Journal of Neuroimmunology 90 (1998) 137–142

Journal of Neuroimmunology

Evolution of the immune response in the central nervous system following infection with Borna disease virus

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Received 23 December 1997; revised 3 March 1998; accepted 5 March 1998

Abstract

Borna disease virus infection of Lewis rats results in an immune-mediated disease associated with transient meningoencephalitis and persistent viral infection. In the acute phase of disease, perivascular immune cell infiltrates consisted of CD4 + and CD8 + T cells, macrophages and NK cells with peak expression of mRNAs encoding the cytokines IL1α, IL2, IL6, TNFα, and IFNγ. In the chronic phase of disease, numbers of NK cells, B cells and activated microglia increased in the brain parenchyma with peak expression of IL4 mRNA. These data were consistent with a switch from a Th1-like, cellular immune response to a Th2-like, humoral immune response.
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Keywords: Cytokine; Th2; Neurotropic virus; Encephalitis; Persistent infection; Apoptosis

1. Introduction

Borna disease virus (BDV) is nonlytic and persists in the central nervous system (CNS) despite a vigorous immune response. Infection of adult Lewis rats results in a transient meningoencephalitis accompanied by a multiphasic, immune-mediated behavioral syndrome, Borna disease (BD) (Hirano et al., 1983; Narayan et al., 1983; Stitz et al., 1995). Whereas acute BD is characterized by a robust cellular immune response (Stitz et al., 1995), the chronic phase is attended by a vigorous humoral immune response (Briese et al., 1995; Hatalski et al., 1995).

The mechanisms by which BDV evades the immune response to persist within the CNS have not been determined. The work described here was initiated to identify alterations in the immune response throughout the course of BD including composition of immune infiltrates, specialized cell death, cytokine mRNA expression and immunoglobulin isotype. Data from these experiments support a shift in the immune response in the CNS from a Th1-like, cellular immune response to a Th2-like, humoral immune response.

2. Materials and methods

2.1. Animal infection

Sixty-three 6-week old male Lewis rats were anesthetized and infected intranasally with 3.6×10^4 focus forming units of BDV. Six male Lewis rats were used as controls.

2.2. Immunohistochemical analysis of immune infiltrates

At different times after infection (0, 4, 5, 6, 10 and 15 weeks post-infection), rats were perfused with 4% paraformaldehyde in phosphate buffer. Immunohistochemistry was performed as detailed elsewhere (Hickey et al., 1983) using monoclonal antibodies (mAbs) specific for rat immune markers OX19 (Dallman et al., 1982), R73 (Hunig et al., 1989), W3/25 (White et al., 1978), OX8 (Brideau et al., 1980), 3.2.3 (Chambers et al., 1989), I169 (Kimura et al., 1984), OX6 (McMaster and Williams, 1979), ED2 (Dijkstra et al., 1985) and OX42 (Robinson et al., 1986).

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PII: S0165-5728(98)00076-9

Immunohistochemical measures were scored by two observers (Table 1).

2.3. Labeling of cells undergoing specialized cell death

Cells with fragmented DNA were labeled by terminal deoxynucleotidyl transferase (TdT) dUTP–biotin nick end labeling (TUNEL) (Gavrieli et al., 1992) using streptavidin–biotin–horseradish peroxidase and ABC-Elite kit (Vector Labs) with True-blue peroxidase substrate (Kirkegaard and Perry Laboratories).

2.4. Relative quantitation of cytokine mRNA in BD-rat brain

Rat IL1α, IL6, TNFα and IFNγ cDNAs clones were generated by oligo (dT) primed reverse transcription of total RNA from BD-rat brain and PCR using specific primers (Shankar et al., 1992). Other clones obtained as gifts were: rat IL4, IL2 (McKnight et al., 1989, 1991), cyclophilin (Danielson et al., 1988) and TGFβ-1 (Qian et al., 1990).

RNase protection assays (RPA) and Northern hybridization analyses were performed for detection of specific mRNA species in BD-rat brains. RNA was extracted from at least three rat brains at different times post-infection (0, 4, 5, 6, 10 and 15 weeks post-infection) (Chirgwin et al., 1979). RPAs (Hod, 1992) were performed using 10 μg of total rat brain RNA, 0.4 μg/ml RNase A and 8.3 units/ml RNase T1 (Ambion) with 0.5 fmole each [^{32}P]-labeled antisense RNA probe (5×10^5–9×10^5 cpm/ng for cytokines and 1×10^5 cpm/ng for cyclophilin). The relative abundance of each RNA was determined by normalizing for probe size and cyclophilin signal intensity. Northern

Table 1
Characterization of immune infiltrates in BD-rat brain

Antibody	Cell/Marker	Location	Normal	Pre-acute[a]	Acute[b]	Chronic[c]
OX-19[d]	T cells	Meninges	−	+	+ + +	+ +
		Vessels	−	+	+ + +	+ +
		Parenchyma	−	−	+	+
W3/25[d]	CD4 + T cells	Meninges	−	+	+ + +	+ +
		Vessels	−	+	+ + +	+ +
		Parenchyma	−	−	+ +	+
OX-8[d]	CD8 + T cells	Meninges	−	+	+ +	+
		Vessels	−	+	+ +	+
		Parenchyma	−	−	+ +	+ +
3.2.3[d]	NK cells	Meninges	−	+	+ +	+
		Vessels	−	+	+ +	+
		Parenchyma	−	−	+	+ +
ED2[d]	Macrophages	Meninges	−	+	+ + +	+ +
		Vessels	−	+	+ + +	+ +
		Parenchyma	−	−	+	+
I169[e]	MHC-I	Meninges	−	+	+ + +	+
		Vessels	−	+	+ + +	+
		Parenchyma	−	−	+ +	+ + +
OX-6[e]	MHC-II	Meninges	+	+	+ + +	+ + +
		Vessels	+	+	+ + +	+ + +
		Parenchyma	−	+	+ +	+ +
αIgG[e]	IgG	Meninges	−	−	+	+
		Vessels	−	+	+	+
		Parenchyma	−	−	+ +	+ + +
Igκ-mRNA[d]	B cells	Meninges	−	−	+	+
		Vessels	−	+	+	+ +
		Parenchyma	−	−	+	+ + +

[a] $n=4$, 3.5 weeks post-infection, prior to onset of clinical disease.
[b] $n=8$, 4–6 weeks post-infection.
[c] $n=6$, 10–15 weeks post-infection.
[d] BD-rat brain sections were analyzed immunohistochemically using antibodies to detect specific immune markers. Stained tissues were scored based on the number of cells per field that were positive. The number of positive cells was defined in the average of five randomly chosen 100× fields: + + +, > 30 cells; + +, 10–30 cells; and +, two to nine cells per field.
[e] Staining was rated as: + + +, intense/complete; + +, intense/restricted; and +, faint/limited.

hybridization analyses employed 10 μg samples of total rat brain RNA (Zeta probe, BioRad).

2.5. Relative quantitation of serum IgE

A sandwich ELISA was established to measure the relative abundance of IgE antibodies in serum samples. Microtiter wells were coated with immunoglobulin purified from goat anti-rat IgE serum (Bethyl Labs) using T gel (Pierce). The wells were then incubated sequentially with 1:1 serial dilutions of serum samples, biotin-conjugated monoclonal mouse anti-rat IgE antibodies (Caltag Labs), ABC-Elite solution (Vector Labs) and 3,3′-5,5′-tetramethylbenzidine (Sigma). Serum IgE titer was defined as the reciprocal serum dilution yielding an optical density of 0.05 at 450 nm.

3. Results

3.1. Characterization of CNS infiltration

Immunohistochemistry was performed on brains from BD-rats at different times post-infection (Table 1). T cells, NK cells and macrophages were first identified in perivascular and meningeal infiltrates at 3.5 weeks post-infection, before the onset of clinical disease (pre-acute). Perivascular and meningeal infiltrates in pre-acute and acute BD consisted of T cells (CD4 + T cells > CD8 + T cells), NK cells and macrophages. Numbers of T cells, macrophages and NK cells in the perivascular and meningeal infiltrates were reduced in chronic BD (Table 1). CD4 + and CD8 + T cells, NK cells and macrophages were present in the parenchyma in both acute and chronic BD (Table 1). These findings extend previous work published by Deschl et al. (1990).

3.2. Apoptosis of infiltrating cells

To explore the mechanism for loss of infiltrating cells between 4 and 15 weeks post-infection, TUNEL was performed to visualize cells undergoing specialized cell death (Gavrieli et al., 1992). During the time of most marked infiltration (4 weeks post-infection), labeling by TUNEL was minimal (Fig. 1A). However, by 6 weeks post-infection, TUNEL(+) cells were readily detected in perivascular, meningeal and parenchymal infiltrates (Fig. 1B). At 15 weeks post-infection, fewer cells in the perivascular infil-

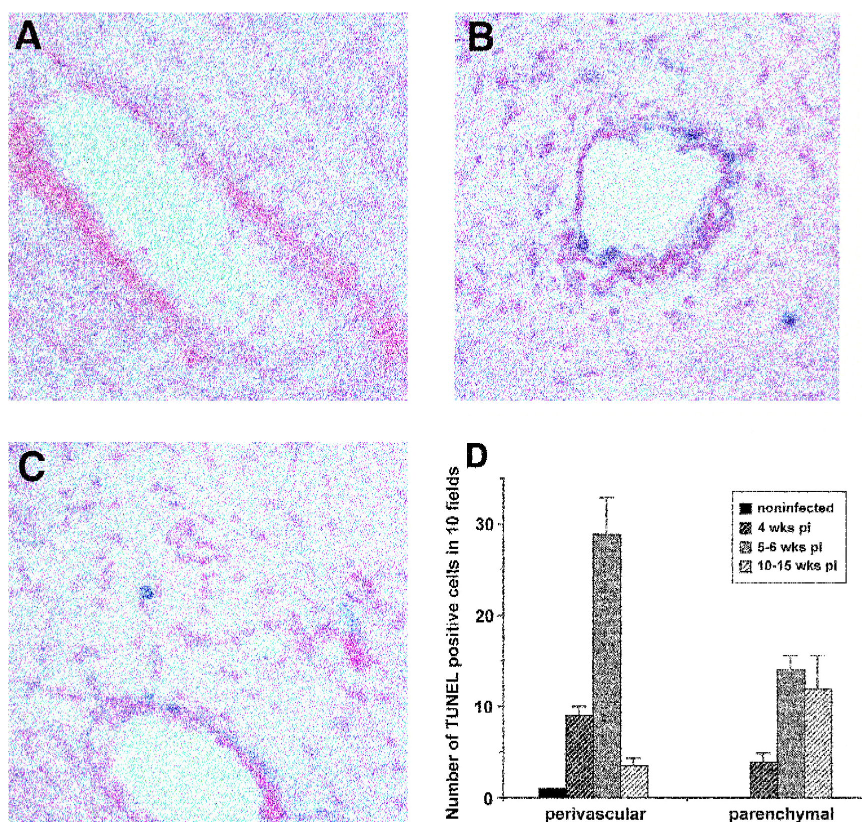

Fig. 1. Specialized cell death in the BD-rat brain detected by terminal deoxynucleotidyl transferase (TdT) dUTP–biotin nick end labeling (TUNEL). BD-rat brain sections showing labeling in parietal cortex from representative rats at 4 (A), 6 (B) and 15 (C) weeks post-infection were labeled using the method of TUNEL to detect DNA fragmentation. Nuclei of labeled cells are blue, tissue is counterstained with contrast red. Photographed at 100 × magnification. The total number of cells labeled by TUNEL in 10 randomly chosen fields from mid-sagittal brain sections was quantified at different times post-infection in the perivascular infiltrates and brain parenchyma (D). Error bars represent the S.E.M. ($n = 2$ for noninfected; $n = 3$ for 4 weeks post-infection; $n = 5$ for both 5–6 and 10–15 weeks post-infection).

trates and meninges were labeled by TUNEL (Fig. 1C). Fig. 1D indicates the number of TUNEL(+) cells at different times post-infection.

3.3. Changes in cytokine mRNA expression during BD

Cytokine mRNA expression was determined in brain samples from animals at different times post-infection (Fig. 2). Consistent with earlier reports (Shankar et al., 1992), in acute BD there was a surge in expression of mRNAs encoding the cytokines IL1α, IL2, IL6, TNFα and IFNγ (Fig. 2A). After peaking at 5 weeks post-infection, expression of these mRNAs declined over the following 10 weeks. TGFβ1 mRNA expression peaked at 5 weeks post-infection, but did not decrease substantially thereafter (Fig. 2B). IL4 mRNA expression increased from 4 to 5 weeks post-infection and continued to increase to 15 weeks post-infection (Fig. 2B).

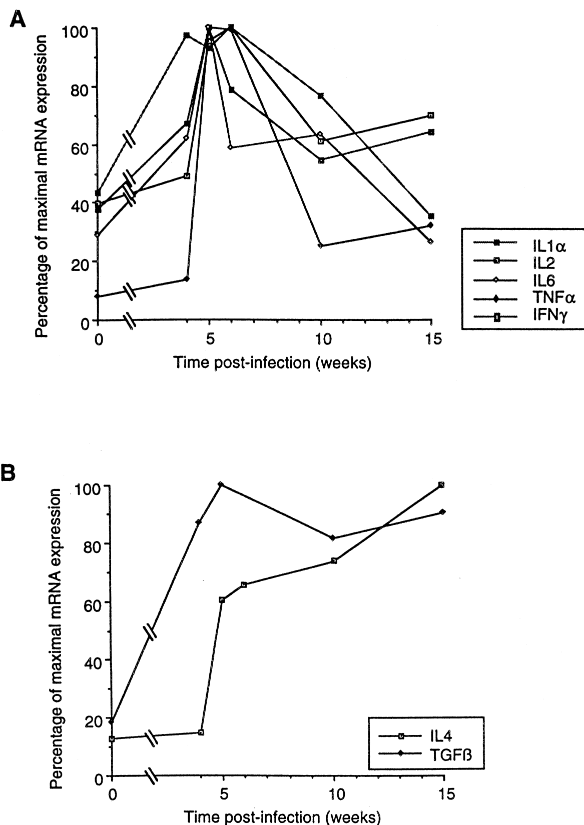

Fig. 2. Timecourse for expression of cytokine mRNAs in BD-rat brain. The levels of individual mRNAs were quantified by densitometric analysis of RNase protection assay or Northern hybridization products, normalization to cyclophilin mRNA signal intensity and compared as a percent of maximal expression. Pro-inflammatory cytokine mRNAs: IL1α, IL2, IL6, TNFα, and IFNγ (A); anti-inflammatory cytokine mRNAs: IL4 and TGFβ1 (B) are compared. For all experiments at least three rat brain RNA samples were analyzed per timepoint with the exception of 10 weeks post-infection at which time two samples were analyzed. The relative cytokine abundance in noninfected rat brains is represented at 0 weeks post-infection. Peak expression of each cytokine mRNA was significantly different from control values ($p < 0.05$).

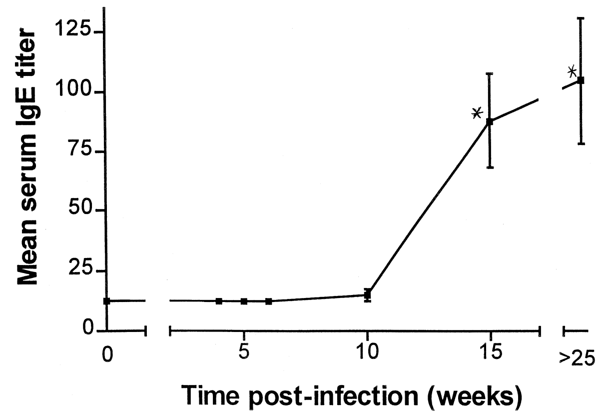

Fig. 3. Detection of IgE in serum of BD-rats. BD-rat sera from different times post-infection were assayed by enzyme-linked immunosorbent assay using antibodies directed against rat IgE. Each point symbolizes the mean of at least 4 samples with error bars designating the S.E.M. Noninfected IgE titer is represented at 0 week post-infection.

3.4. Changes in serum IgE isotype levels throughout the course of BD

IgE was not detected in the first 10 weeks following infection (serum IgE titer of infected animals equivalent to non-infected animals); however, by 15 weeks post-infection and after 25 weeks post-infection, nearly all rats had increased levels of IgE serum antibodies (Fig. 3).

4. Discussion

Regulation of the immune response to viral infections is likely to be an important mechanism for limiting immune-mediated destruction of tissue in the CNS. Here we have analyzed several parameters of the neuroimmune response to determine how alterations in this response over the course of disease may be important for limiting destruction of neural cells during persistent infection.

All cell types present in the perivascular and meningeal infiltrates declined in the chronic phase of disease (Table 1). One possible mechanism for the reduction in CNS immune infiltrates is cell death by apoptosis (Gavrieli et al., 1992). There is precedent for apoptosis-mediated regression of CNS inflammation in experimental allergic encephalomyelitis (Pender et al., 1991). The observation of TUNEL positive cells in perivascular cuffs suggests that this mechanism may be operative in BD.

The immune response observed in BD rats can be described as primarily cellular in the acute phase and humoral in the chronic phase. Such a shift from a cellular to a humoral immune response may be driven by cytokines produced by Th1 and Th2 CD4 + T lymphocytes, respectively. Levels of pro-inflammatory cytokine mRNAs were

highest in acute BD (Fig. 2A). CD4+ Th1 cells produce IFNγ, TNFα and IL2 and are responsible for recruitment and activation of CTL as well as stimulation of antigen presenting cells. The peak expression of these cytokine mRNAs in acute BD suggests the presence of Th1 cells in the CNS and is consistent with infiltration of immune cells into the CNS (Table 1). However, CD8+ T cells, NK cells and resident neural cells cannot be excluded as potential sources of cytokine mRNAs.

Cytokines which can down-regulate the cellular immune response include IL4 and TGFβ. TGFβ1 mRNA expression in the BD-rat brain was elevated in acute BD and remained high in chronic BD (Fig. 1B). In BD, increased levels of TGFβ may play a role in depressing CTL and the Th1 cellular immune response. IL4 mRNAs were present at highest levels in the chronic phase of BDV infection (Fig. 2B); concurrent with regression of CNS infiltration. IL4 is secreted by CD4+ Th2 cells and is consistent with increased numbers of B cells and enhanced antibody production in the CNS in the chronic phase of disease (Table 1).

The antibody isotype studies provide an additional line of evidence for a shift to a Th2 response during BDV infection. Progression into the chronic phase of disease was associated with an increase in IgE serum antibodies (Fig. 5). Immunoglobulin switching to the IgE isotype is triggered by IL4 and is an indicator of the Th2 immune response (Lebman and Coffman, 1988). Additionally, in the chronic phase of disease there was increased humoral immunity directed against BDV (Briese et al., 1995; Hatalski et al., 1995).

It is intriguing to speculate that this shift in the immune response between the acute and chronic phases of BD may be initiated as a protective measure to avert a potentially destructive immune response within the brain, an outcome that facilitates survival of both host and pathogen.

Acknowledgements

We are grateful for the oligonucleotides and probes provided by Dr. B. Dietzschold, Dr. A.N. Barclay, Dr. A.J. McKnight, and Dr. J.G. Sutcliffe. We thank Dr. W.E. Robinson Jr. and Ann Lewis for their valuable contributions. This work was supported in part by NS-29425 (CGH and WIL) and NS-27321 (WFH).

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