Cerebrospinal Fluid

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Examination of cerebrospinal fluid (CSF) in the context of multiple sclerosis (MS), is valuable for several reasons. First, routine diagnostic evaluation of CSF cell counts and various forms of immunoglobulin determination are important to differentiate MS from other diseases. Second, because MS most probably is an organ-specific inflammatory disease and CSF is often the closest one can get to the target organ, examination of this fluid may allow basic studies on the immunopathogenesis of the disease, and indications of different aspects of inflammation should be considered when evaluating treatments aimed at reducing central nervous system inflammation. This article describes measurements taken at the cellular level in blood and CSF, of myelin-antigen autoreactive B- and T-cell responses, as well as cytokine production. Patients with MS display greatly increased numbers of cells in the CSF that produce antibodies against a variety of myelin antigens, such as myelin basic protein, proteolipid protein, and myelin-oligodendrocyte glycoprotein. Such antibodies may promote demyelination, and autoreactive B cells may enhance antigen presentation to T cells. There is also an increased number of T cells in MS, which in response to a broad range of myelin antigens and peptides, produce cytokines. The production of interferon-γ, belonging to the T helper-1 type of cells, may have a disease up-regulatory role, while production of other cytokines, such as transforming growth factor β, may counteract disease. Accurate measurements of cellular production of cytokines will be important in the design and monitoring of immunotherapy.

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In spite of the recent developments in neuroradiological techniques, examination of the cerebrospinal fluid (CSF) still plays an important role in the diagnosis, as well as in more research-directed aspects, of multiple sclerosis (MS). Furthermore, CSF is very often the closest to the target for inflammatory attack one can reach. I will first very shortly review certain examinations of the CSF we think are appropriate in the diagnosis and differential diagnosis of MS, and then discuss cellular reactivities against myelin antigens and expression of different cytokines.

Routine Cerebrospinal Fluid Examinations

It should be well known to every neurologist, but I think it is important to emphasize, that counting of the mononuclear cells in CSF is appropriate to do, and if there are more than 50×10^6 cells/L, one should consider diagnoses other than MS. The intrathecal immunoglobulin IgG synthesis is another still important aspect of MS. Other immunoglobulin isotypes are also of interest, but their determinations have provided little with regard to diagnostic measures. We calculate IgG index equal to IgG CSF:IgG plasma/albumin CSF:albumin plasma [1]. This index is, in our hands, increased in approximately 70% of MS patients. Oligoclonal IgG bands present in the CSF, and absent or fewer in the plasma, we find in approximatley 95% of MS patients. In many materials, lower percentages are found that, however, might be due to less accurate methods to find these bands. To detect oligoclonal IgG we use isolectric focusing, transfer to nitrocellulose membranes, and immuostaining [2]. It is important to always run the CSF in parallel with plasma, since there are conditions where there are similar bands both in plasma and CSF, a pattern that does not indicate intrathecal immunoglobulin synthesis. Thus, presence of oligoclonal IgG bands in the CSF supports the diagnosis of MS. However, it is also important to emphasize that CSF oligoclonal IgG bands are not specific for MS. There are certain other conditions where we frequently find these bands, such as borreliasis, neurosyphillis, human immunodeficiency virus (HIV) infection, and various forms of connective tissue diseases.

Myelin Antigen Autoreactive B- and T-Cell Responses and Cytokine mRNA Expression in CSF and Blood

It has been problematic throughout the years to find antibody responses to putative target autoantigens in MS. This might well be due to binding of such antibodies to target autoantigens in vivo. To bypass this problem we have isolated mononuclear cells from CSF and blood and studied their antibody production in vitro. With this approach we consistently find in patients with

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MS dramatically increased numbers of cells producing antibodies against whole myelin and myelin basic protein (MBP) [3].

Later, we examined a whole series of different myelin antigens. In MS patients there are increases also in numbers of cells producing antibodies against myelin oligodendrocyte glycoprotein (MOG), myelin-associated glycoprotein (MAG), and proteolipid protein (PLP) [4–6]. Such cells are consistently strongly enriched in CSF, close to the target for inflammatory attack. They are, however, not specific for MS. One example of this is nerve trauma. Herein, nerve biopsy provokes an expansion of both B and T cells reactive with myelin antigens [7]. Thus, in MS there are increased numbers of cell secreting antibodies against myelin antigens, strongly enriched in CSF close to the target for inflammatory attack. Although present in high numbers in MS, they may also occur in control patients, but at lower levels, and they may appear secondary to nervous tissue damage. This, however, does not exclude a pathogenetic role of myelin-autoreactive B cells. Conceivably, the autoantibodies may opsonize for macrophage attack and participate in complement-mediated myelinolysis. A second point is that B cells are very effective in presenting antigen. Since they have surface receptors for antigens they may concentrate low amounts of antigens that after processing are presented for autoreactive T cells.

Myelin antigen–directed T cell–mediated autoimmunity has been more difficult to study throughout the years. In T-cell activation the critical interaction between accessory cells and T cells is taking place in the three molecular complex with a peptide antigen bound by a major histocompatibility (MHC) molecule, recognized by the T-cell receptor. This results in activation of the T cell, implying proliferation and/or production of lymphocyte effector molecules, that is, lymphokines/cytokines. To measure T cells, most previous studies have utilized proliferative responses to antigen, but with this outread, most studies have been negative with regard to any increased levels of myelin-reactive T cells in MS. In such studies it might be more relevant to use production of cytokines as outread, due to their important immunoregulatory and effector roles. Recently, two subsets of T-helper cells were defined, that is T helper-1 (TH1) and T helper-2 (TH2) cells. The former produce interleukin-2 (IL-2), interferon-γ (IFN-γ), and lymphotoxin, while the latter produce IL-4, 5, 6, and 10. There are many reasons to believe that a TH1 type of response may be pathogenetically relevant in context with MS [8, 9]. Intra-CNS production of IFN-γ roughly correlates with neurological deficts during experimental autoimmune encephalomyelitis (EAE) [10–12]. T cells that transfer EAE are of the TH1, but not TH2, phenotype [13] and systemic administration of IFN-γ worsens MS disease course [14]. Any disease relevant role of TH2 cells is more unclear; such cells may, however, influence autoreactive B cells.

Additional facts adding to the putative importance of IFN-γ in context with MS are that this cytokine potently activates macrophages, induces MHC antigens, and induces production of other cytokines such as IL-1, tumor necrosis factor-α, and leads to T-cell homing, a phenomenon that in many respects characterizes MS lesions.

Studies of cytokines are not easily accomplished because they often act autocrinely or paracrinely, and seldom endocrinely, so that body fluid levels might very poorly reflect their in vivo production and effects. One should, therefore, preferably determine cellular cytokine production. Using an immunospot method detecting single cells secreting IFN-γ, we some years ago found that MS patients have strongly increased numbers of IFN-γ–producing cells in CSF compared patients with tension headache [15]. To assess the numbers of memory cells that produce IFN-γ (TH1-like cells) in response to antigens, one can add the antigen in question to cultures and enumerate responding cells. Herein, MS patients display strongly increased numbers of cells in blood, enriched in the CSF, producing IFN-γ in repsonse to MBP [15]. May any particular peptide stretch of MBP be preferentially recognized by these autoreactive T cells? We studied this issue in a total number of 70 MS patients and found a very broad response as to MBP peptides recognized. However, the magnitude of the MBP peptide–specific responses were strikingly increased compared with three different control groups [16]. We later showed that several myelin antigens such as PLP, MOG, and MAG are recognized by increased numbers of T cells [4–6]. However, even if similar, cloned T cells in rodents have been shown to transfer EAE, it is difficult by pure phenomenology to ascribe a pathogenetic role to these cells in MS. Their role might be better elucidated by quantitative assessment in context with various therapeutic trials, for instance, during INF-β treatment, which indeed influences MS disease course.

There are also putative disease down-regulatory cytokines, and of those we have focused largely on transforming growth factor-β (TGF-β). Systemic administration of this cytokine ameliorates EAE [17], and cells suppressing EAE effector cells have been shown to produce this cytokine [18]. Disruption of the TGF-β results in a multifocal inflammatory disease, suggesting that this cytokine indeed is an endogenous immunosuppressive molecule [19]. To study a variety of cytokines, including TGF-β, we have used an in situ hybridization method [20, 21] in which synthetic radiolabeled oligonucleotide probes are applied to cells from CSF and from blood. After autoradiography, cytokine-expressing cells can easily be counted in dark-field mi-

croscopy. Herein, patients with MS have increased numbers of cells in blood expressing IFN-γ, IL-4, and TGF-β compared with controls. In the CSF, there is an approximately 10-fold enrichment of these types of cytokine-expressing cells [22]. We have not yet made extensive comparisons with clinical MS variables. However, we have found that patients with slight or low disability have considerably higher numbers of TGF-β–expressing cells in blood than those with severe disability, while the opposite is true for IFN-γ–expressing cells. By using this methodology to study memory cells that respond to myelin antigens and produce specific cytokines, we have shown that MS patients display increased numbers of cells producing IFN-γ, IL-4, and TGF-β in response to both PLP and MBP, with an enrichment in the CSF. In individual cases the relative expression of specific cytokines is often dissociated [23–25].

In conclusion, (1) cytokines have potent effector and immunoregulating roles; (2) the cytokine spectrum and level of production is decisive for the outcome of inflammatory disease; (3) myelin autoantigens induce cytokine production in vitro, and there is an increased expression of cytokine expressing cells in MS patients also in vivo. These observations will have importance for the basic understanding of MS, as well as for design and monitoring of various kinds of immunotherapy.

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