Age-related effects of interleukin-1β on polymorphonuclear neutrophil-dependent increases in blood–brain barrier permeability in rats

D. C. Anthony, S. J. Bolton, S. Fearn and V. H. Perry

Department of Pharmacology, University of Oxford, Oxford, UK

Summary

In adult rats, 50,000 units of recombinant interleukin-1β (IL-1β) injected into the brain parenchyma produced an intense meningitis and disruption of the blood–CSF barrier by 4 h. No increase in vascular permeability to horseradish peroxidase or leukocyte recruitment was observed at the site of injection. By contrast, in juvenile rats, 100 units of IL-1β injected into the striatum gave rise to a large increase in blood–brain barrier permeability and recruitment of polymorphonuclear neutrophils into the tissue around the injection site by 4 h. This effect was also accompanied by a marked meningitis. The injection of 100 units of IL-1β into neonatal (2-h-old) rats gave rise to an increase in permeability of vessels to serum proteins in the meninges, but no increase in vascular permeability was observed at the injection site. The IL-1β-induced increases in vessel permeability in the meninges, parenchyma, and choroid plexus were polymorphonuclear neutrophil dependent, since leukocyte depletion by irradiation or polymorphonuclear neutrophil anti-serum pre-treatment eliminated the response in the juvenile animals and in the adults. Seventy-five thousand units of murine tumour necrosis factor-α injected into the parenchyma of both adults and juvenile animals failed to induce an increase in blood–brain barrier permeability or polymorphonuclear neutrophil recruitment, but did give rise to a mild meningitis. These findings demonstrate clear differences in the responsiveness of different CNS compartments to IL-1β. Furthermore, while tumour necrosis factor-α and IL-1β might have been expected to exhibit similar pro-inflammatory effects in the CNS, this is not the case. We also show, for the first time, that age has a significant effect on the response to a cytokine. The ‘window of susceptibility’ to an inflammatory stimulus in juvenile rats, if paralleled in humans, may be a major factor in the increased susceptibility of children to trauma or to infectious insults to the CNS.

Keywords: blood–brain barrier; cytokines; interleukin-1β; meningitis; tumour necrosis factor-α; ‘window of susceptibility’

Abbreviations: BBB = blood–brain barrier; HRP = horseradish peroxidase; IL-1β = interleukin-1β; PMN = polymorphonuclear neutrophil; TNF-α = tumour necrosis factor-α

Introduction

It is becoming increasingly apparent that many neurological diseases, such as Alzheimer’s disease, Parkinson’s disease, prion diseases and AIDS-related dementia, all have an inflammatory component (Perry et al., 1995). Interest in CNS inflammation has, therefore, increased dramatically in recent years. However, our understanding of the role of the principal inflammatory mediators in the CNS micro-environment remains incomplete. Cytokines not only participate in many normal physiological processes, such as the growth and differentiation of leukocytes, but are also implicated in the pathology associated with inflammation (Callard and Gearing, 1994) and there is a wealth of evidence suggesting that soluble interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) are involved in CNS inflammation: IL-1β receptors are present throughout the brain (Cunningham and De Souza, 1993; Luheshi et al., 1993); resident CNS cells—the astrocytes, microglia and neurons—synthesize IL-1β (Woodroffe, 1995), which plays an important role in T-cell activation and induces the expression of adhesion molecules for leukocytes on endothelial cells (Wang et al., 1994); and astrocytes, microglia and endothelial cells have been shown to produce TNF-α (Woodroffe, 1995). In multiple sclerosis,
the presence of TNF-α and IL-1β in the CSF correlates with disease activity (Hauser et al., 1990), and in animals sensitized to develop experimental allergic encephalomyelitis, intraperitoneal injection of IL-1α promoted disease (Martin and Near, 1995), whereas the injection of soluble IL-1 receptor antagonist delayed the onset and shortened the duration of disease. A role for TNFα is also implicated in experimental allergic encephalomyelitis: e.g. Rolipram, a type IV phosphodiesterase inhibitor that suppresses the production of TNF-α, is an effective treatment (Sommer et al., 1995). During infectious meningitis and cerebral malaria the presence of TNF-α has been correlated with the outcome of the infection (Kwiatkowski et al., 1990).

In general, the brain parenchyma is resistant to polymorphonuclear neutrophil (PMN) recruitment following challenge with cytokines (Andersson et al., 1992b; Perry et al., 1995). However, damage of the blood–brain barrier (BBB) concomitant PMN accumulation is associated with some pathologies such as cerebral ischaemia, trauma or bacterial encephalitis, to which juveniles are particularly susceptible (Tuomanen, 1994). Recent studies have noted that when lipopolysaccharide is administered to the CNS parenchyma of 1-week-old mice, there is an inflammatory response, reminiscent of a peripheral inflammatory response, that is not usually seen in the CNS of adult animals (Lawson and Perry, 1995). This suggests that age-related differences may exist in the response of the CNS parenchyma to pro-inflammatory mediators.

Previous studies have tended to consider the blood–CSF barrier or the blood–retinal barrier as being analogous to the BBB, however, clear distinctions exist between these barriers (Andersson et al., 1992b), and our own previous results have indicated that cytokines might be expected to have differing effects on the vasculature at different sites in the CNS (Andersson et al., 1992b). Injection of IL-1β or TNF-α into the vitreous humour of the eye results in the development of an acute inflammatory response associated with haemorrhage, oedema, and delays in conduction of visually evoked potentials from the eye (Brosnan et al., 1989; Martiney et al., 1990). Similarly, injections of IL-1β or TNF-α into the ventricles induce significant PMN migration into the CSF and increased vascular permeability to serum proteins (Quagliarello et al., 1991; Kim et al., 1992; Megyeri et al., 1992). Such an approach for study of parenchymal BBB function may, therefore, be misleading. The specialized structural features of the BBB include tight junctions between endothelial cells, reduced numbers of cytoplasmic vesicles, high mitochondrial content and an absence of pores and fenestrations. These features are not conserved to the same degree in the endothelium of the blood–CSF barrier and the blood–retinal barrier (Claudio et al., 1994).

In the present study, using horseradish peroxidase (HRP) (a widely used tracer of increased brain vascular permeability to serum proteins) and histochemistry, we investigate the effects of intra-cerebral injection of a range of doses of IL-1β or TNF-α on vessel permeability and on leukocyte recruitment in the different CNS compartments at different times after the challenge. In addition, the response to IL-1β or TNF-α injection in animals of different age is examined.

**Material and methods**

### Animals

Lewis rats, between 2 h and 3 months old, and 3-month-old spontaneously hypertensive rats (Charles River, UK) were used. Neonatal animals were suckled until they were 3 weeks old. Three-month-old Balb/c mice were obtained from the Sir William Dunn School of Pathology, Oxford. Standard laboratory pelleted formula and water were provided ad libitum. In each experiment, at least three animals were used per group to examine the dose response to cytokine, and to investigate the time course and age related effects. Home Office approval was obtained for all the animal experiments described in this study.

### Reagents

Human recombinant IL-1β was obtained from R&D Systems (Abingdon, UK) and from the National Institute for Biological Standards and Controls (NIBSC, Potters Bar, UK). Murine and human recombinant TNF-α were obtained from R&D Systems and from Serotec (Kidlington, UK), respectively. Intra-cerebral injections of the same number of units of both IL-1β products and both TNF-α products gave similar results. All other reagents were obtained from Merck (Poole, UK) and were of AnalaR grade unless otherwise stated. The PMN depleting antiserum was a gift from Dr M. T. Trevethick (Glaxo, Ware, UK).

### Sterotaxic injections

Neonatal rats were anaesthetized with halothane (Rhône Mérieux, UK) and their heads were placed in a plastic foam holder in such a way that the head was firmly held and the rosta-caudal and mediolateral axes were in a level plane. Older rats, from 3 weeks of age, were anaesthetized with avertin (1 ml/100 g) and placed in a stereotaxic frame. Injections (1 µl) of a range of doses of either IL-1β or TNF-α were placed stereotaxically into the striatum, which was chosen as a large region of brain parenchyma distant from the meninges. An incision was made in the scalp to expose bone and a 2-mm diameter burr hole was drilled through the skull to allow the tip of a finely drawn calibrated glass capillary tube to be inserted (external diameter at the tip = 50 µm). The coordinates for the injection site in 3-month-old animals were: bregma, +1.2; lateral, +3.0 mm; and depth 4.5 mm. The animals recovered from the anaesthetic, before being killed at times ranging from 2 h to 1 week. In the younger animals, the injection coordinates were scaled down to ensure that the cytokine was delivered to the appropriate site. Cytokine (1 µl) was also injected into the
skin of the ear using the same injection cannula. The pro-inflammatory cytokines were dissolved in a solution of 0.1% bovine serum albumin in phosphate buffered saline. Control injections of the bovine serum albumin in phosphate buffered saline solution were also carried out.

**Polymorphonuclear neutrophil depletion**

Both 3-week-old animals and 3-month-old animals were gamma irradiated with 9 Gy ($^{137}$Cs source, Graviton RX30/55M, Graviton Ltd) to deplete blood leukocytes. These animals were maintained for 4 days with oxytetracycline (10 mg/100 ml) (Terramycin, Pfizer, UK) added to the drinking water before the cytokine injections were performed. In addition, animals were specifically PMN depleted with an anti-PMN serum (Trevethick et al., 1994), and macrophages were identified using the monoclonal antibodies ED1, which stains most macrophage populations including recruited monocytes, but not quiescent microglia, and ED2, which is a marker for perivascular macrophages in the CNS (Graeber et al., 1989).

**Perfusion and tissue preparation**

After various survival times, the rats were deeply anaesthetized with sodium pentobarbitone. The animals were then transcardially perfused with 100 ml of saline (heparinized) followed by 200 ml of Karnovsky’s fixative (1.25% gluteraldehyde and 1.25% paraformaldehyde in phosphate buffer). The brain was removed, fixed for a further 4 h, and cryoprotected in 30% sucrose overnight at 4°C before being embedded in Tissue-Tek (Miles Inc, Elkhart, USA) and quickly frozen in liquid nitrogen.

**Assessment of blood–brain barrier permeability**

Thirty minutes before being killed, the animals were injected intravenously with type II HRP (Sigma Chemical Co., St Louis, USA), $10^4$ U/kg, as a tracer of increased BBB permeability. The animals were then perfusion-fixed. Coronal, free-floating, sections were cut for HRP localization by a modified Hanker-Yates method (Perry and Linden, 1982). HRP has been used extensively as a tracer of altered vessel permeability, and does not increase endothelial transport during normal conditions (Claudio et al., 1990; Hawkins et al., 1990). Cumulative increases in BBB permeability were detected by immunostaining for extravasated serum IgG.

**Identification of leukocytes**

Cresyl violet stained, 50 µm thick, sections were examined for signs of neuronal damage and for the presence of leukocytes (phagocytic cells could also be identified in the Hanker-Yates treated sections as they are peroxidase positive). Leukocytes referred to as ‘marginated’ in the text, were those cells that appeared to be adherent to the luminal side of the vascular endothelium. Cells described as ‘cuffed’ appeared to be on the abluminal side of vessels, and leukocytes referred to in the text as being ‘recruited’, were those cells that had crossed the vascular endothelium, the basement membrane, and were clearly present in the tissue. Immunohistochemistry was used to confirm the presence and distribution of specific cell populations. Frozen, 10 µm thick serial sections were cut from PLP fixed tissue (2% paraformaldehyde, lysine, periodate and 0.05% glutaraldehyde) (Matyszak and Perry, 1995) and mounted on gelatin-coated glass slides. Antigens were detected using a three-step indirect method (Hsu et al., 1981). Polymorphonuclear neutrophils were identified using the anti-neutrophil serum used to PMN deplete the animals (Trevethick et al., 1994), and macrophages were identified using the monoclonal antibodies ED1, which stains most macrophage populations including recruited monocytes, but not quiescent microglia, and ED2, which is a marker for perivascular macrophages in the CNS (Graeber et al., 1989).

**Quantification of polymorphonuclear neutrophil numbers**

Polymorphonuclear neutrophils, identified by their nuclear morphology, were counted in 50 µm thick, cresyl violet stained, sections from regions immediately adjacent to the injection site. Two non-overlapping fields, containing the highest density of recruited cells within the parenchyma, were chosen and the number of PMNs were calculated as an average number per mm$^2$ for each animal.

**Analysis**

The data are presented as the mean ± SD and where statistical analysis has been employed, Student’s $t$ test was applied.

**Results**

**Controls**

Both 3-week-old and 3-month-old animals received intracerebral injections of vehicle (1 µl, 0.1% bovine serum albumin in PBS) and were killed at 4 h or at 24 h after injection. No increase in vascular permeability or recruitment of leukocytes into the tissue was observed.

**Effects of recombinant human IL-1β on BBB leakage**

**Dose-response experiments**

Adult, 3-month-old Lewis rats were injected in the brain parenchyma with a range of doses, from $5 \times 10^4$ U (0.25 µg) to 1U (5 pg), of IL-1β. These animals were killed after 4 h, as preliminary experiments had shown this to be the time at which maximal increases in vessel permeability could be observed (see below). The injection of $5 \times 10^4$ U of IL-1β produced a pronounced meningoencephalitis and associated leak of the blood–CSF barrier within the meninges (Fig. 1A). The leukocyte recruitment to the meninges was predominantly...
Fig. 1 Vessel permeability. Coronal sections illustrating the changes in vessel permeability to HRP following injections of cytokine (arrow in A shows the position of the injection site, which is in a comparable position in all the sections illustrated). Dark staining indicates regions of increased vessel permeability. (A) A 3-month-old animal 4 h after the injection of IL-1β (50 000 U). Note the absence of any detectable increase in vessel permeability to HRP around the injection site, and the pronounced increase in the permeability of the blood–CSF barrier within the meninges, 3.5 mm from the injection site. (B) A 2-hour-old neonate 4 h after the injection of IL-1β (100 U). HRP can be seen in the area surrounding the lateral ventricles and in the meninges. There was little or no increase in vessel permeability around the injection site. (C) A 3-week-old animal 4 h after the injection of IL-1β (100 U). Note the marked increase in vessel permeability in the parenchyma of the injected hemisphere. (D) A 3-week-old animal 4 h after the injection of IL-1β (100 U). Gamma irradiation (9 Gy) was performed 4 days earlier. No leakage of the BBB can be seen. (E) A 3-month-old animal 24 h after the injection of TNF-α (75 000 U). No increase in BBB permeability in the parenchyma or in the meninges was observed. (F) A 3-week-old animal killed 24 h after the injection of TNF-α (75 000 U).
PMN, identified by their nuclear morphology when stained with cresyl violet (Fig. 2A). An increase in the permeability of the BBB to serum proteins was observed around the larger penetrating vessels in the superficial layers of the cortex (Fig. 2B). These vessels were also filled with marginating PMNs. However, no increase in BBB permeability was observed around the injection site in the centre of the striatum some 3.5 mm from the penetrating vessels in the overlying cortex. In adjacent cresyl violet stained sections, no margination, cuffing or recruitment of leukocytes was observed at the injection site (Fig. 2C). The presence of marginated cells in vessels did not always correspond to increases in vessel permeability. No overt signs of neuronal degeneration were present. A similar pattern was observed with 10⁴ U, 10³ U, and 10² U of IL-1β, but the meningitis produced with 10² U of IL-1β was sub-maximal. Even doses as low as 10 U and 1 U of IL-1β produced a modest but observable meningitis. The meningitis produced with 5×10⁴ U IL-1β was observed around the entire circumference of a coronal section passing through the injected region. 100 U of IL-1β produced a meningitis and an increase in the permeability of the blood–CSF barrier that was confined to the injected hemisphere. The dose of IL-1β chosen to use in all further experiments was 100 U.

Following a recent report that spontaneously hypertensive rats are more susceptible to BBB damage following chronic focal ischaemia (Liu et al., 1993), 100 U of IL-β was injected into the striatum of 3-month-old spontaneously hypertensive rats, which were then killed after 4 h. No increases in BBB permeability were observed that differed in any way from the response of normal 3-month-old Lewis rats.

The influence of age on the response
 BBB permeability changes and leukocyte margination, cuffing and recruitment were examined 4 h after intra-cerebral injection of 100 U IL-1β in animals at the following postnatal ages: 2 h, 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 2 months and 3 months.

In the 2-h-old neonates, HRP was detected in the area
surrounding the lateral ventricles and in the meninges (Fig. 1B), with an associated leukocyte recruitment. However, the number of leukocytes recruited to the meninges was relatively small compared with the number recruited to the meninges in adult animals. There was little or no increase in vessel permeability around the injection site. In 3-week-old animals a dramatic difference in the response to IL-1β was noted. At this age, intra-cerebral injection of 100 U IL-1β produced a marked increase in the permeability of the BBB to HRP in the striatum around the injection site (Fig. 1C). Although leakage of HRP had occurred throughout the striatum in the injected hemisphere, the contralateral side remained unaffected. In addition, leukocytes, which were found to be almost exclusively PMNs, were recruited to the area surrounding the injection site (Fig. 2D), but not to the contralateral side. The larger parenchymal vessels, which were surrounded by more HRP staining, appeared to be the principal site of PMN margination, cuffing and subsequent recruitment. These vessels also appeared to be dilated relative to those present in the contralateral side. Despite the numbers of PMNs present in the brain parenchyma, there was no sign of neuronal degeneration on examination of the cresyl violet stained sections.

In the brains of animals between 2 h and 3 weeks old, the pattern was transitional: the vessels in the striatum gradually became more susceptible to the effects of IL-1β. At 4 and 5 weeks of age, the pattern of BBB leakage and leukocyte recruitment to the tissue was similar to the response evoked in a 3-week-old animal, but by 6 weeks the parenchymal vessels appeared to be becoming more resistant to the action of IL-1β. At 7 weeks of age, some HRP was localized in the parenchyma following IL-1β, although it was minimal and was confined mainly to the regions surrounding the larger vessels, where some leukocyte margination and cuffing could be seen. Two-month-old animals did not display an increase in permeability to serum proteins in the parenchyma following IL-1β. The meningial leakage of protein was more pronounced in the 2-month-old animals than in the 3-month-old animals, although the pattern was broadly similar. In all instances, the BBB and blood–CSF barrier disruption at 4 h was associated with a concomitant recruitment of PMNs into the tissue.

To determine how sensitive the juvenile, 3-week-old, animals were to IL-1β, lower doses were injected. Even 5 pg (1 U) produced a focal increase in BBB permeability and some recruitment of PMNs.

**Time-course experiments**

**Juvenile.** Because HRP may be used to probe the status of BBB integrity at specific time points, rather than to observe an accumulated effect, experiments were performed to examine PMN recruitment and permeability changes over time. In addition, the number of PMNs recruited was quantified. Three-week-old animals were killed at 2, 4, 6, 12, 24, 48 and 72 h after intra-cerebral injection of 100 U IL-1β into the striatum. At 2 h after intra-cerebral injection of IL-1β, there was no observable increase in BBB permeability or leukocyte recruitment (Fig. 3). At 6 h after the injection, there was still some leakage of serum proteins across the BBB; however, this was much less marked than at 4 h. The number of recruited PMNs present was greater at 6 h than at 4 h. By 12 h, the PMN recruitment was maximal (Fig. 3), and the distribution was homogeneous throughout the entire injected hemisphere. No recruitment was observed in the contralateral hemisphere. At 24 h, the number of PMNs present in the brain was still large, although it was less than at 12 h. From 48 h to 72 h the numbers of PMNs present declined dramatically: some pyknotic nuclei were clearly visible.

**Adult.** In 3-month-old animals, the vessels in the brain parenchyma, around the injection site, never leaked HRP. From 6 to 12 h the number of PMNs present in the brain parenchyma increased (Fig. 3). The cells were apparently being recruited from the larger vessels in the cortex. By 24 h, PMNs were evenly distributed over a few millimetres on either side of the injection site, although fewer cells were present compared with the numbers recruited in 3-week-old animals. Detection of extravasated IgG over time, unlike HRP, gives a cumulative indication of increases in vessel permeability. IgG staining of sections from animals killed 24 h after intra-cerebral injection of IL-1β revealed that some...
IgG had become extravasated in the injected hemisphere, suggesting that some changes in vessel permeability had occurred over the course of the experiment.

**Effect of irradiation and polymorphonuclear neutrophil anti-serum treatment**

Three-month-old and 3-week-old animals were gamma irradiated with 9 Gy. These animals then received intra-cerebral injections of IL-1β 4 days later. No meningitis or leakage of the BBB was observed in any of the animals (Fig. 1D). Interestingly, there was a marked vessel dilation in the injected hemisphere close to the site of injection, which was independent of the presence of PMNs. The examination of blood smears confirmed the elimination of leukocytes from the blood. As the leukocyte recruitment was predominantly of PMNs, the effect of intra-cerebral injection of IL-1β was tested on animals that had been specifically PMN depleted by a PMN anti-serum. No increase in vessel permeability or recruitment of leukocytes was observed in 3-week-old animals at 4 h after intra-cerebral injection. Again, analysis of blood smears confirmed the presence of a marked neutropenia.

**Effects of murine recombinant tumour necrosis factor-β on BBB leakage**

Given the results obtained with IL-1β, it was decided to restrict consideration of the effects of TNF-α to specific ages (3-month-old and 3-week-old animals) and time points (4 h, 24 h, 72 h and 1 week). Adult, 3-month-old, Lewis rats were injected with either 10^3 U (20 ng) of human recombinant TNF-α or 7.5x10^4 U (0.5 µg) of murine recombinant TNFα. The injection of either the human or the murine recombinant TNF-α did not elicit any increase in BBB permeability in the parenchyma or in the meninges at any of the experimental time points (Fig. 1E).

There is a high level of homology between rat, mouse and human TNF-α. However, in case the absence of a significant effect was a consequence of species specificity, we also injected 3-month-old Balb/c mice with 7.5x10^4 U murine recombinant TNFα. Once again, no increase in vessel permeability and no difference in leukocyte response was observed 24 h after the injection.

Despite the failure of TNF-α to increase vessel permeability, a number of responses was observed following TNF-α injection. There was some evidence of vascular occlusion, since the perfusions were often incomplete. Monocyte margination and monocyte recruitment in the meninges was observed at 24 h. A few PMNs had also been recruited. Phagocytic macrophage-like cells were also seen to be adhering to the larger vessel walls in the parenchyma around the injection site, but there was no associated leakage of serum protein. These cells were ED1 and ED2 negative. As a biological control for the integrity of the TNF-α, 7.5x10^4 U of the murine recombinant TNF-α was injected into the skin of three adult Lewis rats. This produced a marked PMN recruitment in all animals consistent with previous reports (Groves et al., 1995).

The injection of 7.5x10^4 U of TNF-α into the striatum of a 3-week-old juvenile rat also failed to induce leakage of serum protein into the parenchyma after 4 h. After 24 h and 48 h, there was evidence of a little leakage of tracer to regions surrounding the larger vessels in the parenchyma (Fig. 1F). However, by 72 h no increases in permeability were noted. In contrast to the adult animals at 24 h, small numbers of PMNs were seen to be marginalized to some of the vessels in the parenchyma adjacent to the injection site, although relatively few compared with the effects of IL-1β. There was also some evidence of vascular occlusion, which was similar to that seen in the adult animals.

**Discussion**

The principal findings of this study may be summarized as follows. First, the CNS parenchyma of an adult rat is remarkably resistant to inflammation induced by IL-1β and to TNF-α, in contrast to the effects when these cytokines are injected into the eye, ventricles, and skin (Martin et al., 1988; Groves et al., 1995). Secondly, a ‘window of susceptibility’ exists in the developing CNS, between about 2 and 6 weeks of age: the injection of IL-1β into the striatum of a 3-week-old Lewis rat gives rise to an intense PMN recruitment and to increases in vessel permeability around the injection site, but these effects are not seen in neonates or in young adults. Thirdly, the increases in vessel permeability were neutrophil-dependent. These points will be discussed in turn.

**Responses of adult CNS to inflammatory cytokines**

To study the effects of IL-1β and TNF-α on the BBB permeability and leukocyte recruitment, we injected a small volume of cytokine with finely drawn microcapillaries stereotactically (thereby reducing the associated trauma to a minimum) directly into the CNS parenchyma. Horseradish peroxidase, which does not cross the intact BBB, was used as the tracer of increased vessel permeability (Hawkins et al., 1990; Martiney et al., 1990; Claudio et al., 1994). Horseradish peroxidase histochemical localization permitted visualization of the effects of the cytokines on the BBB in different CNS compartments (brain parenchyma, meninges, etc.). Previous studies (e.g. Martiney et al., 1990; Quagliarello et al., 1991; Kim et al., 1992; Megyeri et al., 1992) have not directly addressed the effects of IL-1β or TNF-α on vessel permeability in the CNS parenchyma. Brosnan and co-workers (Martiney et al., 1990; Claudio et al., 1994) have shown that injection of IL-1β into the eye increases the permeability of the vessels forming the blood–retinal barrier.
Large gaps between endothelial cells were observed at sites of increased permeability. These events were also associated with haemorrhage and recruitment of monocytes and PMNs. Injection of IL-1β and IL-1α into the CSF has been shown to induce PMN emigration into the CSF, and blood–CSF barrier permeability increases were observed by assaying the CSF for intravenously administered \[^{[25]}\text{I}\]BSA (bovine serum albumin) (Quagliarello et al., 1991). Our own results show that the parenchymal vessels appear to be remarkably resistant to permeability changes that can be induced by IL-1β in other parts of the CNS—meninges, retina, choroid plexus—and in peripheral tissue (Martin et al., 1988). Furthermore, in comparison with the meninges, very few leukocytes were recruited to the parenchyma in the IL-1β-treated animals. These observations are supported by the results of Andersson et al. (1992b), in Balb/c mice, where the injection of IL-1β into the hippocampus was demonstrated to induce a rapid leukocyte recruitment in the choroid plexus and ventricular system, but not to the parenchyma itself. They were also able to show that the introduction of as much as 2 μg lipopolysaccharide, a potent inflammmogen, to the brain parenchyma will give rise to only minimal PMN recruitment in the first few days (Andersson et al., 1992a).

In contrast to the effects of IL-1β, intra-cerebral injection of TNF-α provoked little response in this study. Some leukocyte recruitment was observed in adult rats, which was primarily monocytic. Strikingly, no great increase in vascular permeability was observed at any time after challenge at the doses employed. Following TNF-α injections (10^3 U) into the eye, Claudio et al. (1994) also noted a predominantly monocytic response. In addition, they observed some endothelial cell vacuolation, which is indicative of cytotoxicity. Others have shown that when TNF-α is injected into the eye there is a subsequent increase in vascular permeability (Rosenbaum et al., 1988). Wright and Merchant (1992) have performed intra-cerebral injections of human recombinant TNF-α: 6×10^4 U provoked leukocyte adherence and capping from 4 h to 48 h. There was an area of leukocyte recruitment in the parenchyma in the region surrounding the injection site at 48 h. Polymorphonuclear neutrophils were observed to be the predominant cell type. However, their injections were performed with a 28 gauge blunt-ended injection cannula, and a 5 μl volume was administered. It seems likely that substantially more trauma was inflicted by that injection protocol, since even their vehicle-injected animals exhibited a low level of vascular capping, comprising PMNs and macrophages, from 4 to 48 h. In our own experiments, no leukocyte adherence, capping, or recruitment was ever observed in the vehicle treated animals at any time after injection.

**Window of susceptibility**

The discovery of the ‘window of susceptibility’ to IL-1β, in a 3-week-old rat, was a wholly unexpected finding. It cannot simply be attributed to an immature BBB, since it is known that a tight BBB is formed prior to parturition (Mollgard and Saunders, 1986; Butt et al., 1990). Investigating pial vessels, Butt et al. (1990) have shown that the electrical resistance, a measure of cellular and paracellular permeability to ions, of vessels in a rat of 21-days gestation is no different from rats 33 days after birth. Furthermore, as animals injected with IL-1β 2 h after parturition display neither increased parenchymal vessel permeability nor PMN recruitment following intra-cerebral injection of IL-1β, the development of the adult ‘resistant’ character is not merely a progressive relationship with age.

The susceptibility of individuals to bacterial infection of the CNS and meninges is highly dependent on age (Tuomanen, 1994). Increasing age appears to protect the individual: nearly three-quarters of all cases of meningitis occur in children under 5 years old. The nature of the infective organism giving rise to meningitis also appears to be age dependent (Tuomanen, 1994). Meningitis or encephalitis often develops following a prolonged period of high level bacteraemia that leads to the seeding of the brain parenchyma. The details of this invasive step are unknown. However, it is known that a high circulating concentration of endotoxin is associated with increased expression of IL-1β in the CNS (van Dam et al., 1992). If a ‘window of susceptibility’ to IL-1β exists in humans, as it does in juvenile rats, increased BBB permeability arising from the presence of IL-1β in the brain parenchyma may facilitate bacterial invasion. Furthermore, the severity of any infection that develops in the CNS is likely to be increased during the ‘window of susceptibility’, when endogenous microglial IL-1β production might cause further BBB disruption. A therapy antagonizing the effects of IL-1β may prove particularly beneficial during the period of increased susceptibility of the BBB to IL-1β. The ‘window of susceptibility’ may also be of significance in children with severe head injuries or with cerebral malaria.

The increase in permeability of the BBB induced by IL-1β may enable non-CNS antigens to enter the brain parenchyma. It has been postulated that some CNS immune-mediated diseases, such as multiple sclerosis, may be initiated by an immune reaction to non-CNS antigens that have, in the past, become sequestered behind the BBB (Matyszak and Perry, 1995). It has been shown that adenovirus, herpes simplex virus and paramagnetic monocrystalline iron oxide nanoparticles may be deposited in the parenchyma following transient mannitol-induced osmotic opening of the BBB (Muldoon et al., 1995).

**BBB permeability is polymorphonuclear neutrophil dependent**

The precise mechanisms by which IL-1β induces increases in vessel permeability and leukocyte recruitment were not
addressed by this study. However, it is clear from our PMN depletion experiments that the observed increases in IL-1β-induced vessel permeability are dependent on an interaction between endothelial cells and PMNs, and not due to the action of IL-1β on endothelial cells alone. Although IL-1β did cause vessel dilation in the absence of PMNs, interleukin-1β is not a chemo-attractant, but is known to be an inducer of C-X-C chemokines—in particular interleukin-8 (IL-8) in humans and cytokine induced neutrophil chemo-attractant in rodents—which are potent and specific neutrophil chemo-attractants (Yamasaki et al., 1995). Bell et al. (1996) have demonstrated that intra-cerebral injection of recombinant murine macrophage inflammatory protein 2 and IL-8 will overcome the resistance of adult mouse brain to PMN recruitment and cause BBB breakdown. Therefore, we may speculate that during the ‘window of susceptibility’, chemokines are induced in the CNS parenchyma of young animals that are not found in the resistant adult CNS. Much like adult rat parenchymal vessels, new-born (2 h to 1 week) rat parenchymal vessels in the CNS are resistant to the effects of IL-1β. In the same way, neonatal mice are resistant, unlike 1-week-old mice, to the effects of intra-cerebral lipopolysaccharide injection (Lawson and Perry, 1995). Since we have demonstrated that increases in CNS–vessel permeability are neutrophil dependent, the resistance of neonatal rats to IL-1β may be accounted for, at least in part, by the relative neutropenia and inefficient PMN migration that is present in neonates (Kanwar and Cairo, 1993).

In summary, juvenile rats exhibit a ‘window of susceptibility’ to the proinflammatory effects of IL-1β, which could not have been predicted from our knowledge of BBB development. We also show that the parenchymal BBB and the blood–CSF barrier respond differently to inflammmogens, and that neutrophil-endothelial cell interactions mediate the IL-1β-induced increases in vessel permeability. In the light of these observations, different approaches to the management of children with conditions resulting in CNS inflammation need to be considered.

Acknowledgement
This work was funded by Neures Ltd, Abingdon, Oxon, UK.

References


Received June 13, 1996. Revised October 31, 1996.

Accepted November 18, 1996.