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慢性活動性EBV感染症(CAEBV)といわれてきた疾患
――EBV陽性T，NKリンパ増殖症(EBV-T/NK-LPDs)

新井文子*

要旨

・慢性活動性EBV感染症(chronic active Epstein-Barr virus infection：CAEBV)は，当初は抗EBV抗体高値に伴う全身の炎症症状，すなわち発熱，肝機能障害，リンパ節腫脹などが持続する疾患として報告された。
・その中でEBV感染細胞がTもしくはNK細胞で，かつクローナルな増殖をみる例を，皮膚に病変が限局する二つの疾患，種痘様水疱症，蚊刺過敏症，およびEBV陽性血球貪食性リンパ組織球症と合わせて，EBV陽性T，NKリンパ増殖症(EBV-positive T or NK-cell lymphoproliferative diseases：EBV-T/NK-LPDs)とし，リンパ系腫瘍として取り扱う提案がされている。本稿でもそれに基づき記載する。
・EBV-T/NK-LPDsは本邦を中心とする東アジアに報告が集中している。
・ありふれたウイルスがなぜ一部のヒトでT，NK細胞に持続感染し，腫瘍発症に至るのかその仕組みは解明されていない。
・EBV-T/NK-LPDsの診断には，末梢血中のEBV-DNA定量検査とEBV感染細胞の同定検査が必要である。
・EBV-T/NK-LPDsの多くは，臓器障害，より悪性度の高い腫瘍への進行，もしくは血球貪食症候群を発症し，適切な治療がなされないと致死的経過をとる。
・唯一の根治療法は同種造血幹細胞移植である。

疾患概念の変遷

慢性活動性EBV感染症(chronic active Epstein-Barr virus infection：CAEBV)とは，伝染性単核球症(infectious mononucleosis：IM)様の慢性炎症症状が持続する疾患としてVirelizierらにより1978年に最初に報告された[1]。当初は慢性化したIMと考えられていたが，1988年にJonesらによりT細胞にEBVが感染し，かつクローナルに増殖している症例があることが報告された[2]。その後も同様の報告が続き，2008年に改訂された

*東京医科歯科大学大学院医歯学総合研究科血液内科〔〒113-8519 東京都文京区湯島1-5-45〕 ARAI Ayako

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WHOのリンパ腫分類ではT細胞性腫瘍の一つとして記載された[3]。

さらに，NK細胞への感染例や，皮膚に特徴的な病変を生じる2疾患，種痘様水疱症(hydroa vacciniforme-like eruption)，蚊刺過敏症(hypersensitivity to mosquito bites)，そしてEBV陽性血球貪食性リンパ組織球症(EBV-positive hemophagocytic lymphohistiocytosis)はEBVがT，NK細胞に感染し，クローナルな増殖を認め，経過中リンパ腫を発症するなどCAEBVと同じような経過をたどることから，これらを一つの疾患単位とし，EBV陽性T，NKリンパ増殖症(EBV-positive T or NK-cell lymphoproliferative diseases；EBV-T/NK-LPDs)として統一する提案がなされている[4]。

CAEBVという診断名について

CAEBVという疾患名はIM様の炎症症状が持続し，抗EBV抗体上昇を伴う疾患を，あくまでEBVの感染症という視点でとらえたものである。しかし，前述のようにCAEBVの中にはEBVのTもしくはNK細胞への感染と，それらのクローナルな増殖を認める例があり，適切な治療を行わないと進行し致死的となることから，EBV-T/NK-LPDsという腫瘍として病名を改めることが提案されている[4]。

一方，IMの中には，症状が数カ月遷延するものがある。さらに最近では，急性期にEBVがT細胞へ感染し，形態的に異型性を伴いリンパ腫との鑑別が困難な例が報告されている[5]。今までCAEBVとして報告された中には，このようなIMの症例が含まれている可能性がある。IMの診断のポイントは，正確な病歴聴取とVCA-IgM抗体検査から初感染の所見を得ることである。IMの多くは自然軽快する。正しい診療のため，これらの鑑別は非常に重要である。

この他に，CAEBVとして報告されたものには，B細胞にEBVの感染を認めるものもある。米国から報告されてきたCAEBVはこのタイプが多いが，低γグロブリン血症などT，NK細胞感染型と異なった所見を示し，T，NK細胞に感染をみるものとは異なった疾患と考えられる[6]。

以上のように，従来CAEBVと診断され，報告されてきた症例には少なくとも以上の三つの病態が含まれている可能性がある。今後はこれらを区別して解析することが，それぞれの病態解明のために必要である。

本稿では，EBV-T/NK-LPDs，すなわちEBVの感染細胞がTもしくはNK細胞と同定され，かつクローナルな増殖を示す疾患について述べる。

疫学

EBV-T/NK-LPDsの頻度はきわめてまれである。正確な頻度は不明であるが，CAEBVとしての全国調査によると，本邦での2005～2009年の新規患者数は平均して1年に23.8人であった(難治性疾患克服研究事業 H21-難治-一般-094「慢性活動性EBV感染症の実態解明と診断法確立に関する研究」報告書より)。また，近年，成人患者の報告は増えており，特に2009年には50%以上が成人例で，なかには80歳代の例もあった。世界各地から報告例があるが，発症には地域性があり日本など東アジアに報告が集中している。このことから発症には何らかの遺伝的な背景が関与していると考えられている。

発症機序と病態

EBVがB細胞を不死化，腫瘍化させることはよく知られている。しかし，EBVがT細胞の腫瘍化にも関与しているかどうかは証明されていない。EBVは，日本では20歳以上の成人の90%が感染を受けているごくありふれたウイルスである。そもそも，なぜこのようなありふれたウイル

スが一部の人では重篤な疾患の原因になるのだろうか。

EBVの感染標的細胞は通常はB細胞であるが，その感染受容体であるCD21はT細胞，NK細胞には発現していないといわれている。しかし，わずかであるがT細胞にも発現しているという報告[7]や，B細胞との接触による免疫学的シナプスによりCD21がNK細胞へ発現しEBVが感染し得るという報告[8]がなされている。また，IMの急性期にEBVがT細胞やNK細胞に感染し得ることも報告[9]されており，感染自体は起こり得ると考えられる。

また，EBVがT，NK細胞に感染すると，それらはB細胞同様不死化することが最近明らかになってきた。私たちはT細胞株に*in vitro*でEBVを感染させると，抗がん薬etoposideへの感受性が低下することを見いだした(第18回欧州血液学会総会発表)。同じように*in vitro*でEBVをNK細胞株に感染させるとdoxorubicinによるアポトーシスも抑制される[10]。

さらに，EBV-T/NK-LPDs患者の細胞ではactivation-induced cytidine deaminase(AID)といわれる分子の発現が高いことも報告されている[11]。AIDとは，積極的に遺伝子に変異をいれる蛋白質であり，B細胞における免疫グロブリンのクラススイッチや体細胞超変異に関与している。AID発現の亢進は遺伝子に変異が起こりやすくなることを示唆しており，腫瘍の進展に関連している可能性がある。

大島らはEBVが感染したT，NK細胞では遺伝子変異が蓄積し，オリゴクローナルからモノクローナルな増殖をきたした結果，EBV-T/NK-LPDs，リンパ腫発症にいたる発症モデルを提唱している[12]。実際にEBV-T/NK-LPDsから節外性NK/T細胞リンパ腫鼻型やアグレッシブNK細胞リンパ腫へ進展した例の報告は，その説を裏付けている[4]。

以上を総合するとEBVはB細胞腫瘍のみならずT，NK細胞腫瘍の原因にもなり得ると考えられ，このことからも，EBV-T/NK-LPDsという疾患は，感染症，炎症性疾患としてではなく，EBVによるT細胞，NK細胞の腫瘍として扱うことは合理的である。

EBV-T/NK-LPDsの臨床像

発熱を伴う慢性の臓器障害がEBV-T/NK-LPDsの臨床像である。つまり，炎症と腫瘍，二つの所見を示すことが大きな特徴である。

1 炎症症状

もっとも多い症状は発熱である。その他，多発リンパ節腫脹，肝障害などを示すことが多い。血管炎を生じ，それに伴う臓器障害をきたすことや，ぶどう膜炎を合併することもある。よって患者は消化器内科，膠原病内科の門をまず叩くことが多い。

一部の患者では特徴的な皮膚症状を認める。その一つが蚊(ヒトスジシマカ)に刺された後，局所の高度の炎症に加え，高熱をきたす，いわゆる蚊刺過敏症である。刺された場所は潰瘍化し，約1カ月かけて瘢痕を残して治癒する。ヒトスジシマカの唾液成分に対するEBV感染細胞の高度な反応が原因と考えられている。感染細胞の種類と発症は関連はない。また，日光に当たる皮膚に，炎症や水疱を繰り返すことがあり，種痘様水疱症といわれる。これらの皮膚症状は小児例・若年例に多く，思春期に軽快するが感染細胞が除去されることはない。

最近の研究によって，EBV-T/NK-LPDs患者では血中のインターロイキン-6，TNF-α，インターフェロン-γなどの炎症性サイトカイン濃度が上昇していることが明らかとなっている。これらのサイトカインは感染細胞が産生し，炎症の原因となるほか，マクロファージを刺激して，血球貪食症候群をきたすと考えられている[13]。

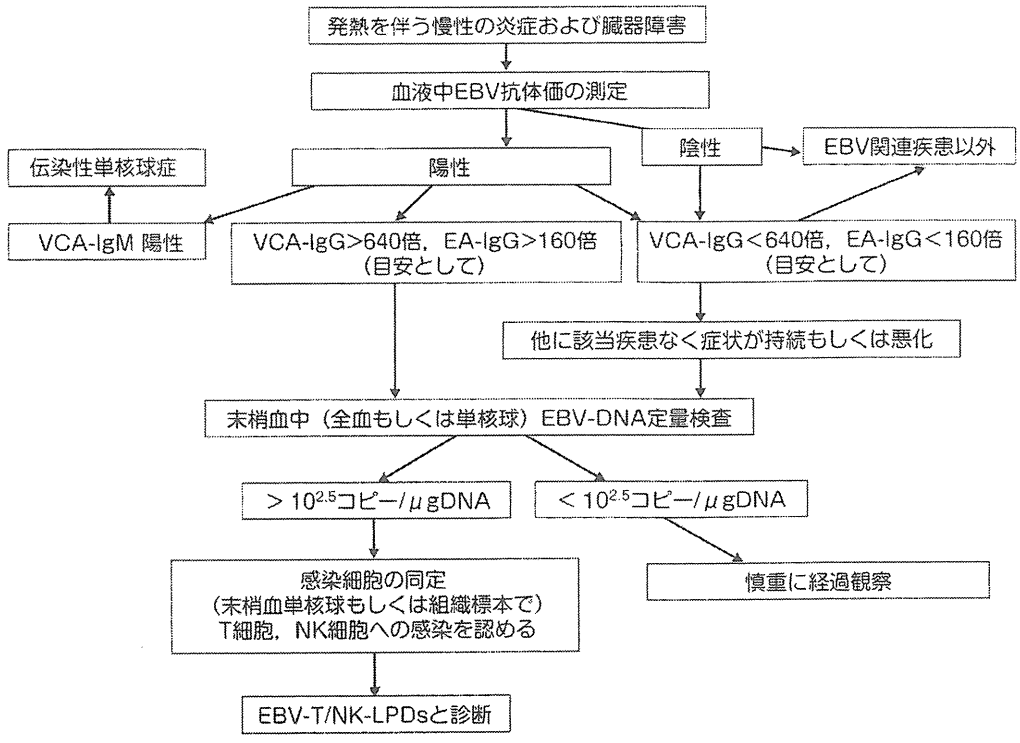

図　EBV陽性T，NKリンパ増殖症診断のフローチャート（EBウイルス感染症研究会診断指針および文献[4]より）

2 腫瘍症状

EBV感染細胞の増殖と浸潤により臓器障害を起こす。リンパ節，肝臓，脾臓などのリンパ系組織のみならず，皮膚，肺，心筋，腸管，中枢および末梢神経など，あらゆる臓器が標的となり得る。EBV-T/NK-LPDsは腫瘤を形成することは多くない。また，浸潤細胞は異型性に乏しい。よって病理組織のみでの診断は困難であることが多い。

予後は非常に悪い。2012年の木村らの108例（1～50歳）の解析では，観察中央値46カ月で44%の症例が重症臓器不全で死亡している[4]。さらに，因子別の予後解析では，発症時年齢が8歳未満の症例の15年生存率は59.7%であったのに対し，8歳以上では27%と有意な差が認められた。筆者らの20歳以上の成人例の解析でも，21例中，移植例2例を含む12例（57%）が死亡しており，特に発症時年齢が50歳以上では75%が発症から平均8カ月で死亡していた[14]。これらの結果から，EBV-T/NK-LPDsは年齢の高い患者ほど予後が悪いと推測される。

診　断

EBV-T/NK-LPDsの診断は容易ではない。筆者らの解析では，成人例では発症から治療開始までの平均期間は20カ月と長かった[14]。その主な原因は，疾患の認知度が低いこと，病理診断が困難であることが考えられる。

本疾患の診断のためのフローチャートを図に示す。以下の順に検査を進めていく。

1 抗体検査

前述の臨床症状から EBV-T/NK-LPDs を疑ったら，まず EBV 抗体検査を行い，既感染であることを確認する。EBV-T/NK-LPDs では，抗 VCA-IgG のみならず抗 EA-IgG 抗体が高値を示すことが多い。一方，抗 EBNA 抗体は陰性もしくは低下とされるが特異的ではない。抗 VCA-IgM 抗体陽性を確認し，IM を除外することは重要である。

2 ウイルス DNA 量測定

抗体が陽性かつ IM が除外されたら，次に末梢血中の EBV の DNA 量を測定する(EBV-DNA 定量検査)。全血もしくは単核球分画での測定を行う。EBV-T/NK-LPDs では EBV 感染細胞が末梢血中に検出されるため，$10^{2.5}$コピー/μgDNA 以上を示す[15]。これは診断に必須の検査であるが，2013 年現在，保険適用外である。多くの検査会社で外注検査として受け付けている。

末梢血中で EBV が増加していて，EBV-T/NK-LPDs の疑いが高くなったら，確定検査には EBV 感染細胞の同定，つまり，どのタイプのリンパ球分画に EBV が感染しているのか(通常通り B 細胞か，それとも T 細胞もしくは NK 細胞か)を調べる。組織標本を用いて *in situ* hybridization 法(*in situ* hybridization of Epstein-Barr virus-encoded mRNA(EBER))と免疫染色法を行い EBV 陽性細胞の表現型を検討するか，末梢血リンパ球を各分画に分け，それぞれの EBV-DNA 量を解析する。後者は研究施設での解析となる。前述のように，EBV-T/NK-LPDs では EBV 感染細胞は T もしくは NK 細胞であり，その確認をもって診断となる。

治　療

EBV-T/NK-LPDs の予後は不良で，慢性に進行し適切に治療されないと致死的経過をとる。本疾患は，炎症と，腫瘍の二つの性質を持つため，両者のコントロールを目的とした治療を行う。炎症に対しては，prednisolone，cyclosporine A，VP-16 の併用療法がもっともよく行われており，症状の制御に有効である[16]。一方で，感染腫瘍細胞を除去し得る有効な化学療法は確立されていない。

しかし，造血幹細胞移植の有効性が指摘されており，木村らの 108 例の解析では，移植施行例の 15 年生存率は 60.6%であったのに対し，未施行例では 25.7%と移植例が有意に生存率が高かった[17]。さらに，2010 年に河らは大阪母子保健総合医療センターの移植例を後方視的に解析し，骨髄非破壊的移植を行った患者では 90%以上の 3 年生存率を得たと報告している[18]。今後は長期予後も含めた多数例での移植成績の解析に加え，至適移植時期の検討，さらには移植が困難な症例に対する治療法の開発が必要である。

おわりに

CAEBV と報告されてきた疾患の中から，EBV が T，NK 細胞に感染し，かつ感染細胞がクローナルに増殖しているものを，同じく EBV 感染 T，NK 細胞のクローナルな増殖をみる蚊刺過敏症，種痘様水疱症と合わせて EBV-T/NK-LPDs とし，一つの疾患として解説した。今後は，これがはたして一つの疾患なのか，感染細胞や発症年齢などで臨床像に差があるのか，さらに詳細な検討が必要である。EBV-T/NK-LPDs の解析は，EBV による T，NK 腫瘍発症機構の解明のみならず EBV 陽性 T，NK 細胞腫瘍というカテゴリーの確立，さらには治療法開発にもつながり得る。本邦を中心とした東アジアの研究者に期待される役割は大きい。

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Current Studies on Chronic Active Epstein-Barr virus Infection in Japan

Running title: Chronic Active EBV Infection

Shigeyoshi Fujiwara, MD, PhD[1], Hiroshi Kimura, MD, PhD[2], Ken-ichi Imadome, PhD[1], Ayako Arai, MD, PhD[3], Eiichi Kodama, MD, PhD[4], Tomohiro Morio, MD, PhD[5], Norio Shimizu, PhD[6], and Hiroshi Wakiguchi, MD, PhD[7].

[1]Department of Infectious Diseases, National Research Institute for Child Health and Development, Tokyo; [2]Department of Virology, Nagoya University Graduate School of Medicine, Nagoya; [3]Department of Hematology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo; [4]Department of Internal Medicine/Division of Emerging Infectious Diseases,Tohoku University Graduate School of Medicine, Sendai; [5]Department of Developmental Biology and Pediatrics, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo; [6]Department of Virology, Division of Medical Science, Medical Research Institute, Tokyo Medical and Dental University, Tokyo; [7]Department of Pediatrics, Kochi Medical School, Kochi University, Kochi.

Correspondence: Shigeyoshi Fujiwara, MD, PhD, Department of Infectious Diseases, National Research Institute for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan. Phone: +81-3-3417-2457. Fax: +81-3-3417-2419. Email: fujiwara-s@ncchd.go.jp

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Abstract

Epstein-Barr virus (EBV) infection is usually asymptomatic and persists lifelong. Although EBV-infected B cells have a potential of unlimited proliferation, they are effectively removed by the virus-specific cytotoxic T cells and EBV-associated lymphoproliferative disease develops only in immunocompromised hosts. Rarely, however, individuals without apparent immunodeficiency develop chronic EBV infection with persistent infectious mononucleosis-like symptoms. These patients exhibit high EBV DNA load in the peripheral blood and systemic clonal expansion of EBV-infected T cells or natural killer (NK) cells. Their prognosis is poor with life-threatening complications includinghemophagocyticlymphohistiocytosis, organ failure, and malignant lymphomas. The term chronic active EBV infection (CAEBV) is now generally used for this disease. Geographical distribution of CAEBV is markedly uneven and most cases have been reported from Japan and other East Asian countries. Here wesummarize our current understanding of CAEBV and describe recent progress of CAEBV research in Japan.

Key words: Epstein-Barr virus, chronic active EBV infection, EBV-associated hemophagocyticlymphohistiocytosis, hypersensitivity to mosquito bites, hydroavacciniforme, EBV-associated T/NK-cell lymphoproliferative disease, mouse model, flow-cytometric in situ hybridization.

Introduction

Epstein-Barr virus (EBV) was discovered in cultured cells of Burkitt lymphoma as the first human tumor virus[1]. Since then EBV was found associated with a number of malignancies, including Hodgkin lymphoma, nasopharyngeal carcinoma, and gastric carcinoma[2]. In spite of close association with these malignancies,EBV was shown to be a ubiquitousvirus infecting more than 90% of adult population in the world_ENREF_2. EBV-associated malignancies thus develop in a restricted fraction of hosts through collective effects of various factors, including host genetic background and environmental factors, as well asfunctions of EBV genes. EBV infection in humans is usually asymptomatic and persists lifelong as latent infection, although primary infection later than adolescencefrequentlyresults in infectious mononucleosis (IM). IM is caused by transient proliferation of EBV-infected B cells accompanied by excessive responses of EBV-specific cytotoxic T cells (CTLs). The main target of EBV is B cells and epithelial cells, and EBV has a unique biological activity to transform B cellsand establishimmortalized lymphoblastoid cell lines. Since EBV-transformed cells express at least nine viral proteins including the highly immunogenic EBV nuclear antigens 3 (EBNA3s)and EBNA2 (the latency III type EBV gene expression), they are readily removed by the virus-specific CTLs and the virus does not cause lymphoproliferative disease in normal immunocompetent hosts[3]. In immunocompromised hosts like transplant recipients and AIDS patients, however, EBV-transformed cells are not efficiently removed and may cause EBV-associated B-cell lymphoproliferative disease (LPD).

Rare EBV-infected individuals without apparent immunodeficiency present with persistent or recurring IM-like symptoms including fever, hepatosplenomegaly, lymphadenopathy, and liver dysfunction, as well as high EBV DNA load in the peripheral blood[4-7]. The term chronic active

EBV infection (CAEBV) is now generally used to describe this disease. Patients with CAEBV encountered in Japan and other East Asian countries have poor prognosis and characterized by clonal expansion of EBV-infected T cells or natural killer (NK) cells[8-11]. In contrast, a similar disease with less morbidity and mortality has been reported from Western countries and it is usually associated with proliferation of EBV-infected B cells[12]. In this review, focused on CAEBV as an EBV-associated T/NK-cell lymphoproliferative disease (EBV^+ T/NK-LPD), we summarize the current understanding of the disease and describe our own recent workssubsidized by grants from the Ministry of Health Labour and Welfare of Japan.

Clinical characteristics of CAEBV and other EBV-associated T/NK-cell lymphoproliferative diseases

As described above, IM-like symptoms are the main symptoms of CAEBV[4-7]. Other clinical manifestations include thrombocytopenia, anemia, pancytopenia, diarrhea, and uveitis. Peripheral blood EBV DNA load regularly exceeds $10^{2.5}$ copies/μg DNA[13]. High-level production of various cytokines, including IL-1β, IL-10, and IFN-γ has beendetected in CAEBV patients and are thought to play important roles in inflammatory symptoms of the disease[14-16]. CAEBV can be classified into the T-cell and NK-cell types, depending on which lymphocyte subset is mainly infected with EBV. A survey of Japanese CAEBV patients revealed that the T-cell type is associated with less favorable prognosis than the NK-cell type[17, 18]_ENREF_16. CAEBV was included in the 2008 WHO classification of lymphomas as the systemic EBV^+ T-cell lymphoproliferative disease of childhood[19].

Although the clinical course of CAEBV is chronic, patients often develop fatal complications such as multi-organ failure,disseminated intravascular coagulopathy (DIC), digestive tract

ulcer/perforation, coronary artery aneurysms, and malignant lymphomas, as well as EBV-associated hemophagocyticlymphohistiocytosis (EBV-HLH)[7]. HLH is a hyperinflammatory condition caused by overproduction of cytokines by excessively activated T cells and macrophages. Clinical characteristics of HLH include fever, hepatosplenomegaly, pancytopenia, hypertriglyceridemia, DIC, and liver dysfunction[20].EBV-HLH usually occurs following primary EBV infection and is itself characterized by clonal proliferation of EBV-infected T or NK cells(most often $CD8^+$ T cells)[21, 22]. EBV-HLH can also occur in association with X-linked lymphoproliferative disease (XLP) and XIAP deficiency[23].

Patients with CAEBV may have characteristic cutaneous complications, namely hypersensitivity to mosquito bites (HMB) and hydroavacciniforme (HV), that are themselves distinctEBV^+ T/NK-LPDs characterized by clonal proliferation of EBV-infected T or NK cells. Both HMB and HV can occur independently or in association with CAEBV. HV is a childhood photosensitivity disorder, characterized by necrotic vesiculopapules on sun-exposed areas[24]. EBV DNA levels are elevated in patients' peripheral blood and histochemical analysis of skin lesions revealsinfiltration of T cells expressing the EBV-encoded small RNA (EBER)[25].Although most cases of HV resolve by early adult life, cases overlapping with CAEBV may eventually develop EBV-positive malignant lymphoma that was included in the 2008 WHO classification of lymphoma as the hydroavacciniforme-like lymphoma[19, 26]_ENREF_26_ENREF_19_ENREF_19_ENREF_19_ENREF_27. HMB is characterized by severe local skin reactions to mosquito bites including erythematous swelling with bullae, necrotic ulcerations, and depressed scars[27]. These local reactions may be accompanied by general symptoms such as high fever, lymphadenopathy, and liver dysfunction.Most HMB cases have EBV infection in NK cells in skin lesions and peripheral blood[28, 29]. HMB cases without

systemic symptoms may eventually develop CAEBV[28].

Prospective clinicopathologic analyses of CAEBV and other EBV⁺ T/NK-LPDs

CAEBV, EBV-HLH, HMB, and HV are thus distinct but overlapping entities categorized as EBV^+ T/NK-LPDs. Higher incidence of these diseases in East Asian countries and their occasional coincidence in a single patient imply a common pathogenesis for them[7, 30]_ENREF_7. Kimura and others performed a large-scale prospective study of Japanese EBV^+ T/NK-LPD cases[31]. 108 cases of EBV^+ T/NK-LPDs (80 cases of CAEBV, 15 cases of EBV-HLH, 9 cases of HMB, and 4 cases of HV) were analyzed. The results showed that the clinical profile of EBV^+ T/NK-LPD is closely linked with the lineage of EBV-infected cells. More than half (53%) of EBV-HLH patients had EBV in the $CD8^+$ T-cellsubset, in contrast to the low incidence of EBV infection in this subset in the other EBV^+ T/NK-LPDs. Most patients (89%) of HMB had EBV-infected NK cells, whereas majority (75%) of patients with HV had EBV-infected γδT cells. In a median follow-up period of 46 months, 47 patients (44%) died of severe organ complications and 13 (12%) developed overt lymphoma or leukemia. The age of onset ≥8years and liver dysfunction were risk factors for mortality and transplanted patients had better prognosis. Patients with $CD4^+$ T-cell infection had shorter survival as compared with those with NK-cell infection. Because shorter time from onset to hematopoietic stem cell transplantation (HSCT) and inactive disease at HSCT were associated with longer survival, earlier HSCT in good condition was considered preferable. Among the 108 patients enrolled, four patients developed aggressive NK-cell leukemia (ANKL) and six patients developed extranodal NK/T-cell lymphoma (ENKL). It is thus conceivable that a certain fraction of patients with ANKL and ENKL havedeveloped these malignancies as a consequence of CAEBV[32, 33]_ENREF_34.

Characteristics of adult CAEBV cases

CAEBV has been described mainly as a disease of children and young adults; the mean age of onset was estimated to be 11.3 years[18]_ENREF_25. Recently, however, increasing number of adult patients fulfilling the criteria of CAEBV_ENREF_34are reported. This may be a true increase in the incidence of adult-onset CAEBV or reflect improved recognition of this disease by physicians. Arai and others reviewed 23 cases of adult-onset CAEBV and described their characteristics[34]_ENREF_34. In 87% of adult cases, T cells were infected with EBV, whereas in childhood-onset cases, theT- and NK-cell types were equally frequent. Adult-onset cases appeared rapidly progressive and more aggressive, although the number of patients analyzed was limited. Further investigation with larger number of patients is required to elucidate the characteristics of adulthood CAEBV and its relation to the childhood counterpart.

Recurrence of CAEBV with EBV-infected, donor-derived T cells following HSCT

The relative prevalenceof CAEBV in East Asia and natives of Central and South America implies a genetic background for its pathogenesis. Recently HLA-A*26, an MHC class I allele relatively common in East Asia, was found to be associated with an increased risk for EBV^+ T/NK-LPD[35]_ENREF_37. Although possible involvement of EBV strains with increased propensity to induce T/NK-celllymphoproliferation cannot be formally denied, it is highly unlikely because outbreaks and familial transmission of CAEBV havenot been reported. Arai and others reported an intriguing case of CAEBV who experienced a relapse after bone marrow transplantation[36]. A 35 year-old female patient with CAEBV of the CD8 type had HSCTfrom an unrelated male donor following myeloablative preconditioning with total body irradiation. The serologic HLA types of the patient and the donor were identical, whereasthe DNA typeswere different in two HLA-DR alleles.Although the peripheral blood EBV DNA was undetectable at one month after HSCT and remained so for nearly twelve months, her EBV DNA load increased again

and reached 1.0×10^5 copies/μg DNA. EBV was found primarily in $CD8^+$ T cells again. However, her EBV-infected cells had now an XY karyotype,clearly indicating their donor origin. Sequencing analysis of the variable region of the EBV-encoded *LMP1* gene showed that the virus strain infecting $CD8^+$ T cells were different before and after BMT, suggesting that her repeated episodes of CAEBV were not caused by a rare EBV strain with an unusual biological activity. If we do not suppose that these two consecutive episodes of CAEBV in a single patient occurred just by chance, these findings may suggest that she has a certain genetic background that exerts its direct effectson cellular lineages unrelated to hematopoietic stem cells.

Pathophysiology of CAEBV

Pathogenesis of CAEBV is not understood. Most T and NK cells do not express the EBV receptor CD21 andthe mechanism of their infection with EBV is not clear. Transfer of CD21 from B cells to NK cells through immunological synapse may render the latter cells accessible for EBV[37]. The mechanism by which EBV induces proliferation of T and NK cells is not known either. EBV-induced expression of CD40 and its engagement by CD40L may have a role in the survival of EBV-infected T and NK cells of CAEBVpatients[38]. Since EBV-positive T or NK cells have been occasionally found in the tonsil and peripheral blood of IM patients, ectopic EBV infection in T or NK cells does not necessarily lead to the development of CAEBV[39-41]. Although EBV-infected T and NK cells inCAEBV patients and cell lines derived from them do not express the most immunodominant EBNA3s and EBNA2, they express EBNA1, latent membrane protein 1 (LMP1) and LMP2 (the latency II type EBV gene expression) that are frequently recognized by EBV-specific CTLs[3, 42-45]._ENREF_40 Hosts with normal immune functions are thus expected tohave the capacity to recognize EBV-infected T and NK cells. It is thus conceivable that patients

with CAEBV have a certain defect in immunologic functions that causes inefficient recognitionand/or killing of EBV-infected latency II cells. Indeed, deficiency in cellular immune responses to EBV has been detected in patients with CAEBV[46-48].The defect in T-cell responses to LMP2A might be particularly relevant to this issue[47]. Interestingly, a patient with clinical manifestations similar to CAEBV, although the virus was found in his B cells, was shown to have mutations in the gene encoding perforin, that has a critical role in granule-mediated killing of target cells[49]. None of other patients with CAEBV, however, were shown to have a mutation in the*perforin* gene. Mutations of the genes responsible for XLP, XIAP deficiency, and familial HLH (except for the type 2 that is caused by mutations of *perforin*) have not been reported for patients with CAEBV[7].

Clonal proliferation of EBV-infected T or NK cells in CAEBV and other EBV^+ T/NK-LPDs implies that these diseases have amalignant nature. However, CAEBV is a chronic disease and patients with clonal expansion of EBV-infected T or NK cells may stay in stable condition for years without treatment[18]. Overt malignant lymphoma occurs usually after a long course of disease. Therefore CAEBV may represent, at least in its early phase,premalignant or smoldering phase of EBV-positive leukemia/lymphomas_ENREF_46. Ohshima and others proposed a pathological categorization of CAEBV into a continuous spectrum ranging from a smoldering phase to overt leukemia/lymphoma[50]. Clonality of EBV-infected T or NK cells in CAEBV may not necessarily indicate a malignant phenotype; acquisition of clonality might be a result of other selective processes such as immune escape.

Mouse xenograft models for EBV^+ T/NK-LPD

Animal models for EBV^+ T/NK-LPD have not been available, rendering researches on their pathogenesis and therapy difficult. Imadome and others transplanted peripheral blood

mononuclear cells (PBMC) isolated from patients with CAEBV and EBV-HLH to immunodeficient mice of the NOD/Shi-*scid*/IL-2Rγ^{null}(NOG) strain, and successfully reproduced major features of these diseases including systemic monoclonal proliferation of EBV-infected T or NK cells and hypercytokinemia (Fig. 1)[51]. Although many features were common to CAEBV and EBV-HLH model mice, hemorrhagic lesionsin the abdominal and thoracic cavities and extreme hypercytokinemia were unique to the latter model, indicating that these mouse models reflect the differences in the pathophysiology of the original diseases. Importantly, these models revealed an essential role of $CD4^+$ T cells in the engraftment of EBV-infected T and NK cells. In vivo depletion of $CD4^+$ T cells following transplantation effectively prevented the engraftment of EBV-infected cellsof not only the $CD4^+$ lineage but also the $CD8^+$ and $CD56^+$ lineages. Furthermore, OKT-4 antibody given after engraftment was also effective to reduce EBV DNA load in the peripheral blood and major organs (Imadome and others, unpublished results). These results suggest that therapeutic approaches targeting $CD4^+$ T cells may be possible.

Diagnosis and monitoring of CAEBV

Prolonged or relapsing symptoms of IM are the major cluetothe diagnosis of CAEBV. Although elevated serum antibody titers against EBV-encoded antigens are often found, this does not occur always and normal titers of anti-EBV antibodies should not preclude the diagnosis of CAEBV[7]. Diagnostic criteria for CAEBV has been published[13]. Quantitation of peripheral blood EBV DNA is most important for diagnosis and a finding of elevated value should be followed by identification of EBV-infected T or NK cells. Quantitation of EBV DNA is however influenced by many factors and the results can vary in different laboratories[52]. Recently, therefore, an international standard EBV DNA sample for normalization became available fromthe National Institute for Biological

Standards and Controls, USA. Since CAEBV is a chronic disease that may progress to overt malignancy and early HSCT in a better clinical condition is recommended, precise monitoring of patient's clinical parameters is particularly important.

Flow-cytometric in situ hybridization for identification of EBV-infected cells

Diagnosis of CAEBV requires exact phenotypingof EBV-infected cells. This process has been usually performed by immunobeads sorting of PBMC into lymphocyte subsets, followed by measurement of EBV DNA in each subset by quantitative PCR. These processesarehowever time-consuming and require specific skills. Kimura and others developed a new method termed flow-cytometric in situ hybridization (FISH) to phenotype EBV-infected cells (Fig. 2)[53, 54]. They utilized a fluorescently labeledpeptide nucleic acid (PNA) probe complementary to EBER and succeeded in detecting EBER by flow cytometry. Following reaction with antibodies specific to surface markers, peripheral blood mononuclear cells were permeabilized and subjected to in situ hybridization with the PNA probe. EBER probes and surface-bound antibodies were then detected simultaneously by flowcytometry. EBV-infected cells with a certain phenotype can be directly counted by FISH that is less laborious thanthe current method. They demonstrated that FISH can be applied for the diagnosis of EBV^+ T/NK-LPD and showed that EBV infects mainly γδT cells in HV[53-55].

miRNA as a potential biomarker of CAEBV

microRNA (miRNA) is a small non-coding RNA of 18-25 nucleotides and plays a critical role in the regulation of cellular proliferation, differentiation, and apoptosis through negatively regulating mRNA translation[56]. miRNAs are encoded not only by cells but also by viruses; EBV is actually the first virus shown to encode miRNAs[57]. Two clusters of EBV-encoded miRNAs have been identified; miR-BHRFs and miR-BARTS[58]. Kawano and others reported that plasma levels of

miR-BART1-5p, 2-5p, 5, and 22 are significantly higher in patients with CAEBV than in those with IM and healthy controls[59]. Plasma miR-BART 2-5p, 4, 7, 13, 15, and 22 levels were significantly elevated in CAEBV patients with active disease than in those with inactive disease. miR-BART13 levels could discriminate between patients with active disease and those with inactive disease with a clear cut-off value. Similarly, plasma miR-BART2-5p and 15 levels could clearly discriminate between patients with complete remission from others. Importantly, plasma EBV DNA levels did not show any significant correlation with these clinical parameters. These results suggest that EBV-encoded miRNAs in plasma may be a useful biomarkerfor the diagnosis and monitoring of CAEBV.

Therapy of CAEBV

Various therapeuticshave been tried for the treatment of CAEBV, including antiviral, chemotherapeutic, and immunomodulatory drugs, with only limited success. These regimens induced sustained complete remission in only exceptional cases and HSCT is at present the only curative therapy for CAEBV[60]. Current event-free survival rate for CAEBV patients following HSCTis estimated to be 0.561 ± 0.086[61].Very recently, Kawa and colleagues reported an excellent results of HSCT following non-destructive pretreatment (RIST)[62]. For18 pediatric patients with CAEBV who were treated with RIST, three-year event-free survival was 85.0±8.0% and three year overall survival rate was 95.0±4.9%. HSCT is thus the therapy of choice for CAEBV. However, HSCT is still accompanied by substantial risk and CAEBV patients have high risks for transplantation-related complications[18]. It is therefore desirable to develop novel therapies that do not depend on HSCT. Preclinical studies of two candidate drugs for CAEBV have been carried out recently and gave hopeful results.

Bortezomib, known as an inhibitor of 26S proteasome[63], also has an inhibitory effect on the cellular transcription factor NF-κB. Since the survival and proliferation of EBV-transformed B cells are critically dependent on NF-κB activity, bortezomib has been shown to induce apoptosis in these cells[64]. Iwata and others investigated the effect of bortezomib on EBV-infected T-cell lines including those derived from CAEBV[65]. Bortezomib induced apoptosis in all human T-cell lymphoma cell lines examined whether or not they were infected with EBV. In addition,bortezomib induced the expression of EBV lytic-cycle genes BZLF1 and gp350/220 as has been reported for EBV-infected B-cell lines[66]. Bortezomib also induced apoptosis specifically in EBV-infected T- or NK-cells cultured ex vivo from patients with EBV^{+}T/NK-LPDs.

Valproic acid is a widely used anti-epileptic drug and is also known as a potent histone deacetylase (HDAC) inhibitor. HDAC inhibitorshave potent anticancer activities with proven efficacy in various human malignancies. Valproicacid induces lytic infection in EBV-infected B-lymphoblastoid and gastric carcinoma cell lines and thereby potentiatethe effects of chemotherapeutic agents both in vitro and in vivo[67]_ENREF_19. Iwata and others examined the effect of valproic acid on EBV-infected T and NK-cell lines[68]. They found that this agent induces apoptosis in human EBV-infected T and NK cells. Use of the drug with the NF-κB inhibitor bortezomib showed an additive effect. In contrast to the previous results with EBV-infected B-cell lines, valproic acid did not induce lytic infection in the virus-infected T and NK-cell lines, indicating that the apoptosis-inducing effect of valproic acid is not dependent on induction of EBV lytic cycle.

Perspective

Significant progress has been made in the research of many aspects of CAEBV, including