J. Anat. (1995) 187, pp. 603–611, with 15 figures Printed in Great Britain

Expression and upregulation of transferrin receptors and iron uptake in the epiplexus cells of different aged rats injected with lipopolysaccharide and interferon-gamma

JIA LU, CHARANJIT KAUR AND ENG-ANG LING

Department of Anatomy, Faculty of Medicine, National University of Singapore

(*Accepted 17 April 1995*)

ABSTRACT

The expression of transferrin receptors marked by the monoclonal antibody OX-26 and localisation of iron was studied in epiplexus cells in the lateral ventricles of different aged rats subjected to the challenge of the bacterial toxin, lipopolysaccharide, and treatment with interferon-gamma. Transferrin receptor expression in epiplexus cells was extremely low in normal rats, but was vigorously elevated in rats receiving intraperitoneal injections of lipopolysaccharide (LPS); other antigens such as complement type 3 receptors immunostained with OX-42 and macrophage antigen marked by ED-1 were also notably augmented. After 6 successive intraperitoneal injections of interferon-gamma, the expression of transferrin receptors showed only a moderate increase. Using Perls' staining method, some iron-containing epiplexus cells were observed in early normal postnatal rats, but their number was markedly decreased with age. After LPS administration, a moderate increase in the number of iron-containing epiplexus cells was observed in different aged rats. The significance of the upregulation and vigorous expression of transferrin receptors on epiplexus cells is speculative. One possible explanation would be that they facilitate an increase in the acquisition of iron by these cells for storage, oxidative killing and/or immunological activation. The localisation of iron in the choroid plexus in the lateral ventricles suggests that the latter may function as an important storage organ for iron. Present results also suggest the presence of an efficient transport system for the transfer of iron across the interface of the blood and cerebrospinal fluid.

Key words: Rat; choroid plexus; epiplexus cells; transferrin receptors; iron uptake; lipopolysaccharide; interferon gamma.

INTRODUCTION

Transferrin receptors are present on the surface of a variety of cell types and they are known to mediate the internalisation of iron-saturated transferrin by receptor-mediated endocytosis (Taylor & Morgan, 1990; Roberts et al. 1993). The receptors are highly expressed on specialised cell types which require iron to fulfil specific functions (Laskey et al. 1988). It is known that iron is an essential element partaking in many enzymatic functions and is required for oxidative metabolism (Roskams & Connor, 1992). In the central nervous system, transferrin receptors are widely distributed (Hill & Switzer, 1984; Jefferies et al. 1984): on the endothelial cells of brain capillaries (Jefferies et al. 1984), cortical neurons (Jefferies et al. 1984; Giometto et al. 1990), oligodendrocytes (Lin & Connor, 1989; Giometto et al. 1990) and the choroid plexus (Giometto et al. 1990). Studies of neural tissues in vitro have also shown the presence of transferrin receptors on brain capillaries (Pardridge et al. 1987), neurons (Oh et al. 1986) and oligodendrocytes (Espinosa de los Monteros & Foucaud, 1987). Besides the above-mentioned cell types, transferrin receptors have also been shown to occur on murine and human tissue macrophages in vitro (Hamilton et al. 1984; Taetle & Honeysett, 1988). It was suggested that transferrin receptors may facilitate the acquisition of iron by macrophages for storage and oxidative killing (Deiss, 1983). Moreover, the expression of transferrin

Correspondence to Dr Jia Lu, Department of Anatomy, Faculty of Medicine, National University of Singapore, Singapore 0511.

receptors may also reflect the immune activation of macrophages (Hamilton et al. 1984).

The present study aimed to establish whether the epiplexus cells associated with the choroid plexus considered to be the macrophages in the cerebrospinal fluid (CSF)-ventricular system (Carpenter et al. 1970; Sturrock, 1978; Ling, 1979, 1981, 1983; Maxwell & McGadey, 1988; Kaur et al. 1990; Maxwell et al. 1992; Lu et al. 1993*a*, *b*) are endowed with transferrin receptors in vivo. Since the expression of transferrin receptors is known to be regulated by different stimulations (Hamilton, 1982; Neckers et al. 1984), we also sought to determine if the regulation of transferrin receptors on epiplexus cells would be influenced by the bacterial toxin, lipopolysaccharide (LPS) and the cytokine, interferon-gamma; both are known to exert different effects on these cells including the upregulation of major histocompatibility complex (MHC) class I and II antigens (Lu et al. 1994*a*, *b*). In addition to the localisation of transferrin receptors, this study also examined the possible contents of iron in epiplexus cells in both normal and LPS-treated rats. This information is deemed necessary as a step to understand fully the regulation of iron in the CSF-ventricular system and the brain as a whole.

MATERIALS AND METHODS

A total of 76 Wistar rats of either sex were used in this study. They were divided into 3 groups. (1) *Normal immunohistochemistry*; 24 rats aged 1 d, 7 d, 14 d and 7 wk were used. (2) *Lipopolysaccharide (LPS) injections*. This group consisted of 36 rats aged 1 d, 7 d and 6 wk. Each rat was given 2 intraperitoneal (i.p.) injections of LPS. The dosage for the first injection of LPS was 1 μgl/g body weight in normal saline (10 mg LPS in 1 ml saline; 0.55: B5, Sigma, L2880). In order to enhance the effects of LPS, all rats were given a second or booster injection 3 d after the first injection. Since the mortality rate was rather high (> 50%), the dosage for the second injection was half that of the first. The control rats received injections of an equal volume and frequency of normal saline. The rats were killed at 7 d, 14 d or 7 wk of age for the respective age groups, i.e. 7 d after the first LPS injection. (3) *Interferon-gamma (IFN-γ) injections*. This group consisted of 16 rats aged 1 and 7 d. Under ether anaesthesia, the rats were given single daily intraperitoneal (i.p.) injection of 1 μl/g body weight of rat recombinant IFN-γ (10^5 U in 1 ml sterile distilled water, Gibco BrL, 3283 SA) for 6 consecutive days. Control rats received injections of an equal volume and frequency of sterile distilled water. The rats were killed at 7 and 14 d of age.

Immunohistochemical localization of transferrin receptors

From the above-mentioned 3 groups of animals, a total of 50 rats were deeply anaesthetised with ether and perfused with Ringer's solution until the liver and lungs were clear of blood. This was 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 15–20 min, after which the brain was removed and fixed in a similar fixative for another 2 h. The brain was then kept in 0.1 M phosphate buffer containing 20% sucrose overnight at 4 °C. 40 μm frozen sections of the cerebrum which included the two lateral ventricles and their contained choroid plexuses were cut and rinsed in phosphate-buffered saline (PBS) containing 0.1% Triton X-100. The sections were then incubated at room temperature for 18–20 h using one of the following monoclonal antibodies: OX-26 (Sera-Lab, MAS 262b), OX-42 (Sera Lab, MAS 370b) diluted 1:100 with PBS and ED-1 (Serotec MCA 341) diluted 1:400 with PBS. The antibodies OX-26 and OX-42 were for the detection of transferrin receptors and complement type 3 receptors, respectively, while ED-1 is a specific macrophage marker (Dijkstra et al. 1985). In control incubations, some sections were incubated in a medium by omitting the primary antibodies. The sections were then washed and subsequently incubated in biotinylated antimouse IgG (diluted 1:100 with PBS) for 1 h, washed and then placed in the avidin–biotin peroxidase complex (PK-4002, Vector Laboratories) for 1 h at room temperature. The peroxidase was visualised by using nickel-enhanced 3,3′-diaminobenzidine (DAB) solution. The sections were counterstained with 1% methyl green, dehydrated and mounted in Permount.

Detection of iron

A total of 26 rats from the first and second groups were perfused with Ringer's solution under deep anaesthesia with ether and, 3.5 or 7% chloral hydrate. The Ringer's prewash lasted until the liver and lungs were clear of blood. This was followed by perfusion with 10% formalin. The brain was removed and 40 μm thick frozen sections of the cerebrum which included the two lateral ventricles and contained choroid

plexuses were cut. Sections were immersed in Perls' solution (1:1, 2% HCl and 2% potassium ferrocyanide) at room temperature for 30 min, rinsed in deionised water and then incubated in 0.5% 3,3'-DAB containing 1% hydrogen peroxide in cold phosphate buffer (pH 7.4) for 15 min according to the method of Hill & Switzer (1984). The sections were then rinsed in deionised water and subsequently counterstained with 1% methyl green, dehydrated and mounted in Permount.

All sections which included the two lateral ventricles and their contained choroid plexuses from each rat were examined by light microscopy to assess the immunoreactivity and frequency of epiplexus cells semiquantitatively.

RESULTS

Immunohistochemical localisation of transferrin receptors

Normal immunohistochemistry. The immunoreactivity of epiplexus cells with OX-26 was extremely low in rats of different ages, with the occurrence of only sporadic immunopositive cells. With OX-42 and in normal rats of different age groups, the epiplexus cells associated with the choroid plexus were strongly immunostained. The majority of the cells occurred singly. The immunostaining of epiplexus cells with ED-1, however, was weaker than that with OX-42 and, the number of ED-1 immunoreactive cells was much smaller. Moreover, some parts of the choroid epithelium were also weakly stained with OX-26.

Administration of LPS (Table 1). In rats killed at 7 d of age, OX-26 positive epiplexus cells were rarely observed in the control rats but their number increased considerably in the LPS-injected rats. With OX-42 or ED-1, epiplexus cells were intensely stained both in control and LPS-injected rats. In the latter, the immunoreactivity of epiplexus cells appeared to be stronger.

In rats killed at 14 d of age, OX-26 positive epiplexus cells were hardly detectable in the control rats (Fig. 1) but they showed a dramatic increase in number following LPS injections (Fig. 2). In some parts of the choroid plexus, immunopositive epiplexus cells which appeared in clusters were observed (Fig. 2). With OX-42 or ED-1, immunoreactive epiplexus cells were widely distributed on the choroid plexus in the control rats. Following LPS injections, there was an upsurge of OX-42 and ED-1 positive cells which tended to occur in clusters.

In rats killed at 7 wk of age (adult), OX-26 immunoreactive epiplexus cells were rarely observed in the control animals (Fig. 3), but they increased considerably in the LPS-injected rats (Fig. 4). With OX-42, epiplexus cells were intensely stained both in control (Fig. 5) and LPS-injected rats (Fig. 6) although in the latter the number of epiplexus cells was clearly greater. With ED-1, immunoreactive epiplexus cells were weakly stained in the control rats (Fig. 7). Following LPS injections, however, their immunoreactivity was noticeably enhanced (Fig. 8). Moreover, the numbers of OX-42 and ED-1 positive epiplexus cells were comparatively more than OX-26 positive cells in both control and LPS-injected rats.

In rats of different age groups given LPS injections, the immunoreactivity of the choroid epithelium with OX-26 appeared to be enhanced (Figs 2, 4) when compared with their corresponding control animals (Figs 1, 3).

Administration of IFN-γ (Table 2). With OX-26, only a few immunoreactive epiplexus cells were observed in the control rats (Fig. 9). However, following 6 successive injections of IFN-γ, the number of immunoreactive cells appeared to show a moderate increase (Fig. 10). Epiplexus cells were immunostained with OX-42 and ED-1 both in control and IFN-γ-injected rats. The OX-26 immunoreactivity of choroid plexus did not show any alteration after IFN-γ treatment.

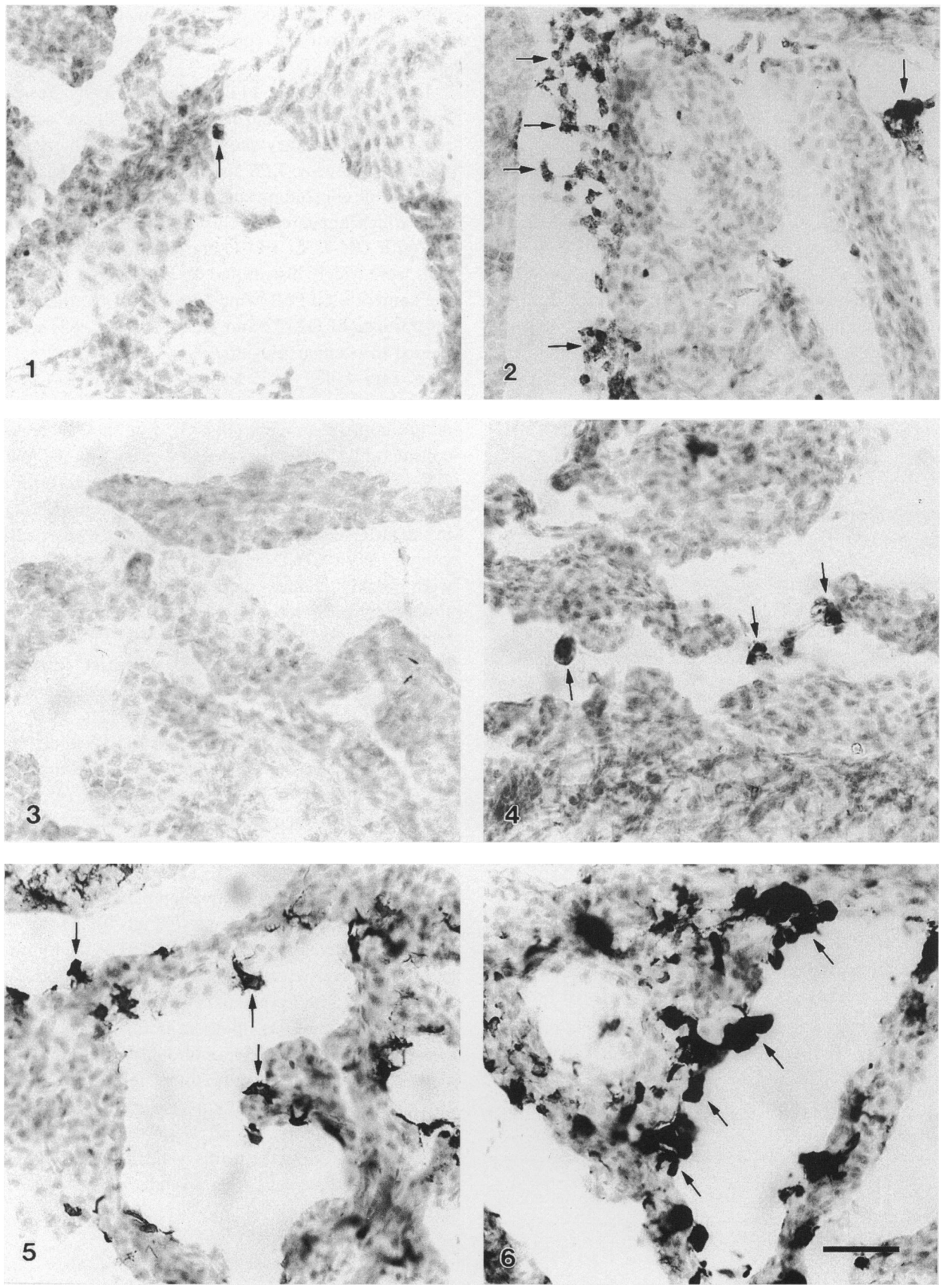
Control incubation. In all sections incubated in a medium by omitting the primary antibodies, immunoreactive epiplexus cells were absent.

Table 1. *Immunoreactivity and frequency of epiplexus cells in rats receiving 2 i.p. injections of lipopolysaccharide (LPS) and normal saline (NS)*

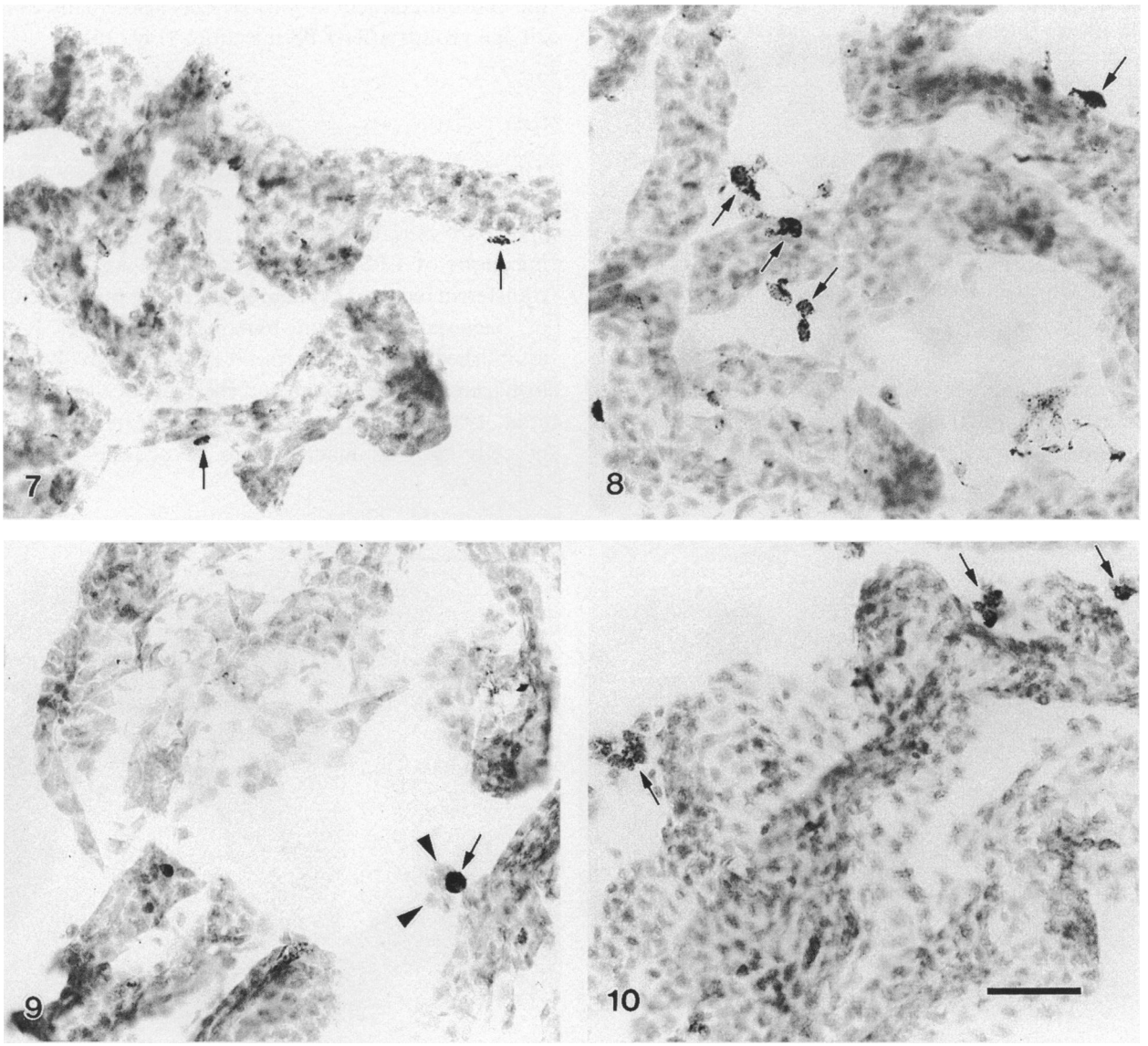
Treatment	Age at killing	OX-42	ED-1	OX-26
NS1, NS2	7 d	+ ~ + +	+	±
LPS1, LPS2	7 d	+ +	+ +	+
NS1, NS2	14 d	+ +	+	±
LPS1, LPS2	14 d	+ + +	+ +	+ +
NS1, NS2	7 wk	+ +	+	±
LPS1, LPS2	7 wk	+ + +	+ +	+

Localisation of iron

Only occasional iron-containing epiplexus cells were observed in early normal postnatal rats and they became barely detectable with age. The epithelium of



Figs 1, 2. Epiplexus cells (arrows) immunostained with OX-26 in the lateral ventricles in 14-d-old rats killed after i.p. injections of normal saline (Fig. 1) and LPS (Fig. 2). A marked accumulation of OX-26 positive cells is observed in the LPS-injected rat (Fig. 2). Only 1 epiplexus cell (arrow) is stained in the control animal (Fig. 1). The choroid epithelium is weakly stained with OX-26 in the control rat (Fig. 1); its immunoreactivity appears to be enhanced in the LPS-injected rat (Fig. 2).



Figs 7, 8. ED-1 immunostained epiplexus cells (arrows) in the lateral ventricles in 7-wk-old rats killed after i.p. injections of normal saline (Fig. 7) and LPS (Fig. 8). The epiplexus cells in the LPS-injected rat are more intensely stained.

Figs 9, 10. Choroid plexus in the lateral ventricles in 14-d-old rats killed after 6 successive i.p. injections of sterile distilled water (Fig. 9) and IFN-γ (Fig. 10). The number of OX-26 immunoreactive epiplexus cells (arrows) in the IFN-γ-injected rat shows a moderate increase (Fig. 10) when compared with that of the control (Fig. 9). Arrowheads, unlabelled epiplexus cells. Bar, 50 μm (Figs 7–10).

the choroid plexus also displayed positive staining of iron in rats of different ages (Fig. 11). The labelling of iron content of the choroid epithelium, however, was extremely uneven, some areas being heavily stained while in others the epithelial cells were only weakly tinted. Some fine iron-positive granules were distributed randomly in the choroid epithelial cytoplasm (Fig. 11). Following LPS injections, the number of iron-containing epiplexus cells showed a moderate increase (Figs 12–14), whereas the staining of epiplexus cells for iron varied considerably, some cells being intensely stained while others were only weakly tinted or virtually unstained (Figs 12–14). In postnatal rats receiving LPS injections, epiplexus cells intensely

Figs 3, 4. Choroid plexus in the lateral ventricles in 7-wk-old rats killed after i.p. injections of normal saline (Fig. 3) and LPS (Fig. 4). A few round and amoeboid epiplexus cells (arrows) are intensely immunostained with OX-26 in the LPS-injected rat (Fig. 4), but they are hardly detectable in the control rat (Fig. 3). The choroid epithelium is lightly tinted with OX-26 in the control rat (Fig. 3) but its immunostaining is clearly enhanced in the LPS-injected rat (Fig. 4).

Figs 5, 6. OX-42 immunostained epiplexus cells (arrows) in the lateral ventricles in 7-wk-old rats killed after i.p. injections of normal saline (Fig. 5) and LPS (Fig. 6). The immunoreactive cells in the control rat (Fig. 5) are much fewer than in the LPS-injected rat (Fig. 6). Bar, 50 μm (Figs 1–6).

Table 2. *Immunoreactivity and frequency of epiplexus cells in 7-d-old rats receiving 6 successive i.p. injections of interferon-γ (IFN-γ) and sterile distilled water*

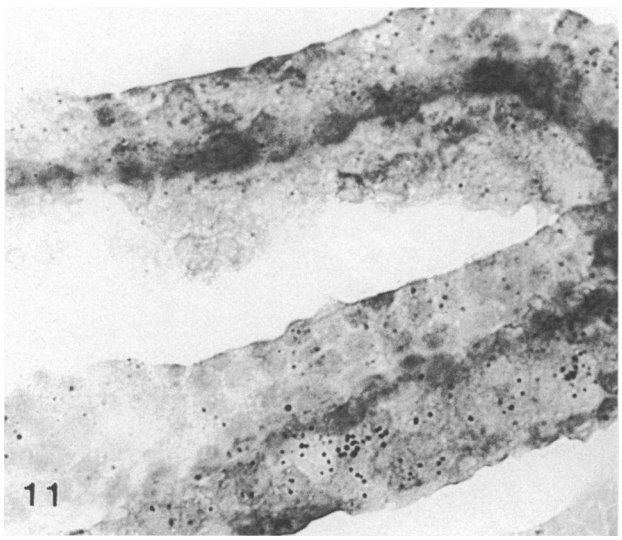
Treatment	OX-42	ED-1	OX-26
Distilled water	++	+~++	±
IFN-γ	++	+~++	±~+

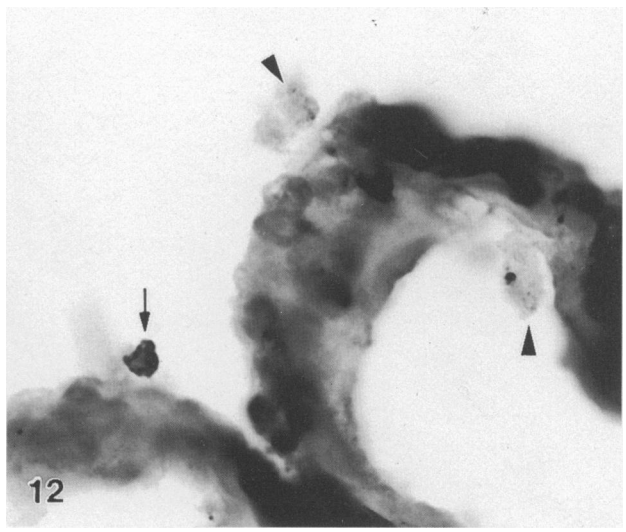
+++, very strong reaction with large number of cells; ++, strong reaction with many cells; +, moderate reaction with some cells; ±, very weak reaction with very few cells; −, no reaction.

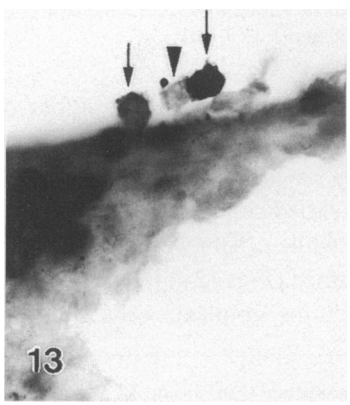
stained for iron were common, while in the adult rats given the same treatment, the lightly tinted iron-containing cells prevailed. The staining intensity of the choroid epithelium for iron was also enhanced in all age groups after LPS injections (Fig. 15).

DISCUSSION

The most striking feature in the present study was the upregulation of transferrin receptor expression on epiplexus cells in different aged rats after the i.p. injections of LPS. The significance of an enhanced transferrin receptor expression following the challenge of bacterial endotoxin remains speculative. It is established that transferrin receptors are involved in iron transport for growth and survival of cells (Laskey et al. 1988) and they presumably facilitate the ability of cells such as macrophages to acquire iron for







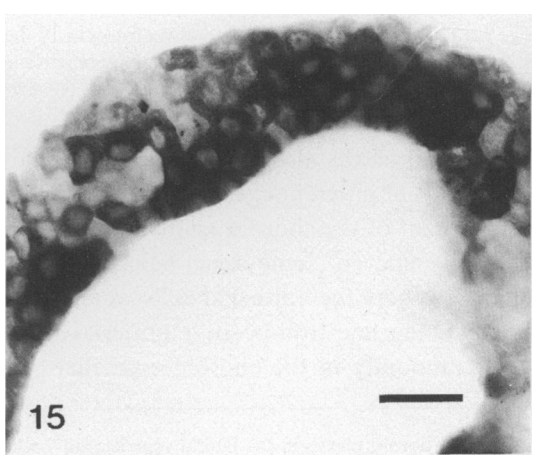


Fig. 11. Choroid plexus in the lateral ventricle of a 7-d-old normal rat. The choroid epithelium is unevenly stained for iron. Discrete iron-containing granules are widely distributed in the choroid epithelial cells. Iron-containing epiplexus cells are hardly observed.

Figs 12–14. Choroid plexus in the lateral ventricles of 7-d-old rats after i.p. injections of LPS. Some epiplexus cells are intensely stained for iron (arrows); others are only weakly tinted (arrowheads).

Fig. 15. Choroid epithelium is intensely stained for iron in a 7-d-old rat after i.p. injections of LPS. Bar, 25 μm (Figs 11–15).

storage and oxidative killing (Deiss, 1983). Transferrin receptors also appear to be involved in cellular recognition (Lazarus & Baines, 1985). In immunological responses, transferrin receptors serve as a useful marker of immune activation of macrophages (Hamilton et al. 1984). Transferrin receptor expression is described as being at a low level on resident tissue macrophages (Hamilton et al. 1984). In the present study, the expression of transferrin receptors on the epiplexus cells in normal rats was hardly detectable, suggesting that these cells are relatively quiescent under normal conditions. The rapid and vigorous expression of transferrin receptors on epiplexus cells in response to LPS treatment is probably a reflection of their activation both in phagocytosis and immune regulation known to be the potential functions of the cell type (Lu et al. 1993*a*, *b*, 1994*a*, *b*).

Another noteworthy feature of the CSF–ventricular system after LPS injection was the upsurge and accumulation of OX-26 positive epiplexus cells especially in rats killed at the age of 14 d. It has been reported that an inflammatory stimulus may induce a burst of cell division by monocyte precursors in bone marrow before their progeny cells, i.e. monocytes, are released into the blood circulation (Hamilton et al. 1984). Transferrin receptor expression is known to be associated with cell proliferation (Raivich et al. 1991). When this is taken into consideration along with the present results, it is suggested that the upsurge of OX-26 immunopositive epiplexus cells after LPS injections may have resulted from the active proliferation of the monocyte precursors. Since epiplexus cells are considered to be derived from blood monocytes (Carpenter et al. 1970; Sturrock, 1978; Ling, 1979, 1981, 1983; Maxwell et al. 1992; Lu et al. 1994*a*), a greater influx of the latter would have contributed to the increase in the number of OX-26 immunopositive epiplexus cells (Lu et al. 1994*b*). Thus the elevated expression of transferrin receptors on epiplexus cells is probably a remnant of the recent proliferative activity of their precursor cells. The increase in the population of epiplexus cells after LPS injections may also be partly due to the proliferation of local epiplexus cells. This is because some epiplexus cells were observed to be labelled by bromodeoxyuridine, a marker of cell proliferation, following LPS injection in our preliminary study (Lu et al. unpublished data). The present argument, however, does not exclude the possibility that more local epiplexus cells were induced to express transferrin receptors after LPS injection.

It has been shown in in vitro study that IFN-γ stimulates the increase of transferrin expression by macrophages (Taetle & Honeysett, 1988). IFN-γ is known to be a potent activator of macrophage functions, such as the increase of major histocompatibility complex class II antigen expression (Lu et al. 1994*a*). However, a reverse effect on macrophage transferrin receptor expression has also been reported (Byrd & Horwitz, 1993). Our present study showed only a moderate increase in the expression of transferrin receptor on epiplexus cells after 6 successive i.p. injections of IFN-γ indicating that IFN-γ contributes only a minor role to the regulation of transferrin receptors on epiplexus cells.

In agreement with a previous study (Giometto et al. 1990), the present results also showed OX-26 immunoreactivity in the choroid epithelium. It is therefore suggested that iron may be taken up via receptor-mediated endocytosis by the choroid epithelial cells or transported across the choroid epithelium into the CSF.

It is well established that iron is an essential cofactor for many cellular enzymes and plays a vital role in cell functions. In the present study, occasional iron-containing epiplexus cells were observed in early normal postnatal rats. Since iron requirement is necessary for cell proliferation and growth (Taylor & Morgan, 1990), it is possible that iron is required by the epiplexus cells for similar functions. In this connection, it is surprising to note the low level of transferrin receptor expression on epiplexus cells of normal postnatal rats in which only sporadic OX-26 cells were detected. It is tempting to speculate that the uptake of iron in the epiplexus cells in postnatal rats is not only mediated by transferrin receptors but also perhaps by nonspecific endocytosis. The subsequent diminution of iron-containing epiplexus cells with age may be attributable to the low level of transferrin receptor expression and a reduced requirement of iron by the epiplexus cells so that only cells which showed a sufficient level of iron were detected. On the other hand, after i.p. injections of LPS the number of iron-containing epiplexus cells showed a moderate increase. It is known that iron plays a major role in macrophage interactions with intracellular and extracellular pathogens, possibly serving as a catalyst in the generation of toxic oxygen metabolites used in antimicrobial defence (Britigan et al. 1991). It remains to be determined if the iron-containing epiplexus cells when challenged by the bacterial toxin would partake in a similar defensive role in the ventricular system.

Previous studies (Dwork et al. 1988; Benkovic & Connor, 1993) reported that iron is undetectable in the choroid plexus although a high level of transferrin was detected in the choroid epithelium. In contrast, our results showed an intense staining of iron in the

choroid epithelium. It is therefore suggested that the choroid plexus could also serve as an iron storage organ regulating the iron level in the ventricular CSF and the brain as a whole. The close association of iron-containing epiplexus cells and the choroid epithelium with accumulated iron suggests that they act as an efficient transport system for the mobilisation of iron between the blood and CSF. The discrepancy between our results and previous findings (Dwork et al. 1988; Benkovic & Connor, 1993) may be due to species variation or different staining methods used.

Finally, the present study showed the labelling of OX-42 and ED-1 in the epiplexus cells in all age groups. ED-1 is known to be a specific marker for the mononuclear phagocytes (Dijkstra et al. 1985). The fact that epiplexus cells are intensely stained with this antibody substantiates the monocytic lineage of this cell type. The vigorous expression of CR 3 receptors following LPS treatments indicates the possibility of their involvement in a receptor-mediated endocytosis.

ACKNOWLEDGEMENTS

This study was supported by research grants (RP920365; RP930332) from the National University of Singapore.

REFERENCES

Benkovic SA, Connor JR (1993) Ferritin, transferrin and iron in selected regions of the adult and aged rat brain. *Journal of Comparative Neurology* **338**, 97–113.

Britigan BE, Serody JS, Hayek MB, Charniga GM, Cohen MS (1991) Lactoferrin uptake by mononuclear phagocytes. *Journal of Immunology* **147**, 4271–4279.

Byrd TF, Horwitz MA (1993) Regulation of transferrin receptor expression and ferritin content in human mononuclear phagocytes. *Journal of Clinical Investigation* **91**, 969–976.

Carpenter SJ, McCarthy LE, Borison HL (1970) Electron microscopic study of epiplexus (Kolmer) cells of the cat choroid plexus. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **110**, 471–486.

Deiss A (1983) Iron metabolism in reticuloendothelial cells. *Seminars in Hematology* **20**, 81–90.

Dijkstra CD, Döpp EA, Joling P, Kraal G (1985) The heterogeneity of mononuclear phagocytes in lymphoid organs: distinct macrophages subpopulations in the rat recognized by monoclonal antibodies ED1, ED2 and ED3. *Immunology* **54**, 589–599.

Dwork AJ, Schon EA, Herbert J (1988) Non-identical distribution of transferrin and ferric iron in human brains. *Neuroscience* **27**, 333–345.

Espinosa de los Monteros A, Foucaud B (1987) Effect of iron and transferrin on pure oligodendrocytes in culture; characterization of a high-affinity transferrin receptor at different ages. *Developmental Brain Research* **35**, 123–130.

Giometto B, Bozza F, Argentiero V, Gallo P, Pagni S, Piccinno MG, et al. (1990) Transferrin receptors in the rat central nervous system. *Journal of the Neurological Sciences* **98**, 81–90.

Hamilton TA (1982) Regulation of transferrin receptor expression in concanavalin-A stimulated and Gross virus transformed rat lymphoblasts. *Journal of Cellular Physiology* **113**, 40–46.

Hamilton TA, Weiel JE, Adams DO (1984) Expression of the transferrin receptor in murine peritoneal macrophages is modulated in the different stages of activation. *Journal of Immunology* **132**, 2285–2290.

Hill JM, Switzer RC (1984) The regional distribution and cellular localization of iron in the rat brain. *Neuroscience* **11**, 595–603.

Jefferies WA, Brandon MR, Hunt SV, Williams AF, Gatter KC, Mason DY (1984) Transferrin receptor on endothelium of brain capillaries. *Nature* **312**, 162–163.

Kaur C, Ling EA, Gopalakrishnakone P, Wong WC (1990) Response of intraventricular macrophages to crotoxin-coated microcarrier beads injected into the lateral ventricle of postnatal rats. *Journal of Anatomy* **168**, 63–72.

Laskey J, Webb I, Schulman HM, Ponka P (1988) Evidence that transferrin supports cell proliferation by supplying iron for DNA synthesis. *Experimental Cell Research* **176**, 87–95.

Lazarus AH, Baines MG (1985) Studies on the mechanism of specificity of human natural killer cells for tumour cells: correlation between target cell transferrin receptor expression and competitive activity. *Cellular Immunology* **96**, 255–266.

Lin HH, Connor JR (1989) The development of the transferrin-transferrin receptor system in relation to astrocytes, MBP and galactocerebroside in normal and myelin-deficient rat optic nerves. *Developmental Brain Research* **49**, 281–293.

Ling EA (1979) Ultrastructure and origin of epiplexus cells in the telencephalic choroid plexus of postnatal rats studied by intravenous injection of carbon particles. *Journal of Anatomy* **129**, 479–492.

Ling EA (1981) Ultrastructure and mode of formation of epiplexus cells in the choroid plexus in the lateral ventricles of the monkey (*Macaca fascicularis*). *Journal of Anatomy* **133**, 559–569.

Ling EA (1983) Scanning electron microscopic study of epiplexus cells in the lateral ventricles of the monkey (*Macaca fascicularis*). *Journal of Anatomy* **137**, 645–652.

Lu J, Kaur C, Ling EA (1993*a*) Intraventricular macrophages in the lateral ventricles with special reference to epiplexus cells: a quantitative analysis and their uptake of fluorescent tracer injected intraperitoneally in rats of different ages. *Journal of Anatomy* **183**, 405–416.

Lu J, Kaur C, Ling EA (1993*b*) Uptake of tracer by the epiplexus cells via the choroid plexus epithelium following an intravenous or intraperitoneal injection of horseradish peroxidase in rats. *Journal of Anatomy* **183**, 609–617.

Lu J, Kaur C, Ling EA (1994*a*) Immunophenotypic features of epiplexus cells and their response to interferon gamma injected intraperitoneally in postnatal rats. *Journal of Anatomy* **185**, 75–84.

Lu J, Kaur C, Ling EA (1994*b*) Upregulation of surface antigens on epiplexus cells in postnatal rats following intraperitoneal injections of lipopolysaccharide. *Neuroscience* **63**, 1169–1178.

Maxwell WL, McGadey J (1988) Response of intraventricular macrophages after a penetrant cerebral lesion. *Journal of Anatomy* **160**, 145–155.

Maxwell WL, Hardy IG, Watt TA (1992) Changes in the choroid plexus, responses by intrinsic epiplexus cells and recruitment from monocytes after experimental head acceleration injury in the non-human primate. *Acta Neuropathologica* **84**, 78–84.

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

Neckers LM, Yenokida G, James SP (1984) The role of the transferrin receptor in human B-lymphocyte activation. *Journal of Immunology* **133**, 2437–2441.

Oh T, Markelonis GJ, Royal GM, Bregman BS (1986)

Immunocytochemical distribution of transferrin and its receptor in the developing chicken nervous system. *Developmental Brain Research* **30**, 207–220.

PARDRIDGE WM, EISENBERG J, YANG J (1987) Human blood–brain barrier transferrin receptor. *Metabolism* **36**, 892–895.

RAIVICH G, GRAEBER MB, GEHRMANN J, KREUTZBERG GW (1991) Transferrin receptor expression and iron uptake in the injured and regenerating rat sciatic nerve. *European Journal of Neuroscience* **3**, 919–927.

ROBERTS RL, FINE RE, SANDRA A (1993) Receptor-mediated endocytosis of transferrin at the blood–brain barrier. *Journal of Cell Science* **104**, 521–532.

ROSKAMS AJ, CONNOR JR (1992) Transferrin receptor expression in myelin deficient (md) rats. *Journal of Neuroscience Research* **31**, 421–427.

STURROCK RR (1978) A developmental study of epiplexus cells and supraependymal cells and their possible relationship to microglia. *Neuropathology and Applied Neurobiology* **4**, 307–320.

TAETLE R, HONEYSETT JM (1988) γ-Interferon modulates human monocyte/macrophage transferrin receptor expression. *Blood* **71**, 1590–1595.

TAYLOR EM, MORGAN EH (1990) Developmental changes in transferrin and iron uptake by the brain in the rat. *Developmental Brain Research* **55**, 35–42.