Letter to the Editor

Multicentric Castleman’s disease with impaired lymphocytic apoptosis

Dear Editor,

Multicentric Castleman’s Disease (MCD) is a rare non-malignant lymphoproliferative disorder presenting systemic symptoms such as fever, night sweats, fatigue, anemia, effusions and multifocal lymphadenopathy. Human herpes virus type 8 (HHV-8) is known to cause MCD in HIV patients and IL-6 and VEGF may contribute to non-clonal lymphoproliferation and unique manifestations, but the etiology of non-HIV MCD is not clear. Here, we describe the first case of MCD with increased CD3⁺/CD8⁺/CD4⁺ CD8⁺ double-negative T (DNT) cells and impaired lymphocytic apoptosis.

A 37-year old woman with a history of type-I diabetes and Grave’s disease was admitted to our hospital for evaluation of diffuse ground grass opacities, small nodules in both lung fields with hilar and mediastinal lymphadenopathy in chest radiographs and computed tomography (Fig. 1a, b). On admission, she had fatigue, dyspnea and slight fever with normocytic anemia, polyclonal hypergammaglobulinemia, and elevations of C-reactive protein and soluble IL-2 receptor (1410 U/ml). None of bacteria, fungi, or viruses including HIV, EBV, and HHV-8 was detected in her peripheral blood. In contrast, anti-nuclear antibody anti-SSA, and rheumatoid factor were positive, in addition to endocrine disorder-related autoantibodies, but she did not show any findings that suggest rheumatoid disorders.

To make a definite diagnosis, a surgical lung and mediastinal lymph node biopsy was performed. Histopathological findings showed infiltration of polyclonal and IgG4-negative plasma cells (Fig. 1c, d), and plasma IL-6 (23.1 pg/ml) and VEGF (667 pg/ml) levels were elevated. These findings supported the diagnosis of MCD. However, she showed no neuropathy, skin abnormality, neuropathy or edema, and clonal plasma cells were not detected in her bone marrow, negating the POEMS syndrome. We next focused on her unique family history. Of note, her mother and brother also had autoimmune endocrine disorders, suggesting that genetic basis may underlie her autoimmune endocrine disorders and MCD. Autoimmune lymphoproliferative syndrome (ALPS) is another rare non-malignant lymphoproliferative disorder associated with increased DNT cells (Fig. 2) and apoptosis defect of lymphocytes (Fig. 3), usually due to inherited genetic abnormality in the apoptosis-related pathway. Therefore, we enumerated DNT cells, which are hallmark of ALPS that shows both familial autoimmune disorders and nonmalignant proliferation of lymphocytes. Interestingly, flow cytometry of her peripheral mononuclear cells showed increased proportion of DNT cells (4.9% in total T cells) beyond the criterion of ALPS (>2.5%). Increased proportions of DNT cells were also observed in her mother and brother as well as autoimmune endocrine disorders, confirming familial predisposition of a DNT cell expansion. In addition, she showed increased survival of phytohemagglutinin-activated T cells after incubation in the presence of anti-Fas antibody and methylprednisolone, but not 50 μM C2-ceramide, suggesting the impairment of Fas-induced apoptosis. We also found elevated plasma IL-18 level (866 pg/ml), which is another accessory criterion of ALPS (>500 pg/ml). However, in spite of her several ALPS-like features, her clinical features were not sufficient to be diagnosed as ALPS because mutation in FAS gene was not detected in her lymphocytes.

MCD is a polyclonal lymphoproliferative disorder with systemic manifestations due to increased IL-6. In patients with HIV, HHV-8 infection may lead to MCD, however, the etiology of non-HIV MCD has not been clarified to date. In small numbers of patients, plasma cell myeloma contributes to development of MCD, especially in the POEMS syndrome, but in majority of patients with MCD, the pathogenesis including the origin of IL-6 production is not known. Our present case had MCD with ALPS-like features, suggesting the possibility that lymphoproliferation driven by impaired lymphocytic apoptosis led to the clinical features of MCD, although increased proportion of DNT cells and mutations in the apoptosis-related genes have not been reported in MCD.

It has been reported that Fas/Fas ligand-deficient lpr and gld mice show an increase of DNT cells, autoimmunity, and lymphoproliferation with massive and systemic enlargement of lymph nodes. Histological evaluation of lymph nodes of these mice shows blurring of nodal architecture with lymphocytic proliferation of histiocytes and plasma cell. In addition, the histological feature of enlarged lymph node in ALPS is reactive follicular hyperplasia, often with focal progressive transformation of germinal center and polyclonal plasma-cytosis. The histological findings of our case, which showed the features of MCD including polyclonal interfollicular expansions of plasma cells, may resemble with those seen in Fas/Fas-ligand deficient lpr and gld mice, suggesting the involvement of impaired lymphocytic apoptosis in the pathogenesis of MCD in our case.

Our patient was not diagnosed as having ALPS in spite of her several ALPS-like clinical features. The lack of mutations in the FAS gene was one of the main reasons why her clinical characteristics did not fulfill the criteria of ALPS, however, it has been reported a genetic abnormality other than FAS mutation in majority of patients with ALPS. In fact, mutations in FAS ligand, caspase-8,-10 and neuroblastoma RAS were found in patients with ALPS. In the present case, although all possible gene mutations have not been analyzed,
increased count of DNT cells observed not only in our case but also in her family members suggests a genetic abnormality other than FAS mutation may underlay the pathogenesis of the patient. The clinical features of our case suggest the possible relationship between MCD and ALPS. However, there are some differences between MCD and ALPS. The majority of ALPS patients have mutations in apoptosis-related genes such as FAS, whereas it is not clear whether MCD patients have these mutations. In addition, a raised DNT cell subset has not been reported in MCD. Furthermore, serum IL-6 level is normal or mildly elevated in ALPS. However, MCD has many variants, as several different histological patterns show, and ALPS, the syndrome may also have many variants, as several known or unknown gene mutations show. In this regard, MCD-ALPS overlap may exist, and the present case would be included in the category.

This patient has been treated with oral corticosteroid, because corticosteroid is used as the treatment for both ALPS and MCD. However, efficacy of predonisolone has been insufficient for lymphadenopathy and pulmonary infiltration in our patient, and optimal treatment for impaired lymphocyte apoptosis, including ALPS, remains to be controversial. Immunosuppressive drugs and immunotherapy for B lymphocytes with anti-CD20 rituximab has been attempted for ALPS, but their clinical efficacy and safety should be further investigated.

**Fig. 1.** HRCT of the chest at diagnosis shows bilateral centrilobular nodules, thickening of the interlobular septa and mediastinal lymphadenopathy (a, b). Histological findings of the lung (c) and mediastinal lymph node (d) show infiltration of polyclonal and IgG4-negative plasma cells around the bronchovascular bundles and in the interfollicular areas of the nodes (H&E A ×40, B ×200).

**Fig. 2.** Double-negative T (DNT) cells in the peripheral blood mononuclear cells (MNCs). Four-color flow cytometry showed increased proportion (4.9%) of CD4⁺CD8⁻ DNT cells in the αβTCR⁺CD3⁺ fraction (Gate X3). In control MNCs from 3 healthy individuals, proportions of DNT cells were less than 0.1% (data not shown).

**Fig. 3.** Relative cell survival after apoptosis assay. Peripheral blood mononuclear cells taken from the patient with ALPS and 2 control healthy individuals were incubated in the RPMI supplemented with 10% fetal bovine serum and 2 U/mL IL-2. During incubation, cells were stimulated by phytohemagglutinin (PHA) for 6 h at days 0 (1 μg/mL) and 12 (0.1 μg/mL) and washed. At day 18, cells were collected and anti-Fas antibody (1 μg/mL), methyl-predonisolone (mPSL; 100 μM), C2 ceramide (50 μM), or control medium was added. After further incubation in the presence of these reagents for 18 h, proportions of survived cells were evaluated using trypan blue, and percentages of cell survival in the presence of indicated reagents relative to cell survival after incubation with control medium were calculated.
In conclusion, the first case of MCD with increased DNT cells and impaired lymphocytic apoptosis described here provides the further evidence for understanding the pathogenesis of MCD. Although the present case suggests possible involvement of impaired lymphocytic apoptosis in the pathogenesis of MCD, we need more cases to clarify the relationship between MCD and ALPS as well as optimal therapy for these patients in the future.

Conflict of interest
The authors have no conflict of interest to declare.

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