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Apoptosis in brain-specific autoimmune disease

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Recent neuropathological studies of experimental autoimmune encephalomyelitis have focused attention on the high number of cells in the lesions that show typical morphological features of apoptosis. Surprisingly, it has turned out that the vast majority of apoptotic cells are T lymphocytes and that they actually represent the antigen-specific T-cell population responsible for the induction of the disease. Taken together, these data suggest that clearance of autoimmune inflammation in the nervous system is accomplished by the destruction of the antigen-specific T-cell population within the lesions. This may explain the low level of central nervous system specific T-cell memory formation, as well as previously unexplained phenomena of 'epitope spreading', in autoimmune inflammation of the nervous system.

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Introduction

Cell death is a key event in physiological, as well as pathological, processes of the immune system. It can be mediated by two principally different mechanisms: apoptosis and necrosis. Apoptosis refers to a series of morphological changes that occur in dying cells and that are different from the changes seen in necrosis [1]. Unlike necrosis, which mediates cell death by disruption of the plasma membrane and lytic degradation of cytoplasmic organelles, a cell undergoing apoptosis loses volume, the cytoplasm condenses, and the nucleus shows condensation and clumping of the chromatin in parallel with DNA fragmentation. In the final stage, cell fragments pinch off forming so called 'apoptotic bodies'. Biochemically, activation of intracellular enzyme systems leads to primary DNA fragmentation, which is later followed by cell lysis [2]. Overall, the cell is lysed without liberation of proinflammatory degradation products. Before total degradation of apoptotic cells occurs, these cells are taken up by phagocytes thereby limiting the spilling of proinflammatory cytoplasmic contents into the surrounding tissue [3].

To date, many studies have focused on the role of apoptosis in physiological conditions of the immune system, such as positive or negative selection of T cells in the thymus [4]. However, relatively little is known about the patterns and mechanisms of cell death in immune mediated inflammatory lesions in target organs. In this review, we will discuss recent data, obtained from the well defined model of experimental autoimmune encephalomyelitis (EAE), that suggest that local cell death may play a major role in the regulation of the inflammatory process.

Basic principles of brain inflammation, as revealed by the study of EAE

The central nervous system (CNS) is continuously surveyed by the immune system. $CD4^+$ T lymphocytes, which are activated in the circulation, can pass through the blood–brain barrier irrespective of their specific target antigens [5,6]. Yet, most of these T cells will not find the specific antigen in the nervous system and will therefore be rapidly cleared from the CNS parenchyma [6]. However, when T cells that are directed against an autoantigen of the CNS, such as myelin basic protein (MBP), confront their antigen on antigen-presenting cells (APCs) in the perivascular space, they will home and will be further activated in the nervous tissue (Fig. 1). This leads to a cascade of secondary events, such as the upregulation of the production of cytokines and chemokines [7,8•,9], endothelial adhesion molecules [10–12], and further induction of the expression of MHC class II antigens on local APCs [13,14]. These events facilitate the entrance of a secondary wave of leukocytes into the CNS, leading to disease and local tissue damage. Acute EAE is a monophasic disease followed by spontaneous recovery, yet, in certain animal species, a chronic progressive or relapsing disease may develop.

Although the individual steps that operate in immune surveillance and the induction of brain inflammation are well understood, relatively little is known about the local mechanisms responsible for clearance of inflammation and the subsequent induction of tolerance. Recent evidence from both *in vitro* and *in vivo* studies of T-cell apoptosis, however, suggests that cell death in the CNS, besides playing a role in target-tissue destruction,

Abbreviations

APC—antigen-presenting cell; **CNS**—central nervous system; **EAE**—experimental autoimmnue encephalomyelitis; **IL**—interleukin; **MBP**—myelin basic protein; **TCR**—T-cell receptor; **TGF**—transforming growth factor; **V**—variable.

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is an essential aspect of the local regulation of the inflammatory reaction.

Mechanisms of apoptosis induction in T cells *in vitro*

A number of mechanisms have been found to induce apoptosis of T cells *in vitro*. Early studies by Wyllie [15] revealed that corticosteroids are strong inducers of apoptosis in T cells. This also occurs in encephalitogenic (MBP-specific) T cells, which can be synchroniously driven into apoptosis when glucocorticosteroids are added *in vitro* at a late stage after antigen-specific stimulation (R Gold, M Schmied, U Tontsch, H-P Hartung, H Wekerle, unpublished data). A second mechanism responsible for T-cell death is so-called 'propriocidal regulation' [16], implying that T cells become apoptotic when they encounter a strong antigenic challenge. Unlike apoptosis induced by glucocorticoids, antigen-induced apoptosis occurs preferentially in the S phase of the cell-cycle progression of T cells [17]. Moreover, apoptosis of T-cell hybridomas triggered by antigen engagement of the TCR seems to happen irrespective of the presence of glucocorticoids. This only occurs at moderate doses of these stimuli, whereas a high dose of either antigen or glucocorticoids overrides the antagonism, and induction of apoptosis proceeds [18]. A third way to drive T cells into apoptosis *in vitro* is by the addition or withdrawal of certain cytokines. An important factor in the induction of apoptosis is IL-2 [19]. The presence of IL-2 is not important for apoptosis induced by corticosteroids [20], but is essential for antigen-induced apoptosis, probably because it causes T cells to enter the S phase or later stages of the cell cycle, where they are susceptible to apoptosis [16]. Although the presence of IL-2 is required for antigen-induced apoptosis under certain conditions, its deprivation may also drive T cells dependent on IL-2 into apoptosis [21•]. Another interesting cytokine is transforming growth factor (TGF)-β. This cytokine belongs to a family of peptides with potent immunosuppressive effects. It

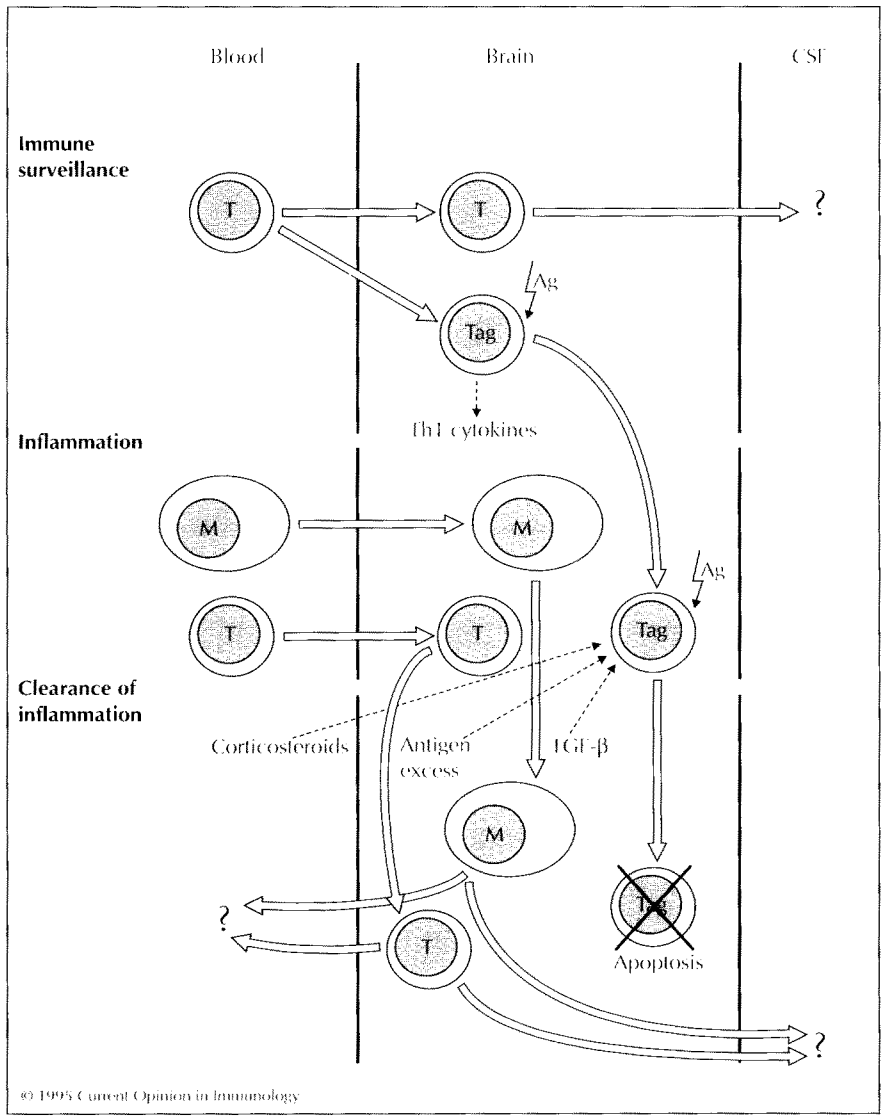

Fig. 1. Hypothetical view of clearance of brain inflammation by apoptosis of T cells during autoimmune disease in CNS. During surveillance of the CNS, activated antigen-specific T cells (Tag) enter the CNS and locally recognize their antigen (Ag). This results in the release of Th1 cytokines, which is followed by entrance of a secondary wave of T cells (T) and macrophages (M). During the inflammatory response, the antigen-specific T cells undergo apoptosis, possibly by combined influences of corticosteroids, antigen and cytokines. CSF, cerebrospinal fluid.

inhibits the proliferation of T cells [22] and is able to downregulate interferon (IFN)-γ induced by MHC class II expression [23]. Studies by Weller *et al.* [21•] revealed that exposure to TGF-β in both IL-2-dependent and IL-2-independent (IL-4-dependent) T-cell lines induced apoptosis.

Clearance of inflammation by apoptosis of T cells in EAE

In 1991, Pender *et al.* [24] described that besides oligodendrocytes, in being target cells in the tissue destruction in EAE, T cells in the lesions were also destroyed by apoptosis. With this description, the authors raised, for the first time, the possibility that local apoptosis of T cells in the target tissue could be a way of downregulating inflammation and installing tolerance. This initial observation of the presence of apoptotic T cells in the CNS of animals with EAE was followed by a more detailed study from Schmied *et al.* [25] who used *in situ* nick translation in combination with immunocytochemistry to show that, at least in acute monophasic EAE, mainly T lymphocytes and not the cells of the CNS parenchyma were undergoing apoptosis. Furthermore, in this study, quantitation suggested that apoptosis is a very efficient mechanism of T-cell elimination in the lesions. At the time of recovery from the disease, up to 49% of all T cells present showed nuclear changes. These data also indicated that within a 24 hour period, two to sixfold more than the total T-cell population present in the inflammatory infiltrates were destroyed by apoptosis and, thus, that active inflammation could only been maintained by the recruitment of new T cells from the circulation or by local proliferation. More recently, it became clear that the local destruction of T cells is not restricted to EAE, but can also be found in other inflammatory diseases of the nervous system. For example, apoptotic T cells are present to a similar extent in the lesions of experimental allergic neuritis, an autoimmune mediated inflammatory disease of peripheral nerves [26•], and demyelinating encephalomyelitis induced by coronavirus [27•]. In addition, T-cell apoptosis is frequently encountered in active lesions of multiple sclerosis [28•].

Several mechanisms may operate in the elimination of T cells. During EAE, a strong corticosteroid response is generated, which leads to the generation of high serum levels of glucocorticoids at the onset of remission (Fig. 1). Adrenalectomy aggravates EAE, an effect that can be abolished by steroid replacement [29]. Furthermore, the peak of apoptosis within lesions coincides with the peak of serum glucocorticoid levels. In EAE, however, only T cells that have infiltrated the CNS undergo apoptosis, whereas T cells in meninges and perivascular space virtually completely escape apoptotic destruction [25], thus making a sole corticosteroid effect unlikely to operate *in vivo* .

Other observations argue in favour of antigen-induced apoptosis in EAE. MBP-specific T cells in rodents preferentially express the Vβ8.2$^+$ TCR [30–32]. In EAE, these Vβ8.2$^+$ T cells predominantly localize in the CNS parenchyma [33,34•]; furthermore, in EAE lesions, apoptosis is highest in the Vβ8.2$^+$ MBP-specific T-cell population [35•], indirectly suggesting that antigen-specific cells in particular are susceptible (Fig. 1). Preliminary experiments performed in our laboratories studying the behaviour of prelabeled encephalitogenic T cells *in vivo* indeed suggest that it is predominantly the antigen-specific T-cell population that is eliminated by apoptosis *in situ*.

Liberation of MBP during the course of inflammatory mediated tissue damage may finally lead to a concentration of the specific antigen in the brain extracellular space (Fig. 1) that is sufficient for the induction of T-cell apoptosis *in situ* [36•]. Besides the antigen concentration, the kind of APC may also play a role in antigen-induced apoptosis. During EAE, perivascular cells, infiltrating macrophages, microglial cells and astrocytes may act as APCs. For the latter, in recent years, evidence has been obtained that points to a downregulatory role in EAE [37]. These *in vivo* observations are in line with the *in vitro* finding that apoptosis of T lymphocytes at late post-activation stage is pronounced when astrocytes are used as APCs, whereas apoptosis is marginal or absent when antigen presentation is accomplished by APCs from the thymus (R Gold, M Schmied, U Tontsch, H-P Hartung, H Wekerle, unpublished data). However, the rate of T-cell apoptosis during EAE in radiation bone marrow chimeric rats is similar to normal rats with EAE, in spite of the inability in these chimeras to present antigen to the transferred T lymphocytes by local astrocytes or microglial cells [38].

As part of the probable multifactorial induction of T-cell apoptosis, a third mechanism involved in T-cell elimination during the course of EAE may be cytokine-mediated apoptosis. In particular TGF-β may be of importance (Fig. 1). When administered during EAE, this cytokine has been shown to improve the clinical course of this disease [39,40]. As seen in the *in vitro* studies by Weller *et al.* [21•], TGF-β may also affect EAE by inducing apoptosis in T cells. TGF-β in brain lesions is not only made by inflammatory cells, such as macrophages [41], but can also be produced by astrocytes [42].

Conclusions

Recent evidence shows that the disposal of autoaggresive T cells during EAE is a very effective mechanism for clearance of CNS inflammation. Apoptosis of T cells might occur through an increase in the production of corticosteroids, the presence or absence of cytokines, through antigen induction, or through combinations of these. Whatever the mechanisms are that finally lead to apoptosis of T lymphocytes in the lesions of EAE, the elimination of these cells in the target organ has profound immunological consequences and may explain characteristics of CNS autoimmunity, which

so far have remained enigmatic. For instance, it may explain why Ohmori *et al.* [43] found that in CNS lesions, proliferation of T cells is discontinued rapidly. Moreover, it is generally known that rodents that have gone through one episode of EAE are protected against attempts to induce a relapse by a second immunization. This is against intuition because, in general, primary immunization leads to the expansion of antigen-specific lymphocyte clones and to differentiation of memory cells.

Although a variety of phenomena (i.e. hormonal conditioning and immunological network interactions) play a role in the resistance to reinduction of EAE, deletion of primary encephalitogenic T cells is definitely an additional contributing factor. This has been best shown in Lewis rats and H-2^u mice where, initially, most encephalitogenic (MBP-specific) T cells use the Vβ8.2^+ gene for their antigen receptors. During the course of disease, however, these originally dominant T-cell clones are progressively lost [44]. This process is accompanied by the gradual loss of reactivity against the dominant encephalitogenic epitopes, MBP sequence 68–88 in Lewis rats [33,45] and Ac1–10 in H-2^u mice [31,32], and a redirected response against additional secondary epitopes [46,47], a phenomenon termed (intramolecular) 'epitope spreading' [48]. We propose that both the low MBP-specific T-cell memory formation, as well as epitope spreading, are a direct result of local death (apoptosis) of the encephalitogenic T cells within the target parenchyma. Accordingly, in the Lewis rat, the first wave of Vβ8.2^+ (MBP peptide 68–88) specific T cells, which are responsible for the clinical EAE episode, would be eliminated from CNS by apoptosis. T cells specific for secondary, 'cryptic' epitopes would be expanded instead. It will have to be established in the future whether the effective elimination of T cells as seen in both EAE as well as in experimental autoimmune neuritis (EAN), is a unique mechanism in nervous system immune reactivity or if it is a general mechanism which also functions in autoimmune or infectious diseases of other organs.

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