JCI The Journal of Clinical Investigation

CD4+ T cells mediate cytotoxicity in neurodegenerative diseases

Stanley H. Appel

J Clin Invest. 2009;119(1):13-15. https://doi.org/10.1172/JCI38096.

Commentary

Neuroinflammation, characterized by activated microglia and infiltrating T cells, is a prominent pathological feature in neurodegenerative diseases. However, whether this inflammation contributes to neuronal injury or is a late consequence of neuronal injury is unclear. In this issue of the *JCI*, Brochard et al. report that CD4+ T cells are cytotoxic in a mouse model of Parkinson disease (PD) (see the related article beginning on page 182). Specifically, invading T lymphocytes contributed to neuronal cell death via the Fas/FasL pathway. The results implicate the adaptive immune system in the pathogenesis of Parkinson neurodegeneration and provide a meaningful rationale for immune-based therapies for PD.

1. Ludwin, S.K., and Raine, C.S. 2008. The neuropathology of MS. In *Multiple sclerosis: a comprehensive text.* C.S. Raine, H.F. McFarland, and R. Hohlfeld, editors. Saunders/Elsevier. New York, New York, USA. 151–177.
2. D'Souza, B., Miyamoto, A., and Weinmaster, G. 2008. The many facets of Notch ligands. *Oncogene.* **27**:5148–5167.
3. Wang, S., et al. 1998. Notch receptor activation inhibits oligodendrocyte differentiation. *Neuron.* **21**:63–75.
4. Popko, B. 2003. Notch Signaling: A rheostat regulating oligodendrocyte differentiation? *Dev. Cell.* **5**:668–669.
5. Hu, Q.D., et al. 2003. F3/contactin acts as a functional ligand for Notch during oligodendrocyte maturation. *Cell.* **115**:163–175.
6. Hu, Q.D., Ma, Q.-H., Gennarini, G., and Xiao, Z.C. 2006. Cross-talk between F3/Contactin and Notch at axoglial interface: A role in oligodendrocyte development. *Dev. Neurosci.* **28**:25–33.
7. John, G.R., et al. 2002. Multiple sclerosis: re-expression of a developmental pathway that restricts oligodendrocyte maturation. *Nat. Med.* **8**:1115–1121.
8. Nakahara, J., Kanekura, K., Nawa, M., Aiso, S., and Suzuki, N. 2009. Abnormal expression of TIP30 and arrested nucleocytoplasmic transport within oligodendrocyte precursor cells in multiple sclerosis. *J. Clin. Invest.* **119**:169–181.
9. Padiath, Q.S., et al. 2006. Lamin B1 duplications cause autosomal dominant leukodystrophy. *Nat. Genet.* **38**:1114–1123.
10. Schwankhaus, J.D., Katz, D.A., Eldridge, R., Schlesinger, S., and McFarland, H. 1994. Clinical and pathological features of an autosomal dominant, adult-onset leukodystrophy simulating chronic progressive multiple sclerosis. *Arch. Neurol.* **51**:757–766.
11. King, F.W., and Shtivelman, E. 2004. Inhibition of nuclear import by the proapoptotic protein CC3. *Mol. Cell. Biol.* **24**:7091–7101.
12. Lock, C., et al. 2002. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. *Nat. Med.* **8**:500–508.
13. El Omari, K., Bird, L.E., Nichols, C.E., Ren, J., and Stammers, D.K. 2005. Crystal structure of CC3 (TIP30): implications for its role as a tumor suppressor. *J. Biol. Chem.* **280**:18229–18236.
14. Zhao, J., et al. 2008. Thirty-kilodalton Tat-interacting protein suppresses tumor metastasis by inhibition of osteopontin transcription in human hepatocellular carcinoma. *Hepatology.* **48**:265–275.
15. Hur, E.M., et al. 2007. Osteopontin-induced relapse and progression of autoimmune brain disease through enhanced survival of activated T cells. *Nat. Immunol.* **8**:74–83.
16. Ranscht, B. 1988. Sequence of contactin, a 130-kD glycoprotein concentrated in areas of interneuronal contact, defines a new member of the immunoglobulin supergene family in the nervous system. *J. Cell Biol.* **107**:1561–1573.
17. Rios, J.C., et al. 2000. Contactin-associated protein (Caspr) and contactin form a complex that is targeted to the paranodal junctions during myelination. *J. Neurosci.* **20**:8354–8364.
18. Wolswijk, G., and Balesar, R. 2003. Changes in the expression and localization of the paranodal protein Caspr on axons in chronic multiple sclerosis. *Brain.* **126**:1638–1649.
19. Jurynczyk, M., Jurewicz, A., Raine, C.S., and Selmaj, K. 2008. Notch3 inhibition in myelin-reactive T cells down-regulates protein kinase C theta and attenuates experimental autoimmune encephalomyelitis. *J. Immunol.* **180**:2634–2640.
20. Stidworthy, M.F., et al. 2004. Notch1 and Jagged1 are expressed after CNS demyelination, but are not a major rate-determining factor during remyelination. *Brain.* **127**:1928–1941.
21. Genoud, S., et al. 2002. Notch1 control of oligodendrocyte differentiation in the spinal cord. *J. Cell Biol.* **158**:709–718.

CD4+ T cells mediate cytotoxicity in neurodegenerative diseases

Stanley H. Appel

Department of Neurology, Methodist Neurological Institute, Houston, Texas, USA.

Neuroinflammation, characterized by activated microglia and infiltrating T cells, is a prominent pathological feature in neurodegenerative diseases. However, whether this inflammation contributes to neuronal injury or is a late consequence of neuronal injury is unclear. In this issue of the *JCI*, Brochard et al. report that CD4+ T cells are cytotoxic in a mouse model of Parkinson disease (PD) (see the related article beginning on page 182). Specifically, invading T lymphocytes contributed to neuronal cell death via the Fas/FasL pathway. The results implicate the adaptive immune system in the pathogenesis of Parkinson neurodegeneration and provide a meaningful rationale for immune-based therapies for PD.

Neuroinflammation in Parkinson disease

Parkinson disease (PD) is a motor system disorder, which is characterized by tremor, rigidity, slowed movements, and impaired balance and coordination and results from the loss of dopamine-producing cells in the brain. The nigrostriatal pathway — a neural pathway connecting the substantia nigra with the striatum — is one of four major dopamine signaling pathways in the brain and is prominently involved in controlling movement. In PD, the nigrostriatal pathway as well as dopaminergic and nondopaminergic neurons are compromised, and this is accompanied by inflammatory changes in microglia (the innate immune cells of the central nervous system) and infiltration of T lymphocytes (cells of the adaptive immune system). It has long been thought that microglial and T cell infiltration are not primary events in the pathogenesis of neurodegeneration but are instead responses to neuronal injury. However, recent studies (discussed below) support an alternative point of view and provide compelling evidence that both activated microglia and T lymphocytes make a significant contribution to neurodegeneration, at the very least by amplifying and exacerbating an ongoing inflammatory process and by triggering extensive neuronal degeneration to develop from a small population of stressed dopaminergic neurons.

The role of microglia in dopaminergic cytotoxicity

Much attention has focused on microglia as one of the mediators of the inflammatory response leading to dopaminergic neuronal injury. Microglia are similar to macrophages and are capable of exhibiting either an M1 proinflammatory phenotype (following activation with LPS), characterized by the secretion of proinflammatory cytokines, NO, and superoxide, or an M2 antiinflammatory phenotype (following incubation with IL-4), characterized by the secretion of neurotrophic factors such as IGF-1 and IL-10 (1). In vitro, LPS-induced microglial activation triggers the release of proinflammatory factors, including NO, H_2O_2, and superoxide, causing neurodegeneration of ventral midbrain dopaminergic neurons

Conflict of interest: The author has declared that no conflict of interest exists.

Nonstandard abbreviations used: ALS, amyotrophic lateral sclerosis; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson disease; α-Syn, α-synuclein.

Citation for this article: *J. Clin. Invest.* **119**:13–15 (2009). doi:10.1172/JCI38096.

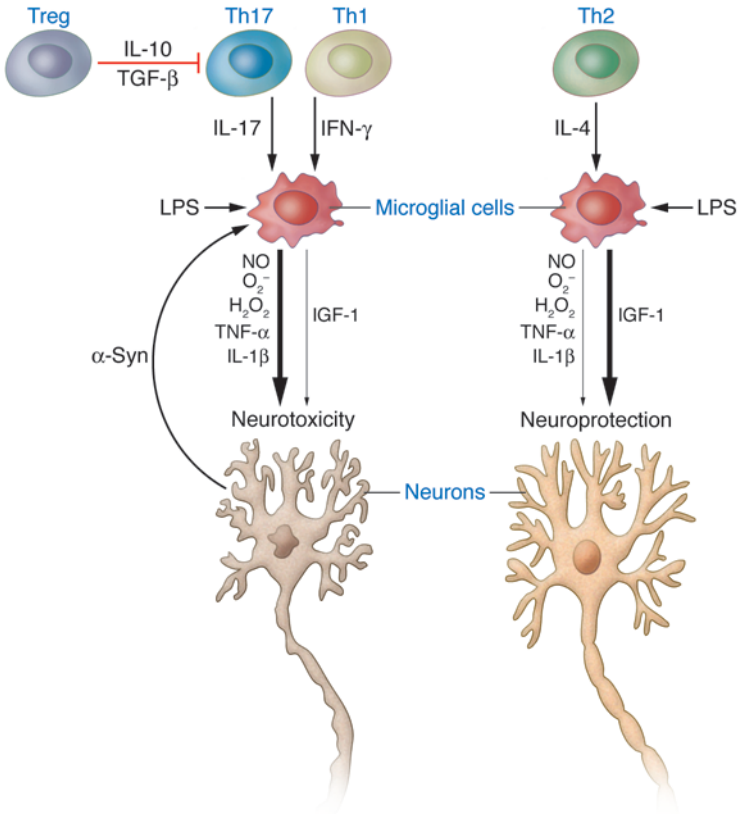

Figure 1
Potential role of T cells in modulating microglia-mediated neurotoxicity. On the left, the presence of Th1 and Th17 cells upregulates (thick lines) the production and release of free radicals from microglia, including NO, superoxide (O_2^-), and hydrogen peroxide (H_2O_2) as well as proinflammatory cytokines such as TNF-α and IL-1β, and downregulates (thin lines) the release of neurotrophic factors such as IGF-1. The result is enhanced neuronal injury, which may trigger the release of increased levels of nitrated/oxidized α-Syn and enhance microglia-mediated neurotoxicity. Tregs can suppress the proinflammatory effects of Th17 cells. On the right, Th2 cells release IL-4, upregulating the release from microglia of neuroprotective IGF-1, downregulating release of free radicals, and resulting in enhanced neuronal protection.

(2). In vivo, elevations of proinflammatory factors have been associated with degeneration of dopaminergic neurons in PD (3), in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine–intoxicated (MPTP-intoxicated) patients (MPTP is a neurotoxin, the administration of which causes PD symptoms) (4), and in MPTP-induced animal models of PD (5). Further, blocking microglial activation with the antibiotic minocycline in the MPTP-induced model of PD prevents dopaminergic neurodegeneration (6).

α-Synuclein as a mediator of microglial activation

The question of what could activate microglia and in turn aggravate neuronal injury has focused attention on the protein α-synuclein (α-Syn), which is expressed predominantly in neurons and is particularly enriched at presynaptic terminals. α-Syn accumulates in mutant forms (7) or as overexpressed wild-type protein (8) in familial PD and in nitrated and oxidized forms in cytosolic aggregates in PD patients (9). Transgenic expression of human α-Syn in mice renders dopaminergic neurons more vulnerable to LPS-induced inflammation, which in turn leads to accumulation of insoluble α-Syn aggregates in nigral neurons (10). In dopaminergic neuronal cultures, dopaminergic neuron cytotoxicity is dependent on the presence of α-Syn and is attenuated in the presence of inhibitors of NO and superoxide released from microglia (10). Thus, altered forms of α-Syn released from stressed neurons appear to participate in a self-propagating cycle, in which microglia are activated, enhancing the release of free radicals and proinflammatory cytokines and further amplifying dopaminergic neurodegeneration.

T cells mediate cytotoxicity in the MPTP-induced mouse model of PD

Although microglia are clearly participants in the pathogenesis of nigral neurodegeneration, the role of T cells has been heretofore less clear. The report by Brochard et al. in this issue of the *JCI* (11) documents the presence of activated microglia and $CD4^+$ and $CD8^+$ T cells in postmortem substantia nigra of PD patients as well as in the MPTP-induced model of PD during the course of neuronal degeneration. Following administration of MPTP, increased numbers of activated microglial cells were noted prior to the appearance of $CD3^+$ T cells and concomitant with astrogliosis, suggesting the possible role of microglia in recruiting T cells to the injured substantia nigra. When MPTP was administered to two different strains of immunodeficient mice that lack mature T lymphocytes (*Tcrb*$^{-/-}$ and *Rag1*$^{-/-}$ mice), T cell infiltration of the substantia nigra pars compacta was markedly reduced and dopaminergic cell injury was attenuated. In the absence of T cells, dopaminergic neurodegeneration was attenuated. Thus, T cells mediate neuronal cytotoxicity in the MPTP-induced murine model of PD. $CD4^+$ T cells are the population of T cells mediating the cytotoxicity, since dopaminergic neuron survival following MPTP administration was increased in *Cd4*$^{-/-}$ mice but not in *Cd8*$^{-/-}$ mice. The resistance to MPTP-induced neurodegeneration observed in *Rag1*$^{-/-}$ mice could be reversed when spleen cells from wild-type or *Ifng*$^{-/-}$ mice, but not from mice bearing mutant FasL, were passively transferred prior to MPTP administration. Thus, in MPTP-mediated dopaminergic cell death, $CD4^+$ T cells require the expression of a functional proapoptotic FasL but not the inflammatory cytokine Ifn-γ.

Previous studies have shown that levels of IFN-γ are significantly elevated in PD patients as well as in the MPTP-mediated mouse model of PD (12). In *Ifng*$^{-/-}$ mice, MPTP-induced loss of substantia nigra pars compacta neurons was attenuated as was microglial activation (12). The importance of Ifn-γ would suggest the involvement of $CD4^+$ Th1 cells, which can secrete Ifn-γ and

activate microglia (13). However, in the current study reported by Brochard et al. (11), the fact that the effect on MPTP-induced neurodegeneration was the same irrespective of whether spleen cells were passively transferred from wild-type or *Ifng*$^{-/-}$ mice (11) suggests that Ifn-γ may not be required for T cell–mediated dopaminergic cell death. Thus Th1 cells, which secrete Ifn-γ, may not be the relevant subpopulation involved in the MPTP-mediated T cell toxicity observed. The demonstration that expression of a functional FasL was required for the CD4$^+$ Th cell–mediated dopaminergic cell death supports the involvement of CD4$^+$ FasL$^+$ T cell populations, which could activate microglia to secrete proinflammatory factors (14). The recently described IL-17–secreting Th17 lymphocytes represent another proinflammatory T cell that could mediate CD4$^+$ T cell–induced cytotoxicity. The main function of IL-17–secreting T cells is to mediate inflammation, by stimulating production of inflammatory cytokines such as TNF-α, IL-1β, and IL-6 (15). Clearly, the identity of the T cells responsible for the enhanced dopaminergic cytotoxicity cannot be definitively determined until this issue is addressed by passive transfer of specific subpopulations of CD4$^+$ T cells. It is of interest that nitrated α-Syn may participate in the T cell–mediated cytotoxicity, since transfer of T cells from mice immunized with nitrotyrosine-modified α-Syn led to a robust neuroinflammatory response, with accelerated dopaminergic cell loss (16). Collectively, the data suggest that CD4$^+$ T cells mediate cytotoxicity, by activating microglia to release free radicals and proinflammatory cytokines and induce dopaminergic neurodegeneration. However, a direct toxic effect of CD4$^+$ T cells on substantia nigra dopaminergic neurons cannot be excluded.

Neuroinflammation can be neuroprotective as well as cytotoxic

Marked microglial activation and lymphocytic infiltration are also present in patients with the neurodegenerative disease amyotrophic lateral sclerosis (ALS) as well as in transgenic mice overexpressing mutant Cu-Zn superoxide dismutase 1 (mSOD1), an animal model of familial ALS (17). Surprisingly, T cells were reported to be neuroprotective in this model, rather than cytotoxic as currently reported in the MPTP-induced murine models of PD (11) (Figure 1). When mSOD1 mice were bred with *Rag2*$^{-/-}$ mice lacking functional T cells or with *Cd4*$^{-/-}$ mice lacking CD4$^+$ T cells, motor neuron disease was accelerated, accompanied by increased mRNA levels of proinflammatory cytokines and NADPH oxidase 2 (NOX2), which is responsible for generating superoxide (18). Levels of trophic factors and glial glutamate transporters were also decreased. Bone marrow transplants reconstituted mice with T cells, prolonged survival, and suppressed cytotoxicity in conjunction with restoring expression of neuroprotective factors and lessening the expression of NOX2. Thus, CD4$^+$ T cells significantly influence the neurodegenerative process in both the MPTP-induced murine model of PD and a transgenic mouse model of familial ALS, mediating cytotoxicity in the former and neuroprotection in the latter.

Conclusion

The development of immunotherapeutic approaches to the treatment of PD will depend on determining which subpopulations of CD4$^+$ T cells are responsible for cytotoxicity and which of these may enhance neuroprotection. With the compelling evidence now provided by Brochard and colleagues (11), that CD4$^+$ T cells mediate cytotoxicity in the MPTP-induced mouse model of PD, Th1 and Th17 cells become potential targets in efforts to minimize the hostile neuronal microenvironment. IL-12 enhances expression of the transcription factors STAT4 and T-bet, which regulate lineage commitment and development of CD4$^+$ Th cells from naive T cells. TGF-β, in addition to IL-6 or IL-21, enhances expression of the transcription factor RORγt and promotes development of Th17 from naive T cells, the expansion of which is enhanced by IL-1 and IL-23 (19). Suppressing these differentiating signals as well as the Th1 and/or Th17 cells themselves has now become a potentially meaningful approach to immunotherapy for PD. An alternative approach might focus on Tregs, which appear capable of regulating immune responses mediated by other T cell subtypes, including Th17 cells. Therefore, increasing numbers of Tregs in order to suppress proinflammatory T cells might also foster a more neuroprotective environment. Most significantly, these and other immune-based strategies now have a cogent rationale, given the demonstration that T cells themselves are orchestrating cytotoxic events in the MPTP-induced mouse model of PD (11) and possibly in human PD itself.

Acknowledgments

The author is supported by the Muscular Dystrophy Association and the NIH.

Address correspondence to: Stanley H. Appel, Department of Neurology, Methodist Neurological Institute, 6560 Fannin St., #802, Houston, Texas 77030, USA. Phone: (713) 441-3765; Fax: (713) 793-7271; E-mail: sappel@tmhs.org.

1. Mantovani, A. 2004. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol.* **25**:677–686.
2. Le, W., et al. 2001. Microglial activation and dopaminergic cell injury: an in vitro model relevant to Parkinson's disease. *J. Neurosci.* **21**:8447–8455.
3. McGeer, P.L., Itagi, S., and Akiyama, H. 1988. Rate of cell death in parkinsonism indicates active neuropathological process. *Ann. Neurol.* **24**:574–576.
4. Langston, J.W., et al. 1999. Evidence of active nerve degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyidine exposure. *Ann. Neurol.* **46**:598–605.
5. Liberatore, G., et al. 1999. Inducible nitric oxide synthase stimulates dopaminergic neurodegeneration in the MPTP model of Parkinson disease. *Nat. Med.* **5**:1403–1409.
6. Wu, D.C., et al. 2002. Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyidine mouse model of Parkinson disease. *J. Neurosci.* **22**:1763–1771.
7. Polymeropoulos, M.H., et al. 1997. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science.* **276**:2045–2047.
8. Farrer, M.J., et al. 2004. Comparison of kindreds with parkinsonism and alpha-synuclein genomic multiplications. *Ann. Neurol.* **55**:174–179.
9. Martinez-Vicente, M. 2008. Dopamine-modified alpha-synuclein blocks chaperone-mediated autophagy. *J. Clin. Invest.* **118**:777–788.
10. Gao, H.-M., et al. 2008. Neuroinflammation and oxidation/nitration of α-synuclein linked to dopaminergic neurodegeneration. *J. Neurosci.* **28**:7687–7698.
11. Brochard, V., et al. 2009. Infiltration of CD4$^+$ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. *J. Clin. Invest.* **119**:182–192.
12. Mount, M.P., et al. 2007. Involvement of interferon-gamma in microglia-mediated loss of dopaminergic neurons. *J. Neurosci.* **27**:3328–3337.
13. Mosmann, T.R., et al. 1986. Two types of murine helper T cell clone. 1. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* **136**:2348–2357.
14. Park, D.R., et al. 2003. Fas(CD95) induces proinflammatory cytokine responses by human monocytes and monocyte-derived macrophages. *J. Immunol.* **170**:6209–6216.
15. Kebir, H., et al. 2007. Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. *Nat. Med.* **13**:1173–1175.
16. Benner, E.J. 2008. Nitrated α-synuclein immunity accelerates degeneration of nigral dopaminergic neurons. *PLoS ONE.* **3**:e1376.
17. Henkel, J.S., Beers, D.R., Siklos, L., and Appel, S.H. 2006. The chemokine MCP-1 and the dendritic and myeloid cells it attracts are increased in the mSOD1 mouse model of ALS. *Mol. Cell. Neurosci.* **31**:427–437.
18. Beers, D.R., Henkel, J.S., Zhao, W., Wang, J., and Appel, S.H. 2008. CD4+ T cells support glial neuroprotection, slow disease progression, and modify glial morphology in an animal model of inherited ALS. *Proc. Natl. Acad. Sci. U. S. A.* **105**:15558–15563.
19. Mills, K.H.G. 2008. Induction, function, and regulation of IL-17-producing T cells. *Eur. J. Immunol.* **38**:2636–2649.