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H. 知的所有権の出願・取得状況（予定を含む）

該当なし。

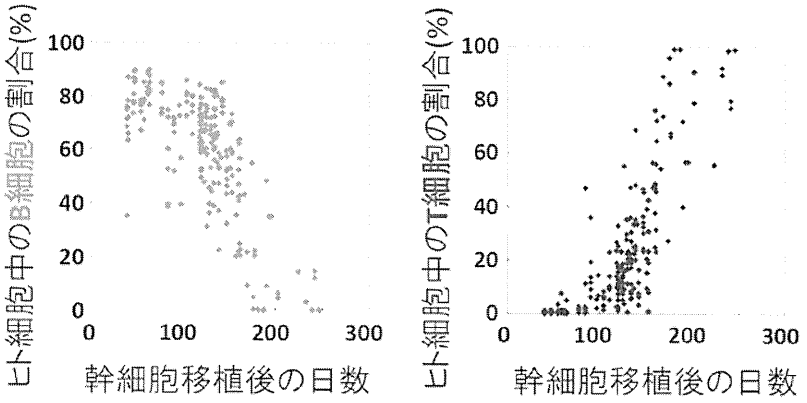

図 1．造血幹細胞移植後の NOG マウス末梢血中ヒト細胞における B 細胞及び T 細胞の割合の経時変化.

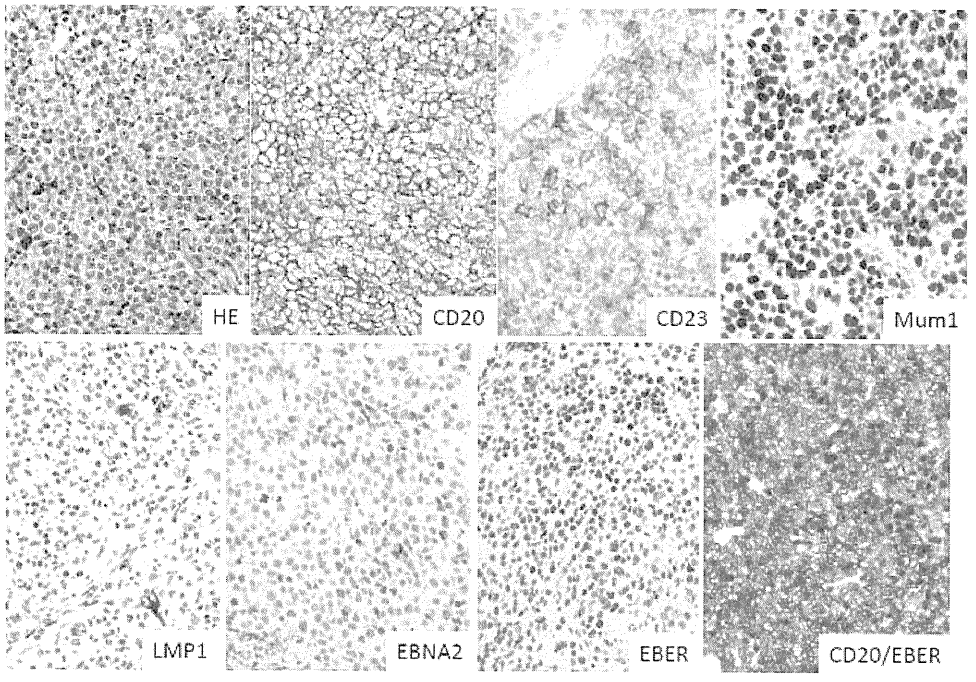

図 2. EBV 感染ヒト化マウスに発生した DLBCL.

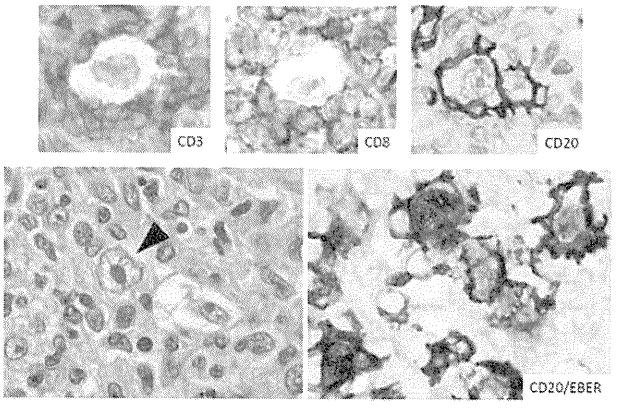

図 3. EBV 感染ヒト化マウスに生じたホジキン様リンパ腫. アロウヘッドはホジキン様細胞を示す。

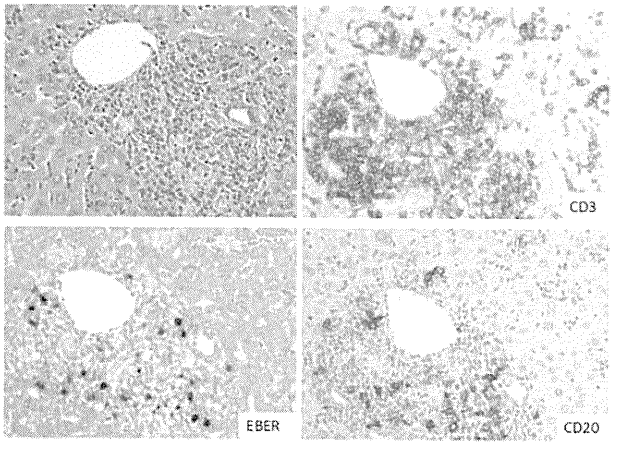

図 4. EBV 感染ヒト化マウスに生じた伝染性単核症様リンパ増殖.

厚生労働科学研究費補助金（エイズ対策研究事業）
分担研究報告書

新規高度免疫不全マウスの樹立とNF-κBを標的とした抗悪性リンパ腫薬の評価

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研究要旨　エイズリンパ腫の病態解析と新規治療法の開発に供するために、新規高度免疫不全マウスの作成とこれらのマウスを用いたエイズ関連悪性リンパ腫のマウスモデルの樹立を試みている。新規高度免疫不全マウス(NOD/Rag-2/Jak3 欠損マウスまたは Balb-c/ Rag-2/Jak3 欠損マウス)腹腔内にヒト Primary effusion lymphoma (PEL)細胞株を移植することにより、PEL マウスモデルを樹立した。これらマウスを用いて Diethyldithiocarbamate と berberine に NF-κB 阻害を介する抗リンパ腫作用があることが判明した。Diethyldithiocarbamate と berberine が、PEL ばかりでなく他の NF-κB 活性が亢進しているエイズ関連悪性リンパ腫の予防と治療に有効である可能性が示唆された。

A. 研究目的

本研究の目的は、エイズ関連悪性リンパ腫のマウスモデルを作成し、エイズ関連リンパ腫の標準的な治療法、新規治療法の開発に供することである。本年度は、特に HIV-1 感染者にかなり特異的に発症し予後不良の Primary effusion lymphoma (PEL)の治療モデルを用いて、Diethyldithiocarbamate と berberine の抗腫瘍効果について検討を行った。また、新規無毛高度免疫不全マウスの作成を行った。

B. 研究方法

ヒト Primary effusion lymphoma (PEL)細胞株 (BCBL-1,TY-1, BC-1, BC-3) に Diethyldithiocarbamate (DDTC) または berberine を添加し、MTT 法によりその効果を調べた。

NF-κB 阻害作用は、Western blot 法、プロモーターアッセイなどにより解析した。

高度免疫不全マウス NOD/Rag-2/Jak3 欠損マウス及び Balb-c/ Rag-2/Jak3 欠損マウスは、NOD マウスまたは Balb/c マウスに Rag-2 欠損マウス（熊本大学生命資源研究・支援センターから供与）または Jak-3 欠損マウス（理化学研究所 RCAI 斉藤隆博士から供与）を 10 世代交配し、更に Rag-2 マウスと Jak3 欠損マウスを交配して作成した。NOJ マウス腹腔に PEL 細胞株 BCBL-1 を移植して PEL モデルマウスを作成し、更に薬剤投与の有効性を検証した。

（倫理面への配慮）

免疫不全マウスの作成及び移植実験等の動

物実験は、熊本大学動物実験委員会の承認を得た上で「熊本大学動物実験指針」に従い実施した。動物実験は、「熊本大学動物実験指針」を遵守し、極力動物の苦痛軽減に配慮して行っている。動物実験における実験処置に対する倫理基準では、カテゴリーB（動物に対してほとんど不快感を与えないと思われる実験）レベルの実験であり、解析時には「動物の処分方法に関する指針」を遵守して、頸椎脱臼により安楽死させた。

C. 研究結果

1) PI の抗 PEL 効果の検討

Diethyldithiocarbamate (DDTC)及び berberine の抗腫瘍効果を MTT 法により検討した。その結果、DDTC と berberine の両者に強い抗 PEL 効果が認められた。DDTC または berberine 添加により Annexin V 陽性細胞の増加と caspase 3 活性の増加が認められたことから、これらの薬剤は、PEL にアポトーシスを誘導する事が判明した。

2) PI の作用機序

PEL 細胞株に DDTC または berberine 添加後、NF-κB p65 の発現を検討した。その結果、両者において NF-κB 活性化抑制が認められた。従って、PI は NF-κB 阻害作用を呈することで抗腫瘍効果を発揮する事が示唆された

3) PEL 発症マウスモデルの樹立

高度免疫不全マウス腹腔に PEL 細胞株 BCBL-1 を移植して PEL 発症マウスモデルを作成した。BCBL-1 1×10^7 個を腹腔内に移植したところ 3 週間後には腹水の増加と肺・肝・脾臓に転移が認められた。

4) PI による PEL マウスモデルの治療

PEL 発症マウスモデルに DDTC または berberine 腹腔内投与した。その結果、DDTC または berberine 投与群では、明らかな腹水量の低下と転移の抑制が認められた。。

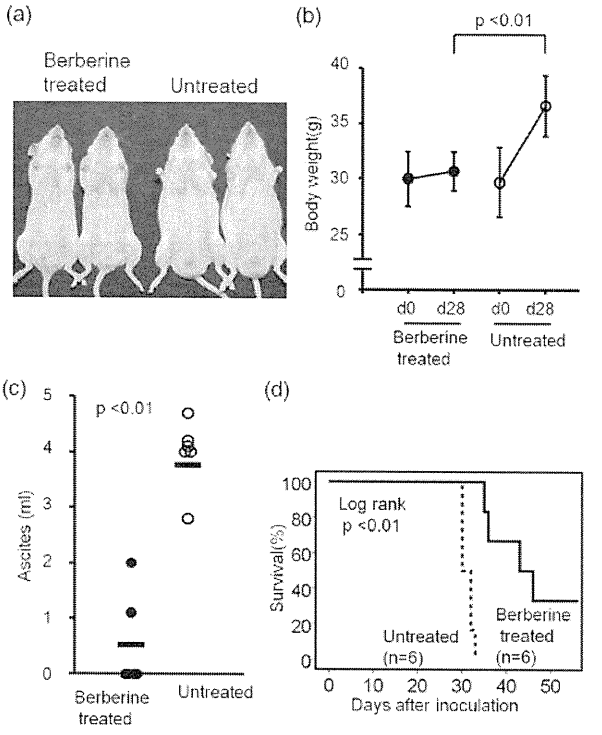

図 1．Berberine の抗 PEL 作用．Berberine 投与により PEL マウスの腹水貯留は抑えられた。

D. 考察

エイズ関連悪性リンパ腫のマウスモデルを用いて、DDTC 及び berberine が PEL の治療に有効である可能性を示した。これらの薬剤は、NF-κB 阻害作用を介して PEL に Caspase 依存性アポトーシスを誘導していることが示唆された。

HIV-1 感染者では高頻度に悪性リンパ腫が発症し、HIV-1 感染者の長期予後を規定する重要な合併症となっている。以前は、HIV-1 のコントロールがされていない免疫不全の状態での脳原発悪性リンパ腫やびまん性大細胞性リンパ腫の合併が多かった。最近は、HIV-1 感染がコントロールされている症例においてバーキットリンパ腫やホジキンリンパ腫が発症す

る例が増えており注意が必要である。これらの悪性リンパ腫の半数以上が EB ウイルス（Epstein-Barr virus: : EBV）感染が原因とされている。HHV-8 や EBV 感染による NF-κB 活性化が悪性リンパ腫発症の主因のひとつとされており、NF-κB はこれらの悪性リンパ腫治療の分子標的として注目を浴びている。本研究では、DDTC 及び berberine に抗 NF-κB 活性があることを証明した。これらの薬剤は、エイズ関連悪性リンパ腫の治療のみならず、予防にも有効である可能性が示唆された。

E. 結論

エイズ関連悪性リンパ腫のマウスモデルを用いて、DDTC 及び berberine に抗 PEL 効果があることを示した。本マウスモデルは、今後エイズ関連悪性リンパ腫の新たな治療法の開発に役立つことが期待される。また、これらの薬剤は抗 NF-κB 効果を示すことから、PEL のみでなく NF-κB が活性化している他の悪性リンパ腫（ホジキン病や EBV を起因とする非ホジキンリンパ腫など）の予防と治療に有効である可能性が示された。

F. 健康危機情報

該当なし。

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H. 知的所有権の出願・取得状況（予定を含む）

1. 特許申請：
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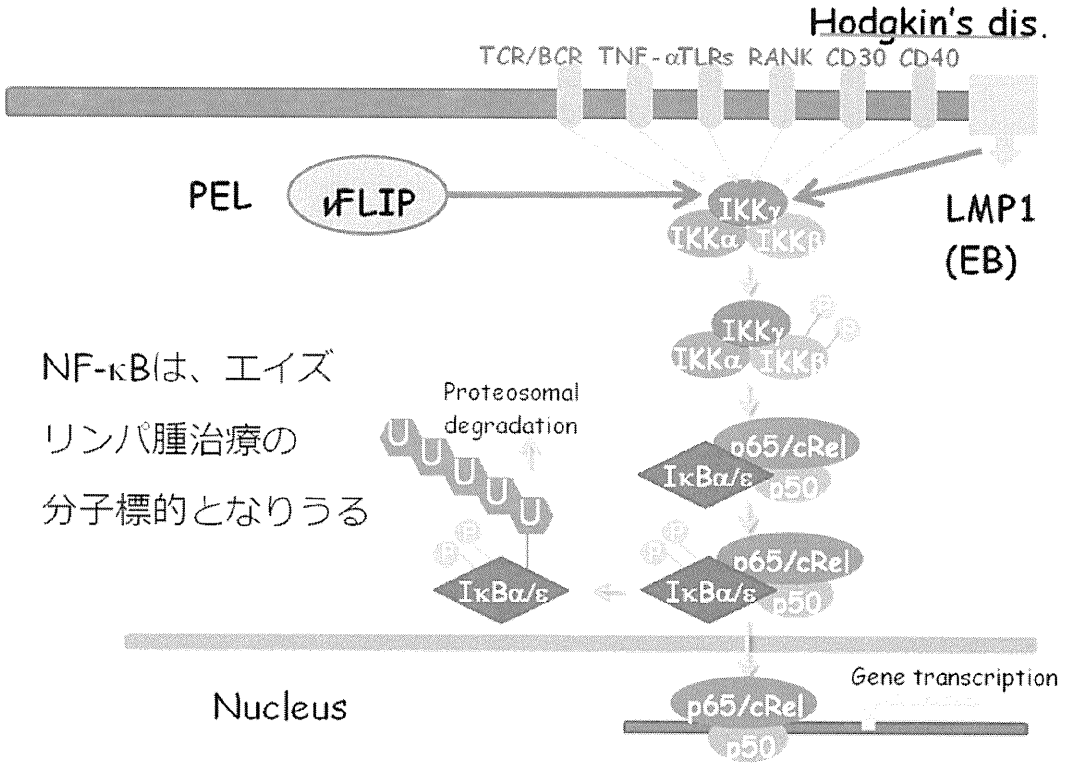
2. 実用新案登録：なし
3. その他：なし



エイズ関連悪性リンパ腫における **NF-κB** の活性化

HIV-1 感染者においては、Epstein-Barr ウィルスや HHV-8 感染を起因とする悪性リンパ腫に罹患しやすい。これらのウィルス感染を起因とする悪性リンパ腫においては、NF-κB 経路が活性化していることから、NF-κB 経路の阻害薬が治療と予防に有効であることが示唆される。

別紙４

研究成果の刊行に関する一覧表

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Review Article Open Access

Diagnosis and Treatment of AIDS-Related Primary Central Nervous Lymphoma

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Summary

Primary central nervous system lymphoma (PCNSL) is an intracranial tumorous lesion that develops in acquired immunodeficiency syndrome (AIDS) patients. PCNSL is very rare, but its prevalence has increased with the expansion of human immunodeficiency virus (HIV) infections. It is diagnosed by imaging or biopsy. Serum and cerebrospinal fluid (CSF) have been analyzed for the presence of diagnostic biomarkers for PCNSL. The presence of Epstein-Barr virus in the CSF is a widely used biomarker, but it has a low positive predictive value. B-cell activation-related cytokines, tumor-specific DNA methylation, and microRNAs have been reported as new candidate biomarkers. Previously, the prognosis of AIDS-related PCNSL was very poor. However, highly active antiretroviral therapy emerged in the late 1990s, which continuously inhibits viral replication and facilitates the recovery of immunity in HIV-infected persons, improving the prognosis of PCNSL patients in combination with whole-brain irradiation. In this review, we focus on the diagnosis of and biomarkers for AIDS-related PCNSL and advancements in its treatment.

Keywords: Primary central nervous system lymphoma; AIDS; HIV infection; Biomarkers; Highly active antiretroviral therapy; Whole-brain irradiation

Introduction

Acquired immunodeficiency syndrome (AIDS) is caused by human immunodeficiency virus (HIV) infection. HIV infects CD4-positive T lymphocytes and destroys the immune system by reducing the number of these lymphocytes. The resulting reduced immunity facilitates the occurrence of opportunistic infections, such as Pneumocystis pneumonia, cytomegalovirus retinitis, and toxoplasma encephalopathy. In AIDS patients, the risk of opportunistic malignant tumors, such as Kaposi's sarcoma, uterine cervical cancer, and non-Hodgkin's lymphoma (NHL), also increases. NHL in HIV-infected persons is divided into systemic NHL and primary central nervous system lymphoma (PCNSL). PCNSL is characterized by the localization of lesions only in the central nervous system (CNS). AIDS is diagnosed when these specific opportunistic infections and malignant tumors develop in HIV-infected persons. AIDS was previously a fatal disease; however, in the late 1990s, highly active antiretroviral therapy (HAART) was developed and improved the prognosis of HIV-infected persons. The combination of several anti-HIV agents continuously inhibits HIV replication in the body, facilitating the recovery of the immune system.

PCNSL was a very rare tumor before the outbreak of HIV, accounting for <1% of intracranial tumor cases and 1–2% of NHL cases [1]. The outbreak of HIV in the early 1980s increased the incidence of AIDS-related PCNSL because HIV-infected persons have an increased risk of developing PCNSL. The incidence rate of AIDS-related PCNSL was much higher than that of non-HIV related PCNSL (3-8 per 1,000 person-years among HIV-infected persons vs. 5-10 per 1,000,000 person-years) [2-4]. The incidence of non-HIV-related PCNLS also increased. Currently, PCNSL are estimated to account for 1-5% of all brain tumors.

In the pre-HAART era, the prognosis of AIDS-related PCNSL was very poor [5]; however, after the development of HAART, several studies reported an improvement in the prognosis of AIDS-related PCNSL patients [6-8]. In addition, the number of PCNSL patients markedly decreased with the advancement of HAART. In the report by Diamond et al., the absolute incidence rate of PCNSL among AIDS patients declined from 8.4 per 1,000 person-years pre-HAART to 1.1 per 1,000 person-years post-HAART [9]. Other studies also reported a dramatic reduction in its incidence [2,3,7,10-13] and prevalence [14] in the HAART era. However, AIDS-related PCNSL develops in cases with markedly reduced immunity [15], and its treatment and diagnosis are frequently difficult because of the presence of multiple opportunistic infections and malignant tumors or the poor condition of the system, reducing diagnostic and therapeutic choices. In this review, we focus on the diagnosis, treatment, and biomarkers for AIDS-related PCNSL together with the progress in elucidating the developmental mechanism.

Diagnostic procedure

In HIV-infected persons, PCNSL forms intracranial masses at a frequency second to toxoplasma encephalopathy [16]. Generally, PCNSL develops when immunity markedly declines in patients previously diagnosed with AIDS [15]. Systemic NHL may develop even though the number of CD4-positive T lymphocytes is maintained; however, the count is <50 cells/μL in most cases of PCNSL [17,18]. To differentiate PCNSL from systemic NHL, an investigation of the whole body is necessary on diagnosis. When the presence of an intracranial mass is suspected on the basis of the symptoms, a computed tomography (CT) or magnetic resonance imaging (MRI) scan of the brain is performed; however, MRI has a higher diagnostic value than CT [19]. In a retrospective analysis of 28 patients with AIDS-related PCNSL, MRI revealed 66 lesions, whereas CT showed 45 lesions, indicating the superior sensitivity of MRI [20]. On CT, shadows of masses with

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a contrast effect are observed, but toxoplasma encephalopathy also shows a similar feature in AIDS patients [21]. On MRI, enhancement is observed similarly to that in CT. Multiple masses with irregular margins and heterogeneous enhancement are detected in many cases [20,22]. ^{18}F-fluorodeoxyglucose-positron emission tomography (FDG-PET) is also useful for diagnosis. In a review of 166 PCNSL cases, only FDG-PET detected abnormalities, while no abnormalities were detected with various other examinations in 8% of cases [23]. The association of FDG-PET findings with malignancy and the activity levels of lymphoma lesions have also been reported [24,25]. When no diagnosis can be made by either method or there is no response to experience-based treatment for toxoplasmosis, a brain biopsy is taken. Diagnosis by brain biopsy is definite, but it is not a necessarily safe procedure. It is difficult to perform when the systemic condition is poor or depending on the anatomical position of the lesion. The histologic type was diffuse large B-cell lymphoma (DLBCL) in ~90% of cases [26], but T-cell lymphoma and anaplastic large cell lymphoma were noted in rare cases. Epstein-Barr virus (EBV) was absent in PCNSL lesions in cases that retained immunity, but EBV infection was confirmed in almost all cases of AIDS-related PCNSL [27-29].

Diagnosis of PCNSL based on EBV-DNA detection in the CSF

EBV is detected in AIDS-related systemic NHL tissue in ~50% of patients, whereas it is detected in almost all patients with AIDS-related PCNSL. Therefore, many studies on the possibility of diagnosing AIDS-related PCNSL by detecting EBV in the CSF have been performed. In studies performed in the 1990s involving AIDS patients, EBV-DNA detection in the CSF employing PCR showed a high sensitivity (83–100%) with regard to the diagnosis of AIDS-related PCNSL [30-35]. The specificity was also high (91–100%), and it has been reported that EBV-DNA was only detected in the CSF of a small number of HIV-infected persons, excluding patients with PCNSL and CNS invasion by systemic NHL. On the basis of these findings, EBV-DNA detection in the CSF was previously regarded as a basis for diagnosing PCNSL in cases in which a biopsy could not be performed, despite PCNSL being suspected on imaging. However, in later studies, the detection of EBV-DNA in the CSF using PCR was not rare in HIV-infected persons, being detected in ~20% of patients [36-38], indicating the presence of a problem regarding the specificity of this technique (~80%). EBV-DNA was also detected in the CSF of ~4% of non-HIV-infected patients with cerebral diseases and without typical symptoms of viral infection of the CNS (such as bacterial meningitis and cerebral hemorrhage) [39]. Since EBV was more frequently detected in the CSF than other herpes viruses and the JC virus, EBV may be readily reactivated subclinically in the CNS regardless of the presence or absence of HIV infection [36,37,39]. EBV-induced encephalitis and myelitis and CNS lymphomatoid granulomatosis (LYG) have also been reported in HIV-infected persons, and all of these patients were positive for CSF EBV-DNA [40-42]. Attention should be paid to the fact that EBV-DNA is detected in the CSF of not only patients with tumors but also those with EBV-induced infectious diseases of the CNS. In a patient with CNS LYG, findings similar to those of PCNSL were noted on MRI (Figure 1). The clinical characteristics of this patient are summarized in Table 1.

The ratio of PCNSL patients to subjects in whom EBV-DNA is detected in the CSF, i.e., the positive predictive value (PPV), is not high. Ivers et al. reported that the PPV was only 29% [38]. In addition to the specificity problem described above, reduced prevalence may also be involved in the low PPV [14]. The development of HAART in the late 1990s facilitated the recovery of immunity by continuously inhibiting HIV replication, which may have reduced the prevalence of AIDS-related PCNSL, thereby decreasing the PPV.

Comparisons between the quantitative and qualitative detection of

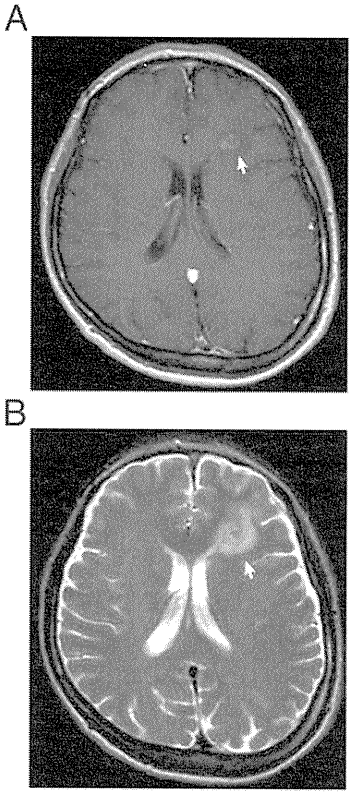

Figure 1: MRI findings of CNS LYG in an HIV-infected person. A: Gadolinium-enhanced T1-weighted MRI reveals a ring-enhanced lesion resembling PCNSL (arrow). B: T2-weighted MRI.

Age (years)		42
Sex		Male
CD4 cell count (/μL)		33
Plasma HIV-RNA (copies/mL)		410000
CSF	EBV-DNA (copies/mL)	1300
Biopsy specimen	EBV*	(+)
	IgH gene rearrangement	(-)

*Epstein-Barr virus (EBV) was detected using EBV-encoded RNA *in-situ* hybridization. CSF, cerebrospinal fluid; IgH, immunoglobulin heavy chain.

Table 1: Clinical characteristics of CNS LYG in an HIV-infected patient.

Biomarkers	References		
Cytokines	Interleukin	IL-10	[32,51]
	Immune activation markers	soluble CD27*1	[53]
	Chemokine	CXCL13*1	[52]
DNA	Tumor-specific DNA methylation	DAPK, p16, MGMT, p14, p73, etc*2	[63]
RNA	miRNAs	miR-17-92 cluster and miR21*1	[79]

*1These biomarkers were tested in the CSF of non-HIV-related PCNSL patients. *2 Tumor-specific methylated DNA in the CSF was not investigated. CSF, cerebrospinal fluid; IL-10, interleukin-10; miRNAs, microRNAs.

Table 2: Biomarker candidates in the CSF for diagnosing AIDS-related PCNSL

EBV-DNA in the CSF have been made [43]. An improvement of the specificity and PPV by setting the cut-off value of quantitative detection at 10,000 copies/mL has been reported, but the report included only 2 cases of AIDS-related PCNSL; therefore, it cannot be concluded whether this cut-off value is appropriate. To establish the optimum cut-off value, it is necessary to analyze a large number of cases. On the basis of the above findings, the absence of EBV-DNA in the CSF detected using PCR provides useful information to exclude AIDS-related PCNSL because of the high detection sensitivity of this technique. However, in the current situation, with a reduced prevalence and low PPV, it is considered that a highly specific biomarker is necessary to diagnose AIDS-related PCNSL. Potential biomarkers for diagnosing AIDS-related PCNSL are discussed below (Table 2).

AIDS-related PCNSL and cytokines

Cytokines are intercellular signal transmission molecules that regulate the differentiation, proliferation, and activation of immunocytes. They are mainly secreted by immunocytes and act on receptors expressed on the surface of target cells. The action of cytokines is then exhibited through specific signal transduction pathways in the target cells. Typical cytokine proteins include the tumor necrosis factor (TNF), interferon, and interleukin (IL) families. These cytokines form complex networks that additively or antagonistically control the immune system. The abnormal expression of various cytokines in HIV-infected patients has been reported. Although HAART reduces the plasma HIV-RNA level to below the detection limit, the circulating cytokine levels are not completely normalized [44,45]. The abnormal activation of B cells by these cytokines is considered to be involved in the development of B-cell lineage NHL.

The serum levels of cytokines involved in B-cell activation and cytokine-related immune activation markers and the risk of developing AIDS-related systemic NHL have been investigated in detail. The elevation of the serum levels of IL-6, IL-10, CXCL13, soluble TNF-R1, soluble CD27, and soluble CD30 prior to systemic NHL development in HIV-infected persons has been reported [46-48]. Of these studies, Breen et al. simultaneously investigated the risk of PCNSL and systemic NHL, but they did not identify any cytokines involved in the risk of developing PCNSL [48]. On the other hand, studies on cytokine gene polymorphisms and the risk of AIDS-related NHL showed that the risk of PCNSL decreased in the presence of either the IL-10 rs1800871 T allele or the IL-10 rs1800872 A allele [49]. These IL-10 promoter polymorphisms were involved in the reduction of IL-10 serum levels [50].

In addition to those in serum, it has been investigated whether cytokines and cytokine-related immune activation markers in the CSF serve as biomarkers for diagnosing PCNSL. In patients with AIDS-related PCNSL, the elevation of IL-10 levels in the CSF, which is secreted by Th2 cells and promotes antibody production by B cells, has been reported [32,51]. The levels of CXCL13, with B-cell-selective chemotactic activity [52], and soluble CD27, which belongs to the TNF receptor superfamily [53], were elevated in patients with non-HIV-related PCNSL. However, the rise in their levels was not tumor-specific. The levels of IL-10 in the CSF were also elevated in HIV-infected patients with cryptococcal meningitis and HIV encephalopathy [54]. In a report examining the levels of soluble CD27 in the CSF, no significant difference was noted between patients with PCNSL and those with inflammatory disease of the CNS [53]. Therefore, although B-cell-related cytokines are involved in the pathogenesis of AIDS-related PCNSL, it may be difficult to differentiate PCNSL and inflammatory lesions by measuring the levels of cytokines in the CSF.

AIDS-related PCNSL and tumor-specific DNA methylation

Malignant tumors may be induced by viral infection and inflammation; however, abnormalities of oncogenes and anti-oncogenes also have a role in their pathogenesis. These abnormalities are classified into genetic aberrations due to mutations and deletions and epigenetic aberrations due to the chemical modification of bases. Epigenetic gene aberrations act on the regulation of gene transcription, and the most common epigenetic change is the methylation of genomic DNA. In malignant tumor cells, anti-oncogenes are silenced, and the association between this silencing and aberrant methylation of CpG islands in the promoter region has been shown. Actually, many reports have described the aberrant methylation of the promoter regions of various anti-oncogenes, such as Rb and p16, in malignant tumors, and the detection of CpG island methylation of cancer-related genes in tumor cell-derived DNA in PCNSL has also been reported. The majority of these reports focused on 1 to several cancer-related genes [55-62]. Chu et al. investigated the DNA methylation status of 14 anti-oncogenes using methylation-specific PCR in 25 PCNSL patients, including 2 HIV-infected patients. DNA methylation was detected in death-associated protein kinase (DAPK) in 84% of samples, p16 in 64%, and O6-methylguanine-methyltransferase (MGMT) in 52%, showing that methylation was noted in 1 of these 3 genes in 96% of samples [63].

The detection of tumor-specific DNA methylation is considered to be a biomarker for early diagnosis. In addition to the detection of DNA methylation in serum and plasma samples from cancer-bearing patients [64], it was detected in sputum samples from lung cancer patients [65] and urinary sediment samples from prostate cancer patients [66], showing the usefulness of detecting DNA methylation for early diagnosis. Since PCNSL is localized in the CNS, it may be ideal to detect DNA methylation in the CSF, rather than peripheral blood. However, aberrant DNA methylation was detected in the serum [67,68] and plasma [69], in addition to the CSF [68], in >50% of patients with glioma, a malignant tumor arising in the CNS, similarly to PCNSL, and it has been proposed that the detection of DNA methylation in peripheral blood and body fluid samples serves as a biomarker for PCNSL [63]. Furthermore, the detection of DNA methylation is associated with prognosis, in addition to early diagnosis. The presence of MGMT methylation is important to predict the reaction of patients to alkylating agents [70].

The comprehensive analysis of DNA methylation using arrays has recently been performed. Systemic DLBCL was divided into activated B-cell (ABC) and germinal center B (GCB) types, and the gene expression pattern differed between these types [71]. The prognosis of ABC-DLBCL was poor [72]. Differences in the DNA methylation pattern, in addition to differences in the expression pattern, have also been clarified using comprehensive DNA methylation analysis [73]. To investigate differences in the molecular mechanisms between PCNSL and systemic DLBCL, Richter et al. compared comprehensive DNA methylation patterns between 5 PCNSL and 49 systemic DLBCL patients [74]. Although tumor-specific DNA methylation was noted in both groups, there was no significant difference between the groups.

Studies on DNA methylation detection in malignant tumors and its significance have progressed rapidly, but most reports on PCNSL

involved patients with normal immunity [55-60,62,74]. Since no study has focused on AIDS-related PCNSL, the influences of HIV and EBV infections on DNA methylation have not been clarified. Moreover, DNA methylation in AIDS-related systemic NHL has been investigated in only 2 reports [75,76]. The analysis of many AIDS patients is complicated by the simultaneous presence of several opportunistic infections and malignant tumors. Imaging diagnosis is difficult when several lesions are present in the same organ. It is impossible to adopt EBV-DNA detection and cytokine level elevation in the CSF as tumor-specific biomarkers to diagnose PCNSL because of the specificity problem and the concomitant presence of inflammatory disease. The use of tumor-specific DNA methylation as a biomarker is reasonable in this regard, and further advancement of research on HIV infection in this field is expected.

MicroRNAs as new biomarker candidates

MicroRNAs (miRNAs) are a type of non-coding RNA that are not translated into protein. Precursor RNA transcribed from DNA goes through various processing stages, and, finally, miRNAs, ~20–25-base, single-stranded RNAs, are produced. These miRNAs bind to the 3′-end of the non-translated regions of specific messenger RNAs (mRNAs), and gene expression is regulated by the degradation of mRNAs and the inhibition of mRNA translation. miRNAs are important regulators of cell function, such as proliferation, differentiation, and apoptosis, and they can also function as oncogenes or anti-oncogenes. Actually, the abnormal expression of miRNAs in various malignant tumors, including lung and breast cancers, has been reported [77]. Abnormal miRNA expression in AIDS-related PCNSL has also recently been reported [78]. Thapa et al. investigated miRNA expression in lesions from 24 AIDS-related NHL patients, including 5 PCNSL patients, using a real-time PCR approach, and observed that the expression of the miR-17-92 cluster, which encodes 7 miRNAs including miR-19 and miR-92, was commonly enhanced in AIDS-related NHL patients. Baraniskin et al. measured the expression of miRNAs in the CSF of 23 patients with non-AIDS-related PCNSL [79]. Compared to patients with CNS inflammation and other diseases, the levels of miR-21, miR-19, and miR-92a in the CSF were significantly increased in PCNSL patients. It has been reported that PCNSL could be diagnosed at a sensitivity and specificity of 95.7 and 96.7%, respectively, by measuring the expression of these 3 miRNAs. It is also interesting to note that these 2 reports included the same miRNAs. Although only a few studies have assessed the expression of miRNAs in AIDS-related PCNSL, the expression levels of miRNAs in the CSF may represent new candidate biomarkers [80].

Treatment

Other than in AIDS patients, PCNSL may develop in immunocompetent persons, although this phenomenon is rare. In a previous study comparing AIDS-related and non-AIDS-related PCNSL, the median survival time (MST) was 0.9 months (vs. 2.7 months) in the untreated group and 3.0 months (vs. 16.6 months) in the radiation group, apparently showing the poor prognosis of the former [81]. However, these findings may have been due to major differences in the patients' backgrounds, such as the biological properties of tumors, immunity, and concomitant infections, despite both types of tumor being lymphoproliferative disease. As described above, EBV was detected in PCNSL lesions in many AIDS patients, but not in the lesions of patients with normal immunity. The primary treatment for non-AIDS-related PCNSL has progressed from radiotherapy alone to chemotherapy with methotrexate alone, multidrug chemotherapy, or the combination of chemotherapy + radiotherapy. In contrast, AIDS-related PCNSL was treated with radiotherapy alone in most reports, and chemotherapy was performed in fewer reported cases. The prevalence of AIDS-related PCNSL peaked in the 1980s to the early half of the 1990s before the appearance of HAART, and the number of patients rapidly decreased thereafter, influencing the state of treatment.

Radiotherapy was the initial sole treatment for AIDS-related PCNSL, and it prolonged the survival of patients to some extent (Table 3). However, it was not curative, and recurrence frequently occurred inside and outside of the irradiated region. Formenti et al. irradiated the whole brain of 10 AIDS-related PCNSL patients, and achieved temporary complete remission (CR) in 6 patients; 2 patients irradiated with 50 Gy survived for more than 1 year, but those irradiated at a lower dose died within several months due to opportunistic infections and tumor recurrence [82]. Baumgartner et al. reported that 17 and 52% of 29 AIDS-related PCNSL patients irradiated at 30–54 Gy achieved a CR and a partial response (PR), respectively, and the MST was 119 days (vs. 27 days in the non-irradiated group). The cause of death was mostly aggravation of PCNSL in the non-irradiated group and opportunistic infections in the irradiated group [83]. Goldstein et al. irradiated 17 AIDS-related PCNSL patients at 5.4–57 Gy, and the MST was 72 days. The radiation dose was not related to the outcome, but the performance status (PS) before treatment was related (MST: 226 days in the group with a favorable Karnovsky PS vs. 59 days in the poor PS group) [21].

These reports in the 1990s clarified that AIDS-related PCNSL is relatively sensitive to radiotherapy; however, the survival time was only several months, even though the tumor was radiation-sensitive and reduced in size, and most patients died of opportunistic infections. HIV infection-induced immunodeficiency may have markedly influenced the outcome.

With the development of HAART, the therapeutic effect of radiotherapy has also increased. Hoffmann et al. reported that the survival times of groups treated with a combination of HAART and radiation (30–50 Gy) and radiation alone and an untreated group were 1,093, 132, and 33 days, respectively, showing that HAART and radiotherapy were independent factors involved in long-term survival [6]. HAART was shown to be a stronger prognostic factor than radiotherapy in another report [84]. Newell et al. reported that the therapeutic results were significantly more favorable in the group diagnosed after 1993 out of patients observed between 1987 and 1998, and the incidence rapidly decreased after 1997 [7]. In the latest report, 23 PCNSL patients diagnosed between 2002 to 2008 were treated with HAART [8]; 21 patients were treated with radiotherapy alone,

Authors (Year)	Cases	HAART	Radiation dose (Gy)	MST	References
Formenti et al. (1989)	10	(-)	22–50	5.5 months	[82]
Baumgartner et al. (1990)	29	(-)	30–54	119 days	[83]
Goldstein et al. (1991)	17	(-)	5.4–57	72 days	[21]
Hoffman et al. (2001)	7	(-)	30–50	132 days	[6]
	5	(+)	30–50	1093 days	
Skiest et al. (2003)	18	(-)	N.A.	52 days	[84]
	7	(+)	N.A.	>667 days*	
Nagai et al. (2010)	13	(+)	≥30	60 months	[8]

*MST not reached at a median follow-up time of 667 days. N.A., data not available; MST, median survival time.

Table 3: Major radiation therapy results for PCNSL with or without HAART.