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# Early major histocompatibility complex (MHC) class I antigen induction in hypothalamic supraoptic and paraventricular nuclei in trypanosome-infected rats

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## Summary

Sprague-Dawley rats were injected intraperitoneally with a suspension of *Trypanosoma brucei brucei*. An early induction of major histocompatibility complex (MHC) class I antigens as well as an infiltration of macrophage-like cells and cytotoxic T-cells was detected with immunohistochemical techniques in circumventricular organs, such as the median eminence, neurohypophysis, subfornical organ, pineal gland and area postrema. These areas, which lack a blood–brain barrier, correspond to those showing early invasion of trypanosomes. In addition, there was a marked induction of MHC class I in neurons in two hypothalamic nuclei, the paraventricular and supraoptic nuclei. Neurons in these two nuclei are located behind the blood–brain barrier, but project to the neurohypophysis and to the median eminence, thereby exposing their axon terminals to factors circulating in the blood or released locally from invading trypanosomes or from macrophages or cytotoxic T-cells. It is suggested that the alteration in the nerve cell bodies in the hypothalamic nuclei is caused by retrograde axonal signals from these target areas.

## Introduction

The tissue distribution of major histocompatibility complex (MHC) transplantation antigens has been studied because of their crucial role in the interaction between T-cells and their targets (Doherty, 1985). In the central nervous system (CNS) none, or only low levels, of the MHC-coded

antigens are expressed (Lampson, 1987). Brisk induction of these antigens in the brain has been observed in vitro, however, on exposure to viruses and  $\gamma$ -interferon (Fierz et al., 1985; Massa et al., 1986; Suzumura et al., 1986) and in vivo during experimental autoimmune disorders (Fontana et al., 1987) and viral infections (Olsson et al., 1987). In the present study, we have examined the appearance of MHC antigens and subsets of inflammatory cells in the brain of rats infected with *Trypanosoma brucei brucei*. This extracellular hemoflagellate does not seem to penetrate the intact blood–brain barrier (BBB), but can be localized to

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areas in the nervous system which lack such a barrier, i.e. the choroid plexus, circumventricular organs and dorsal root ganglia (see Poltera et al., 1980; Schultzberg et al., 1988). Here we report that MHC class I is induced not only in these organs, but also in two hypothalamic nuclei, i.e. the paraventricular (PVN) and the supraoptic (SO) nuclei.

## Materials and methods

Ten male Sprague-Dawley rats, 6 weeks old (Alab, Stockholm, Sweden), were injected intraperitoneally with a suspension of *Trypanosoma brucei brucei*. The strain, variable antigen type AnTat 1/1, derived from stabilate EATRO 1125, was obtained as a generous gift from Dr. Nestor van Meirvenne, Laboratory of Serology, Institute of Tropical Medicine 'Prince Leopold', Antwerp, Belgium, and was passaged once in rats before use. Each animal was injected with 0.2 ml of a suspension of trypanosomes in a phosphate-saline-glucose buffer, pH 8.0, containing 3000–5000 parasites per  $\mu\text{l}$ . 13, 22 and 30 days after injection the rats were killed, the brains dissected and snap frozen. Three uninfected rats were used as controls. Cryostat sections (8  $\mu\text{m}$  thick) of the brain were fixed in cold acetone ( $-20^{\circ}\text{C}$ ) and processed for immunohistochemistry. Endogenous peroxidase activity was extinguished by treatment with 0.3%  $\text{H}_2\text{O}_2$  prior to incubation with antibodies. The following primary antibodies were used: Ox18, which labels MHC class I (Fukumoto et al., 1982); Ox6, which labels MHC class II (McMaster and Williams, 1979); Ox19, which is a pan T cell marker (rat CD5) (Dallman et al., 1984); Ox8, which labels suppressor/cytotoxic T-cells (rat CD8) (Brideau et al., 1980); W3/25, a marker for T-helper cells (Williams et al., 1977), and some macrophages (rat CD4) (Barclay, 1981). Ox18 was purchased from SeraLab (Crawley Down, U.K.). The other antibodies were purified from tissue culture supernatants of hybridomas obtained from Dr. Allan Williams, Oxford, U.K. (Holmdahl et al., 1985). After incubation with the primary antibodies at room temperature for 60 min, the sections were thoroughly rinsed and incubated for 30 min at room temperature with biotinylated horse

anti-mouse antibodies (Vector Lab., Burlingame, CA, U.S.A.) diluted 1:30 in 0.2% normal rat serum. The sections were then exposed to an avidin-biotin-peroxidase complex (ABC kit, Vector Lab.) for 30 min at room temperature and reacted for peroxidase with 3-amino-9-ethyl-carbazole as a substrate (Kaplow, 1975). The specificity of the staining and the optimal antibody concentrations to be used were tested on sections of lymphoid organs and brain. As controls, sections where the primary antibody was omitted, were used.

## Results

In brains from control rats there was some staining for MHC class I antigens in the leptomeninges and in the choroid plexus. Also the luminal surface of the endothelial cells in the intracerebral vessels showed immunoreactivity (Figs. 2B and 3B). In the circumventricular organs, i.e. the median eminence, the area postrema, the pineal gland, the subfornical organ and the neurohypophysis, there was a weak diffuse immunoreactivity in the tissue (Fig. 3B). The rest of the brain parenchyma showed no staining with MHC class I antibodies.

At 13 days after injection of the trypanosomes a very strong, diffusely distributed, staining with MHC class I antibodies was seen in the circumventricular organs. In addition, there was marked staining of the neurons in the PVN and SO (Figs. 1 and 2A), whereas none, or only a faint immunoreactivity was discerned in these areas in control rats (Fig. 2B). The immunoreactivity occurred in the cytoplasm of the nerve cell bodies (Fig. 2A). A strong immunoreaction for MHC class I was observed in the circumventricular organs also at 22 and 30 days after inoculation (Fig. 3A), and from the median eminence the immunoreactivity had extended dorsally into the surrounding neuropil. An intense staining was seen around the subfornical organ. The strong immunoreaction encountered in the two hypothalamic nuclei was evident also at these later time points, and could easily be seen even at the macroscopical level. In addition, there was a marked generalized increase in immunoreactivity in endo-

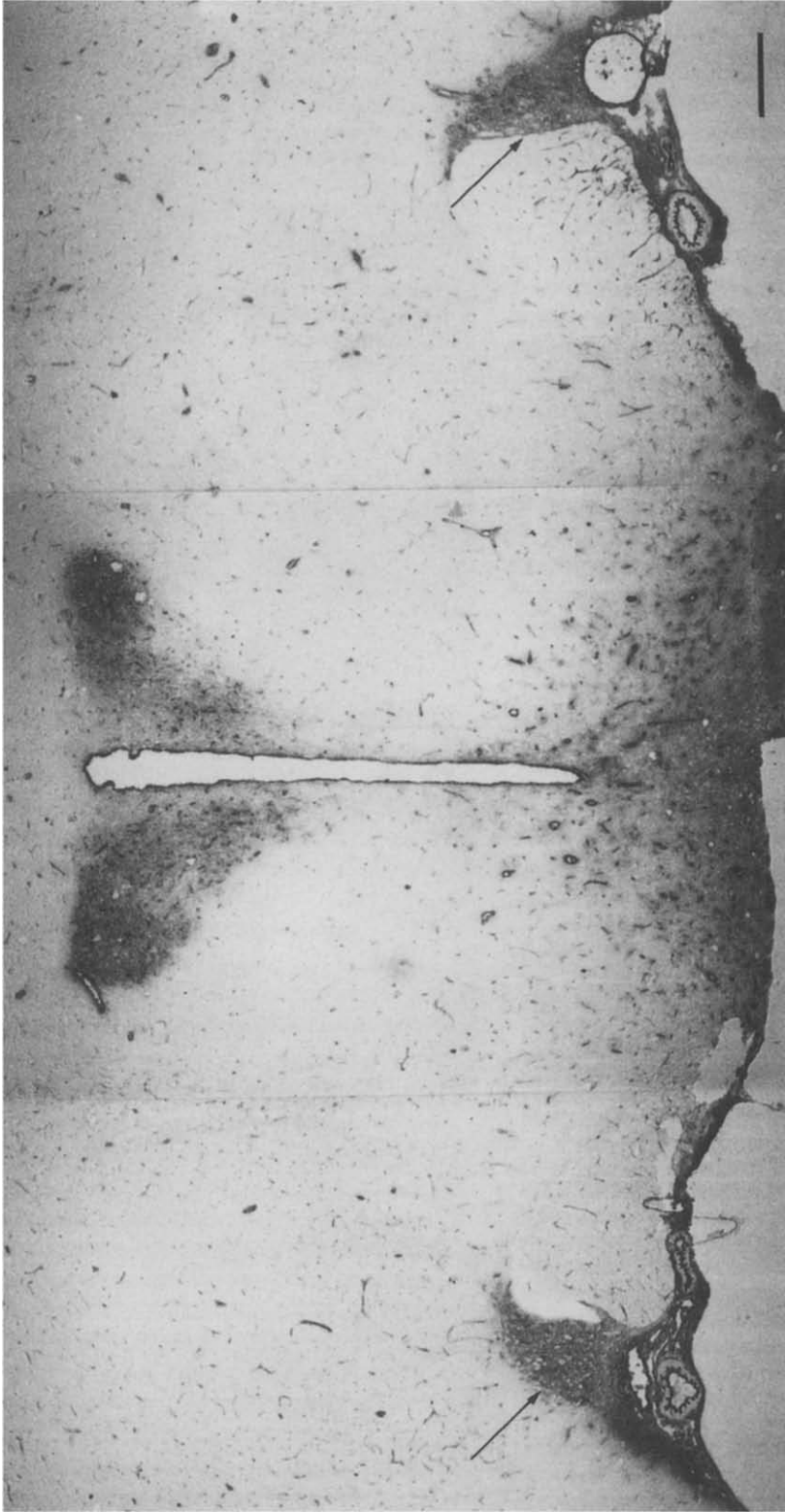
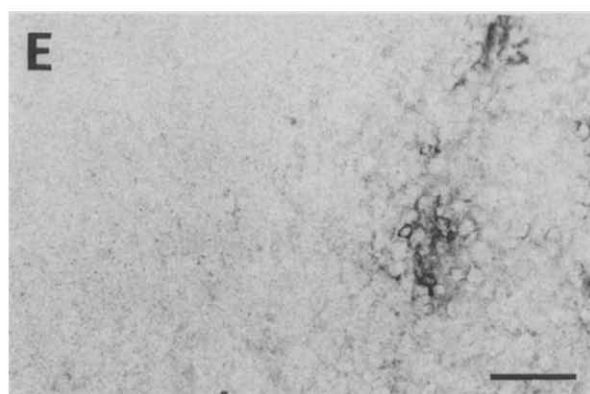
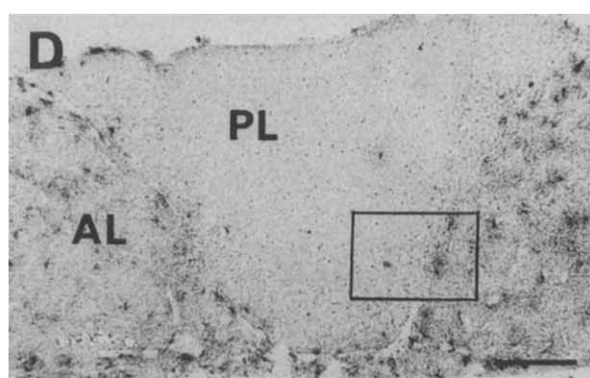
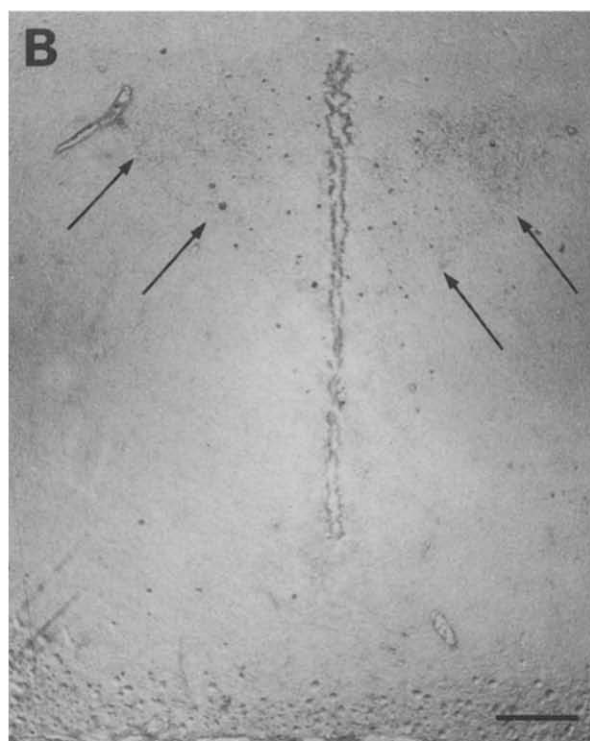
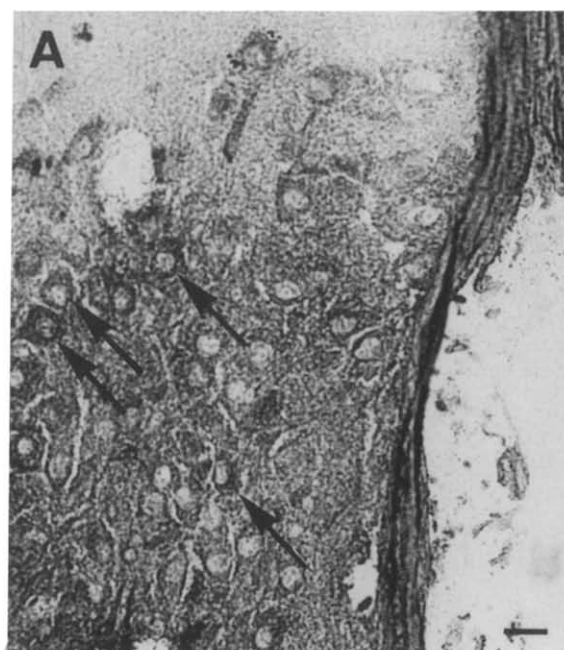


Fig. 1. Immunoperoxidase micrograph of a section of the rat hypothalamus at the level of the paraventricular (PVN) and supraoptic (SO) (arrows) nuclei, after incubation with Ox18 antiserum to MHC class I antigen. The rat was infected with trypanosomes (13 days post-infection). Note the intense MHC class I immunoreaction specifically localized to the PVN and SO. The expression of MHC class I antigen in the PVN from a control rat is shown in Fig. 2*B*. Staining is also observed in the ventral hypothalamus, a region where trypanosomes are found at early stages of the disease. Bar = 250  $\mu$ m.



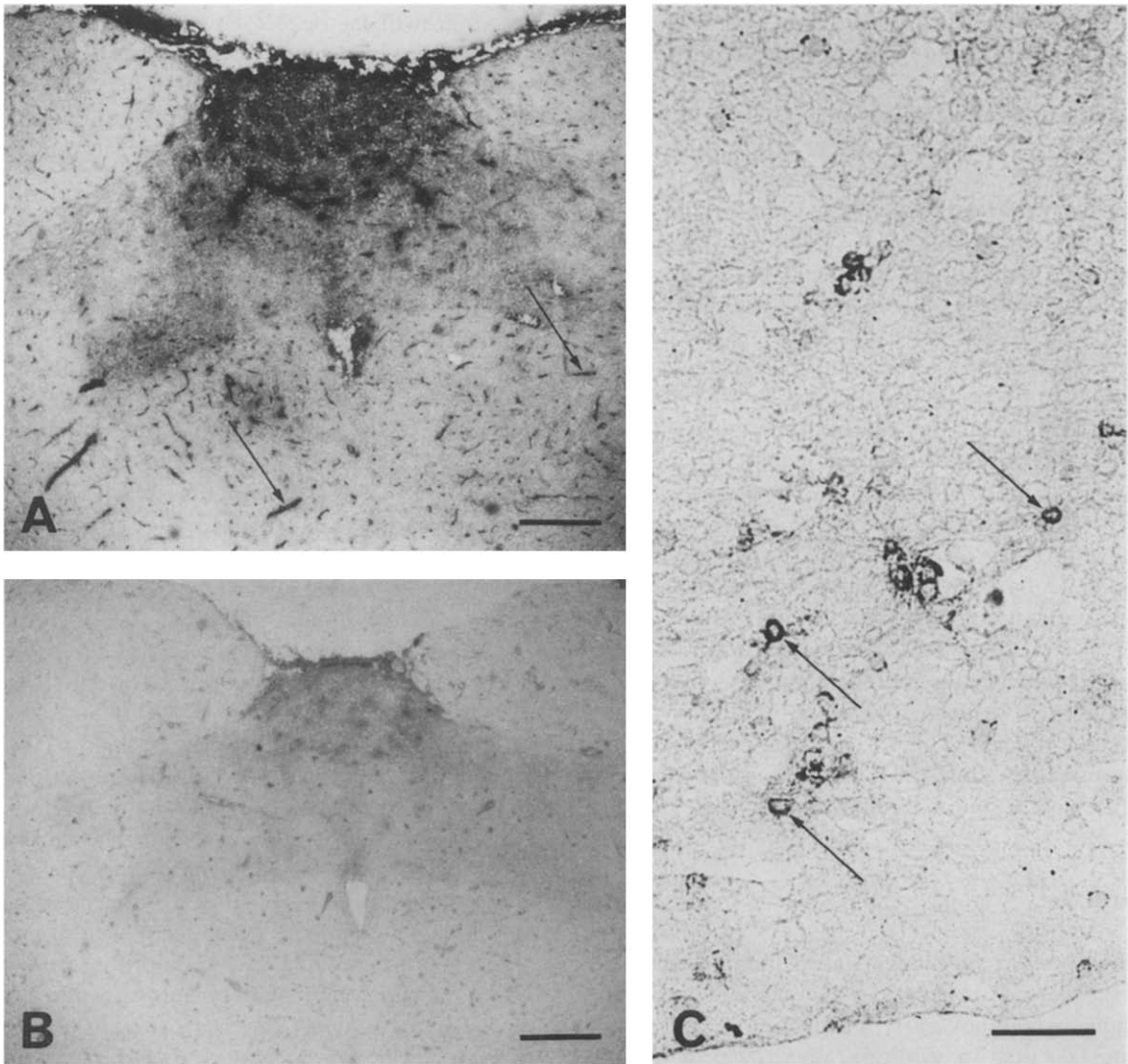


Fig. 3. Immunoperoxidase micrographs of sections of the rat area postrema (*A, B*) and neurohypophysis (*C*), after incubation with antisera to MHC class I antigen (*A, B*) and suppressor/cytotoxic T cells (CD8) (*C*). An intense MHC class I immunoreaction is observed in the area postrema (*A*) of the trypanosome-infected rat (30 days post-infection), as compared with the weak immunoreaction seen in the non-infected rat (*B*). The blood vessels of the infected rat also have a more intense class I immunoreaction (arrows in *A*). A large number of cells labelled with the antiserum for cytotoxic T cells can be seen in the neurohypophysis (arrows in *C*). Bars = 250  $\mu\text{m}$  (*A, B*) and 50  $\mu\text{m}$  (*C*).

Fig. 2. Immunoperoxidase micrographs of sections of the rat hypothalamus (*A, B*) and hypophysis (*C, D, E*), after incubation with antisera to MHC class I (*A, B*) and class II (*C, D, E*) antigens. *A* and *C* show tissue from trypanosome-infected rats (13 and 22 days post-infection, respectively), and *B, D* and *E* from control rats. An intense MHC class I immunoreaction is observed in the supraoptic (SO) nucleus (*A*), some of which is localized in neuronal cell bodies (arrows in *A*). Only a weak class I immunoreaction can be seen in the paraventricular nucleus (PVN) of the non-infected rat (arrows in *B*), and should be compared with the intense reaction observed in the PVN of a trypanosome-infected animal as shown in Fig. 1. A large number of perivascular cells with immunoreaction for MHC class II are found in the anterior lobe of the hypophysis (AL), whereas no or only few such cells can be seen in the neurohypophysis (posterior lobe; PL) (*D, E*) of the non-infected rat. However, several class II-immunoreactive cells (arrows in *C*), as well as a diffuse parenchymal immunoreaction, are evident in the neurohypophysis of the trypanosome-infected rat (13 days post-infection). The MHC class II staining in *C* should thus be compared to that seen in the neurohypophyseal part of *E*, which has the same magnification. *E* is a higher magnification of part of *D* (indicated by a rectangle). Bars indicate 250  $\mu\text{m}$  (*A, B, D*) and 50  $\mu\text{m}$  (*C, E*).

thelial cells both in intracerebral vessels and vessels in the leptomeninges and choroid plexus (Fig. 3A).

Antibodies against MHC class II antigens labelled only a few perivascular cells in the control brains. This was also the case for the circumventricular organs in these brains. After infection, the number of MHC class II-positive perivascular, macrophage-like cells had increased somewhat in the whole brain. The number of positive cells was especially large in the choroid plexus and in the circumventricular organs (Fig. 2C-E). The hypothalamic nuclei showed no more increase in the number of labelled macrophage-like cells than the rest of the brain parenchyma. There was no staining of neurons. Lymphocytes of the suppressor/cytotoxic phenotype had appeared in the choroid plexus and the circumventricular organs 13 days after inoculation (Fig. 3C). They were more numerous after 22 and 30 days, and had now also to a limited extent infiltrated the rest of the brain parenchyma. A few such lymphocytes occurred also in the two hypothalamic nuclei. The W3/25 antibody labelled macrophages similar to the O<sub>x</sub>6 antibody, but only an occasional lymphocytic cell.

## Discussion

The present study shows a distinct induction of MHC class I antigens in the circumventricular organs, where an early infiltration of trypanosomes occurs. In addition, there was a strong MHC class I reaction in the SO and PVN at an early stage in the disease. The neurons in these hypothalamic nuclei reside behind the BBB, but project outside this barrier, namely to the neurohypophysis and median eminence, both of which are circumventricular organs. It has already been shown that, after a nerve injury, MHC class I is induced on motor neurons (Maehlen et al., 1988). This induction may be due to signals carried to the nerve cell body by retrograde axonal transport, as has been discussed for the chromatolytic response after axotomy (Kristensson, 1984). The nature of such signals is unknown, but different neurotrophic factors and cytokines may be operative. Target-derived neurotrophic factors such as

nerve growth factor (NGF) have been implicated in the maintenance of neuronal integrity (Thoenen and Barde, 1980). A neurotrophic factor-mediated regulation might be disturbed by the inflammatory process in various ways, e.g. through the action of cytokines such as tumour necrosis factor (TNF) or interleukin-1 (IL-1) (Lindholm et al., 1987); indeed, IL-1 has recently been demonstrated in peripheral nerves (Schultzberg et al., 1987). Cytokines are known to be potent MHC antigen inducers *in vitro* (Hirsch et al., 1983; Wong et al., 1984; Fierz et al., 1985; Lavi et al., 1987; Massa et al., 1987). Both TNF and IL-1 are increased in the blood during trypanosome infections (Askonas and Bancroft, 1984; Le and Vilček, 1987), and these substances may reach the projections of the paraventricular and supraoptic neurons outside the BBB. Furthermore, infiltrating lymphocytes and macrophages in the circumventricular organs could be a local source of cytokines (Kriegler et al., 1988). Substances originating from invading trypanosomes should also be considered.

The biological significance, if any, of MHC class I induction in the hypothalamic neurons is not known. It may represent an increased immune responsiveness, as a defence mechanism during an infectious process, or could be due to one of the other functions of MHC class I antigens, such as in cellular interactions, which have been discussed (Dausset and Contu, 1980). In addition, it may be noted that the hypothalamic neurons, where MHC class I induction was observed, contain a number of neuroendocrine hormones, including oxytocin, vasopressin, corticotropin-releasing factor (CRF), cholecystokinin, dynorphin, angiotensin II, glucagon, luteinizing hormone-releasing hormone (LHRH), thyrotropin-releasing hormone (TRH), somatostatin and neurotensin (Silverman and Pickard, 1983; Armstrong, 1985). Disturbances in these substances may explain some of the symptoms observed in trypanosomiasis. It has been suggested that the immune system serves a sensory function by recognizing foreign antigens and, in response, releasing molecules (cytokines) to which the nervous system may react (Blalock, 1984); for example, systemic administration of recombinant IL-1 may cause a release of CRF from the hypothalamus (Berkenbosch et al., 1987). The role of

MHC class I molecules in these events needs further analysis.

In conclusion, we have shown that trypanosome infection causes the induction of MHC class I antigens in the PVN and SO, possibly due to inflammatory substances in the target areas producing retrograde axonal signals.

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