論 文 要 旨

Histopathologic differences between human T-lymphotropic virus type 1 (HTLV-1)-positive and HTLV-1-negative polymyositis

（ HTLV-1 陽性多発性筋炎と HTLV-1 陰性多発性筋炎における病理組織学的差異の検討 ）

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【序論および目的】

Human T-lymphotropic virus type 1 (HTLV-1) is a causative agent of adult T-cell leukemia (ATL). HTLV-1 is reported to be associated with a particular type of chronic progressive myelopathy: HTLV-1-associated myelopathy/tropical spastic paresis (HAM/TSP). Shortly after, HTLV-1 infection was linked to other inflammatory diseases such as uveitis, arthritis, bronchoalveolitis, Sjögren syndrome, and myositis. Epidemiologic studies show a high incidence of seropositivity for HTLV-1 among polymyositis (PM) patients. However, whether HTLV-1 is a direct causative agent or it plays a role in the pathogenesis of PM remains unknown. In addition, HTLV-1 infection is closely related to inclusion body myositis (IBM). PM and IBM are 2 types of inflammatory myopathies. IBM can be distinguished from PM by the presence of rimmed vacuoles, cytoplasmic inclusions, and amyloid deposits in addition to inflammatory changes. To determine the clinical and histopathological differences between HTLV-1-positive and HTLV-1-negative PM, we retrospectively evaluated patients with PM not associated with any other disease and conducted a comparative study.

【材料および方法】

Of all patients who underwent muscle biopsy during the previous 10 years at Kagoshima University Hospital, South Kyushu, Japan, 68 patients were reported to be diagnosed with inflammatory myopathy; of these patients, 21 PM cases were carefully selected for this study, and re-evaluated using the modified criteria introduced by Dalakas and Hohlfeld (2003) by Immunofluorescence double staining for CD8 and MHC-1. We evaluated HTLV-1 positivity through serology, confirmed it by nested PCR using DNA extracted from muscles, and then assessed the tissue viral load. Meticulous histopathological examination was performed using routine histochemical for (H&E), modified Gomori trichrome, PAS, Sudan black, myosin ATPase, NADH-tetrazolium reductase, CCO, AMP deaminase, acid phosphatase, and SDH and immunohistochemical staining, for CD4, CD8,CD20, CD68, NCAM, MHCn, Perforin, HSP47, thrombomodulin. Selected cases were examined by electron microscopy.

【結果】

Nested PCR using DNA extracted from muscle and serological evaluation showed that among the 21 patients with PM, 11 were HTLV-1-positive and 10 were HTLV-1-negative. There were no significant differences between groups regarding age, sex or serum CK levels. However, the duration of illness was significantly longer in the HTLV-1-positive group ($p = 0.0043$).

At the level of histopathology, both groups demonstrated classic features of PM, following significant differences were found in the HTLV-1-positive PM group compared to the HTLV-1-negative PM group;

- Endomysial inflammatory infiltrates were more prominent ($p = 0.0008$).
- Necrotic fibers were less frequently seen ($p = 0.0201$).
- Regenerative activities were more prominent for MHCn-positive staining fibers ($p = 0.045$) and for NCAM-positive fibers ($p = 0.063$).
- Partial or complete CCO reduction was more frequent ($p = 0.0031$) .
- Electron microscopy demonstrated mitochondrial morphological abnormalities in HTLV-1-positive PM and the percentage of mitochondria with morphological abnormalities correlated with the counts of CCO reduced or negative fibers ($p = 0.0304$).

【考察および結論】

In this study, the incidence of PM was 31% (21/68), which is higher than that of other types of inflammatory myopathies. This might be because this study was conducted on patients with myositis in Kagoshima and Okinawa prefectures, where HTLV-1 is endemic and usually shows the PM phenotype. Therefore, the association of PM with HTLV-1 may not be coincidental.

Regarding the clinical course, patients of HTLV-1-positive PM run a more protracted course, because they have a significantly longer duration of illness (from time of onset of symptoms until the of biopsy). Beside the differences in histopathology, significantly higher endomysial infiltration of CD8+ and CD4+ T-cells was observed in HTLV-1-positive PM; some of the infiltrating CD4+ cells in muscle lesions are considered to be the HTLV-1 genome-harboring cells. We also observed the accumulation of CD8+ T-cells around aberrant normal myofibers. This suggests that myositis in patients with HTLV-1 infection is not caused by a direct viral infection to muscle fibers, but rather by an immune reaction between HTLV-1-infected CD4+ cells and HTLV-1-specific CD8+ cytotoxic T lymphocytes. Regarding necrosis and regeneration, the results ran contrary to our expectations and the general rule that regeneration follows necrosis—this is why we preferred to assign them as regenerative processes (for the purpose of mending or maintaining partially damaged muscle fibers). That up-regulation of regenerative processes might be the cause of the longer protracted course of HTLV-1-positive PM. We found a reduction in CCO activity in HTLV-1-positive PM, this attracted our attention, and we examined the mitochondrial ultrastructure. Mitochondrial morphological abnormalities were more frequently observed in HTLV-1-positive than in HTLV-1-negative patients or in normal control subjects. Therefore, these mitochondrial morphological abnormalities are more likely to be connected with HTLV-1 infection. In particular, we found a significant correlation between these morphological abnormalities and the numbers of CCO-deficient or CCO-negative fibers.

In summary, we observed the clinical and morphological differences of muscle biopsy specimens between cases of HTLV-1-positive and HTLV-1-negative PM. Although these differences were significant, they were not specific to HTLV-1-positive PM, because they were also observed (although less frequently) in HTLV-1-negative PM. These results suggest that HTLV-1 is responsible for modifying the clinical course and the histopathological features of myofibers observed in the present study.

(Clinical and Experimental Neuroimmunology Vol. 2, No. 1　2011 年　印刷中)

論文審査の要旨

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Histopathologic differences between human T-lymphotropic virus type 1 (HTLV-1)-positive and HTLV-1-negative polymyositis

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成人T細胞白血病（ATL）やHTLV-1関連脊髄症（HAM）、HTLV-1関連ぶどう膜炎はHTLV-1感染により引き起こされる疾患としてその疾患概念が確立しており、関節炎や筋炎、シェーグレン症候群などの炎症性疾患との関連についても疑われている。しかしながら、特に多発性筋炎については関連を示唆する多くの報告があるものの、まだ一疾患単位として確立していない。本研究では、鹿児島大学で筋生検により多発性筋炎と診断された症例について、HTLV-1陽性者と陰性者の病理組織学的所見を比較することにより、HTLV-1陽性多発性筋炎の病理組織像の特徴を明らかにすることを目的としている。過去の炎症性筋疾患68例のうち、封入体筋炎、皮膚筋炎、ミトコンドリア筋症、筋病理所見に影響するHAMや膠原病などの合併症のある例を除外した21例について、HTLV-1感染の有無を、筋組織より抽出したDNAのnested PCRと血清抗体価により判定し、HTLV-1陽性多発性筋炎11例、HTLV-1陰性多発性筋炎10例に分け、病理組織学的に検索した。その所見を陽性例と陰性例で比較するとともに、臨床所見との相関を検討した。

その結果、本研究で以下の知見が明らかにされた。

1）陽性例では臨床経過が遷延性であった。

2）陽性例では壊死線維は有意に少なく、炎症細胞浸潤がendomysium により多くみられた。

3）再生像を示す線維は陽性例で高頻度であった。

4）炎症細胞のサブセットに差は見られなかった。

5）陽性例でチトクロームCオキシダーゼ染色の完全欠損、部分欠損を示す筋線維が高頻度に見られ、電顕によりミトコンドリアの形態異常が確認された。

これらの結果より、HTLV-1の有無により生検筋の病理組織所見に有意差を認めたことは、HTLV-1が多発性筋炎の病像を修飾する因子の1つであると結論している。

本研究では HTLV-1 感染の有無により多発性筋炎の病理組織像に違いが生じていることを初めて明らかにするとともに、ミトコンドリアの機能異常、形態変化を病巣で証明しており、HTLV-1関連疾患の病態の理解に重要な知見を加えている。よって本研究は学位論文として十分な価値を有するものと判定した。

最終試験の結果の要旨

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主査および副査の５名は、平成23年2月15日、学位申請者 アブダッラ ハザム モハメド 君に面接し、学位申請論文の内容について説明を求めると共に、関連事項について試問を行った。具体的には、以下のような質疑応答がなされ、いずれについても満足すべき回答を得ることができた。

質問 1) In PM, expression of MHC-I is necessary and its association with $CD8^+$ cells is characteristic. What about MHC-II in PM?

（回答）MHC II expression is positive in some PM cases and is correlated to histological features such as inflammatory infiltrates, increased connective tissue, and its evaluation is based on the frequency of positive staining rather than to the degree of the MCH II expression.

質問 2) Did you recognize the relationship between $CD8^+$ cells and NCAM-positive muscle fibers?

（回答）No, however in our study, NCAM and endomyseal $CD8^+$ cells were higher in HTLV-1 positive PM and we postulated a close relationship between $CD8^+$ and $CD4^+$ cells.

質問 3) Are there neutrophils or macrophages in the infiltrates in HTLV-1-positive PM?

（回答）By immunohistochemistry neutrophils are absent, while macrophages accounts for 21% in the endomysium and 14% in the perimysium in the HTLV-1-positive PM, and for 19% in the endomysium and 16% in perimysium in the HTLV-1-negative PM group.

質問 4) May decreased activity of CCO and mitochondrial abnormalities be age-related?

（回答）The possibility to be age related was excluded by intact other mitochondrial enzymes (SDH and AMP deaminase), its association with morphological abnormalities (age related abnormalities is not associated with structural abnormalities), its high percentage (range 8–61%; mean 30%) while in age related is rarely more than 5% and its absence in normal control with the same age and in HTLV-1 negative group.

質問 5) What is the pathway by which $p13^{II}$ induce mitochondrial abnormalities?

（回答）$p13^{II}$, a non structural accessory hydrophilic protein, it can traverse the cell and mitochondrial membrane freely and accumulates in the inner mitochondrial membranes a close proximity to CCO; causes mitochondrial morphological abnormalities by altering membrane permeability changing its permeability; followed by loss of matrix and cristae with its enzyme component.

質問 6) Is there any correlation between CCO deficiency and clinical manifestation?

(回答) No, as CCO activity was more frequently seen in HTLV-1 positive cases, but there was no difference in the clinical manifestations except in the duration of illness but without difference in the severity of the clinical manifestation.

質問 7) How high about 29% of PM cases with CD8 MHC-1 complex (definite PM) in this study?
(回答) It is a high percentage as we used a retrospective pathway which gave us a strict results depending on steroid therapy which is the most reliable method. In addition, the number of cases in our cohort is small, other study is conducted on 270 cases and demonstrated in 5% of cases.
質問 **8)** Is IBM related to HTLV-1 or not?
(回答) Many studies showed that IBM is related to HTLV-1by demonstration of anti-HTLV-I antibodies in the patients' sera. Biopsies showed IBM features, while PCR and in situ hybridization revealed HTLV-I proviral DNA in T cells, but not in muscle fibers.
質問 **9)** What about $CD4^+$ distribution in the endomysium and perimysium?
(回答) $CD4^+$ cells accounts for 30% and 31% in the endomysium and for 48% and 44% in the perimysium in HTLV-1-positive and negative PM groups, respectively.
質問 **10)** What are changes observed in patients denoting their improvement at the level of histopathology or other follow up methods?
(回答) At the level of histopathology it was not assessed since no need for biopsy repetition but assessment was done by improvement of patients' clinical symptoms and improvement of EMG abnormalities.
質問 **11)** Can you explain more about necrosis and regeneration in muscle tissue?
(回答) When fibers injury occurs, fibers tried to stop it and repair by intarcellar mechanisms but if it failed, necrosis may occur which may be: a) Focal necrosis in some but not all fibers. b) Segmental necrosis of a limited number of sarcomeres. c) Degeneration of the entire fiber. d) Fields of necrosis involving several muscle fibers. While regeneration may be: a) continuous repair for a segment (continuous with undamaged portion) by help of satellite cells or b) discontinuous repair to replace the entire fiber by myoblasts fused together forming a long basophilic ribbon.
質問 **12)** In PM, are muscle fibers expressing other epitopes instead of MHC-1 to react with CD8 cells?
(回答) Recently, PM was connected to many other epitopes like IL-15, sIL-2R, sTNF-R1, TLR-3, TLR-7, IL-17, IL-6, CCL20 and IFN-γ.
質問 **13)** As HTLV-1 acts as an immune modulating factor via some cytokines and chemokines, did you assessed them in this study?
(回答) No, we didn't assess any of them as our study is comparative histological descriptive study.
質問 **14)** Could you explain why all cases were negative for ANA?
(回答) Because we have already deleted cases of PM associated with collagen disorder in which ANA may be positive.
質問 **15)** What is the source of proviral load in biopsied tissue specimen?
(回答) $CD4^+$cells infiltrating muscle tissue as all efforts were failed to proof that muscle tissue is a target for HTLV-1 virus.
質問 **16)** Do cases with high proviral load show any specific features?
(回答) No, proviral load was not correlated with any clinical or histological data.
質問 **17)** What about HAM/TSP and interstitial pneumonia associating with your cases?
(回答) Cases of PM with HAM/TSP were excluded as they may show neurogenic atrophy or disuse atrophy and thus interfere with interpretation, while none of cases was associated with interstitial pneumonia.
以上の結果から、５名の審査委員は申請者が大学院博士課程修了者としての学力・識見を有しているものと認め、博士（医学）の学位を与えるに足る資格を有するものと認定した。