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T-cell apoptosis in autoimmune diseases: termination of inflammation in the nervous system and other sites with specialized immune-defense mechanisms

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We have studied T-cell apoptosis in animal models of human autoimmune disorders of the nervous system and in other tissues devoid of specialized immune-defense mechanisms. Our data suggest that the CNS has high potential for elimination of T-cell-dependent inflammation, whereas this mechanism is less effective in the PNS, and is almost absent in other tissues such as muscle and skin. Interestingly, several conventional and novel immunotherapeutic approaches, such as glucocorticosteroid and high-dose antigen therapy, induce T-cell apoptosis in situ. In vitro experiments suggest different scenarios for the mechanisms by which specific cellular and humoral elements in the nervous system synergize and sensitize T cells for apoptosis in vivo. We also discuss regulatory, proapoptotic mechanisms, such as the Fas–FasL system and galectin-1, that have been utilized in other tissues to mediate immune protection.

The concept of immune privilege was originally defined as the protection of tissue grafted to certain sites and was later extended to describe the seclusion of particular areas of the body from the systemic immune compartment. However, this concept was abandoned more than a decade ago when it was shown that immune surveillance is indeed operative in such tissues. Even the CNS, previously considered the prototypic privileged site, is constantly patrolled by activated T lymphocytes, which can induce profound damage when they ‘find’ their specific antigen in the context of appropriate restriction molecules (reviewed in Ref. 2). This particular example illustrates the fact that specialized anatomic barriers, such as the blood–brain barrier or the absence of lymphatic drainage, do not necessarily guarantee the absence of immune-mediated damage in these sites. Furthermore, it has become clear that T-cell trafficking within the CNS can be uncoupled from the development of diseases and that under certain conditions, delayed-type hypersensitivity reactions can give inflammatory lymphocytes access to the CNS (Ref. 7) and even provide therapeutic approaches. Examples of other tissues that utilize specialized immunological defense mechanisms are the retina, the cornea, the anterior chamber of the eye, the testis, and the liver. However, it is likely that in every tissue multiple factors operate that ensure rapid and gentle elimination of inflammation.

In 1972, Kerr and colleagues used morphological criteria to define apoptosis as a specialized form of cell death. Typically, chromatin condensation and cell shrinkage occur in parallel but the integrity of the cell membrane is preserved much longer. Traditionally, apoptotic cells were identified by electron microscopy, but new techniques, such as in situ tailing assays, have recently become available that detect biochemical events associated with oligonucleosomal DNA fragmentation in apoptotic cells. Although apoptosis is a purely morphological term, it has an array of pathological and functional implications. The importance of apoptosis for the immune system has been reviewed in detail by Cohen and colleagues who have clearly differentiated apoptosis from programmed cell death, which is merely a functional term. Since the plasma membrane itself is not a primary target of apoptosis but rather provides specific signals for phagocytic cells, release of proinflammatory cytokines is avoided. It is conceivable that in vulnerable organs, such as the CNS, the parenchyma is extremely susceptible and has low capacity for regeneration. Theoretically, apoptosis would provide an ideal, non-inflammatory mechanism to terminate the autoimmune T-cell attack in vulnerable tissues, assuring a minimum of detrimental bystander damage to the local parenchyma. Do immunologically protected sites take advantage of this mechanism of cell death?

Apoptosis of T lymphocytes in inflamed nervous tissue but not in muscle or skin

Experimental autoimmune encephalomyelitis (EAE) and neuritis (EAN) serve as animal models for the human diseases multiple sclerosis (MS) and Guillain-Barré syndrome. Both models can be induced by immunization with myelin antigen (active disease) or by intravenous transfer of CD4+ T lymphocytes specific for myelin antigens (adoptive-transfer (AT) model). Pender and colleagues were the first to draw attention to apoptosis as a possible mechanism of cell destruction in inflammatory brain lesions of Lewis rat.
T-cell apoptosis and the nervous system

Immunocytochemical characterization of apoptotic cells in Lewis rat autoimmune encephalomyelitis (EAE).

Fig. 1. Immunocytochemical characterization of apoptotic cells in Lewis rat autoimmune encephalomyelitis (EAE). (A) W3/13-positive T cells in the spinal cord. Some of these are in different stages of apoptotic cell death (arrows). In advanced stages of apoptosis, membrane immunoreactivity has been lost (open arrow). (B) 0.5-μm-thick plastic sections after pre-embedding immunocytochemistry for αβ T-cell receptor. Open arrow denotes an apoptotic cell. (C and D) Electron micrographs showing apoptotic T cells with positive staining for αβ T-cell receptor in (C) or common leukocyte antigen (D). Scale bars, 5 μm (A and B), 1 μm (C and D). (C and D) Reproduced from Ref. 21 with kind permission of the Am. J. Pathol.

Fig. 2. T-cell apoptosis in vivo. Apoptosis during the natural course of experimental autoimmune encephalomyelitis (EAE) or neuritis (EAN) that was induced by cell transfer (A) or by immunization with myelin basic protein (MBP) or peripheral nerve myelin (AN) (B). Ordinate shows percentage of apoptotic T cells (mean ± SD).

Possible mechanisms of T-cell apoptosis in vivo

Elution of antigen-specific invading T cells

The inflammatory infiltrate in EAE is composed of primary antigen-specific T cells, which are responsible
for the induction of inflammation, and of other non-
anantigen-specific T cells, which are secondarily
recruited into the lesions at later stages when the
immunoinflammatory reaction is fully developed39.
To delineate better the mechanisms of apoptosis in
vivo, it is important to know whether antigen-specific
cells alone or also additionally recruited bystander T
cells are destroyed within the CNS. T cells isolated
from established EAE lesions contain only a minor frac-
tion of MBP-specific cells (see discussion in Ref. 21),
suggesting that antigen-specific cells are preferentially
removed from inflamed CNS tissue. Furthermore,
local proliferation of T cells, particularly antigen-
specific, T cells, occurs only to a very low extent in
the lesions39. Preferential apoptosis of MBP-reactive
cells could account for these observations31. To investigate
this further, Pender and colleagues induced EAE by a
T-cell clone using the Vβ8.2 T-cell receptor, which is the
predominant T-cell-receptor element in MBP-
induced EAE of the Lewis rat32. They then charac-
terized apoptosis and usage of the T-cell receptor in
lymphocytes isolated and enriched from spinal cord33–35.
They found that the frequency of Vβ8.2+ T-cells was
sevenfold higher in the apoptotic population than in
non-apoptotic T cells. Their findings were substanti-
ated by studies with T-cell lines that recognize oval-
bumin (OVA), an antigen not present in the Lewis rat,
and by experiments in which MBP- and OVA-specific
T cells were transferred. These experiments confirmed
that the in vitro reactivity was preserved only for non-
CNS antigens like OVA. Furthermore, Pender and col-
leagues could not detect recirculation of Vβ8.2+ T cells
in peripheral lymphoid organs. This work suggests via
indirect evidence that, in EAE, antigen-specific T cells
that utilize the Vβ8.2 T-cell receptor are the prime tar-
get for destruction by apoptosis. These interesting
results might be biased by the altered density of col-
lapsed apoptotic cells11, which might preclude quanti-
tative recovery during gradient centrifugation. Indeed,
the overall frequency of T-cell apoptosis in EAE is
much lower than previously reported in histological
studies39. Furthermore, there is evidence that other T-
cell receptors besides Vβ8.2 are involved in reactivity
to MBP (Ref. 33). These T cells could also be recruited
to the CNS in EAE and would be overlooked in inves-
tigations that focus only on Vβ8.2+ T cells.

These studies permit two potential interpretations.
First, the nervous system might effectively eliminate T
lymphocytes by antigen-specific mechanisms. Second,
the CNS tissue might create a hostile environment
that drives all T cells into apoptosis as soon as they
infiltrate the parenchyma. In ongoing analyses, this
question is being addressed by the induction of EAE
through the transfer of MBP-specific T cells. These
cells contain a stable genomic marker and thus allow
unequivocal differentiation between the MBP-reactive
and the secondarily recruited T cells in the lesions
(J. Bauer and H. Lassmann, pers. commun.).

Mechanisms identified in cell-culture experiments

Since T-cell apoptosis appears to be rather organ
specific, it is conceivable that local resident cells
might render T cells susceptible to apoptosis directly
or through soluble factors. Furthermore, local cells
might cooperate with systemic humoral factors to
deliver a proapoptotic stimulus. With regard to the
CNS, astrocytes and microglia cells might have a cen-
tral role. Astrocytes can present autoantigens to
autoimmune T-cell lines36, but astrocytes are only par-
tially competent antigen-presenting cells that are
unable to trigger the complete program of T-cell
activation11. We studied antigen-driven effects of
astrocytes on T cells and evaluated the role of steroid
hormones40. Interestingly, astrocytes exerted a suppres-
sive effect on T-cell activation and this was mediated
by cell contact. Glucocorticoids markedly augmented
T-cell apoptosis when added to T-cell-astrocyte
cultures during late stages of T-cell activation on
day 3. However, glucocorticoids had no effect when
added at earlier time points or when thymus cells were
used as antigen presenters. The effect was specific
since it could be inhibited by blockade of the cytosolic
steroid receptor through the action of RU486. Other
immunomodulatory compounds such as lipooxins,
TGfβ, or inhibition of nitric-oxide synthase did not

![Fig. 3. Immunocytochemical characterization of apoptotic cells in Lewis rat experimental
autoimmune neuritis (EAN). (A) B115-1-positive T cells in the sciotic nerve that show nuclear
morphology characteristic of apoptosis (arrow). Open arrow denotes advanced stage of apop-
tosis with loss of membrane immunoreactivity. (B) Double labeling for DNA fragmentation
(black) and expression of ED-1, a macrophage marker antigen (red). After antigen therapy
macrophages engulf apoptotic DNA. (C and D) Double labeling for DNA fragmentation
(black) and expression of B115-1, a T-cell marker antigen (red) in control animals that have
received an irrelevant myelin protein (C) or after antigen therapy with i.v. administration of
recombinant P2 protein (D). (E and F) Higher magnifications of the regions indicated by stars
in C and D respectively. A reduction in inflammatory T cells and increase in apoptotic frag-
ments is visible after antigen therapy. At higher magnification, cells double-stained for T-cell
antigen (red) and DNA fragmentation (black) are visible (arrows in E and F). Terminal stages
of apoptosis have lost membrane immunoreactivity (open arrows). Scale bars, 10 μm (A, B,
and F) and 50 μm (C and D).](image)
modulate T-cell apoptosis in that system, indicating that these factors are probably not involved. Recently, similar activation of the apoptotic pathway of T cells was noted when freshly isolated microglia that lacked the surface expression of costimulatory molecules were used as antigen-presenting cells39. These results argue for a scenario in which nonprofessional antigen-presenting cells prime T cells for an apoptotic stimulus, which might then be delivered by hormonal influences of the microenvironment or systemic changes. Interestingly, steroid hormones might be produced locally40 or released systemically during tissue injury. Crosslinking of the Fas antigen by its trimeric ligand initiates a signaling cascade that leads to apoptotic cell death (reviewed in Ref. 47). The Fas system is also involved in the pathogenesis of liver diseases, where crosslinking of Fas receptors on hepatocytes leads to severe apoptotic liver damage that can be inhibited by therapeutic agents such as linomide48. Interactions that use the Fas–FasL system have now been demonstrated in corneal epithelium and in the retina49 and invading Fas-positive tumor cells can be eliminated by apoptosis in situ. Apart from an active role of Fas–Fasl, in maintaining immune privilege in grafted testis50 and prevention of rejection of islet allografts51, FasL-bearing cells have now been demonstrated in brain from MS patients and in other inflammatory CNS diseases52,53. Dowling et al.54 showed co-localization of expression of Fasl and apoptotic nuclei. Thus, it is conceivable that the Fas–Fasl system might be involved in apoptosis. Special reference should also be made to galectin-1 and its role in warranting immune protection in the liver. Galectin-1 belongs to the family of β-galactoside-binding proteins and has been shown to eliminate activated T cells by apoptosis54. Obviously, this was mediated by interactions that used N-glycans that were expressed on the CD45 molecule. Galectin-1 is a major lecin on hepatic sinusoidal endothelial cells (reviewed in Ref. 55). Therefore patrolling activated lymphocytes that might accumulate in this organ (reviewed in Ref. 55) could be eliminated after interacting with galectin on sinusoidal cells. It is unknown whether similar galectins are expressed in the nervous system.

To date, detailed studies on T-lymphocyte apoptosis during recovery from inflammation are lacking for autoimmune diseases that involve other specialized and immunologically protected sites such as experimental autoimmune neuritis.

Therapeutic induction of T-cell apoptosis in autoimmune diseases: studies in EAN

Therapeutic regimens in patients with immune-mediated disorders of the nervous system aim at reducing inflammation and accelerating recovery.
Since high-dose glucocorticosteroids are the mainstay as therapeutically active compounds in this group of optic neuritis and acute relapses of MS, we investigated whether intravenous steroid pulse-therapy could induce T-cell apoptosis in situ. This was studied in the model of AT-EAN that was induced in Lewis rats by transfer of activated, P2-specific T lymphocyte clones. To delineate whether the effect of steroid hormones is stage specific, two pulses of glucocorticosteroids (10 mg kg\(^{-1}\) body wt\(^{-1}\)) were administered either after the first appearance of symptoms or at the maximum of the disease. Because of the rapid elimination of apoptotic cells, rats were sacrificed 6 h later. At both time points there was a massive reduction of T-cell infiltration and an increase of T-cell apoptosis that was four-to fivefold higher than the rate of apoptosis that occurred spontaneously in control animals. This underscores the fact that the elimination of inflammatory T cells in situ is indeed one mechanism of action in high-dose glucocorticosteroid therapy and is not stage specific. Similar results were recently reported from Penders’ group, which studied the effect of glucocorticosteroids in EAE (Ref. 5).

Another approach that has been developed for treatment of experimental autoimmune disorders is antigen-specific therapy. It is based on the observation that T-cell receptor re-engagement at an appropriate antigen-specific therapy. It is based on the observation that T-cell receptor re-engagement at an appropriate

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**Selected references**

1. Medawar, P. (1948) Br. J. Exp. Pathol. 29, 58–69
2. Wekerle, H. et al. (1986) Trends Neurosci. 9, 273–277
3. Fabre, Z., Raine, C.S. and Hart, M.N. (1994) Immunol. Today 15, 218–228
4. Shekian, P. and Benveniste, E.N. (1996) J. Immunol. 157, 1819–1822
5. Perry, V.H. et al. (1995) Curr. Opin. Neurol. 8, 636–641
6. Konner, H. et al. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 11066–11070
7. Andersonson, P.E.E., Perry, V.H. and Gordon, S. (1995) Nat. Neurosci. 8, 169–186
8. Sampson, J.H. et al. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 10299–10304
9. Streilein, J.W. (1995) Science 270, 1158–1159
10. Kerr, J.R.F., Wyllie, A.H. and curvature, A.W. (1972) Br. J. Cancer 26, 239–257
11. Wyllie, A.H. et al. J. Exp. Med. 119, 453–501
12. Gold, R. et al. (1994) Lab. Invest. 71, 219–235
13. Cohn, J.J. et al. (1992) Rev. Immunol. 10, 267–293
14. Sabat, J. et al. (1991) Immunol. Today 12, 131–136
15. Hartung, H.P., Stoll, G. and Toroya, K.V. (1993) in Jerne-N60: Neuroradiology (3rd edn) (Dyck, F.J. et al., eds), p. 418, W.B. Saunders
16. Lassmann, H. et al. (1991) Rev. Neurol. (Paris) 147, 563–781
17. Martini, R., McFarland, H.F. and McFarlin, D.E. (1992) J. Neuroimmunol. 33, 153–167
18. Penders, M.P. et al. (1993) J. Neuroimmunol. 38, 83–87
19. Penders, M.P. et al. (1992) J. Autoimmun. 5, 403–410
20. Penders, M.P. et al. (1993) J. Autoimmun. 64, 344–452
21. Nguyen, B.R., McCombe, P.A. and Penders, M.P. et al. (1994) J. Neuroimmunol. 48, 143–152
22. Barac-Latas, V., Wege, H. and Lassmann, H. (1995) Reg. Immunol. 8, 355–357
23. Grova, K. et al. (1994) Brain 117, 1111–1122
24. Zeltl, U. et al. (1990) Acta Neuroophthalmol. 6, 365–367
25. Burck, W. et al. (1991) Eur. J. Immunol. 21, 847–853
26. Schneider, C. et al. (1994) J. Neuroimmunol. Exp. Neurol. 33, 319–326
27. Dolakah, M.C. (1991) New Engl. J. Med. 325, 1487–1498
28. Creutz, H.E. et al. (1999) Lab. Invest. 64, 125–170
29. Glueck, K. et al. (1992) Lab. Invest. 66, 24–62
30. Buccheri, J. et al. J. Neuroimmunol. 73, 839–849
31. Chihab, M. et al. (1994) Brain 117, 279–284
32. Tait, Z., McCombe, P.A. and Penders, M.P. (1994) Eur. J. Immunol. 24, 2809–2817
33. Tait, Z., McCombe, P.A. and Penders, M.P. (1995) J. Immunol. 157, 967–973
34. Gold, R. et al. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 5850–5854
35. Fontana, A., Her, W. and Wekerle, H. (1984) Nature 317, 273–276
36. Weber, F. et al. (1994) Brain 117, 59–69
37. Gold, R. et al. (1996) Brain 119, 651–659
38. Ford, A.L. et al. (1996) J. Exp. Med. 184, 1733–1745
39. Yu S. et al. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 203

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**Conclusions and perspectives**

Disposal of autogressive T cells in the nervous system is a very effective mechanism for termination of lesions in the CNS. Our data strongly suggest that destruction of T cells through apoptosis in inflammatory lesions is a phenomenon that occurs in sites that possess specific immune-defense mechanisms and not a general feature for clearance of autoimmune lesions. It might also explain some poorly understood features of CNS autoimmunity. First, the absence of T-cell proliferation in lesions of the nervous system might be caused by progression of apoptosis. Furthermore, T-cell apoptosis might eliminate the first wave of MBP-reactive T cells, which are responsible for clinical disease (184–190), and thus prevent the expansion of dominant autoggressive clones, and might form the basis for intramolecular epitope spreading39 and exposure of cryptic epitopes during later stages of disease. T-cell apoptosis is a sophisticated, naturally occurring mechanism that confers protection of vulnerable sites from tissue damage. At present, we do not know exactly how this is achieved in vivo, but we have now begun to uncover the mechanisms in order to take advantage of its therapeutic applications.

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The calcium channel and the organization of the presynaptic transmitter release face

Ellis F. Stanley

Calcium influx through ion channels located on the release face of the presynaptic nerve terminal gates the release of neurotransmitters by the fusion of the secretory vesicle and the discharge of its contents. Recently, several lines of research have indicated that the relationship between the Ca\textsuperscript{2+} channel and the release site might be more complex than dictated simply by its role as an ion conduit. The evidence suggests that the channel and the transmitter-release mechanism exist as a multimolecular entity and that this interaction has functional consequences, not only on the mechanism and properties of transmitter release, but also on the behavior of the presynaptic Ca\textsuperscript{2+} channel itself.

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**Molecular elements of the release face**

In channels

Prior to the realization that there were multiple types of Ca\textsuperscript{2+} channel it was already recognized that presynaptic Ca\textsuperscript{2+} channels were not the same as those in muscle since transmitter release was not blocked by agents that inhibit Ca\textsuperscript{2+} influx in muscles\textsuperscript{1}. The N-type Ca\textsuperscript{2+} channel was the first distinct type of Ca\textsuperscript{2+} channel that was demonstrated to be involved in transmitter release largely on the basis of blockade with the specific toxin ω-conotoxin GVIA (ω-Ctx–GVIA) (Refs 5,6). More recently it has proved possible to localize N-type Ca\textsuperscript{2+} channels directly at the transmitter release face\textsuperscript{7,8} and to characterize their single channel properties in situ\textsuperscript{9} (see below). A variety of types of Ca\textsuperscript{2+} channel are utilized for transmitter release in different nerve terminals (reviewed in Ref. 11), including the P/Q-type that is blocked by the spider toxin ω-agatoxin-IVA (ω-Aga-IVA). Several studies have attempted to determine quantitatively the relative importance of different types of channel in specific nerve terminals simply on the basis of the fractional block of transmitter release using these toxins. However, this strategy should be pursued with caution since it is fraught with difficulties not only in interpretation (see Ref. 11) but also because the sensitivities of the presynaptic channels to these ligands cannot always be presumed\textsuperscript{12}.

Many other ion channels and ligand-gated ionotropic receptors have been suggested to be present on...