J. Anat. (1994) 184, pp. 285–296, with 21 figures Printed in Great Britain

Upregulation and induction of surface antigens with special reference to MHC class II expression in microglia in postnatal rat brain following intravenous or intraperitoneal injections of lipopolysaccharide

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(*Accepted 27 August 1993*)

ABSTRACT

The effects of bacterial lipopolysaccharide (LPS) on the expression of surface antigens including major histocompatibility complex (MHC) and complement type 3 (CR3) receptors on microglial cells in the corpus callosum in postnatal rat brain were investigated. When LPS was injected intravenously (i.v.) in 1-d-old rats, the immunostaining of callosal amoeboid microglial cells with OX-18 directed against MHC class I antigen was enhanced 24 h after the injection in comparison with the controls. The expression of MHC class II (Ia) antigen on the same cell type as shown by its immunoreactivity with OX-6 was also elicited especially after 2 intraperitoneal (i.p.) injections of LPS. Thus 7 d after a single i.p. injection of LPS into 1-d-old rats, only a few OX-6 positive cells showing a moderate staining reaction were observed in the corpus callosum. The immunoreactivity diminished 14 d after the injection. However, in rats receiving 2 successive i.p. injections of LPS at 1 and 4 d of age and killed 7 d after the 1st injection, a significant number of intensely stained OX-6 positive amoeboid microglial cells were observed in the corpus callosum. The expression of MHC class II antigens induced by 2 injections of LPS was sustained at least until d 14 when the callosal ramified microglial cells, known to be derived from gradual metamorphic transformation of amoeboid microglia, still exhibited intense immunoreactivity with OX-6. The effect of LPS on the expression of CR3 on amoeboid microglial cells was not obvious after a single injection, but the immunoreactivity with OX-42 was also augmented in rats given 2 i.p. administration of LPS into rats at 1 an 4 d of age. It is concluded from this study that the expression of MHC class I and class II antigens on amoeboid microglial cells in corpus callosum was upregulated and induced respectively after i.v. or i.p. injection of LPS into early postnatal rats. Although relatively fewer in number when compared with OX-18 and OX-42 positive cells, it is suggested that the OX-6 positive cells would have the potentiality to function in antigen presentation in the postnatal rat brain when challenged by the endotoxin.

Key words: Endotoxin, amoeboid microglia, MHC antigens, CR3 receptors.

INTRODUCTION

The central nervous system (CNS) has long been considered an immunologically privileged site (Barker & Billingham, 1977; Wikstrand & Bigner, 1980), the reason being that the major histocompatibility complex (MHC) class I and class II (Ia) antigens essential for initiation of immunological responses are absent or very low in normal CNS (Sasaki et al. 1989; Streit et al. 1989; Morioka & Streit, 1991; Perry & Gordon, 1991; Sasaki & Nakazato, 1992). This concept now appears untenable in view of recent demonstrations of MHC antigens on microglial cells in inflammation (Gehrman et al. 1993), intracerebral grafts (Poltorak & Freed, 1989; Finsen et al. 1991) and experimentally induced neurodegeneration (Streit et al. 1988, 1989; Konno et al. 1989; Morioka & Streit, 1991; Kaur & Ling, 1992; Ling et al. 1992; Finsen et al. 1993). MHC

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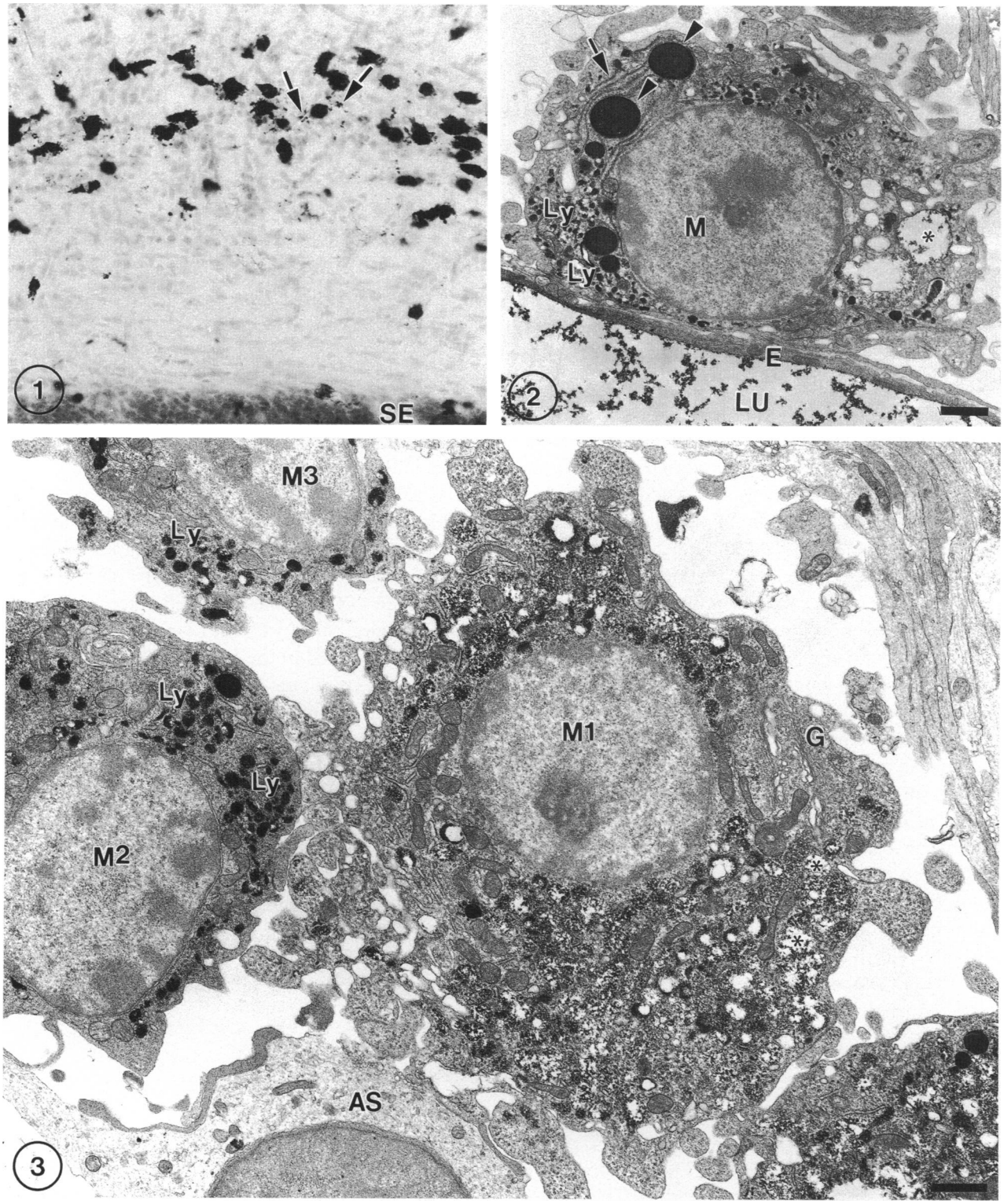


Fig. 1. Corpus callosum above the lateral ventricle 24 h after an i.p. injection of HRP in 1-d-old rat. Amoeboid microglial cells are heavily loaded with HRP reaction product. Some free HRP particles (arrows) are present in the interstitial spaces. SE, subependyma of lateral ventricle. ×270.

Fig. 2. Amoeboid microglial cell (M) closely adherent to the outer wall of a blood vessel in the corpus callosum in a 7-d-old rat, 6 h after an i.v. injection of HRP. The lumen of the blood vessel (LU) is filled with HRP reaction product. HRP is also sequestered in the cytoplasmic vacuoles (asterisk) and the abundant lysosomes (Ly) of the AMC. E, endothelial cell; arrow, rough endoplasmic reticulum; arrowheads, lipid droplets. Bar, 1 μm.

Fig. 3. Three amoeboid microglial cells (M1, M2 and M3) in the corpus callosum in a 1-d-old rat, 6 h after an i.v. injection of HRP. The cells are filled with HRP localised in the lysosomes (Ly), vacuoles (asterisks) and dispersed in the cytoplasm. G, Golgi apparatus; AS, astrocyte. Bar, 1 μm.

Table 1. *Immunoreactivity of amoeboid microglial cells in corpus callosum after a single i.v. injection of LPS and NS into 1-d-old rats*

Treatment	Interval after injection	OX-18	OX-6	OX-42
NS	1 h	++	−	++
LPS	1 h	++	−	++
NS	6 h	++	−	++
LPS	6 h	++	−	++
NS	24 h	++	−	++
LPS	24 h	+++	+	++

+++ very strong reaction; ++ strong reaction; + moderate reaction; − no reaction. NS, normal saline; LPS, lipopolysaccharide.

antigens have also been detected on microglial cells in pathological human brain (Sasaki & Nakazato, 1992) and in human neurodegenerative disorders, e.g. Alzheimer's disease (McGeer et al. 1987, 1993; Dickson et al. 1993) and Parkinson's disease (McRae & Dahlström, 1992). On treatment with γ-interferon (IFN-γ), microglial cells also exhibited an increased expression of MHC class II antigen (Sethna & Lampson, 1991).

In our earlier studies (Ling et al. 1990, 1991; Ling & Wong, 1993), we had demonstrated in the corpus callosum in normal postnatal rats a large number of macrophagic amoeboid microglial cells (AMC) showing intense immunoreactivity for MHC class I antigen and type 3 complement receptors. The expression of MHC class II antigen, however, was hardly detectable or virtually absent in these cells. Relevant to the present study is the use of bacterial lipopolysaccharide (LPS) which is known to cause a dramatic increase in Ia expression in mouse peritoneal macrophages (Ziegler et al. 1984). LPS also induced the expression of MHC class I and class II antigens in several other tissues (Halloran et al. 1988; Jephthah-Ochola et al. 1988). In this study, we sought to find out whether the expression of MHC antigens on AMC in the postnatal rat brain could be upregulated or elicited when exposed to LPS achieved by either i.p. or i.v. administration. This is because our earlier studies (Kaur et al. 1986; Xu et al. 1993) had already established that foreign substances, e.g. horseradish peroxidase or rhodamine introduced by these routes would readily be circulated to the cerebral vessels where they would enter the corpus callosum by transendothelial transport and subsequently be endocytosed by the preponderant AMC. With this premise, it was reasoned that LPS when administered by these routes could also gain access into the same site where the residential AMC would be challenged by the endotoxin.

MATERIALS AND METHODS

HRP injection

In order to confirm our previous studies (Kaur et al. 1986; Xu et al. 1993) that tracers injected by the i.p. or i.v. route could gain access into the postnatal corpus callosum in which the amoeboid microglial cells are heavily populated, horseradish peroxidase (HRP) was administered in this study into 1 and 7 d old Wistar rats.

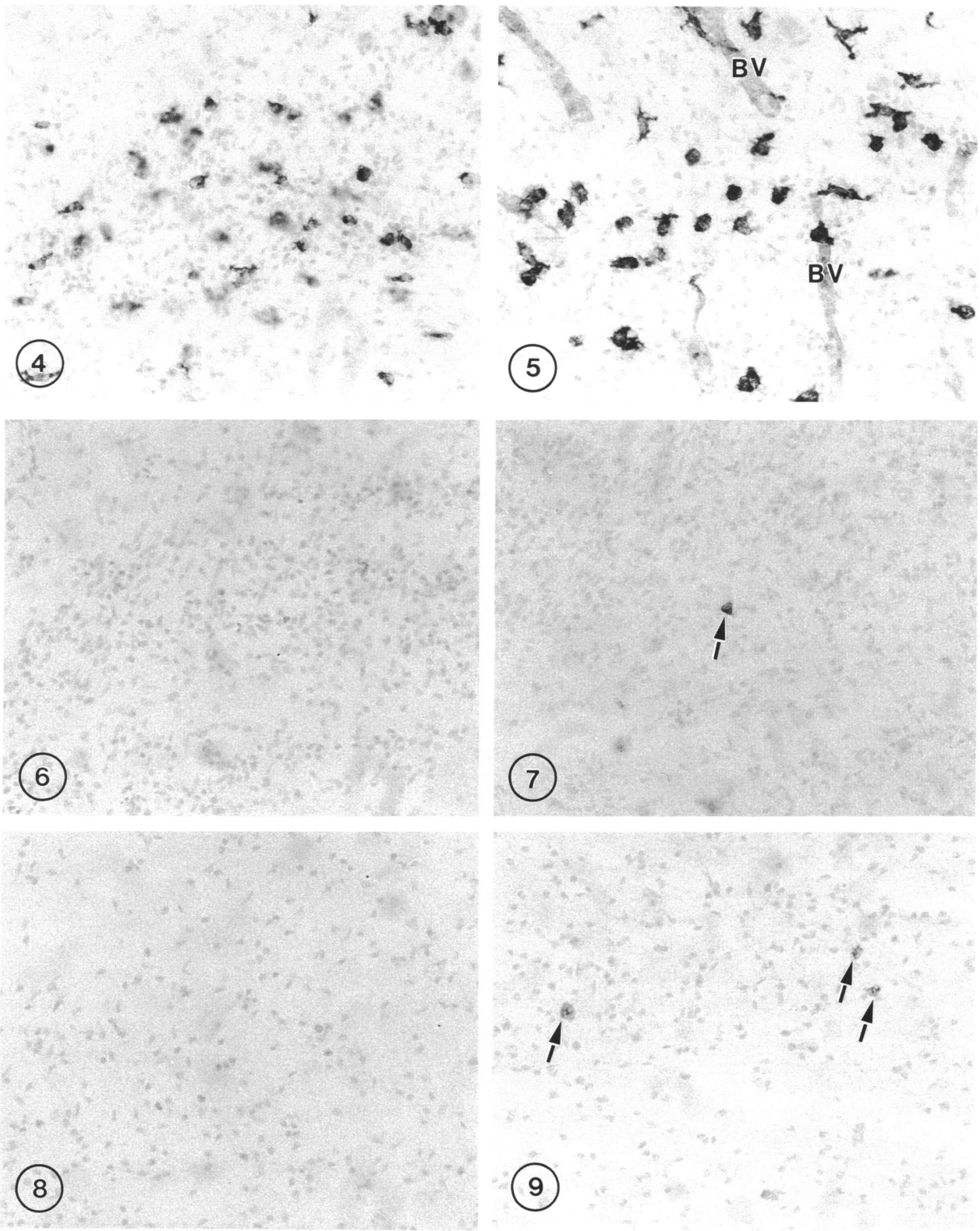
Under ether anaesthesia, each rat was given an i.p. or a single injection of HRP via the left external jugular vein. HRP (type VI, Sigma P8375) in normal saline (8 mg HRP in 50 µl saline, i.e. 16%) was given at 4 µl/g body weight of the animals. The rats were killed 6 and 24 h after the injection for light (LM) and electron microscopy (EM). Tetramethyl benzidine (TMB) and 3,3′-diaminobenzidine (DAB) were used as substrates for LM and EM respectively. The details of tissue processing have been described previously (Kaur et al. 1986).

LPS injection

A total of 50 rats (Wistar) aged 1 and 7 d were used in this experiment. Lipopolysaccharide from Escherichia coli (055: B5, Sigma, L2880) was prepared in normal saline at 10 mg/ml.

Intravenous injection. Under ether anaesthesia, postnatal rats aged 1 d were given a single i.v. injection of 10 µl LPS via the left external jugular vein. For control, rats of the same age were given an equal volume of normal saline injection. The rats were killed 1, 6 and 24 h after injection. Animals with longer survival intervals were not studied because newborn rats subjected to i.v. injection following skin incision for the exposure of the external jugular vein were always cannibalised by the mother rats.

Intraperitoneal injection. Rats aged 1 d were injected with 10 µl LPS intraperitoneally. Control rats received an equal volume of NS injection. In order to enhance the effects of LPS, some of the rats were given a second or booster injection 3 d later, i.e. at 4 d of age. Since the mortality rate was rather high ($\sim$ 50% of the rats died after the 1st treatment), the dosage of LPS for the 2nd injection was half that of the 1st. For comparison, the control rats also received a 2nd injection of normal saline. The rats were killed 1, 2, 7 and 14 d after the 1st injection.



Figs 4, 5. OX-18 positive amoeboid microglial cells in the corpus callosum 24 h after an i.v. injection of saline (Fig. 4) and LPS (Fig. 5) in 1-d-old rats. Note that the immunoreactivity of AMC in the LPS-injected rat (Fig. 5) is markedly augmented when compared with that of the saline control rat (Fig. 4). The blood vessels (BV) in the LPS-injected rat (Fig. 5) are also stained. ×270.

Figs 6, 7. Immunostaining with OX-6 in the corpus callosum, 24 h after an i.v. injection of saline (Fig. 6) and LPS (Fig. 7) in 1-d-old rats. Sporadic OX-6 positive cells (Fig. 7, arrow) occur in the LPS-injected rat but none in the control rat (Fig.6). ×270.

Figs 8, 9. Immunostaining with OX-6 in the corpus callosum, 7 d after a single i.p. injection of saline (Fig. 8) and LPS (Fig. 9) into 1-d-old rats. The corpus callosum in the LPS-injected rat (Fig. 9) shows some OX-6 positive cells (arrows) which are absent in the saline control rat (Fig. 8). ×270.

Rats aged 7 d were given an i.p. injection of 20 µl LPS. As in the 1-d-old rats, some of the rats also received a 2nd or booster injection (10 µl LPS) 3 d following the 1st injection. The corresponding control rats were also given a 2nd injection of normal saline at the same time interval. The animals were killed 2 and 7 d following the 1st injection.

At least 3 rats were killed at each of the time intervals in the respective experimental groups. To minimise individual variation, animals belonging to the same litter were used for any particular experimental procedure.

Immunohistochemistry

Under ether anaesthesia, the rats were perfused with Ringer's solution followed by an aldehyde fixative composed of a mixture of periodate-lysine-paraformaldehyde containing 2% paraformaldehyde according to the method of McLean & Nakane (1974). The perfusion lasted for 10–15 min. After perfusion, the brain was removed and fixed in the same fixative for 2 h and was then kept in 0.1 M phosphate buffered saline (PBS) containing 10% sucrose overnight at 4 °C. Coronal frozen sections of the cerebrum were cut at 40 µm and rinsed in PBS; they were then incubated overnight at room temperature with one of the following monoclonal antibodies diluted 1:100 with PBS: OX-18 (Sera Lab, MAS 10 lb), OX-6 (Sera Lab, MAS 043b) and OX-42 (Sera Lab, MAS 370b). The monoclonal antibodies were for the detection of MHC I, MHC II (Ia) and complement type 3 receptors respectively. Subsequent antibody detection was carried out using biotinylated antimouse IgG (rat adsorbed) and Vectastain ABC kit (PK-4000, Vector Laboratories) with 3,3′-diaminobenzidine as peroxidase substrate. The sections were counterstained with 1% methyl green, dehydrated and mounted in Permount.

RESULTS

HRP injection

Light microscopy

In 1 and 7 d old rats receiving i.p. injection of HRP and killed 24 h later, amoeboid microglial cells (AMC) in the corpus callosum above the lateral ventricles were heavily loaded with HRP reaction product (Fig. 1). The majority of the stained AMC were round (Fig. 1). Free HRP reaction particles were observed in the interstitial spaces (Fig. 1). AMC in the cavum septum pellucidum were also strongly labelled with HRP. In the cerebral cortex, the only HRP-labelled elements were some endothelial cells and perivascular elements closely associated with capillary walls. Macrophages in meninges were intensely labelled with HRP.

Electron microscopy

Ultrastructural study showed that the AMC in the corpus callosum had endocytosed a massive amount of HRP, 6 h after i.v. injection in 1 and 7-d-old rats (Figs 2, 3). The high electron dense HRP reaction product was localised in the abundant lysosomes and vacuoles (Figs 2, 3). Some HRP reaction particles were freely dispersed in the cytoplasm (Fig. 3).

LPS injection

Intravenous injection (Table 1)

At 1 and 6 h after LPS injection into 1-d-old rats, the AMC in the corpus callosum were strongly stained with OX-18 and OX-42. The immunoreactivity was comparable to that of the control rats given saline injection. Most of the intensely stained AMC were

Table 2. *Immunoreactivity of microglial cells in corpus callosum after a single i.p. injection of LPS and NS into 1-d-old rats*

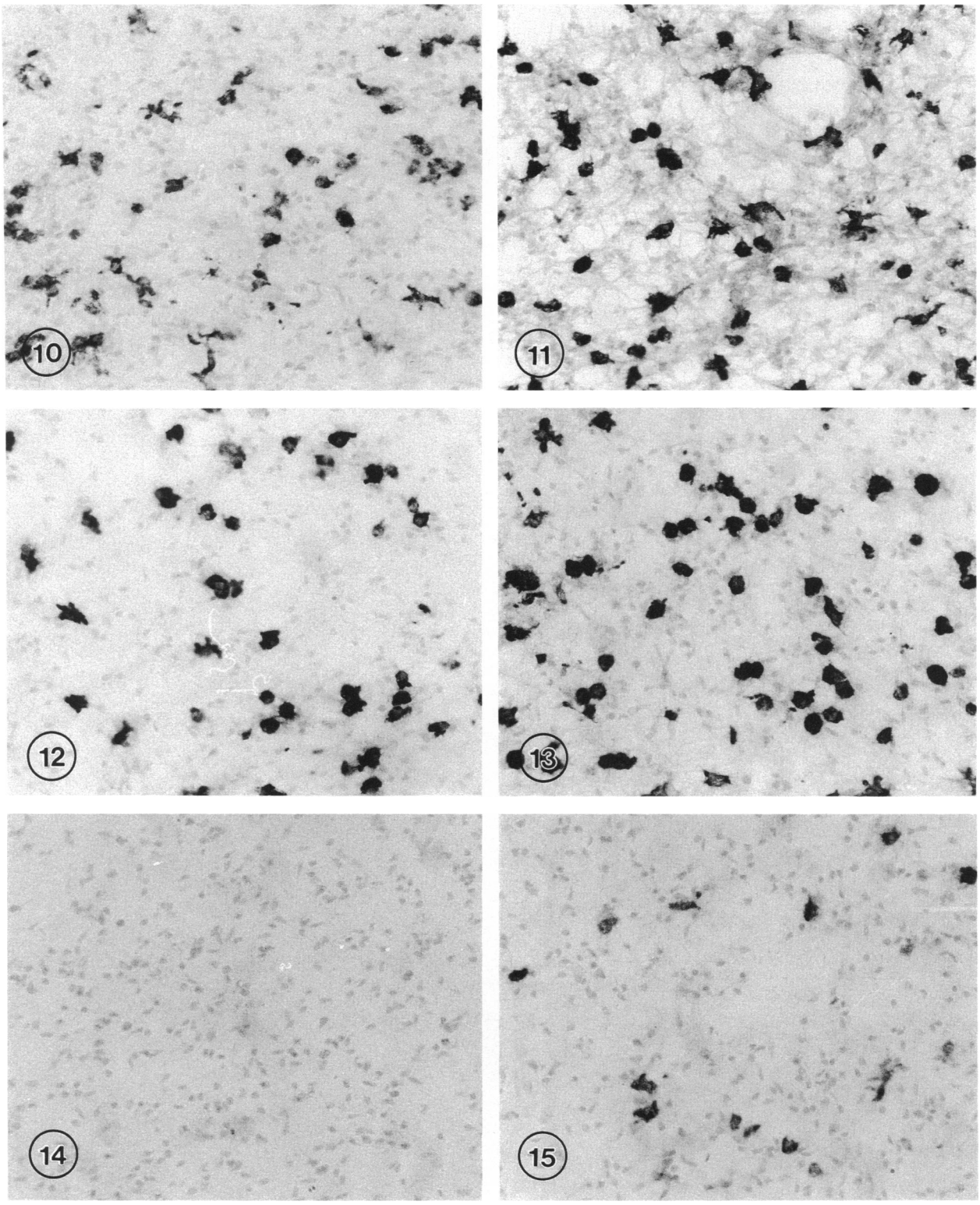
Treatment	Interval after injection	OX-18	OX-6	OX-42
NS	1 d	++	−	++
LPS	1 d	++	−	++
NS	2 d	++	−	++
LPS	2 d	++	−	++
NS	7 d	++	−	++
LPS	7 d	++	+	++
NS	14 d	±	−	+
LPS	14 d	±	−	+

++ strong reaction; + moderate reaction; ± weak reaction; − no reaction.

Table 3. *Immunoreactivity of microglial cells in corpus callosum in rats after 2 successive i.p. injections of LPS and NS at 1 and 4 d of age*

Treatment	Interval after 1st injection	OX-18	OX-6	OX-42
NS1, NS2	7 d	++	−	++
LPS1, LPS2	7 d	+++	++	+++
NS1, NS2	14 d	±	−	+
LPS1, LPS2	14 d	++	++	+++

+++ very strong reaction; ++ strong reaction; + moderate reaction; ± weak reaction; − no reaction.



Figs 10, 11. Rats receiving 2 successive i.p. injections of saline (Fig. 10) and LPS (Fig. 11) at 1 and 4 d of age and killed 7 d after the first injection. Note that the amoeboid microglial cells in the LPS-injected rat are more intensely stained with OX-18 (Fig. 11) when compared with those of the saline control rat (Fig. 10). ×270.

Figs 12, 13. Rats receiving 2 successive i.p. injections of saline (Fig. 12) and LPS (Fig. 13) at 1 and 4 d of age and killed 7 d after the 1st injection. The immunostaining of amoeboid microglial cells for OX-42 in LPS-injected rat (Fig. 13) appears to be stronger than that of the saline control rat. ×270.

round with some bearing stout processes. In saline control rats, OX-6 positive cells were absent. Similarly, they were undetectable in rats killed 1 and 6 h after LPS injection.

In rats killed 24 h after LPS injection, however, the OX-18 immunoreactivity in AMC was obviously enhanced when compared with the control (Figs 4, 5). Furthermore, the endothelial cells of the callosal blood vessels were also more intensely stained than in the saline control rats (Fig. 5). A remarkable feature of the corpus callosum 24 h after i.v. injection of LPS was the staining of a few randomly scattered OX-6 positive AMC (Fig. 7). The latter displayed a moderate immunoreaction and most of them were round (Fig. 7). As in the earlier intervals, none of the callosal AMC in the control rats showed immunoreactivity with OX-6 (Fig. 6).

Intraperitoneal injection

Single LPS injection into 1-d-old rats (*Table 2*). At 1, 2 and 7 d after the injection of LPS, the immunostaining of AMC with OX-18 and OX-42 did not show any obvious difference from that in the control rats of the corresponding survival intervals. In both the LPS and saline-injected rats, the number of labelled cells showed a steady increase with age. The immunostaining of callosal endothelium with OX-18 was greatly enhanced 1 and 2 d after LPS injection when compared with the control animals. This, however, appeared to subside in rats killed 7 d after LPS injection. OX-6 positive cells were absent in all control animals (Fig. 8) as well as in rats given LPS injection and killed 1 and 2 d later. A variable number of AMC moderately stained with OX-6, however, occurred 7 days after a single LPS injection (Fig. 9). The cells varied from round to oval with short processes.

In rats killed 14 d after injection, the immunoreactivity of the callosal microglial cells with OX-18 was greatly reduced in both the LPS and saline-injected rats. The stained cells which appeared to be reduced in number at this age assumed a branched appearance typical of ramified microglial cells. As with OX-18, the intensity of immunostaining of these cells with OX-42 was noticeably weakened with a concurrent reduction in their number. In general, the OX-42 positive cells appeared more numerous than OX-18 positive cells and their immunoreactivity was also more intense. OX-6 positive cells were absent 14 d following a single LPS injection.

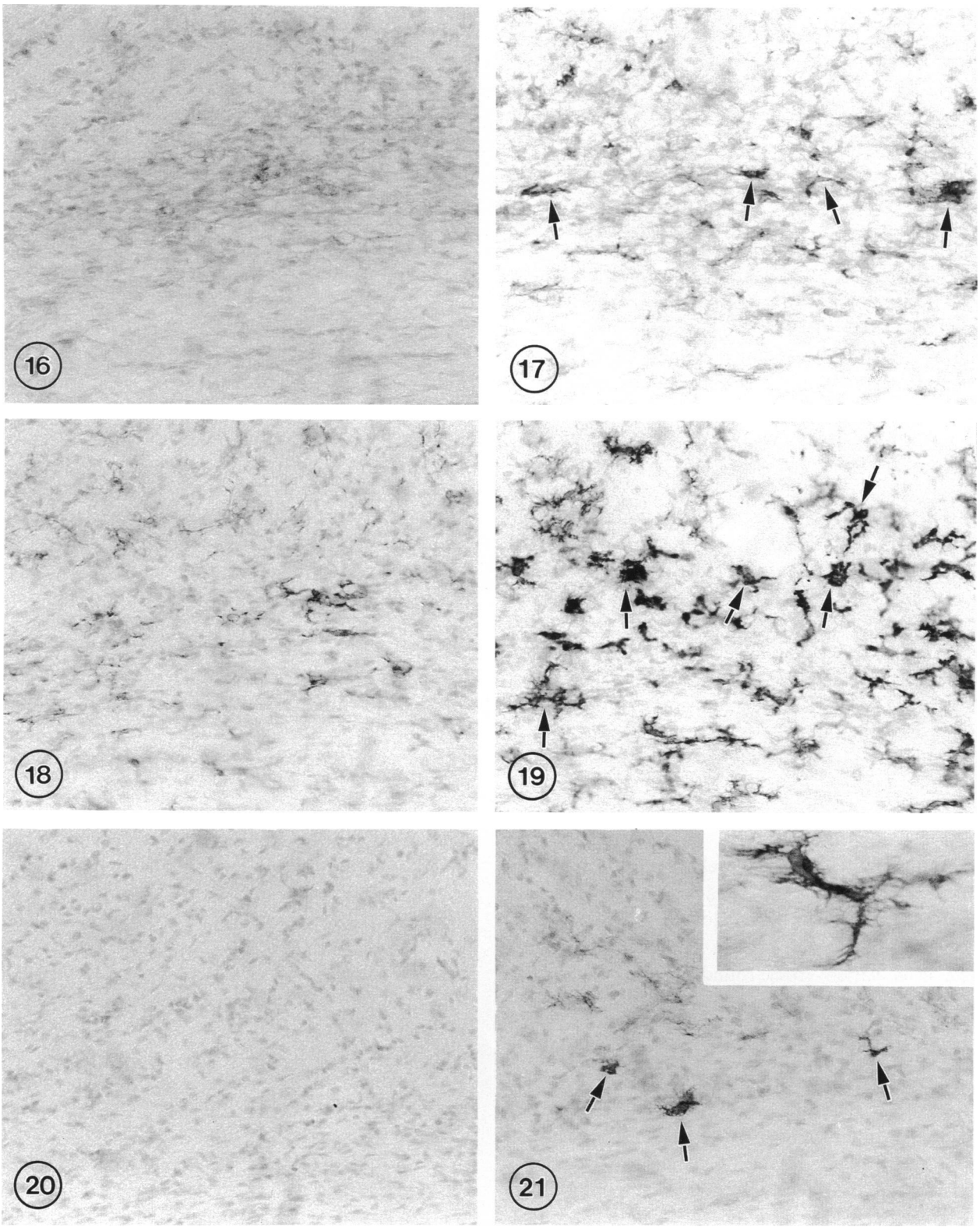
Successive LPS injections into rats at 1 and 4 d of age (*Table 3*). At 7 d after the first injection, the immunoreactivity of AMC with OX-18 was stronger than that in the control rats receiving saline injections (Figs 10, 11). The intensity of immunostaining of AMC with OX-42 in LPS-injected rats appeared to be slightly increased when compared with control animals (Figs 12, 13). The most remarkable feature after 2 injections of LPS was the appearance of many randomly distributed OX-6 positive AMC in the corpus callosum (Figs 14, 15). Although relatively fewer in number when compared with OX-18 and OX-42 positive cells, the OX-6 positive cells were intensely stained (Fig. 15); they were mostly oval with stout processes (Fig. 15).

At 14 d after the first LPS injection, the immunoreactivity of microglial cells, now appearing ramified, was significantly enhanced with OX-18 and OX-42 when compared with the corresponding control rats (Figs 16–19). Intensely stained OX-6 positive cells were common in the corpus callosum of the LPS-injected rats but absent in the control (Figs 20, 21). Most of the OX-6 positive cells were ramified with long extending processes (Fig. 21).

Single i.p. injection of LPS into 7 d rats (*Table 4*). In animals killed 2 d after the injection of LPS, the callosal AMC were darkly stained with OX-18. The immunoreactivity was more intense than that of the control animals. There were also more labelled cells in the LPS-injected rats. Most of the OX-18 positive cells were oval, bearing a variable number of short processes, but some displayed long ramified processes. With OX-42, there was no obvious difference in the immunoreactivity of AMC between the control and LPS-injected rats. OX-6 positive cells were absent in both groups. In animals killed 7 d after the injection of LPS, the immunoreaction of microglial cells with OX-18, OX-6 and OX-42 was comparable in control and LPS-injected rats.

Successive LPS injections into 7 and 10 d rats (*Table 5*). At 7 d after the first injection, OX-18 immunoreactivity of microglial cells in the corpus callosum was enhanced when compared with that in the corresponding control animals. Some OX-6 positive cells, although with weak immunoreactivity, were

Figs 14, 15. Rats receiving 2 successive i.p. injections of saline (Fig. 14) and LPS (Fig. 15) at 1 and 4 d of age and killed 7 d after the 1st injection. Several intensely stained OX-6 positive cells are seen in Figure 15 after 2 LPS injections. They are absent in the corresponding saline control rat (Fig. 14). ×270.



Figs 16, 17. Rats receiving 2 successive i.p. injections of saline (Fig. 16) and LPS (Fig. 17) at 1 and 4 d of age and killed 14 d after the 1st injection. The ramified microglial cells (arrows) in the corpus callosum in the LPS-injected rat exhibit a strong immunoreaction with OX-18 (Fig. 17). They are barely detected in the saline control rat (Fig. 16). ×270.

Figs 18, 19. Rats receiving 2 successive i.p. injections of saline (Fig. 18) and LPS (Fig. 19) at 1 and 4 d of age and killed 14 d after the 1st injection. The ramified microglial cells (arrows) in the LPS-injected rat display a much stronger immunoreactivity with OX-42 (Fig. 19) when compared with those in the saline control rat (Fig. 18). ×270.

Figs 20, 21. Rats receiving 2 successive i.p. injections of saline (Fig. 20) and LPS (Fig. 21) at 1 and 4 d of age and killed 14 d after the 1st injection. OX-6 positive ramified microglial cells (arrows) are consistently observed in the LPS-injected rat (Fig. 21). Inset in Figure 21 shows a typical ramified microglial cell immunostained with OX-6. OX-6 positive cells are absent in the corresponding saline control rat (Fig. 20). ×270. Inset, ×540.

Table 4. *Immunoreactivity of microglial cells in corpus callosum after a single i.p. injection of LPS and NS into 7-d-old rats*

Treatment	Interval after injection	OX-18	OX-6	OX-42
NS	2 d	+	−	++
LPS	2 d	++	−	++
NS	7 d	±	−	+
LPS	7 d	±	−	+

++ strong reaction; + moderate reaction; ± weak reaction; − no reaction.

Table 5. *Immunoreactivity of microglial cells in corpus callosum in rats after 2 successive i.p. injections of LPS and NS at 7 and 10 d of age*

Treatment	Interval after 1st injection	OX-18	OX-6	OX-42
NS1, NS2	7 d	±	−	+
LPS1, LPS2	7 d	+	±	+

+ moderate reaction; ± weak reaction; − no reaction.

randomly distributed in the corpus callosum. The labelled OX-6 positive cells were extremely ramified with long branching processes. They were absent in the control animals. The immunoreactivity of microglial cells with OX-42 paralleled that in the saline control rats.

DISCUSSION

The view that the CNS is an immunologically privileged site (Barker & Billingham, 1977; Wikstrant & Bigner, 1980; Head & Griffin, 1985) requires reappraisal in view of recent findings of the presence of cells bearing MHC antigens in the CNS in normal and under pathological or experimental conditions, (Tribolet et al. 1984; Hayes et al. 1987; McGeer et al. 1987; Kajiwara et al. 1991; McRae & Dahlstrom, 1992; Sasaki & Nakazato, 1992). On treatment with γ-interferon (IFN-γ) in vitro, microglia became Ia-positive and functioned as antigen-presenting cells (Frei et al. 1987), but it remains uncertain whether the activated microglia exhibiting Ia antigen in vivo would also act as antigen-presenting cells (Streit et al. 1988). Hickey & Kimura (1988) and Hickey et al. (1992) suggested that perivascular and parenchymal microglial cells would probably partake in such a role in the CNS of mammals. The expression of MHC class II antigen by microglial cells has been demonstrated in the white matter in the normal human brain (Hayes et al. 1987; Sasaki and Nakazato, 1992). It is noteworthy that very few, if any, microglial cells display MHC antigens in the normal rat brain (Perry et al. 1993). Our previous study (Ling et al. 1991) showed that although considerable MHC class I antigen was detected in the amoeboid microglial cells in the corpus callosum in early postnatal rats, the presence of MHC class II (Ia) antigen, however, was barely detectable.

The present study showed that MHC class I antigen was a sensitive marker for stimulation by LPS. When the latter was injected i.v. into 1 d rats, the immunoreactivity of AMC with OX-18 was intensified 24 h later when compared with the control animals, indicating an upregulation of the antigen. The upregulation of MHC I on AMC was also evident 2 d following an i.p. injection of LPS into 7 d rats. The elevation of MHC class I expression was clearly accentuated and sustained by 2 successive i.p. injections of LPS. Another interesting finding following LPS injection was the increase in the immunostaining of endothelial cells with OX-18, at 24 h and at 2 d after an i.v. injection, but this subsided with time. The significance of this is unclear. Relevant to the present findings is the work of Jephthah-Ochola et al. (1988) who demonstrated that in mice given 2 i.p. injections of LPS, there was a definite increase in class I and class II MHC products in many tissues including kidney, liver, heart, lung and pancreas. The present result further amplifies this in that the expression of MHC class I antigen on AMC in postnatal rat brain is also enhanced by LPS.

The most remarkable finding in the present study is the induction of class II MHC antigen expression on callosal AMC especially after 2 i.p. injections of LPS. The induction of MHC class II antigen by LPS seems to take a longer interval when compared with that for MHC class I antigen. The novel expression of MHC II antigen was more readily elicited when LPS was injected i.v. than by the i.p. route into 1 d rats. This could be due to a higher blood concentration of LPS in i.v.-injected rats. At 7 d after a single i.p. injection of LPS into 1 d rats, the OX-6 positive callosal AMC showed only a moderate staining reaction and this diminished 14 d after the injection. However, when a 2nd or booster injection was administered in rats at 4 d of age, the amoeboid microglial cells exhibited a stronger immunoreaction. The number of OX-6 positive AMC was also significantly greater than that of rats receiving a single LPS injection. The expression of MHC class II antigens by 2 successive injections of LPS was sustained at least until d 14 when the callosal microglial cells, which had transformed into the

ramified form, were still intensely stained. When the 2 experimental age groups of rats were compared, it appears that with increasing age the animals become less sensitive to LPS. This is based on the observation that in 7 d rats given a single i.p. injection of LPS, OX-6 positive cells were undetectable 7 d after the administration, but were observed in 1 d rats subjected to the same treatment. Only when 2 successive injections of LPS were given in rats at 7 and 10 d of age did the expression of MHC class II antigen on microglia become detectable. Despite this, the OX-6 positive microglia were fewer and their immunostaining was weaker when compared with rats receiving 2 injections at 1 and 4 d.

It appears from other studies that it is more difficult to induce the expression of MHC class II antigen than class I antigen. For example, Jephthah-Ochola et al. (1988) reported that increased class I expression could be induced by a single LPS injection, whereas class II induction required a 2nd injection. Ziegler et al. (1984) observed that i.p. injection of LPS (1 injection) into LPS-responder mice caused a dramatic increase in Ia expression only in the peritoneal macrophage population harvested 1 wk after injection. Our results are in agreement with theirs with respect to the frequency of LPS given and the time interval allowed for a sufficient induction of MHC antigen expression on AMC.

The effect of LPS on type 3 complement receptors (CR3) is less obvious and inconsistent. Only in rats receiving 2 i.p. injections of LPS at 1 and 4 d of age and killed 14 d after the 1st injection, the immunoreactivity of microglial cells with OX-42 was much stronger than that in the control rats. A possible explanation for this may be that CR3 receptors are less susceptible to LPS stimulation, although individual variation is another factor to be considered. It is speculated that the enhanced immunoreactivity with OX-42 on microglial cells after 2 injections of LPS into rats at 1 and 4 d of age may be related to a possible increase in CR3 receptor-mediated endocytosis.

Lipopolysaccharides (endotoxins) are localised on the surface of all gram-negative bacteria (Rietschel & Brade, 1992). They are known to elicit disease symptoms ranging from fever to irreversible shock and death. Paradoxically, the same endotoxins that threaten human health can enhance the body's overall immune resistance to bacterial and viral infections and cancer (Rietschel & Brade, 1992). Stimulation of macrophages appears to be a general property of LPS extracted from gram-negative bacterial cell walls (Doe et al. 1978). Free endotoxins interact with and activate macrophages which then secrete many mediators to initiate or amplify both specific and nonspecific immune responses (Rietschel & Brade, 1992). Several studies had demonstrated that LPS could upregulate the MHC antigen expression either in vitro or in vivo (Ziegler et al. 1984; Jephthah-Ochola et al. 1988). It needs to be mentioned that down-regulation of macrophage Ia expression by LPS has also been reported for in vitro studies (Yem & Parmely, 1981; Steeg et al. 1982). Results in this study tend to support the involvement of LPS in upregulation of MHC antigen expression, although the underlying mechanisms remain speculative. LPS is known to induce human and murine peripheral T lymphocytes to produce IFN-γ (Blanchard et al. 1986; Le et al. 1986), which has been shown to enhance the expression of MHC class II molecules in a large number of cells including macrophages (Skoskiewicz et al. 1985; Momburg et al. 1986; Ijzermans & Marquet, 1989). The effect of LPS on the upregulation of MHC antigens can be completely inhibited by a monoclonal antibody against IFN-γ (Wentworth & Ziegler, 1987; Halloran et al. 1988; Jephthah-Ochola et al. 1988), indicating the involvement of IFN-γ in MHC induction. It remains unclear whether T lymphocytes are responsible for the release of IFN-γ. Wentworth & Ziegler (1987) investigated the effect of LPS on Ia expression in T cell deficient mice by using the congenitally athymic mice which showed a dramatic increase in the expression of Ia by peritoneal macrophages 7 d after the injection. The authors therefore concluded that increased macrophage Ia expression could occur in the absence of mature functioning T cells. Similar results were observed by Halloran et al. (1988) who concluded that LPS increased MHC expression by inducing a population of non-T cells to release IFN-γ. A hypothesis on a probable sequence of events leading to the induction of MHC expression by LPS in nude and normal mice was put forward by Jephthah-Ochola et al. (1988), i.e. LPS, through its action on macrophages, induces NK-like cells (and in normal mice possibly T cells as well) to produce IFN-γ and other mediators, which in turn upregulate MHC expression. TNF is also considered to be involved in the induction of MHC, particularly class I (Jephthah-Ochola et al. 1988). However, the ability of TNF to induce MHC class II expression requires synergy with IFN-γ (Pujol-Borrell et al. 1987).

It is unequivocal from this study that LPS injected i.p. or i.v. can enhance and elicit the expression of

MHC class I and class II antigens on AMC in postnatal rat brain. This is in accord with our earlier findings (Xu et al. 1993) that fluorescent tracer when administered i.p. or i.v. into 1 and 7 d rats is readily circulated to the cerebral vessels where it gains access into the corpus callosum to be taken up by the callosal AMC. The accessibility of postnatal corpus callosum to exogenous materials is substantiated in the present study by the labelling of AMC with HRP. It seems justifiable, therefore, to assume that LPS when introduced by the same routes could also enter the same site. In other words, it is possible that the callosal AMC would be subjected to stimulation by LPS in circulation. It remains to be elucidated whether LPS acts directly or indirectly through IFN-γ which is known to induce Ia-negative ramified microglial cells and amoeboid microglial cells to become Ia-positive cells in in vitro studies (Frei et al. 1987; Sasaki et al. 1989). The i.v. infusion of IFN-γ or its intracerebral or cerebrospinal fluid injection also causes the expression of MHC class II antigen in rat brain (Steiniger & van der Meide, 1988; Lassmann et al. 1989; Sethna & Lampson, 1991). These results, when taken together with those mentioned above, suggest that LPS might act through IFN-γ in the upregulation of the expression of MHC antigens on AMC in the present study. The induction of MHC class II expression on AMC by LPS appeared to be less effective in 7 d rats compared with the younger animals. One possibility for this change may be that the cells had become more differentiated at this age and they were probably less sensitive to LPS stimulation. Another possible explanation might be related to a reduced permeability of the callosal blood capillaries to LPS. This is because the permeability of callosal capillaries to foreign substances is known to reduce with advancing age (Xu et al. 1993). The results in this study may have some clinical implications since they tend to suggest that the brain of the newborn is more readily accessible to exogenous materials, e.g. endotoxins.

The significance of upregulation of MHC antigens on AMC in this study remains to be explored. As mentioned above, on treatment with IFN-γ in vitro, microglial cells became Ia-positive and functioned as antigen-presenting cells (Frei et al. 1987). Whether Ia-positive microglial cells in vivo are capable of presenting antigen to T cells is still uncertain. Hickey & Kimura (1988) reported that perivascular microglial cells in CNS presented antigen in vivo. In the present study only a small proportion of the callosal microglia were OX-6 positive following 2 successive injections of LPS, but from a speculative point of view, these OX-6 positive cells would have the potential to function in antigen presentation in the postnatal rat brain when challenged by the endotoxin.

ACKNOWLEDGEMENTS

This study was supported by a research grant (RP920315) from the National University of Singapore. The secretarial assistance of Ms Diljit Kaur in the preparation of the manuscript is gratefully acknowledged.

REFERENCES

Barker CF, Billingham RE (1977) Immunologically privileged sites. *Advances in Immunology* **25**, 1–54.

Blanchard DK, Djeu JY, Klein TW, Friedman H, Stewart WE (1986) Interferon-γ induction by lipopolysaccharide: dependence on interleukin 2 and macrophages. *Journal of Immunology* **136**, 963–969.

Dickson DW, Lee SC, Mattiace LA, Yen S-HC, Brasnan C (1993) Microglia and cytokines in neurological disease, with special reference to AIDS and Alzheimer's disease. *Glia* **7**, 75–83.

Doe WF, Yang ST, Morrison DC, Betz SJ, Henson PM (1978). Macrophage stimulation by bacterial lipopolysaccharides. II. Evidence for differentiation signals delivered by lipid A and by a protein rich fraction of lipopolysaccharides. *Journal of Experimental Medicine* **148**, 557–568.

Finsen BR, Sørensen T, Castellano B, Pedersen EB, Zimmer J (1991) Leuckocyte infiltration and glial reactions in xenografts of mouse brain tissue undergoing rejection in the adult rat brain. *Journal of Neuroimmunology* **32**, 159–183.

Finsen BR, Jørgensen MB, Diemer NH, Zimmer J (1993) Microglial MHC antigen expression after ischemic and kainic acid lesions of the adult rat hippocampus. *Glia* **7**, 41–49.

Frei K, Siepl C, Groscurth P, Bodmer S, Schwerdel C, Fontana A (1987) Antigen presentation and tumor cytotoxicity by interferon-γ-treated microglial cells. *European Journal of Immunology* **17**, 1271–1278.

Gehrman J, Gold R, Linington C, Lannes-Vieira J, Wekerle H, Kreutzberg GW (1993) Microglial involvement in experimental autoimmune inflammation of the central and peripheral nervous system. *Glia* **7**, 50–59.

Halloran PF, Urmson J, Farkes S, Phillips RA, Fulop G, Cockfield S, et al. (1988) Effects of cyclosporine on systemic MHC expression. *Transplantation* **46**, 68S–72S.

Hayes GM, Woodroofe MN, Cuzner ML (1987) Microglia are the major cell type expression MHC class II in human white matter. *Journal of the Neurological Sciences* **80**, 25–37.

Head JR, Griffin WS (1985) Functional capacity of solid tissue transplants in the brain: evidence for immunological privilege. *Proceedings of the Royal Society of London B* **224**, 375–387.

Hickey WF, Kimura H (1988) Perivascular microglial cells of the CNS are bone marrow-derived and present antigen in vivo. *Science* **239**, 290–292.

Hickey WF, Vass K, Lassmann H (1992) Bone marrow-derived elements in the central nervous system: an immunohistochemical and ultrastructural survey of rat chimeras. *Journal of Neuropathology and Experimental Neurology* **51**, 246–256.

Ijzermans JNM, Marquet RL (1989) Interferon-gamma: a review. *Immunobiology* **179**, 456–473.

JEPHTHAH-OCHOLA J, URMSON J, FARKAS S, HALLORAN PF (1988) Regulation of MHC expression in vivo: bacterial lipopolysaccharide induces class I and II MHC products in mouse tissues by a T cell-independent, cyclosporine-sensitive mechanism. *Journal of Immunology* **141**, 792–800.

KAJIWARA K, HIROZANE A, FUKUMOTO T, ORITA T, NISHIZAKI T, KAMIRYO T, et al. (1991) Major histocompatibility complex expression in brain of rats with graft-versus-host disease. *Journal of Neuroimmunology* **32**, 191–198.

KAUR C, LING EA, WONG WC (1986) Labelling of amoeboid microglial cells in rats of various ages following an intravenous injection of horseradish peroxidase. *Acta Anatomica* **125**, 132–137.

KAUR C, LING EA (1992) Activation and re-expression of surface antigen in microglia following an epidural application of kainic acid in the rat brain. *Journal of Anatomy* **180**, 333–342.

KONNO H, YAMAMOTO T, IWASAKI Y, SUZUKI H, SAITO T, TERUNUMA H (1989) Wallerian degeneration induces Ia-antigen expression in the rat brain. *Journal of Neuroimmunology* **25**, 151–159.

LASSMAN H, VASS K, BRUNNER C (1989) The role of gamma-interferon in inflammatory demyelination. *Journal of Neuropathology and Experimental Neurology* **48**, 365.

LE J, LIN J-X, HENRIKSEN-DESTEFANO D, VILČEK J (1986) Bacterial lipopolysaccharide-induced interferon-γ production: role of interleukin 1 and interleukin 2. *Journal of Immunology* **136**, 4525–4530.

LING EA, WONG WC (1993) The origin and nature of ramified and amoeboid microglia: a historical review and current concepts. *Glia* **7**, 9–18.

LING EA, KAUR C, YICK TY, WONG WC (1990) Immunocytochemical localization of CR3 complement receptors with OX-42 in amoeboid microglia in postnatal rats. *Anatomy and Embryology* **182**, 481–486.

LING EA, KAUR C, WONG WC (1991) Expression of major histocompatibility complex and leukocyte common antigens in amoeboid microglia in postnatal rats. *Journal of Anatomy* **177**, 117–126.

LING EA, KAUR C, WONG WC (1992) Expression of major histocompatibility complex antigens and CR3 complement receptors in activated microglia following an injection of ricin into the sciatic nerve in rats. *Histology and Histopathology* **7**, 93–100.

MCGEER PL, ITAGAKI S, TAGO H, MCGEER EG (1987) Reactive microglia in patients with senile dementia of the Alzheimer type are positive for the histocompatibility glycoprotein HLA-DR. *Neuroscience Letters* **79**, 195–200.

MCGEER PL, KAWAMATA J, WALKER DG, AKIYAMA H, TOOYAMA I, MCGEER EG (1993) Microglia in degenerative neuroglial disease. *Glia* **7**, 84–92.

MCLEAN IW, NAKANE PK (1974) Periodate-lysine-paraformaldehyde fixative a new fixative for immunoelectron microscopy. *Journal of Histochemistry and Cytochemistry* **22**, 1077–1087.

MCRAE A, DAHLSTRÖM A (1992) Immune responses in brains of Alzheimer's and Parkinson's diseases patients: hypothesis and reality. *Review of Neuroscience* **3**, 79–97.

MOMBURG F, KOCH N, MÖLLER P, MOLDENHAUER G, BUTCHER GW, HÄMMERLING GJ (1986) Differential expression of Ia and Ia-associated invariant chain in mouse tissue after in vivo treatment with IFN-γ. *Journal of Immunology* **136**, 940–948.

MORIOKA T, STREIT WJ (1991) Expression of immunomolecules on microglial cells following neonatal sciatic nerve axotomy. *Journal of Neuroimmunology* **35**, 21–30.

PERRY VH, GORDON S (1991) Macrophages and the nervous system. *International Review of Cytology* **125**, 203–244.

PERRY VH, MATYSZAK MK, FEARN S (1993) Altered antigen expression of microglia in the aged rodent CNS. *Glia* **7**, 60–67.

POLTORAK M, FREED WJ (1989) Immunological reactions induced by intracerebral transplantation: evidence that host microglia but not astroglia are the antigen-presenting cells. *Experimental Neurology* **103**, 222–233.

PUJOL-BORRELL R, TODD I, DOSHI M, BOTTAZZO GF, SUTTON R, GRAY D et al. (1987) HLA class II induction in human islet cells by interferon-γ plus tumour necrosis factor or lymphotoxin. *Nature* **326**, 304–306.

RIETSCHEL ET, BRADE H (1992) Bacterial endotoxins. *Scientific American*, August, 26–33.

SASAKI A, LEVISON SW, TING JP-Y (1989) Comparison and quantitation of Ia antigen expression on cultured macroglia and amoeboid microglia from Lewis rat cerebral cortex: analyses and implications. *Journal of Neuroimmunology* **25**, 63–74.

SASAKI A, NAKAZATO Y (1992) The identity of cells expressing MHC class II antigens in normal and pathological human brain. *Neuropathology and Applied Neurobiology* **18**, 13–26.

SETHNA MP, LAMPSON LA (1991) Immune modulation within the brain: recruitment of inflammatory cells and increased major histocompatibility antigen expression following intracerebral injection of interferon-γ. *Journal of Neuroimmunology* **34**, 121–132.

SKOSKIEWICZ MJ, COLVIN RB, SCHNEEBERGER EE, RUSSELL PS (1985) Widespread and selective induction of major histocompatibility complex-determined antigens in vivo by γ interferon. *Journal of Experimental Medicine* **162**, 1645–1664.

STEEG PS, JOHNSON HM, OPPENHEIM JJ (1982) Regulation of murine macrophages Ia antigen expression by an immune interferon-like lymphokine: inhibitory effect of endototoxin. *Journal of Immunology* **129**, 2402–2406.

STEINIGER B, VAN DER MEIDE PH (1988) Rat ependyma and microglial cells express class II MHC antigens after intravenous infusion of recombinant gamma interferon. *Journal of Neuroimmunology* **19**, 111–118.

STREIT WJ, GRAEBER MB, KREUTZBERG GW (1988) Functional plasticity of microglia: a review. *Glia* **1**, 301–307.

STREIT WJ, GRAEBER MB, KREUTZBERG GW (1989) Expression of Ia antigen on perivascular and microglial cells after sublethal and lethal motor neuron injury. *Experimental Neurology* **105**, 115–126.

TRIBOLET NDE, HAMOU MF, MACH J-P, CARREL S, SCHREYER M (1984) Demonstration of HLA-DR antigens in normal human brain. *Journal Neurology, Neurosurgery and Psychiatry* **47**, 417–418.

WENTWORTH PA, ZIEGLER HK (1987) Induction of macrophage Ia expression by lipopolysaccharide and Listeria monocytogenes in congenitally athymic nude mice. *Journal of Immunology* **138**, 3167–3173.

WIKSTRAND CJ, BIGNER DD (1980) Immunobiologic aspects of the brain and human gliomas. *American Journal of Pathology* **98**, 517–567.

XU J, KAUR C, LING EA (1993) Variation with age in the labelling of amoeboid microglial cells in rats following an intraperitoneal or intravenous injection of a fluorescent dye. *Journal of Anatomy* **182**, 55–63.

YEM AW, PARMELY MJ (1981) Modulation of Ia-like antigen expression and antigen-presenting activity of human monocytes by endotoxin and zymosan A. *Journal of Immunology* **127**, 2245–2251.

ZIEGLER HK, STAFFILENO LK, WENTWORTH P (1984) Modulation of macrophage Ia-expression by lipopolysaccharide. I. Induction of Ia expression in vivo. *Journal of Immunology* **133**, 1825–1835.