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Characteristic Cytokine Profile of Individual Cell Populations in the Normal and Toxoplasma Gondii-infected Murine Brain

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During infectious encephalitis a complex cytokine network is activated in the CNS, to which immune cells and brain parenchymal cells may contribute. The *in vivo* cytokine production of individual cell populations in the brain, which is still largely unknown, was studied in normal and *Toxoplasma gondii*-infected BALB/C mice by immunohistochemistry and *in situ* hybridization. In addition, leukocytes were isolated from the brain by Percoll gradient separation. These cells were either analysed for cytokine production by flow cytometry or further purified using the high-gradient magnetic activated cell separation, followed by RT-PCR evaluation of cytokine mRNA transcripts. In the normal brain, microglia/macrophages produced IL-1β, IL-10, and TNF-α. In the *Toxoplasma gondii*-infected brain, these cells showed a *de novo* expression of iNOS. $CD4^+$ and $CD8^+$ T cells produced IL-2, IL-10, TNF-α, and IFN-γ. IL-4 was exclusively produced by $CD4^+$ T cells, whereas $CD8^+$ T cells expressed IL-1β. These experiments reveal a characteristic cytokine profile of individual cell populations in the CNS. The immune-mediated control of *Toxoplasma gondii* in the brain results from the interaction of brain parenchymal cells and immune cells recruited to the CNS and their cytokines.

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Tolerance in EAE: A Central Role for CTLA-4

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Murine relapsing-remitting experimental autoimmune encephalomyelitis (R-EAE) is a $CD4^+$ T cell-mediated demyelinating disease inducible in SJL mice by the injection of PLP139-151. During the first relapse, encephalitogenic T cell responses arise to PLP178-191. I.V. injection of neuroantigen-coupled cells is an effectively prevents or treats R-EAE. Treatment prior to or following priming with PLP139-151 results in the blockade of disease induction. To understand the mechanisms involved in coupled-cell induced tolerance, we have used DO11.10 transgenic mice (ova albumin 323-339 specific). Treatment with OVA323-339-coupled cells in recipients of DO11.10 cells or in the transgenic mice resulted in the rapid activation of antigen-specific cells determined by CD25, CD44, CD62L and CD69 surface expression. This encounter resulted in a large increase in the intracellular expression of CTLA-4, a negative regulator of T cell activation. To test the role of CTLA-4 in tolerance to R-EAE, mice were pre-tolerized to PLP178-191 and primed with PLP139-151. Treatment with anti-CTLA-4 resulted in the reversal of tolerance as determined by DTH. Thus CTLA-4 appears to play an important role in the maintenance of long-term immune tolerance.

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Cytokine Profiles in the Brains of Mice Infected with Semliki Forest Virus Indicate a Switch from an Early Th1 Response to a Later Th2 Response

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Following intraperitoneal inoculation of mice, the alphavirus, Semliki Forest virus (SFV, A7[74]) gains access to the CNS across cerebral endothelial cells and enters neurons and oligodendrocytes (Fazakerley et al., Virology, 195, 627-637, 1993). A predominantly $CD8^+$ inflammatory infiltrate, intrathecal antibodies and upregulation of MHC-I and -II molecules are observed in the CNS (Morris et al., J.Neuroimmunol., 74, 185-197, 1997). $CD8^+$ T-cells are necessary for demyelination (Subak-Sharpe et al. J. Virol. 67, 7629-7633, 1993). In mature CNS cells viral replication is restricted and in the absence of T-cells can persist with no apparent pathological consequences (Amor et al., J.Gen.Virol., 77,281-291, 1996). Measurement of cytokine responses in the CNS using an RNase Protection Assay indicate that a Th1 response is followed by a Th2 response. Transcripts for IL-12, TNFα, and IL-1β rapidly increase (maximal at days 4 and 5) and are followed by rises in transcripts for IL-10 and IL-6 (maximal at day 7). The TNF-α and IFN-γ response profiles are biphasic. The IL-12 and IL-10 levels show an inverse correlation. Levels of all the above cytokines fall as virus is cleared from the brain (day 7 to 10), and levels of TNF-β rise steadily from this time to 3 weeks and remain elevated for weeks post-infection. B-cells are most numerous during this phase. Immunoglobulin isotype levels of anti-viral antibodies confirm that Th1 associated IgG2a responses predominate in the early infection (days 5 to 10). The Th2 associated IgG1 response develops relatively slowly over a period of several weeks.

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Pertussis Toxin Enhances Differentiation and Induces Cytokine Class Switching of Antigen-specific T Cells

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Pertussis toxin (ptx) is part of most protocols for inducing EAE in murine models. Opening of the blood-brain-barrier and inhibition of peripheral T cell anergy have been postulated as possible mechanisms for its actions, but its effects in autoimmune disease remains enigmatic. We have used a model system in which adjuvant-guided type-1 and type-2 immunity to Hen eggwhite lysozyme (HEL) and myelin basic protein (MBP) are selectively induced in BALB/c and B10.PL mice to investigate the effects of ptx on cytokine balances and T cell frequencies. Our results show: a) ptx markedly enhances the frequency of antigen-specific T cells as measured by ELISA spot, b) the amount of cytokine produced per cell is increased. c) ptx elicits a strong type-1 cytokine response when added to incomplete Freund's adjuvants (IFA) as the immunizing protocol. We conclude that another possible mechanism by which ptx facilitates induction of EAE is by enhancing differentiation of autoantigen-specific T cells, increasing cytokine output on the single cell level, and favoring type-1 differentiation of T cells, thereby expanding the number of pathogenic T cells and maximizing the likelihood of the development of EAE.

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Th1/Th2 Balance in Inflammatory Neurologic Diseases

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In order to clarify the Th1/Th2 balance, we studied the ratio of IFN γ -prod ucing cells to IL-4-producing cells among the CD4+ T cells from peripheral blood by flow cytometory as well as serum total and allergen-specific IgE by ELISA in 30 patients with acute monophasic or recurrent myelitis, 64 patients with multiple sclerosis (MS) including 25 patients with optic-spinal form, 33 patients with Guillain-Barre syndrome (GBS), 14 patients with mononeuritis multiplex, 12 patients with chronic inflammatory polyradiculoneuropathy, 32 patients with HAM/TSP, 29 HTLV-I carriers without HAM/TSP, 30 patients with neurodegenerative disorders and 40 healthy controls. Acute myelitis, GBS and mononeuritis multiplex showed a significantly higher frequency of both hyperIgEemia and mite antigen-specific IgE and a lower IFN γ /IL-4 ratio in the CD4+ T cells than did the healthy controls. On the contrary, HAM/TSP and optic-spinal form of MS showed a significantly lower frequency of total and all ergen-specific IgE and a higher IFN γ /IL-4 ratio in the CD4+ T cells than did the healthy controls. Therefore, these findings suggest that acute myelitis, GBS and mononeuritis multiplex are Th2 dominant disease, while HAM/TSP and optic-spinal form of MS are Th1 dominant disease.

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Excitation of Lateral Hypothalamus Shifts the Splenic Th1/Th2 Balance Toward Th1 Dominance

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Lateral hypothalamus (LH) is known as a potent reward/pleasure center. Recently, we indicated that acute stimulation of LH increased splenic NK cell activity. In the present study, we assumed LH might be a center for promoting cellular immunity and investigated whether LH is relevant to Th1/Th2 balance. We electrically stimulated and/or ablated LH areas, potent reward centers, of Wister King Aptektman male rats and investigated splenic $CD4^+$, $CD8^+$ and $CD4^+CD45RC^+$ (Th1 like) cells by flowcytometry after 24 hours post operations and measured, using ELISA, in vitro IFNγ and IL4 productions from non-adherent splenocytes stimulated with anti-CD3ε . Data were analyzed by paired t test. After electrical stimulation of LH, $CD4^+$ and $CD4^+CD45RC^+$ cells increased significantly. After ablation of LH, $CD4^+$ cells significantly decreased. Chronic or severe stress is known to reduce the number of $CD4^+$ cells. These results suggest that LH have anti-stress effects in terms of immunity. On cytokine productions, the LH-stimulated rats showed a marked increase, twice as much as the sham operated ones, in the amount of IFNγ productions and a reduction by half in the amount of IL4 productions, but no significant changes were detected after ablation. These results strongly suggest that excitation of LH increases Th1 activity in the spleen and reward may be relevant to Th1/Th2 balance.