Acta Neuropathol (1999) 97 : 543–546

EXPRESS COMMUNICATION

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Apoptosis of T lymphocytes in acute disseminated encephalomyelitis

Received: 17 December 1998 / Accepted: 11 January 1999

Abstract Apoptosis has been shown to be an efficient mechanism involved in clearance of T lymphocytes from the brains of animals with acute experimental autoimmune encephalomyelitis (EAE), an animal model for human multiple sclerosis. In this report we describe a case of acute disseminated encephalomyelitis following general measles infection. In this disease, which closely mimics the pathology of acute EAE we found a high percentage (30%) of apoptotic T cells. This indicates that in both rodent and human brain clearance of T cell-mediated inflammation follows similar mechanisms.

Introduction

Recent experimental data show that apoptosis of T lymphocytes plays a major role in clearance of T cell-mediated inflammation of the central nervous system (CNS) [1]. Evidence for this comes from experimental models such as experimental autoimmune neuritis (EAN) and experimental autoimmune encephalomyelitis (EAE), where in the latter 30–50% of all T cells in acute CNS lesions are found to be apoptotic [2, 3]. So far, few data are available regarding clearance of inflammatory T lymphocytes in human disease. Although T cell apoptosis is present in multiple sclerosis (MS) lesions, its incidence is low [4, 5]. Reasons for this discrepancy in T cell apoptosis in rodent and human CNS can be (i) disease-related, i.e., due to the chronicity of the inflammatory process in MS, or (ii) a fundamental difference in T cell clearance in the CNS between humans and rodents. To clarify this point, T cell apoptosis has to be studied in an acute, synchronized self-limiting inflammatory disease of the CNS which mimics acute monophasic EAE. Here we describe T cell apoptosis in a case of acute disseminated encephalomyelitis (ADEM) [6], a disease in which viral infections such as measles or chicken pox precedes an acute monophasic inflammatory process in the CNS which closely resembles EAE in rodents. We found that in ADEM brain, similar to acute EAE, up to 30% of all T cells present in the CNS parenchyma were undergoing apoptosis. These results indicate that in rodent and human CNS similar mechanisms operate in clearance of inflammatory T cells.

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Materials and methods

Case report

A 13-year-old boy, 7 days after onset of fever and coughing, and 4 days after the development of an erythematous maculopapulous exanthema, became comatose and was admitted to a department of pediatrics. Measles encephalitis was suspected, and treatment with antibiotics, IV immunoglobulins, and anti-osmotic agents was started. No glucocorticoids were administered. The patient's state rapidly worsened, leading to respiratory failure the day after admission. Artificial respiration and catecholamine treatment of hemodynamic instability were necessary. The next day, a bulbar syndrome developed, and the EEG showed lack of electric activity. Cerebral computerized tomography (CCT) revealed severe diffuse cerebral edema. After transfer to an intensive care unit, invasive monitoring revealed increased intracranial pressure (70 mm Hg). Laboratory data showed increased inflammatory signs and pathological coagulation values. Two days later, i.e., 13 days after the onset of febrile state and 10 days after the outbreak of exanthema, the boy died. Serological tests performed on the day of hospital admission revealed measles virus IgM. General autopsy revealed bilateral pneumonia, and generalized lymphadenitis. The brain showed flattened gyri indicative of cerebral edema, and injected meningeal vessels. No evidence of purulent (bacterial) meningoencephalitis was found.

Neuropathology and immunohistochemistry

Various blocks of cerebrum and cerebellum were fixed with formalin and embedded in paraffin. For routine neuropathological in-

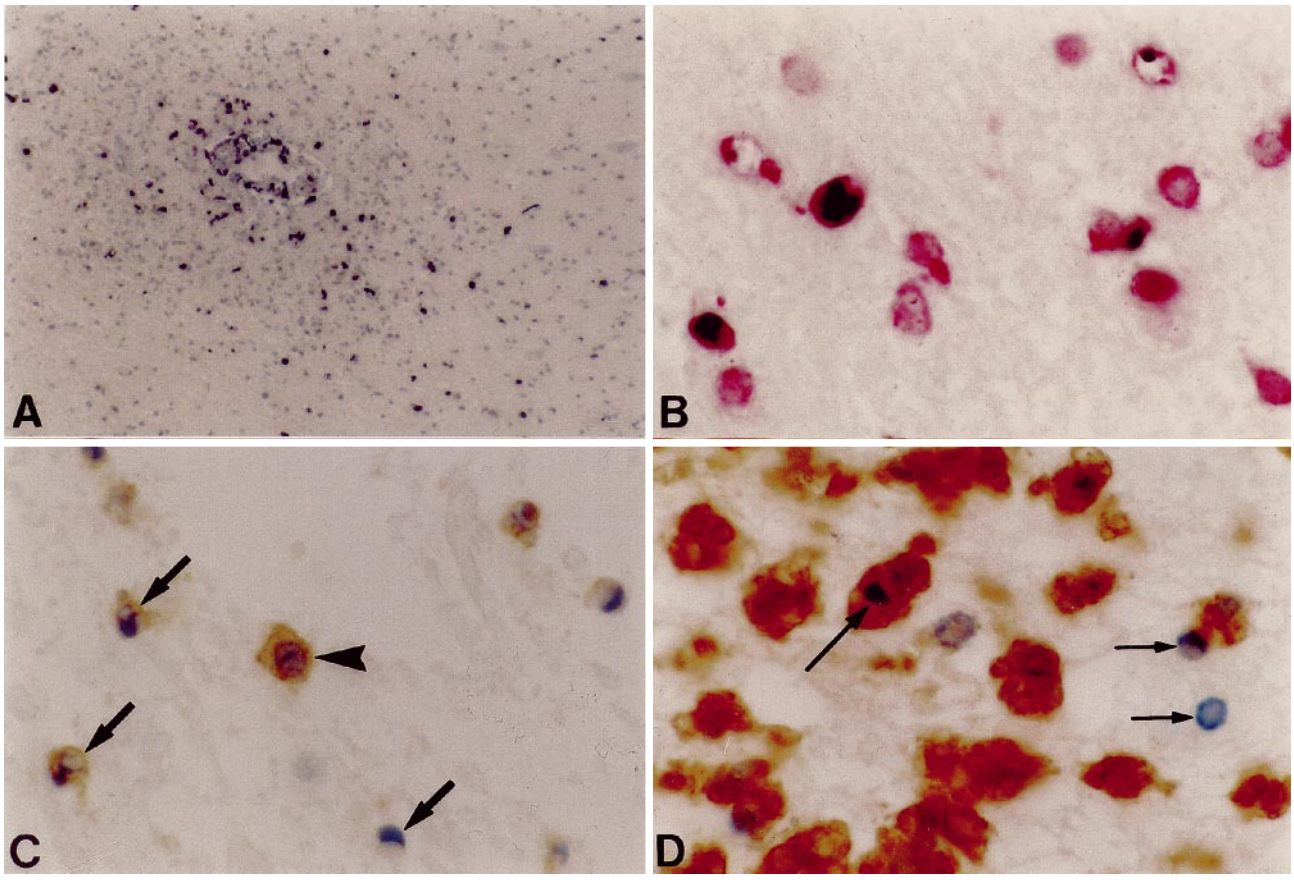

Fig. 1 **A** IST on cerebellum. *Black dots* reveal DNA degradation in inflammatory cells in and around a small blood vessel. **B** Double staining for CLA *(red)* and IST *(black)* shows the presence of DNA degradation in inflammatory cells; cerebellum. **C** A $CD3^+$ T cell *(arrowhead)* with a normal nucleus is surrounded by $CD3^+$ apoptotic *(arrows)* which show darkly stained condensed chromatin. **D** Staining for CD68 in the cerebellum reveals the presence of large numbers of macrophages. The *large arrow* shows a macrophage which has phagocytosed an apoptotic cell. Unstained apoptotic cells *(small arrows)* are seen between the $CD68^+$ cells (*IST* in situ tailing, *CLA* common leukocyte antigen). **A, C** Hematoxylin counterstaining; **A** × 99, **B–D** × 990

vestigation, 3- to 5-µm-thick paraffin sections were stained with hematoxylin-eosin (H&E), Klüver-Barrera (KB) for myelin and Bielschowsky's silver impregnation for axons. Immunocytochemistry was performed with a biotin-avidin technique as described in detail previously [7]. The following primary antibodies were used: monoclonal anti-measles virus (Biogenesis, San Down, N.H.), anti-CD3 (T cell receptor, Dakopatts, Denmark); anti-common leukocyte antigen (CLA, Dakopatts); anti-CD68 (recognizing macrophages, Dakopatts). Apoptotic nuclei were identified by chromatin condensation and nuclear fragmentation.

In situ tailing

DNA fragmentation in apoptotic cells was identified by in situ tailing (IST) as described by Gold et al. [8]. Briefly, 3-µm paraffin sections from spinal cord were deparaffinized, treated with chloroform and air dried. Next, sections were incubated in 50 µl of the reaction mixture (5 µl 10 × tailing buffer, 1 µl digoxigenin labeled nucleotides, 12 U terminal transferase and 44 µl dH_2O). As a secondary step the sections were incubated with an alkaline phosphatase anti-digoxigenin $F(ab')_2$ antibody at a dilution of 1:250. Alkaline phosphatase was visualized with NBT/BCIP. All materials for the IST were obtained from Boehringer Mannheim. Subsequently, the sections were stained with monoclonal antibody against CD3 or CLA as described above.

In situ hybridization for measles virus

Nonradioactive in situ hybridization (ISH) was performed on paraffin sections according to Breitschopf et al. [9] with a DIG-labeled RNA probe (a kind gift from Dr. Schneider-Schaulies, University of Würzburg) recognizing the measles virus nucleocapsid gene [10].

Quantitative evaluation

The percentage of apoptotic cells stained positive for a certain marker was determined by counting labeled non-apoptotic and apoptotic cells, as determined by condensation of the nucleus visualized with hematoxylin counterstain, in three compartments of the CNS (i.e., parenchyma, perivascular space and meninges) of the cerebellum. For each marker a total number of 1000 cells in parenchyma and perivascular space and 500 cells in meninges was counted.

Results and discussion

Routine neuropathological investigation revealed inflammation in meninges as well as perivascular and parenchymal infiltrates of inflammatory cells, domi-

Table 1 Apoptosis in brain with acute disseminated encephalomyelitis. $CD3^+$, CLA^+ and $CD68^+$ cells were counted in cerebellar parenchyma, perivascular space and meninges (*Normal* non-apoptotic cells, *Apo* apoptotic cells, *%* percentage of apoptotic cells, *CLA* common leukocyte antigen)

Marker	Parenchyma			Perivascular space			Meninges		
	Normal	Apo	%	Normal	Apo	%	Normal	Apo	%
CD3	704	298	30	810	190	19	436	71	14
CLA	847	187	19	945	121	11	469	31	6
CD68	988	12	1	992	8	1	494	6	1

nantly present in the white matter. KB stain for myelin and Bielschowsky's silver impregnation showed some perivenous demyelination with sparing of axons. In demyelinated areas, active ongoing demyelination was revealed by the presence of macrophages with early myelin (Luxol-fast blue positive) degradation products. Immunocytochemistry and ISH for measles virus were negative and no intranuclear neuronal inclusion bodies [11] were observed. Thus, the neuropathological findings in this case fulfill the criteria of postinfectious perivenous leukoencephalitis as described [6, 12]. Further examination of the inflammatory cells revealed large numbers of T cells ($CD3^+$, CLA^+) and macrophages (CLA^+, $CD68^+$). In addition to lymphocytes and macrophages, CLA and CD68 immunoreactivity was found on rod-shaped cells with short, thick processes, typical for activated microglia. IST (Fig. 1 A, B) showed the presence of large numbers of cells with DNA fragmentation in the parenchyma, meninges and perivascular space of blood vessels. The condensed homogeneously stained nuclei of these ($CD3^+$, CLA^+) cells (Fig. 1 C) indicated that these cells were apoptotic rather than necrotic. In many cases parts of the nucleus together with parts of the cell cytoplasm had fallen apart in so-called apoptotic bodies. Quantification of macrophages and T lymphocytes revealed that nearly all apoptotic cells belonged to the T cell lineage (Table 1). Only exceptional $CD68^+$ macrophages with apoptotic nuclei were found, the majority of these representing macrophages which had taken up apoptotic cells (Fig. 1 D). Quantitative determination of amount of apoptosis in T cells revealed 30% in the parenchyma (Table 1), while in the meninges and perivascular space, the levels were lower, but still reached 14% and 19%, respectively (Table 1).

A number of studies have described apoptosis of inflammatory cells in animal models of EAE [2, 3, 13] as well as in EAN [14]. In all these studies relatively high numbers of apoptotic T cells (in EAE up to 50% of all T cells) were found in inflammatory lesions. Apoptosis of lymphocytes has been described in human CNS diseases such as MS [4, 5] and subacute sclerosing panencephalitis (SSPE [15]), a rare complication of measles virus infection of the CNS. In the latter, apoptotic lymphocytes were found in the CNS among apoptotic virus-infected cells such as neurons and oligodendrocytes. Unclear is whether apoptosis of T cells in SSPE results from virus infection itself or whether it is induced in CNS-infiltrating lymphocytes for other reasons. Unlike in MS, the level of apoptosis of lymphocytes present in the ADEM brain in the present study equals that found in brain with acute EAE. Several mechanisms are thought to operate in T cell elimination in EAE brain. First, various studies have shown a role for corticosteroids in induction of apoptosis in EAE [16] and in EAN [17]. Corticosteroid-induced apoptosis of T lymphocytes, however, results from treatment with non-physiological doses [16], while abolition of endogenous corticosteroids by adrenalectomy hardly diminishes the level of apoptosis [18]. Before death, this patient was not treated with corticosteroids, indicating that the high level of apoptosis in the CNS was not corticosteroid induced. A second mechanism supposedly involved in apoptosis of lymphocytes in CNS inflammatory lesions is major histocompatibility complex class II-restricted, antigen-specific apoptosis [16]. Our recent studies [3], however, showed that during EAE all lymphocytes, independent of their state of activity or antigen specificity, are eliminated by apoptosis after migration into the CNS. Although the mechanism responsible for the elimination of infiltrating T cells is unknown, the equally high numbers in ADEM and EAE brain suggest that in principle in both rat and human brain a similar apoptosis-inducing mechanism is operating.

Acknowledgements We thank Helene Breitschopf, Angela Kury, Marianne Leisser and Petra Tassotti for expert technical assistence. This work was supported by a grant from the Austrian Science Foundation (FWF 12658-MED).

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