Clin Exp Immunol 1997; **109**:233–241

Haemophilus influenzae and *Streptococcus pneumoniae* induce different intracerebral mRNA cytokine patterns during the course of experimental bacterial meningitis

A. DIAB, J. ZHU, L. LINDQUIST*, B. WRETLIND†, M. BAKHIET & H. LINK *Divisions of Neurology, *Infectious Diseases and †Clinical Bacteriology, Karolinska Institute, Huddinge University Hospital, Stockholm, Sweden*

(Accepted for publication 14 April 1997)

SUMMARY

Using *in situ* hybridization with radiolabelled oligonucleotide probes, we studied the mRNA expression of IL-1β, IL-4, IL-6, IL-10, IL-12, tumour necrosis factor-alpha (TNF-α), TNF-β, interferon-gamma (IFN-γ), and transforming growth factor-beta (TGF-β) in the brain during the lethal course of experimental meningitis in a rat model inoculated intracisternally with *Haemophilus influenzae* type b (Hib) or *Streptococcus pneumoniae* and in uninfected control rats inoculated with the same volume of PBS. The production of IL-1β, IL-4, IL-6 and IFN-γ was also evaluated by immunohistochemistry. In the brain of Hib-inoculated rats, there was marked mRNA expression of IL-1β, IL-6, TNF-α, IL-12 and IFN-γ. IL-1β, IL-6 and TNF-α were up-regulated throughout the observation period at 2, 8 and 18 h post-inoculation (p.i.), with similar patterns of induction. The Th1 cytokines IFN-γ and TNF-β were up-regulated within 8 h p.i. IL-10 and TGF-β were down-regulated at 18 h p.i., while IL-4 was not detected. In contrast, the brain of *S. pneumoniae*-inoculated rats showed lower levels of IL-1β, IL-6 and TNF-α, but higher levels of TNF-β and detectable mRNA expression of IL-4 when compared with Hib-inoculated rats. IL-12, IFN-γ, IL-10 and TGF-β exhibited similar patterns of induction in the brains of Hib- and *S. pneumoniae*-inoculated rats. At 18 h p.i., immunohistochemistry showed similar patterns of IL-1β, IL-4, IL-6 and IFN-γ as mRNA expression in the brains of Hib- and *S. pneumoniae*-inoculated rats. The differences of cytokine profiles induced by the two bacterial strains may imply that different immunomodulating approaches should be considered, depending on etiology.

Keywords Haemophilus influenzae Streptococcus pneumonia brain cytokine meningitis

INTRODUCTION

Despite improvement in antimicrobial therapy, bacterial meningitis is still a serious disease [1,2]. Meningitis caused by *Streptococcus pneumoniae*, the most frequent microorganism in human adult bacterial meningitis, has a fatality rate of about 25% [2,3], while meningitis caused by *Haemophilus influenzae* type b (Hib) remains the most common bacterial etiology of meningitis in children in developing countries [4]. Clinical observations suggest that increased intracranial pressure (ICP), brain oedema and cerebral vasculitis are associated with an unfavourable clinical course [5–9]. The precise pathophysiological mechanisms of the major intracranial complications are poorly understood. Multiple factors including the complement alternative pathway, cytokines, prostaglandins, and platelet-activating factors have been suggested to be involved in the complex nature of the pathophysiological events during bacterial meningitis [10].

Cytokines like IL-1, IL-6 and tumour necrosis factor-alpha (TNF-α) have been proposed as signals that activate centrally controlled responses to systemic injury and infection, but the means by which they communicate with the brain are still uncertain. Whatever the signalling mechanisms are between the periphery and the central nervous system (CNS), the relationship between central and peripheral production of cytokines is, to some extent, reciprocal, since centrally administered IL-1 increases peripheral synthesis of IL-6, apparently via opioid-receptor-mediated pathways [11]. Immune cells such as macrophages, T cells and neutrophils, which can invade the brain after injury or during inflammation, are a rich source of cytokines. However, unlike in peripheral tissues, the entry of myelomonocytic cells into the brain can be delayed for several hours after injury, and neutrophils are rarely detected [12]. Thus, early expression of cytokines within the brain presumably indicates their production by resident brain cells.

Correspondence: Dr Asim Diab, Division of Neurology, Karolinska Institute, Huddinge University Hospital, S-141 68 Huddinge, Sweden.

Although there are multiple mechanisms for cytokine induction in the brain, it is likely that cytokine cascades exist, as is apparent in the sequential induction of TNF-α, IL-1 and IL-6 during CNS infection [13].

Since cytokines act in concert, we assessed the simultaneous induction of several cytokines to understand the background for overlapping and divergent responses to different stimuli by utilizing *in situ* hybridization. IL-1β, IL-4, IL-6, IL-10, IL-12, TNF-α, TNF-β, interferon-gamma (IFN-γ) and transforming growth factor-beta (TGF-β) mRNA expression in response to Hib and *S. pneumoniae*, respectively, was evaluated *in vivo*. We also employed immunohistochemistry to detect IL-1β, IL-4, IL-6 and IFN-γ in individual cells to document actual cytokine production.

MATERIALS AND METHODS

Challenge organisms

Hib strain LCR 528 was originally obtained from cerebrospinal fluid (CSF) of a child with Hib meningitis. Unencapsulated *S. pneumoniae* strain R 6 (number 7607) was an isolate from the blood of a patient with septic infection. The two strains were frozen at −70°C in trypticase soy broth with 10% glycerol pH 7·3 until use. For each experiment, Hib was grown overnight in brain–heart infusion broth, subcultured on chocolate agar plate and allowed to grow for 8 h in late log phase to facilitate maximal capsule expression in the challenge inocula, then centrifuged and resuspended in PBS to an approximate concentration of 10^6 colony-forming units (CFU)/ml as determined by plating 100-fold serial dilution of the organisms on chocolate agar. For *S. pneumoniae*, bacteria were subcultured on blood-agar plates inoculated into brain–heart infusion broth and incubated overnight at 37°C. Pellets were obtained by centrifugation for 20 min at 2500 ***g***. The pellets were washed twice in PBS pH 7·4 and resuspended in PBS to an approximate concentration of 10^6 CFU/ml determined by plating 100-fold serial dilution of the organisms on blood–agar plates. The final inoculum for both strains was 10^6 CFU/rat.

Induction of bacterial meningitis

A total of 72 animals was used. Three-week-old male rats from an outbred Sprague-Dawley strain were anaesthetized with 0·05 ml of thiobutabarbiturate (100 mg/kg) by i.p. inoculation. CSF (50 μl) was removed via intracisternal (i.c.) puncture using a micromanipulator fitted to a 25 G butterfly needle. After CSF removal, 50 μl volumes containing Hib (10^6 CFU) or *S. pneumoniae* (10^6 CFU) or PBS were injected intracisternally into each of six animals simultaneously. After inoculation, meningitis was allowed to progress for defined duration (2, 8 or 18 h), when CSF (50 μl) was resampled for determination of quantitative culture and the rats were killed by exsanguination. CSF diluted 10-fold in physiologic saline, and bacterial concentrations were determined by surface growth on chocolate agar (Hib) or blood agar (*S. pneumoniae*).

In situ *hybridization*

Whole brains were snap-frozen in liquid nitrogen. Cryostat sections (10 μm) were thaw-mounted onto electrically charged glass slides (ProbeOn slides; Fisher Scientific, Pittsburgh, PA), which were stored with silica in sealed boxes at −20°C until hybridization. *In situ* hybridization was performed as described for tissue sections [14]. Synthetic oligonucleotide probes (Scandinavian Gene Synthesis AB, Köping, Sweden) were labelled using ^{35}S-deoxyadenosine-5′-α-(thio)-triphosphate (New England Nuclear, Cambridge, MA) with terminal deoxynucleotidyl transferase (Amersham, Aylesbury, UK). To increase the sensitivity of the method, a mixture of four different ≈48 bp long oligonucleotide probes was used. The oligonucleotide sequences were obtained from GenBank and probes were designed using MacVector software (Table 1). After hybridization, slides were rinsed for 5 × 15 min at 55°C in 1 × SSC, allowed to come to room temperature, dipped in distilled water, dehydrated through gradient ethanol (60%, 70% and 95%), and air dried, dipped in Kodak NTB2 emulsion, and exposed at 4°C for 15–20 days depending on the age of the probe. After developing in Kodak D19, slides were stained with cresyle violet and mounted with Entellan (Merck, Darmstadt, Germany). Coded slides were examined by dark field microscopy at ×10 magnification.

Cells were judged as positive when expressing more than 15 grains with a star-like distribution over their cytoplasm. In cells judged negative, the number of grains was mostly 0–2 per cell, and the grains were scattered randomly over the cell and not distributed in a star-like fashion. The cellular distribution of the grains was always checked under light microscopy at ×20 and/or ×40 magnification (Fig. 1). There were no difficulties in differentiating between cytokine mRNA-positive and -negative cells. Results

Table 1. Survey over cytokine probes used

Probe	Exon	Gene Bank accession number	Complementary to bases
Rat IL-1β	Exon 1	M98820	639–686
	Exon 2		569–616
	Exon 3		278–325
	Exon 4		295–342
Rat IL-4	Exon 1	X16058	83–130
	Exon 2		209–256
	Exon 3		270–317
	Exon 4		331–378
Rat IL-6	probe #2	M26744	139–187
	probe #3		180–223
Mouse IL-10	Exon 1	M37897	79–126
	Exon 2		134–181
	Exon 3		184–231
	Exon 4		402–449
Mouse IL-12 (p40)	Exon 1	M86771	147–194
	Exon 2		595–642
Mouse IL-12 (p35)	Exon 1	M86672	190–238
	Exon 2		706–753
RAT TNF-α	Exon 1	00475	913–960
	Exon 2		2059–2106
	Exon 3		2152–2199
	Exon 4		2316–2363
Rat IFN-γ	Exon 1	M29315	298–345
	Exon 2	M29316	80–125
	Exon 3	M29317	303–350
Human TGF-β1	Exon 3	X02812	1766–1813
	Exon 4		1953–2000
Mouse TNF-β	Exon 1	Y00137	118–165
	Exon 2		202–249
	Exon 3		342–389
	Exon 4		502–549

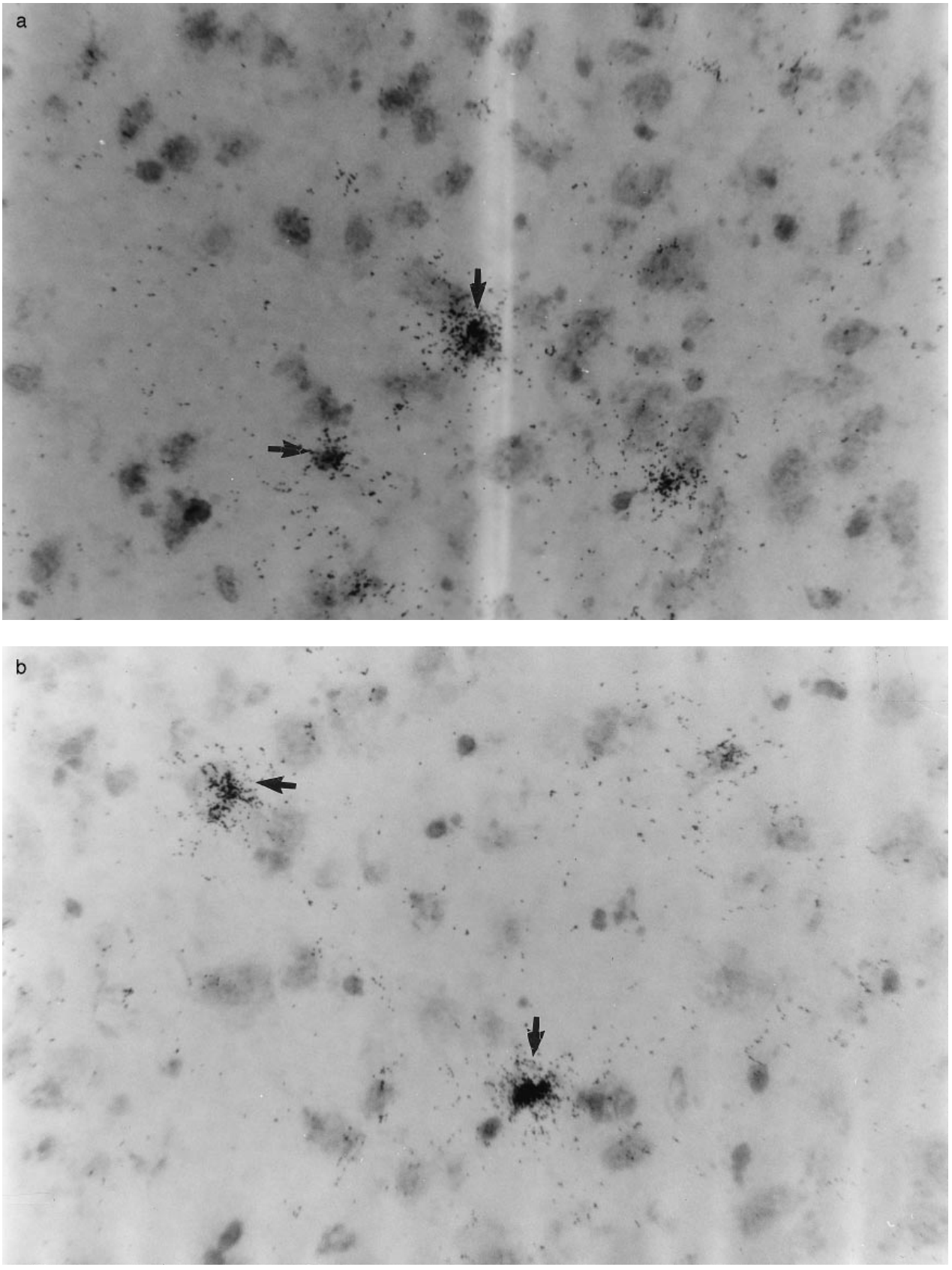


Fig. 1. Autoradiograms of *in situ* hybridization for tumour necrosis factor-alpha (TNF-α) mRNA of brain tissue section from *Haemophilus influenzae* type b (Hib)-inoculated rat killed 2 h p.i. (a) and for IFN-γ mRNA of brain tissue section from *Streptococcus pneumoniae*-inoculated rat killed 18 h p.i. (b). Hybridized cells (arrows) are shown at magnification × 400.

were expressed as numbers per 100 mm^2 tissue section. The tissue section areas were measured by image analysis (Image Analysis System; Seescan, Cambridge, UK).

Immunohistochemistry

Cryostat sections, 10 μm thick, from brains of Hib- and *S. pneumoniae*-infected and uninfected control rats were mounted on gelatin-coated glass slides. Sections were exposed to appropriate dilutions of the following MoAbs: hamster anti-mouse IL-1β (Genzyme, Cambridge, MA) which recognizes rat IL-1β [15], mouse anti-human IL-4 (Genzyme) which recognizes rat IL-14 [16], mouse anti-human IL-6 (Genzyme) which recognizes rat IL-6 [16], and mouse anti-rat IFN-γ [17] (DB1; kindly provided by Peter van der Meide, TNO Centre, Rijswijk, The Netherlands). A biotinylated mouse anti-hamster antibody for detection of IL-1β and biotinylated horse anti-mouse antibody for detection of IL-4, IL-6 and IFN-γ were used as the secondary reagent (Southern Biotechnology, Birmingham, AL) followed by avidin–biotin complex (ABC Vectastain Elite Kit; Vector, Burlingame, CA). Staining was performed using 3-amino-9-ethylcarbazole (Sigma, St Louis, MO) with a haematoxylin counterstain. Omission of the primary antibody served as a negative control. Specificity of the staining was also controlled on sections of peripheral lymphoid organs. The tissue areas were measured by image analysis, and the numbers of stained cells, i.e. cytokine-producing cells, were calculated per 100 mm^2 of tissue area.

Histological techniques

To confirm the development of bacterial meningitis, brains of groups of animals injected with Hib ($n = 6$), *S. pneumoniae* ($n = 6$) or PBS ($n = 6$) were examined histologically. The duration between i.c. inoculation and death was between 5 h and 36 h. The brains were fixed by immersion in 4% neutral buffered formaldehyde solution. The fixed brains were cut to 5 mm thick coronal sections. Following dehydration and paraffin embedding, 5 μm thick sections were made. Haematoxylin–eosin-stained tissues were scored for inflammation of the meninges.

RESULTS

Histopathology of meningitis

At 6 h post-inoculation (p.i.) (but not 2 h p.i.), the leptomeningeal space of all infected animals contained varying amounts of polymorphonuclear leucocytes and mononuclear cells. These cellular meningeal infiltrations were diffusely distributed around the basal cisterns, on the cortical surface, and in the longitudinal fissure of the cerebral hemispheres, with higher density around blood vessels (Fig. 2). Inflammatory cells were also detected in the lumina and perivascular spaces of the small blood vessels entering the uppermost layer of the brain cortex. Cellular infiltrates were not observed in the walls of the greater arteries at the base of the brain. There was no evidence of thrombus formation in meningeal or parenchymal blood vessels. PBS-inoculated control rats did not show any inflammatory cell reaction in the meninges or in the brain parenchyma. CSF cultures were positive in infected animals.

Cytokine mRNA pattern

Induction of IL-1β, IL-6 and TNF-α. For evaluation of proinflammatory cytokines characteristically produced by macrophages (IL-1β and TNF-α) or lymphocytes (IL-6), the cellular mRNA expression of these cytokines was examined in the brain of Hib-, *S. pneumoniae*- and PBS-inoculated rats that were killed at standardized time intervals p.i. In the brain tissue of PBS-inoculated rats, IL-1β and TNF-α mRNA were not detectable, while IL-6 mRNA was detected at very low levels (Fig. 3). In the brain tissue sections of Hib-inoculated rats, mRNA expression for IL-1β, IL-6 and TNF-α showed similar patterns over the study period covering 2, 8 and 18 h p.i. A rapid increase of IL-1β mRNA-expressing cells was recorded 2 h p.i. A drastic increase of IL-1β mRNA-producing cells occurred during the next few hours, and peak production was observed at 18 h p.i. Levels of IL-6 mRNA-expressing cells at 2 h p.i. were low, but increased at 8 h p.i. and showed maximum levels at 18 h p.i. Levels of TNF-α mRNA in Hib-inoculated rats also increased from 2 h to 8 h p.i., and to a maximum by 18 h p.i.

In the brain tissue of *S. pneumoniae*-inoculated rats, patterns of mRNA expression for IL-1β, IL-6 and TNF-α differed from those in Hib-inoculated rats. Slightly elevated levels of IL-1β and TNF-α mRNA-expressing cells were detected 2 h p.i. The levels peaked at 8 h p.i., and declined at 18 h p.i. Levels of IL-6 mRNA-expressing cells in *S. pneumoniae*-inoculated rats were low throughout the observation period and not different from those registered in uninfected control rats.

Induction of IL-12, IFN-γ and TNF-β. Levels of IL-12 and IFN-γ mRNA-expressing cells were very low in the brain of PBS-inoculated control rats. In the brain of Hib-inoculated rats, cells expressing mRNA of IL-12 and IFN-γ showed similar patterns, with a certain induction already at 2 h p.i., a peak at 8 h p.i., and declining levels at 18 h p.i. (Fig. 4). Similar mRNA expression profiles were found in the brain tissue of *S. pneumoniae*-infected rats, although the induction of IL-12 and IFN-γ mRNA noticed at 2 h p.i. was less apparent, while that at 18 h p.i. was more pronounced than in Hib-inoculated rats. Levels of TNF-β mRNA-expressing cells detected in the brains of PBS-inoculated rats were very low (Fig. 4). In the brain tissue of Hib-infected rats, TNF-β mRNA expression was increased at 8 and 18 h p.i. In the brain tissue of *S. pneumoniae*-inoculated rats, TNF-β mRNA expression was increased at all three time points, with maximum levels at 8 h p.i.

Induction of IL-4, IL-10 and TGF-β. Very low levels of IL-4 mRNA-expressing cells were detected in brains of *S. pneumoniae*-inoculated rats (Fig. 5). IL-4 mRNA expression was not detectable in the brains of PBS- and Hib-inoculated rats. Interestingly, IL-10 mRNA was detected at all three time points in the brains of PBS-inoculated control rats. Elevated IL-10 induction was recorded in Hib-infected rat brains at 8 h p.i. At 18 h p.i., IL-10 mRNA expression had declined to levels below that of uninfected control rats. In the brains of *S. pneumoniae*-inoculated rats, IL-10 mRNA induction was elevated at 8 and 18 h p.i. Like IL-10, TGF-β mRNA expression was rather high in the brains of PBS-inoculated control rats over the whole observation period. In the brains of Hib- and *S. pneumoniae*-inoculated rats, the patterns of TGF-β mRNA expression were similar to those of IL-10, although levels below those in PBS-inoculated control rats were registered in Hib-infected rats at 8 and 18 h p.i. This difference did not, however, reach statistical significance.

Immunohistochemistry

Evidence of cytokine-producing cells in the brain at 18 h p.i. We recorded active production of IL-1β and IL-6 in the brain tissue of Hib-inoculated rats, and to a lesser extent in the brain tissue of *S. pneumoniae*-inoculated rats. The levels of production of IFN-γ in the brain tissue of *S. pneumoniae*-inoculated rats were

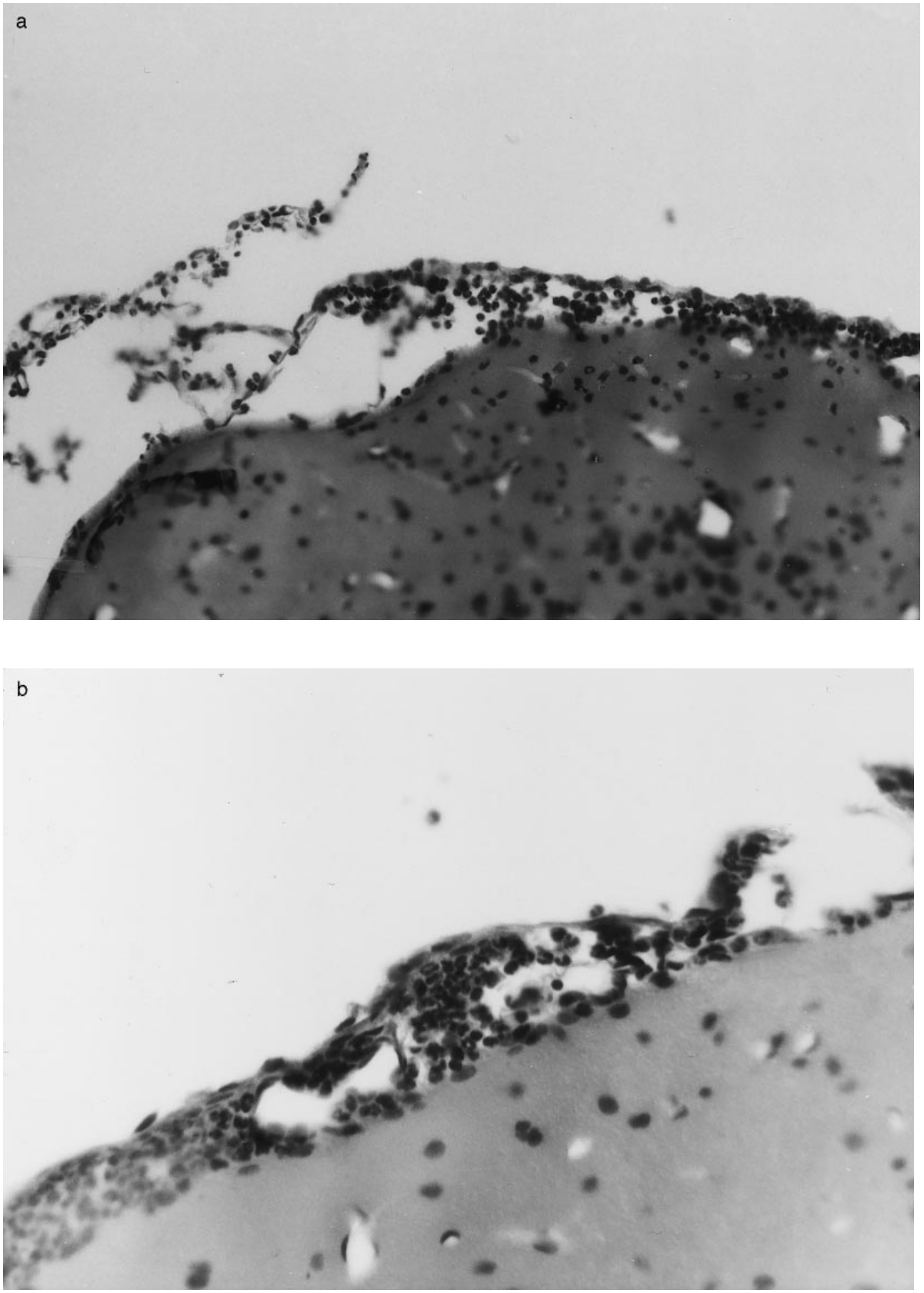


Fig. 2. Micrograph of brain sections. Acute meningitis with polymorphonuclear leucocytes and some mononuclear cells in the leptomeningeal space 18 h post *Haemophilus influenzae* type b (Hib) infection (a) and 18 h post *Streptococcus pneumoniae* infection (b). (Haematoxylin and eosin staining, mag. ×200.)

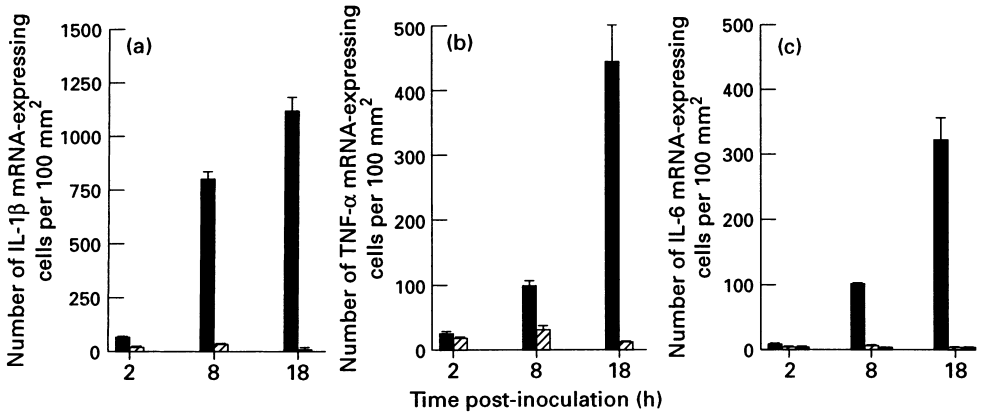


Fig. 3. IL-1β (a), tumour necrosis factor-alpha (TNF-α) (b) and IL-6 (c) mRNA expression in brains of *Haemophilus influenzae* type b (Hib)- (■), *Streptococcus pneumoniae*- (▨) and PBS-inoculated rats (□). Brain sections were obtained at 2 h, 8 h and 18 h p.i. Numbers of cells expressing mRNA detected by *in situ* hybridization were expressed per 100 mm^2 surface area.

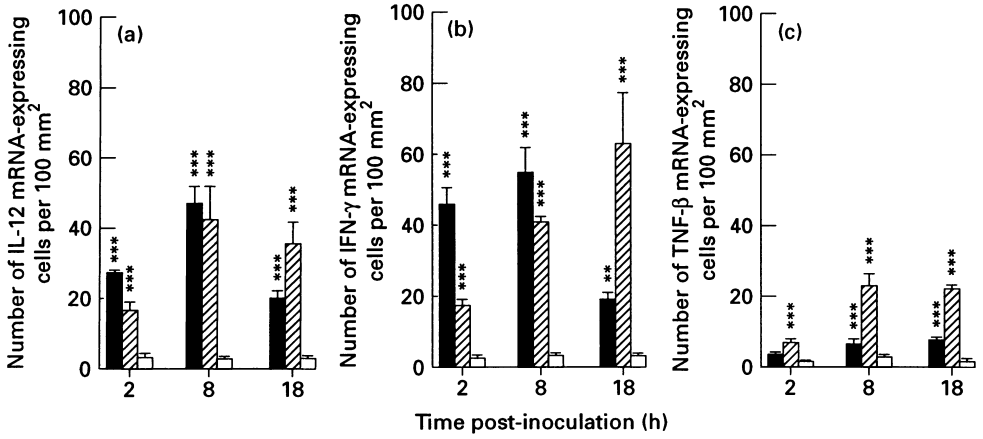


Fig. 4. IL-12 (a), IFN-γ (b) and tumour necrosis factor-beta (TNF-β) (c) mRNA expression in brains of *Haemophilus influenzae* type b (Hib)- (■), *Streptococcus pneumoniae*- (▨) and PBS-inoculated rats (□). The non-parametric Mann–Whitney test was used to evaluate differences between infected and control rats. Bars indicate 1 s.d. *P* values refer to comparisons between Hib-, *S. pneumoniae*- and PBS-inoculated control rat. ***$P < 0{\cdot}001$; **$P < 0{\cdot}01$; *$P < 0{\cdot}05$.

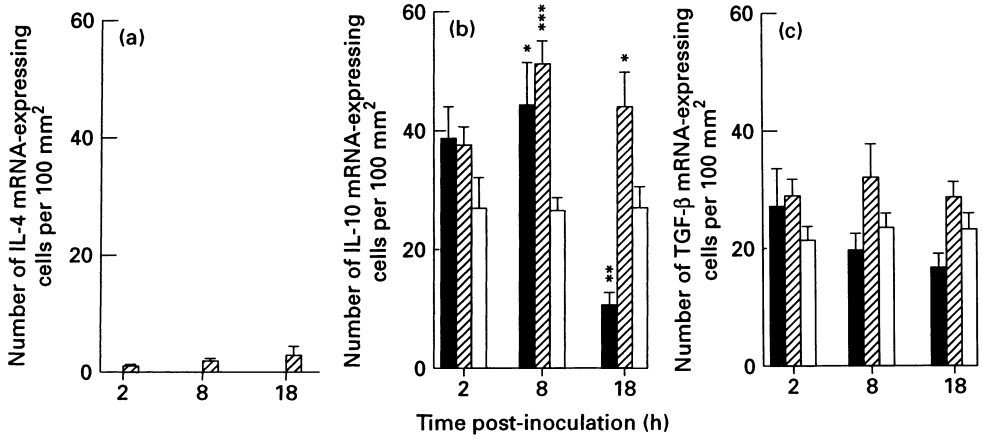
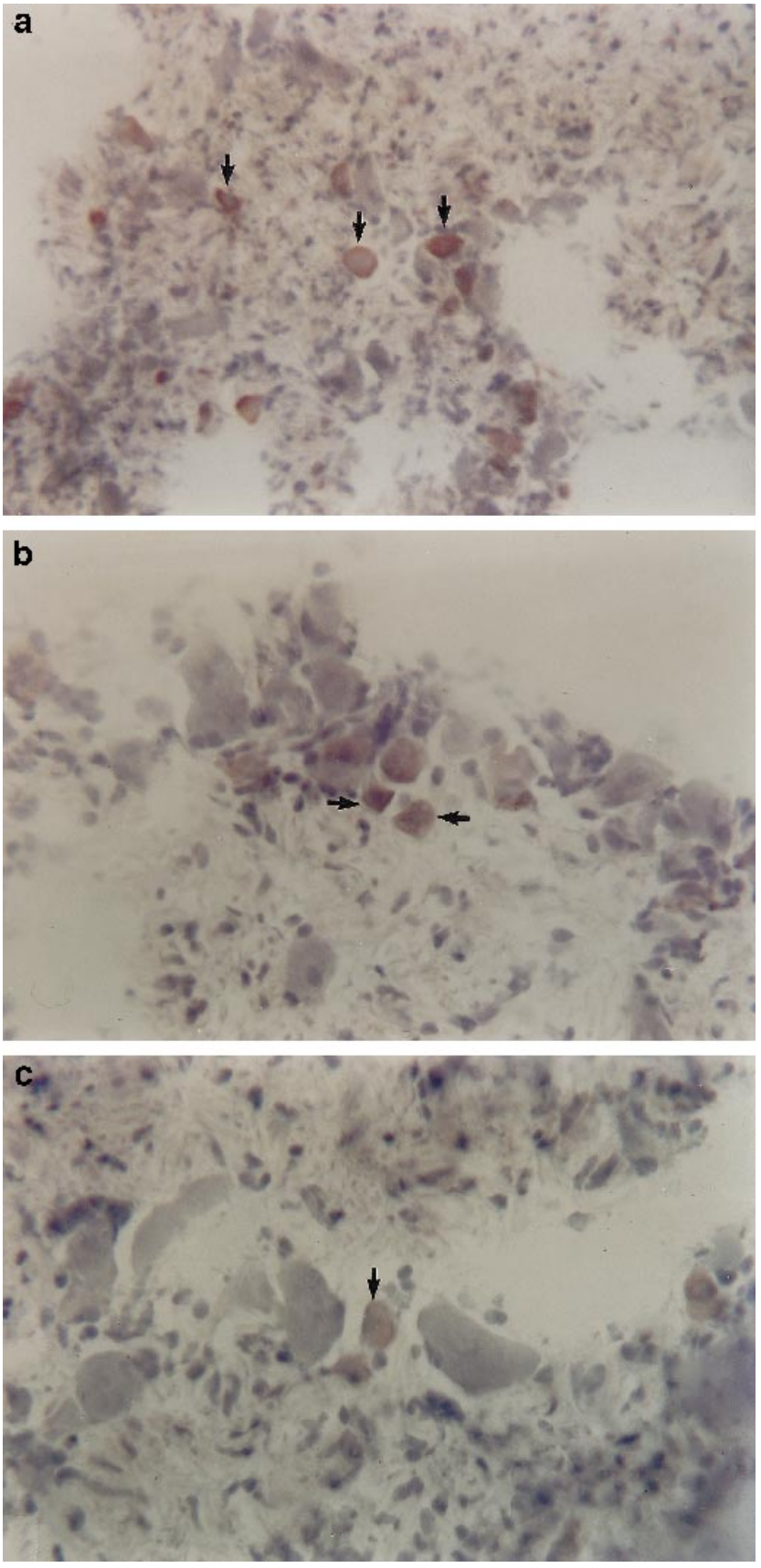


Fig. 5. IL-4 (a), IL-10 (b) and transforming-growth factor-beta (TGF-β) (c) mRNA expression in brains of *Haemophilus influenzae* type b (Hib)- (■), *Streptococcus pneumoniae*- (▨) and PBS-inoculated rats (□). Compare legend of Fig. 4.

Fig. 6. Immunohistochemical staining for IFN-γ-producing cells (arrows) in brain tissue section from *Streptococcus pneumoniae*-inoculated rat killed 18 h p.i (a) and for IFN-γ- (b) and IL-1β-producing cells (arrows) in the brain tissue section from *Haemophilus influenzae* type b (Hib)-inoculated rat killed 18 h p.i. (c). (Mag. ×400.)


a
b
c

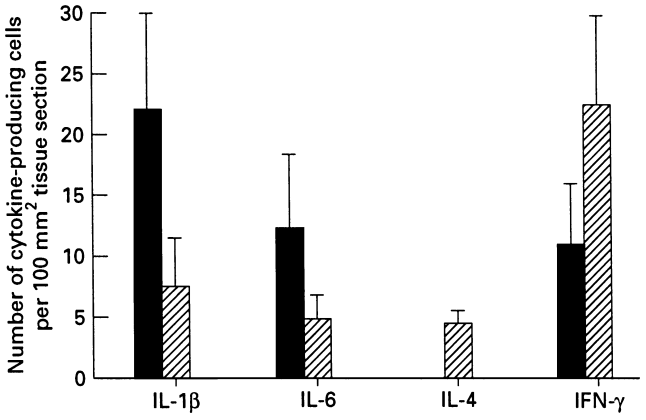


Fig. 7. IL-1β, IL-6, IL-4 and IFN-γ-producing cells in brains of *Haemophilus influenzae* type b (Hib)- (■), *Streptococcus pneumoniae*- (▨) and PBS-inoculated rats (□) 18 h p.i.

considerably higher than those observed in the brain tissue of Hib-inoculated rats (Fig. 6 a,b,c). Production of IL-4 was only slightly elevated in the brain tissue of *S. pneumoniae*-inoculated rats and completely abrogated in the brain tissue of Hib-inoculated rats. We did not detect any production of IL-1β, IL-6, IL-4 and IFN-γ in the brain tissue of PBS-inoculated control rats (Fig. 7).

DISCUSSION

In this study we investigated the early kinetics of several cytokines after infection with either Hib or *S. pneumoniae*. The predominant cytokine messages in Hib-infected rats were those generally associated with a proinflammatory response (IL-1β, IL-6, TNF-α, IL-12, IFN-γ and TNF-β), while *S. pneumoniae*-infected rats responded with mixed pro- and anti-inflammatory (IL-4 and IL-10) responses. In our rat model of Hib and *S. pneumoniae* meningitis, rats mount as vigorous an inflammatory response within the CNS, manifested by positive CSF culture and histopathology of the meninges. It is not known whether the observed differences in intracerebral cytokine mRNA patterns following Hib or *S. pneumoniae* infection reflect differences in clinical symptoms of meningitis induced by Gram-negative bacteria, such as Hib, and Gram-positive bacteria such as *S. pneumoniae*.

Marked increases in expression of IL-1, IL-6 and TNF-α have been observed in rats within 6–8 h after brain damage [18], and mRNA for IL-1β is induced within 15 min of forebrain ischaemia in the rat [19]. The rapid induction of IL-1β, IL-6 and TNF-α mRNA after infection with Hib and *S. pneumoniae* suggests that these cytokines are produced by the intrinsic brain cells that respond promptly to infection, since induction of cytokines from migrating inflammatory cells is necessarily delayed compared with the rapid appearance of IL-1β, IL-6 and TNF-α mRNA-expressing cells after exposure to bacterial inoculation.

Our data revealed a constant induction of TGF-β in the brain of PBS-inoculated control rats, and a lack in change of TGF-β induction in the brain of infected rats. This lack of TGF-β might be related to fatality, since TGF-β has many functions relevant to CNS infection, including regulation of expression of integrins [20], and inhibition of cerebral oedema [21], natural killer (NK) cell proliferation [22] and inflammation [23].

The physiological role of IL-10 produced intracerebrally in bacterial meningitis is not known. It is striking that in Listeria meningitis IL-10 is produced late in the course of the disease compared with other cytokines including TNF-α, IL-6 and IFN-γ. Likewise, the production of IL-10 *in vitro* by lipopolysaccharide (LPS)-stimulated human monocytes is delayed compared with IL-1β, IL-6 and TNF-α [24]. Thus, the observed levels of IL-10 mRNA expression in the present model of fatal meningitis at an early stage of the disease may reflect a regulatory circuit which counteracts the inflammatory process maintained by ongoing production of proinflammatory cytokines. This concept is supported by the recent findings that IL-10 is produced in the CNS of mice having recovered from experimental autoimmune encephalomyelitis rather than during the acute phase of the disease [25].

The very low levels of IL-4 in the brains of the fatal rat model of *S. pneumoniae*-induced meningitis and the absence of IL-4 in the brains of Hib-inoculated rats indicate that IL-4 is not up-regulated in these infections, in contrast to IL-12, which was up-regulated after inoculation with both bacterial strains. IFN-γ and TNF-β were expressed at higher levels in the brains of *S. pneumoniae*-infected rats. Thus, both cytokines were dominant products following *S. pneumoniae* inoculation. The observation of up-regulated Th1 cytokines during meningitis is not unexpected, suggesting that these molecules were associated with inflammation and that the brain may be a site of production of cytokines. Stimulants such as LPS induce the synthesis of large amounts of IL-1β mRNA expressed without significant translation of IL-1β protein [26,27]. In tissue with ongoing inflammatory processes, evaluation of mRNA-positive cells and protein-expressing cells does not necessarily need to be congruent, due to the complexity in the regulation of transcription and translation of cytokine genes and message.

In conclusion, the present data on the dynamics of cytokine mRNA in the CNS during Hib and *S. pneumoniae* meningitis may increase our understanding of cytokine regulation during neuroinflammation. It may furthermore suggest that, depending on etiology, immunomodulation of meningitis should at least in part be handled differently with respect to treatment with anti-cytokine monoclonal antibodies, receptor antagonists or cytokine inducers.

REFERENCES

1 Quagliarello VJ, Schel WM. Bacterial meningitis: pathogenesis, pathophysiology, and progress. N Engl J Med 1992; **327**:864–72.
2 Durand ML, Calderwood SB, Weber DJ. Acute bacterial meningitis in adults. A review of 493 episodes. N Engl J Med 1993; **328**:21–28.
3 Pfister HW, Feiden W, Einhäupl KM. The spectrum of complications during bacterial meningitis in adults: results of a prospective clinical study. Arch Neurol 1993; **50**:575–81.
4 Nottidge VA. Haemophilus influenzae meningitis: a 5-year study in Ibadan, Nigeria. J Infect 1985; **11**:109–17.
5 Paulson OB, Brodersen P, Hansen EL, Kristensen HS. Regional cerebral blood flow, cerebral metabolic rate of oxygen, and cerebrospinal fluid acid-base variables in patients with acute meningitis and encephalitis. Acta Med Scand 1974; **196**:191–8.
6 Nugent SK, Bausher JA, Maxon ER, Rogers MC. Raised intracranial pressure. Its management in *Neisseria meningitidis* meningoencephalitis. Am J Dis Child 1979; **133**:260–2.
7 Horwitz SJ, Boxerbaum B, O'Bell J. Cerebral herniation in bacterial meningitis in childhood. Ann Neurol 1980; **7**:524–8.
8 Igarashi M, Gilmartin RC, Gerald B, Wilbum F, Jabbour JT. Cerebral arteritis and bacterial meningitis. Arch Neurol 1984; **41**:531–5.
9 Pfister HW. Complicated purulent meningitis of the adult: persisting

high mortality caused by vasculitis and increased intracranial pressure. Nervenartz 1989; **60**:249–54.
10 Tuomanen E. Molecular mechanisms of inflammation in experimental pneumococcal meningitis. Ped Infect Dis 1987; **6**:1146–9.
11 De Simoni MG, Sironi M, De Luigi A, Manfridi A, Mantovani A, Ghezzi P. Intracerebroventricular injection of interleukin-1 induces high circulating levels of interleukin-6. J Exp Med 1990; **171**:1773–8.
12 Perry VH, Andersson PB, Gordon S. Macrophage and inflammation in the central nervous system. Trends Neurosci 1993; **16**:268–73.
13 Waage A, Halstensen A, Shalaby R, Brandtzaeg P, Kierulf P, Espevik T. Local production of tumor necrosis factor α, interleukin-1, and interleukin-6 in meningococcal meningitis. J Exp Med 1989; **170**: 1859–67.
14 Dagerlind Å, Friberg K, Bean AJ, Hökfelt T. Sensitive mRNA detection using unfixed tissue: combined radioactive and non-radioactive *in situ* hybridization histochemistry. Histochemistry 1992; **98**:39–49.
15 Wu X, Wittwer AJ, Carr LS, Crippes BA, DeLarco JE, Lefkowith JB. Cytokine-induced neutrophil chemoattractant mediates neutrophil influx in immune complex glomerulonephritis in rat. J Clin Invest 1994; **94**:337–44.
16 Hancock WW, Sayegh MH, Kwok CA, Weiner HL, Carpenter CB. Oral, but not intravenous, alloantigen prevents accelerated allograft rejection by selective intragraft Th2 cell activation. Transplantation 1993; **55**:1112–8.
17 Van der Meide PH, Dubbeld M, Vijverberg K, Kos T, Schellekens H. The purification of rat gamma interferon by use of two monoclonal antibodies. J Gen Virol 1986; **67**:1059–71.
18 Taupin V, Toulmond S, Serrano A, Benavides J, Zavala F. Increase in IL-6, IL-1 and TNF levels in rat brain following traumatic lesion. Neuroimmunol 1993; **42**:177–86.
19 Minami M, Kuraishi Y, Yabuuchi K, Yamazaki A, Satoh M. Induction of interleukin-1 beta mRNA in rat brain after transient forebrain ischemia. J Neurochem 1992; **58**:390–2.
20 Heino J, Ignotz RA, Hemler ME, Crouse C, Massague J. Regulation of cell adhesion receptors by transforming growth factor-beta. J Biol Chem 1989; **264**:380.
21 Pfister HW, Frei K, Ottnad B, Kodel U, Tomasz A, Fontana A. Transforming growth factor β2 inhibits cerebrovascular changes and brain edema formation in the tumor necrosis factor-α independent early phase of experimental pneumococcal meningitis. J Exp Med 1992; **176**:265–8.
22 Su HC, Leite-Morris KA, Braun L, Biron CA. A role for transforming growth factor-beta-1 in regulating natural killer cell and T lymphocyte proliferative responses during acute infection with lymphocytic choriomeningitis virus. J Immunol 1991; **147**:2717–27.
23 Wahl SM, McCartney-Fracis N, Mergenhagen SE. Inflammatory and immunomodulatory roles of TGF-β. Immunol Today 1989; **10**:258–61.
24 De Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Exp Med 1991; **174**:1209–20.
25 Kennedy MK, Torrance DS, Picha KS, Mohler KM. Analysis of cytokine mRNA expression in the central nervous system of mice with experimental autoimmune encephalomyelitis reveals that IL-10 mRNA expression correlates with recovery. J Immunol 1992; **149**: 2496–505.
26 Kaspar R, Gehrke L. Peripheral blood mononuclear cells stimulated with C5a or lipopolysaccharide to synthesize equivalent levels of IL-1β mRNA show unequal IL-1β protein accumulation but similar polyribosome profiles. J Immunol 1994; **153**:277–86.
27 Schindler R, Ghezzi P, Dinarello CA. IL-1 induces IL-1. IV IFN-γ suppresses IL-1 but not lipopolysaccharide-induced transcription of IL-1. J Immunol 1990; **144**:2216–22.