgCl₂, and MnCl₂). Immunohistochemical controls consisted of (a) replacing the primary antibody with PBS and carrying out the staining sequence, (b) substituting the primary antibody with another antibody of the same IgG subclass, and (c) using a secondary biotinylated antibody directed against immunoglobulins from a species other than the one in which the primary antibodies were raised.

**Results**

**Immunohistochemical detection of MHC antigens**

In the operated facial nucleus increased levels of monomorphic determinants of MHC class I antigens could be visualized with monoclonal antibody OX-18, whereas polymorphic determinants recognized by the OX-27 antibody were not detected (Table 1). Staining with antibodies directed against surface glycoproteins of helper and cytotoxic T cells (W3/25, OX-8, respectively), as well as with the pan T cell marker OX-52, was also negative. When the expression of Ia antigens in the axotomized facial nucleus was probed with monoclonal antibody OX-6, a few cells (about 2–3 per section) with a morphology resembling that of microglia were stained. Although the number of Ia-immunoreactive cells was considerably lower than those expressing class I antigens, the time course of appearance of Ia-positive cells was similar to that of cells demonstrating class I immunoreactivity. Other antibodies recognizing determinants of Ia antigens, such as OX-3 and OX-17 did not show staining other than occasional meningeal cells. From 3 to 7 days after axotomy there was a striking increase in OX-18 immunoreactivity in the operated facial nucleus compared to the contralateral unoperated side where reaction product was found only in association with the endothelium of blood vessels (Fig. 1a and b). Stained glial cells were not present in the unoperated nucleus. In contrast, throughout the neuropil of the operated nucleus there was diffuse immunostaining which was more pronounced around individual neurons. At higher magnification the reaction product could be clearly identified on perineuronal glial satellites (Fig. 1c). Typically, the glial nuclei were devoid of staining, and the immunoreactivity appeared to be associated with the cytoplasmic processes closely wrapping neuronal perikarya. Control sections treated as described above showed no staining (Fig. 1d).

**Localization of MHC antigens in comparison with known glial cell markers**

In order to clarify which cell types were expressing class I antigens in response to axotomy, double-immunofluorescence was carried out. Staining with OX-18 in combination with anti-GFAP labelling clearly demonstrated that the OX-18 antibody did not bind to astrocytes (Fig. 2a and b). Similar to class I antigens, GFAP immunoreactivity was noticeably elevated in the operated facial nucleus (Graeber and Kreutzberg, 1986), but the GFAP-positive astrocytic processes presented in a different arrangement around motoneurons. They were localized as many short discrete foot processes in the vicinity of neurons as well as throughout the neuropil, and did not form a continuous perineuronal rim surrounding the neuronal soma. In contrast, when OX-18 immunoreactivity was compared to the staining pattern obtained with the *Griffonia simplicifolia* lectin, complete congruity was observed (Fig. 2c and d). Since selective binding of the lectin to microglial cells has been demonstrated in earlier electron-microscopic studies (Streit and Kreutzberg, 1987), we conclude that the increased expression of monomorphic determinants of MHC class I antigens, as detected by the OX-18 antibody, is a characteristic of microglia.
Fig. 1. Immunostaining with the OX-18 antibody and the avidin-biotin-horseradish peroxidase method. a: In the unoperated facial nucleus only the capillary endothelia show immunoreactivity, ×205. b: Six days after axotomy there is markedly increased presence of class I antigens in the neuropil and, more prominently, around the neurons, ×151. c: High magnification of a single motoneuron reveals perineuronally located reaction product in association with the processes of glial satellites, ×960. d: Immunohistochemical control where the primary antiserum was replaced by PBS lacks staining, ×128.
Fig. 2. Double-immunofluorescence microscopy. a, b: Comparison of the localization of class I surface antigens, detected with OX-18, and intracellular GFAP, respectively. Note the non-overlapping staining patterns demonstrating a lack of class I antigens on astrocytes, ×384. c, d: Comparison of OX-18 immunoreactivity with lectin binding of microglia, respectively, demonstrates overlapping staining patterns, ×467.
Discussion

The present findings afford new evidence with regard to the occurrence and localization of MHC antigens in brain. We have shown that activated microglial cells express high levels of class I antigens during the retrograde reaction in the absence of an immune response. The reported slight upregulation of Ia antigens may be tentatively attributed to microglia based on morphological observations, but final conclusions about the identity of Ia-positive cells must await further studies.

A central issue which has been the subject of long-standing controversy concerns the origin of increased numbers of glial cells in the nucleus of an axotomized peripheral nerve. While there is little doubt that during development microglial cells are derived from blood monocytes (Streit et al., 1988), a consensus about the source of glia after axotomy in adult animals has not yet been attained. Our contention that endogenous glial cells proliferate in response to axotomy stems from extensive light- and electron-microscopic studies in our and other laboratories which failed to show either margination and diapedesis of blood leucocytes, or perivascular infiltrates pathognomonic of brain inflammatory disease (Kreutzberg, 1966; Sjöstrand, 1966; Torvik and Søreide, 1973; Schelper and Adrian, 1980; Søreide, 1981; Streit and Kreutzberg, 1987, 1988; Graeber et al., 1988). In addition, light- and electron-microscopic autoradiography have shown $[^3]$Hthymidine incorporation by perineurally located cells with typical microglial morphology (Kreutzberg, 1966; Graeber et al., 1988). The fact that suicide transport (Wiley et al., 1982) of toxic ricin by facial motoneurons does not produce an inflammatory reaction but a transformation of local microglia into brain macrophages (Streit and Kreutzberg, 1988) provides additional support for our hypothesis. In view of these earlier studies and the results described here the question then arises why endogenous microglial cells express MHC antigens, essential for T cell-mediated immune functions, when no invasion of leucocytes has been observed or can be demonstrated with T cell-specific monoclonal antibodies. It is known that MHC molecules are co-recognized with a foreign antigen on an antigen-presenting cell by cytotoxic or helper T cells leading to a T cell-mediated immune reaction. While in EAE, for example, the introduction of a foreign antigen precedes the inflammatory reaction, there is probably no such event taking place when a peripheral nerve is cut. Furthermore, unlike in EAE, there is no breakdown of the blood–brain barrier detectable with vascular tracers in response to a nerve lesion occurring outside of the CNS (Sjöstrand, 1966). For these reasons it is plausible that the increased expression of class I antigens observed in our paradigm does not result in an immune response, but may instead signify a state of alertness, or immune competence of microglial cells. The increased expression of MHC antigens in the absence of a foreign antigen is thus not sufficient to cause an immune response in brain. Low-level expression of class I antigens in normal rat brain by 'cells of dendritic morphology (DC)' — a term in need of definition for the brain parenchyma — has been described (Matsumoto and Fujiwara, 1986; Matsumoto et al., 1986). It is likely that DC represent a proportion of resting microglial cells bearing a small density of MHC molecules. These resting cells, however, must first receive a stimulus, such as after axotomy, in order to become activated and show such high MHC antigen expression as described in the present study.

Another possibility which has so far received little attention, is that MHC antigens may have functions related to non-immunological events. Although MHC modulation does not seem to play a role during neural differentiation and development (Lampson, 1987), one should consider the possibility of an involvement of these molecules in cell–cell interactions during nerve regeneration. This speculation is attractive in view of protein sequencing data revealing homologies between MHC molecules and immunoglobulin-related structures found on neural tissues, such as the neural cell adhesion molecule, and the myelin-associated glycoprotein (Williams, 1984, 1987). Possibly, these molecules which are members of the immunoglobulin superfamily, may have a common evolutionary origin, and may serve functions related to basic cell surface recognition events.

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