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Sleeping sickness and the central nervous system*

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ABSTRACT

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Chronic African trypanosomiasis is associated with progressive behavioural deficits, for which there is a complex underlying central nervous system (CNS) pathology. This has been extensively studied in man and a range of experimental animals. An initial meningitis, which can occur quite early in the infection, is followed by a breakdown of the choroid plexus, movement of the parasite into certain localized brain areas, and subsequent encephalitis. The encephalitis consists of a chronic, widespread inflammation with perivascular infiltrations of B-cells, plasma cells, inactivated T-cells and macrophages. The blood-brain barrier is damaged and a vasogenic oedema ensues. Astrocytes and microglia become reactive and the cytokine/mediator network is perturbed. The alterations in some of these signalling substances, e.g. the prostaglandins, may induce some of the behavioural changes, e.g. the hypersomnia. The immunopathology in the CNS may be brought about by elevated levels of active substances in the cerebrospinal fluid, caused by parasite infection.

INTRODUCTION

Several species and strains of African trypanosomes affect the central nervous system (CNS). These belong principally to the *Trypanosoma brucei* group, which leaves the circulatory system and penetrates the extracellular spaces in the host tissues. Man is affected by *T. b. rhodesiense* and *T. b. gambiense*. The chronic Gambian form produces the classic neurological manifestations of sleeping sickness, with progressive mental deterioration, reversed sleep pattern, and eventually permanent sleep and coma (Apted 1970). Signs of CNS involvement have only rarely been documented for cattle and other animals in the field (Wellde, Reardon, Chumo, Kovatch, Waema, Wykoff, Mwangi, Boyce & Williams 1989), although this may be due to a lack of appropriate behavioural studies. However, the sleeping sickness state develops in a range of experimental infections in primates and rodents with *T. b. rhodesiense, T. b. gambiense* and *T. b. brucei*, and in cattle also with mixed infections of *T. b. brucei* and *T. congolense* (Masake, Nantulya, Akol & Musoke 1984). Some of these infections, e.g. in rodents, monkeys and goats, have been extensively studied in relation to the changes affecting the nervous system (Jennings & Gray 1983; Schmidt 1983; Mutayoba, Meyer, Osaso & Gombe 1989).

Much effort has been made to elucidate the changes which underline the neurological manifestations. The majority of studies have been made on the animal models. Different medical and experimental aspects of sleeping sickness and the nervous system were summarized by Apted (1970), Greenwood & Whittle (1980), Pentreath (1989) and Hunter & Kennedy

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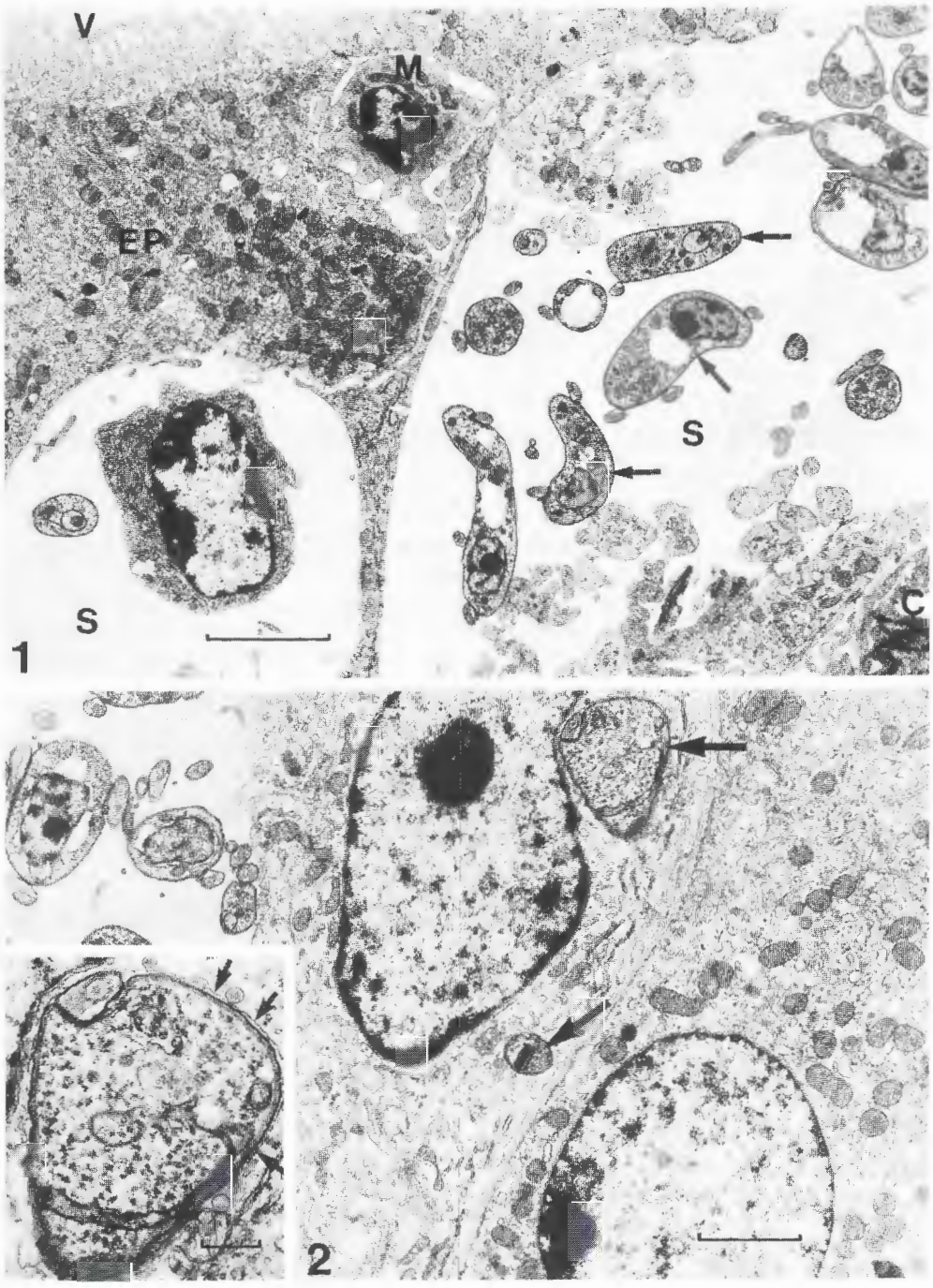

FIG. 1 Electron micrograph of the choroid plexus (third ventricle; V) of mouse 20 d p.i. with *Trypanosoma brucei brucei*. The tissue was preserved by perfusion with 2 % glutaraldehyde, post-fixation in 1 % osmium tetroxide and embedding in Araldite. Large numbers of parasite (some arrowed) occupy the stromal space (S) which has become expanded and vacuolated, between the capillaries (c) and choroid epithelium (EP). The epithelium, normally continuous, is at this point being maintained by a macrophage (M). The scale is 2 μm

FIG. 2 Electron micrograph of the choroid plexus, from the same tissue as described in Fig. 1. This micrograph shows parasites occupying intracellular locations within the stromal cells (arrows). The inset shows details of this. The trypanosome is partially enclosed by membranes belonging to the stromal cell (small arrows). The significance of the penetration of the cells by the parasite is not yet clear, but it is interpreted here as a prelude to cell damage. The scale is 2 μm; inset 0,5 μm

(1992). More recently, comparative accounts of the nervous system in African and South American trypanosomosis have been made (Villanueva 1993; Pentreath 1994b). The purpose of this paper is to discuss certain areas and recent developments which may point the way in understanding the CNS involvement in African sleeping sickness.

STRUCTURAL AND INFLAMMATORY CHANGES

The different strains of *T. brucei* occupy the meninges fairly soon after infection, producing the meningitis. A key stage in the pathology is the injury to the choroid plexus, with trypanosomes filling the stromal spaces and with obvious evidence of damage to the epithelium. This has been noted in several studies with *T. b. gambiense* and *T. b. rhodesiense* in rodents and primates (Van Marck, Le Ray, Beckers, Jacob, Wery & Gigase 1981; Rudin, Poltera & Jenni 1983; Schmidt 1983). Some features of the damage are illustrated in Fig. 1 and 2, which are from our unpublished studies with *T. b. brucei* in mice. The parasites have access to the cerebrospinal fluid (CSF) and, subsequently, via the sub-arachnoidal spaces, to the perivascular extensions (Virchow-Robin spaces) which extend into the brain. It seems likely that this is a determinant in the progression from meningitis to encephalitis, with the development of the perivascular infiltrations (cuffing) in the brain (Greenwood & Whittle 1980; Schmidt 1983; Adams, Haller, Boa, Doua, Dago & Konian 1986). Human CSF is not, however, a very favourable medium for parasite survival (Pentreath, Owolabi & Doua 1992). The infiltrations in the meninges and perivascular cuffs contain large numbers of B-lymphocytes and plasma cells, morular cells (Mott cells) and macrophages (Greenwood & Whittle 1980).

Of particular significance is the hyperplasia and reactive changes in the astrocytes and microglial cells, also associated with the cuffs in both man (Adams *et al.* 1986) and rodents (Stevens & Moulton 1977), which are discussed further below.

The parasite may enter the CNS parenchyma. This does not appear to be a widespread influx, comparable to that in lymphoid or connective tissue, except in some terminal disease states. In the rodent model, trypanosomes selectively enter areas with a reduced blood-brain barrier (e.g. pineal, area postrema and circumventricular organs) at relatively early stages of the infection and it has been suggested that this may lead to the disturbed rhythms and neuro-endocrine dysfunctions (Schultzberg, Ambatsis, Samuelsson, Kristensson & Van Meirvenne 1988). The relationship between the extravascular spread of parasites and the reduced blood-brain barrier might be due to access of signalling substances which promote parasite entry and growth, rather than easier physical access. A likely substance is interferon-γ (IFN-γ), which has been shown to enhance growth of *T. b. brucei* in lymphoid tissue and possibly in dorsal root ganglia (Bakheit 1993), and which may be released from $CD8^+$ T-cells which infiltrate the same brain areas (Schultzberg, Olsson, Samuelsson, Maehlen & Kristensson 1989).

The pituitary is also damaged by the parasites. In sheep and rodents, for example, *T. b. brucei* causes extensive damage to the microvasculature and penetrates extravascularly (Ikede & Losos 1975; Schultzberg *et al.* 1988). *T. congolense* causes focal degenerative changes in the pituitary of cattle, but in contrast to *T. brucei*, does not penetrate the nerve tissue (Abebe, Shaw & Eley 1993).

Recent studies with the rodent model and *T. b. brucei* have additionally shown that there is progressive damage to the blood-brain barrier. Tracer substances, which in normal rats cannot cross the barrier, can pass freely to many brain areas in the late stages of the disease. At the same time, there are progressive decreases in brain-density, increases in brain-sodium and some reduction in brain-potassium levels which show that widespread vasogenic oedema is occurring (Philip, Dascombe, Fraser & Pentreath 1994, unpublished data).

Thus, the data from a number of studies made chiefly on experimental animal models, show that African trypanosomosis causes multiple pathological changes in the structure of the CNS. These descriptions provide some insights into the inflammatory changes in the nerve tissue, which accompany the disease. However, for an understanding of the mechanisms underlying the neurological manifestations, a knowledge of the immune changes in the CNS must also be obtained.

IMMUNE CHANGES

Although the mammalian CNS was traditionally considered an "immune privileged" site, this view is rapidly changing, since it is now appreciated that the tissue can mount effective immune responses (Benveniste 1993). These may frequently be of a suppressive nature, presumably to protect against inflammatory damage. However, there is evidence that a number of infections and disease states (e.g. viral infections and multiple sclerosis) may induce immunopathological changes, with, e.g., over-production of cytokines which, in turn, may lead to aggravated pathology (Benveniste 1993). There is, moreover, much to be learnt about the general nature of the immune changes in nerve tissue, and this restricts the interpretation for sleeping sickness.

A striking situation regarding the brain immunopathology concerns the post-treatment encephalopathy

which occurs in approximately 5 % of late-stage patients treated with melarsoprol. In these cases, there appears to be a violent inflammatory response in the CNS, leading to convulsions, coma and death a few days following treatment. This reaction could be due to the massive release of antigenic material from the parasites killed inside the brain attaching to the brain cells, which become a target for the immune response (Pepin & Milord 1991). Alternatively, the response may be due to the incomplete killing of trypanosomes behind the blood-brain barrier, which then provoke the reactive encephalopathy (Jennings, Hunter, Kennedy & Murray 1993).

Neuro-immune changes are an obvious feature during the acute and chronic infections and studies have been aimed at unravelling these. The cellular changes already summarized above, include perivascular cuffing with B-cells (mainly plasma cells), morular cells, activated T-cells, macrophages and reactive astrocytes and microglia. In the areas of reduced blood-brain barrier which are selectively penetrated by trypanosomes, there may be induction of major histocompatability complex (MHC) class I antigen expression associated with the infiltration of macrophages and T-cells (Schultzberg *et al.* 1989). Other aspects of the immunopathology have recently been discussed by Hunter & Kennedy (1992), and will be only briefly mentioned here. Firstly, immune complexes between trypanosome antigens and antibodies could be deposited in the CNS, leading to complement activation and vascular damage (Poltera 1980; Lambert, Berney & Kazyumba 1981). This suggestion has not, however, been supported by others (see Hunter & Kennedy 1992). Secondly, auto-antibodies may be generated against a range of CNS tissue structures in humans and animals. These include myelin components and gangliosides (Asonganyi, Lando & Ngu 1989; Amevigbe, Jauberteau-Marchan, Bouteille, Doua, Breton, Nicolas & Dumas 1992; Hunter, Jennings, Tierney, Murray & Kennedy 1992b), as well as antibodies against the neurons in different brain regions (Poltera 1980). The antibodies against myelin may be associated with the demyelination observed in some late-stage cases (Dumas & Boa 1988). However, caution must again be exercised, since the auto-antibodies may be the result of the damage caused by the parasites, rather than its cause.

A new area of growing importance concerns the neuroglial cells with immune accessory functions, in particular the astrocytes. These cells may help coordinate immune responses in the CNS via the cytokine/mediator network. Some aspects of their responses in trypanosomiasis are discussed further.

Roles of astrocytes

Astrocytes make up approximately half the volume of grey matter in the CNS and may outnumber the neurons. The cells are multifunctional in ways which are generally supportive or protective of the neurons. If the CNS is damaged by physical, microbial or chemical insults, astrocytes may respond by a setback of reactions called reactive gliosis. This consists of a complex, graded alteration in phenotype with a large range of structural and metabolic alterations (Eddleston & Mucke 1993). Although the significance of many of the reactive changes are at present a mystery, they include altered expression and secretion (generally increased) in a range of growth factors, cytokines and mediator substances, and altered cell contact/recognition properties (e.g. increased MHC class I and class II expression), which have immune-accessory properties (Hertz, McFarlin & Waksman 1990). The astrocytes act in harmony with the endothelial cells, microglia and lymphocytes to regulate the inflammatory responses in nerve tissue.

It is well known that reactive astrogliosis is a hallmark of the chronic inflammation which accompanies African sleeping sickness (Stevens & Moulton 1977; Adams *et al.* 1986; Anthoons, Van Marck, Gigase & Stevens 1989), and it was suggested that the astrocytes might control the CNS inflammatory responses (Pentreath 1989). Studies with mice have shown that astrocyte activation can precede the chronic inflammatory changes and that there is increased expression in the brain (presumed partly in astrocytes) of mRNA for several cytokines which can activate different components of the immune response (Hunter, Gow, Kennedy, Jennings & Murray 1991; Hunter, Jennings, Kennedy & Murray 1992a). These are summarized in Table 1. In relation to these findings it is important to note that difluoromethylornithine

TABLE 1 Cytokine mRNA in brains of mice infected with *Trypanosoma brucei brucei*

Cytokine	Control	Day 21 p.i.	Post-treatment encephalitis
Interleukin-1 α*	+	+	++
Interleukin-2	–	–	–
Interleukin-4	–	–	+
Interleukin-6*	–	+	–
Interferon-γ	–	+	–
Macrophage inflammatory protein-1	–	+	+
Tumour necrosis factor-α*	–	+	++

* Cytokines produced by astrocytes. Data from Hunter *et al.* (1991; 1992a)
\+ Significant increase
– Significant decrease

(DFMO), a successful drug for the treatment of late-stage *T. b. gambiense*, which can produce rapid improvement of the neurological syndrome, has been shown to reduce the astrogliosis caused by some forms of mechanical damage to the brain (Zoli, Zini, Grimaldi, Biagini & Agnati 1993).

Attempts have been made to analyse some of the cellular responses to trypanosomes in culture. Because of the importance of prostaglandins (PG^s) in regulating the cytokine network, and in controlling sleep patterns (Hayaishi 1991), a study was made of their production by mouse fibroblasts and astrocytes *in vitro* (Alafiatayo, Cookson & Pentreath 1994). Disrupted *T. b. brucei* components caused strong stimulation of PGD_2 and PGE_2 production by both cell types, similar to the effects of bacterial endotoxin. The parasite components and endotoxin together, caused further increases in prostaglandin production. Since previous studies of ours had shown that levels of PGD_2, a potent sleep-inducing substance (Hayaishi 1991), were selectively elevated in the CSF of late-stage sleeping sickness patients, it was suggested that they might have been derived from astrocytes activated by the parasite material (Alafiatayo *et al.* 1994).

Mediator substances and neurotransmitters in the brain and CSF

The levels of mediator substances in the brain and CSF can provide a measure of metabolic, disease and immune changes within the CNS. This is also relevant for pyrogenic, somnogenic and disordered states such as occurs in sleeping sickness. The catecholamine- and indolealkylamine-transmitter substances have been investigated in rodent brain because of their well known involvements in the nervous control of sleep mechanisms (Stibbs & Curtis 1987; Amole, Sharpless, Wittner & Tanowitz 1989). The studies, summarized in Table 2, strongly suggest an increased turnover of the catecholamines, dopamine and noradrenaline (because of the increased levels of the breakdown product homovanillic acid), and 5-hydroxytryptamine (increased catabolite 5-hydroxyindoleacetic acid) in several brain regions. No significant alterations in acetylcholine or associated metabolites were detected.

TABLE 2 Alterations in monoamine transmitters and their metabolites in mice with chronic *Trypanosoma brucei brucei* infections

Brain region	Dopamine	Noradrenaline	Homovanillic acid	5-Hydroxytryptamine	5-Hydroxyindoleacetic acid
Cerebellum		↑			
Brainstem	↑	↑		↓	↑
Hypothalamus				↑	↑
Striatum		↑	↑		
Hippocampus		↑	↑		
Cortex				↓	↑

The arrows indicate increases or decreases relative to uninfected (control) animals. [Summarized from Stibbs & Curtis (1987); Amole *et al.* (1989)]

The increases in cytokine mRNA in the brains of infected mice, summarized in Table 1, might be associated with immunopathology and behavioural changes. Overproduction of tumour necrosis factor-α (TNF-α), for instance, can produce inflammatory damage, and elevated interleukin-1 (IL-1) induces sleep and fever. Unfortunately, experimental evidence is not yet available to determine whether the increases in mRNA are associated with increased release of these substances into the extracellular spaces and CSF. In a study of the CSF of late-stage patients from the Cote d'Ivoire, no elevation of IL-1 was detected (Pentreath, Rees, Owolabi, Philip & Doua 1990). However, in the same group of patients there were some very large increases in PGD_2, and it was proposed that this could account for the hypersomnia (Pentreath *et al.* 1990; Hayaishi 1991). This prostaglandin has also been shown to have important roles in immunosuppression (Goodwin & Webb 1980), suppression of luteinizing hormone release (Kinoshita, Nakai, Katakami, Imura, Shimizu & Hayaishi 1982) and hyperalgesia (Malmberg & Yaksh 1992), which are also major symptoms of sleeping sickness.

We have recently extended the studies of prostaglandins in the CSF of sleeping sickness patients to analysis with gas chromatography/mass spectroscopy (GC-MS). This is important because it provides very accurate chemical identification and quantification compared to our previous studies in which radioimmunoassay techniques were used (Pentreath *et al.* 1990). This is an ongoing study for which the methodology is relatively time-consuming, and to date we have obtained results for samples from six patients at the Projet de Recherches Cliniques sur le Trypanosomiase (P.R.C.T.) Daloa, collected as described previously (Pentreath *et al.* 1990). The patients were diagnosed as having CNS involvement according to the criteria of CSF protein > 40 mg% and/or white-blood-cell count > 5/mm^3, and/or the presence of trypanosomes (Table 3). The patients were treated with Arsobal and further CSF samples were taken 4–6

TABLE 3 Prostaglandin levels in the CSF of late-stage *Trypanosoma brucei gambiense* patients

Patient no.	Pre-treatment						Post-treatment					
	Protein (mg%)	Wbc count (cells/mm^3)	Trypan-osoma (45 µℓ CSF)	PGD_2 (ng/mℓ)	PGE_2 (ng/mℓ)	$PGF_{2\alpha}$ (ng/mℓ)	Pro-tein (mg%)	Wbc count (cells/mm^3)	Trypan-osoma (45 µℓ CSF)	PGD_2 (ng/mℓ)	PGE_2 (ng/mℓ)	$PGF_{2\alpha}$ (ng/mℓ)
1729	41	1332	40	–	12,0	388,0	33	36	0	–	12,0	204,0
1730	36	276	3	–	–	119,0	28	38	0	–	–	47,0
1731	48	686	0	195,0	104,0	97,0	26	118	0	46,0	6,9	51,0
1732	33	1138	7	11,0	6,3	44,0	21	30	0	7,6	3,2	21,0
1733	39	656	800	34,0	3,8	40,0	22	28	0	–	–	39,0
1734	52	220	10	2,3	–	5,0	45	68	0	1,6	–	4,3

– not available

weeks later. Prostaglandins were analysed by GC-MS according to the established methods (Barrow & Taylor 1987). The results are summarized in Table 3. PGD_2 and PGE_2 levels in several patients were markedly elevated, with a maximum value of 195 ng/mℓ for PGD_2. The values fell following chemotherapy, but were still high. In health, these prostaglandins may be present in CSF in concentrations of tens of pg/mℓ, or be absent, whereas the present values were ng/mℓ. We also measured $PGF_{2\alpha}$. Less is known, from a functional point of view, about this closely related prostaglandin. Increased levels may be associated with increased metabolism of other prostaglandins. It is markedly increased in the plasma of rats infected with *T. congolense*, and this may account for the suppression of *corpus luteum* function (Mutayoba *et al.* 1989). The values in the CSF of all the sleeping sickness patients were also markedly elevated (pre-treatment range 5,0–388 ng/mℓ) and reduced after chemotherapy (range 4,3–204 ng/mℓ). Again in health, values of the substance are a few pg/mℓ CSF. These studies, although on a small patient sample, provide further strength to the prostaglandins being seriously perturbed by the chronic inflammation in the CNS during late-stage sleeping sickness.

Another area attracting increasing attention, is the interactions between the mediator substances and the trypanosomes. IFN-γ acts as a growth-stimulatory substance for *T. b. brucei*, which may explain the preferential homing of the parasite in the lymphoid tissue (Bakhiet 1993) and the sensory ganglia (Eneroth, Bakhiet, Olsson & Kristensson 1992). There is also indirect evidence that prostaglandins may promote division (Jack, Black, Reed & Davis 1984). In contrast, nitric oxide (NO) and TNF-α are toxic to the parasites (Lucas, Magez, Songa, Darji, Hamers & De Baetselier 1993; Lucas, Magez, De Leys, Fransen, Scheerlinck, Rampelberg, Sablon & De Baetselier 1994; Vincendeau, Daulouede, Veyret, Darde, Bouteille & Lemesre 1992). These features are summarized in Table 4.

TABLE 4 Mediators affecting growth of *Trypanosoma brucei brucei*

Mediator	Effect
Interferon-γ	Promote division
Prostaglandins	Promote division
Nitric oxide	Trypanostatic
Tumour necrosis factor-α	Trypanostatic

Data from Jack *et al.* (1984), Vincendeau *et al.* (1992), Bakhiet (1993), Lucas *et al.* (1993; 1994)

TABLE 5 Trypanosome products implicated in initiating the immune and pathological changes within the central nervous system

Trypanosome products
Variable antigenic coat
Phospholipase A
Proteases (especially cysteine)
Saturated fatty acids (B cell mitogens)
Aromatic amino acid derivatives (e.g. tryptophol)
Endotoxin-like substances*

* Data sources summarized in Tizard *et al.* (1978) and Pentreath (1994a)

Trypanosome products causing pathology

An area of longstanding enquiry is the nature of the biologically active products from African trypanosomes which are responsible for the damage to the host. The studies reviewed by Tizard, Nielsen, Seed & Hall (1978) were undertaken with a view to understanding the inflammation and pathology in a range

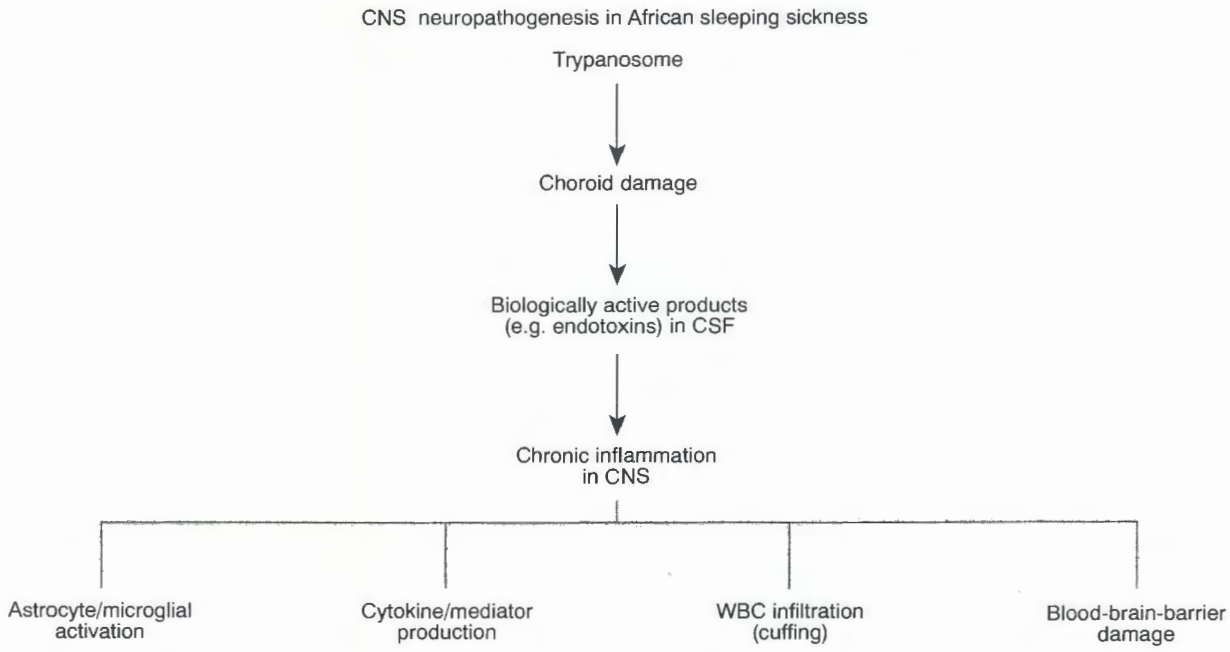

FIG. 3 Suggested sequence of events in the pathogenesis of sleeping sickness in the central nervous system

of blood and other peripheral tissues, but such substances must also be important for initiating the immune and pathological changes within the CNS. Some of the different types of trypanosome products which have been implicated are listed in Table 5.

Many of the clinical and laboratory criteria are similar in trypanosomosis and endotoxaemia. These include the polyclonal enhancement and subsequent immunosuppression, the hypergammaglobulinaemia, complement and kinin activation, and also the fever, headache and hypersomnia. It has been suggested that such aspects of the pathology in sleeping sickness may be due to the presence of non-specific endotoxin-like substances (Greenwood 1974). We therefore studied the levels of the endotoxins in the sera of mice infected with *T. b. brucei*, using the *Limulus* amoebocyte lysate (LAL) test (Alafiatayo, Crawley, Oppenheim & Pentreath 1993). This showed that serum endotoxin levels were elevated during infection, and that some parasite components also contained endotoxin-like activity. Intercurrent bacterial infections were a common feature of the infected animals. It was suggested that the raised endotoxins might be derived from multiple sources, including the parasite, the intercurrent infections, or intestinal and hepatic damage (Alafiatayo *et al*. 1993; Pentreath 1994a).

We have recently extended these endotoxin measurements to the serum and CSF of late-stage patients at the P.R.C.T., Daloa. Our findings (Pentreath, Alafiatayo, Crawley & Oppenheim 1994, unpublished data) have shown that the endotoxin levels are markedly elevated in both fluid compartments, with a close correlation in the raised levels between the two compartments.

CONCLUSIONS

The complex pathology in the CNS of man and animals with chronic African trypanosomosis has multiple components. The mechanisms underlying the changes are starting to be unravelled, in conjunction with the growing understanding of neuro-immune mechanisms and the roles of neuroglial cells. Although much of the neuropathogenesis remains a mystery, several emerging features have prompted us to present the following hypothesis (Fig. 3). In this the breaching of the choroid by the trypanosomes is an important event, because the parasites will then have access to the CSF and Virchow-Robin spaces. With the progressive choroid damage, parasite products, endotoxins and endotoxin-like substances, together with elevated concentrations of cytokine/mediator substances, will accumulate in the CSF. This will be due, in part, to equilibration with the plasma. Relatively small numbers of parasites penetrate the parenchyma, except in the very late and terminal disease stages. The biologically active substances circulating in the CSF will have access, via the junctions between the ependymal cells and the Virchow-Robin spaces, to the parenchyma. These substances, in particular the endotoxin-like substances,

induce astrocyte and microglial activation. This, in turn, leads to the chronic inflammatory state, with lymphocyte/macrophage infiltration, and damage to the blood-brain barrier. The alterations in the cytokine/mediator levels in the brain underly the changes in behaviour with, e.g. hypersomnia induced by the elevated prostaglandins.

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