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ORIGINAL ARTICLE

The clinical characteristics of $CD7^+$ $CD56^+$ acute myeloid leukemias other than M0

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Abstract Immunological phenotyping of acute leukemia have provided enormous and important information for the classification and lineage determination of leukemia. Forty-nine patients with $CD7^+$ $CD56^+$ acute myeloid leukemia (AML) were analyzed. There were 17 patients of M0, which corresponded to myeloid/NK cell precursor acute leukemia, and 32 patients of AML other than M0 (9 each for M1 and M2, one for M3, 3 for M4, 4 for M5 and 6 for M7). Age distribution was similar between these two groups, but $CD7^+$ $CD56^+$ M0 showed significant male predominance than $CD7^+$ $CD56^+$ M1–M7 (M:F = 15:2 vs. 15:17, $P = 0.006$). The disease localization and the hematological manifestations were different, showing fewer white blood cell counts and circulating leukemic blasts, less anemia, less thrombocytopenia and more frequent extramedullary involvement in M0 group. The prognosis was poor in both groups, and there was no statistical difference. These findings suggest that extramedullary involvement of myeloid/NK cell precursor acute

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leukemia is not directly derived from the presence of CD7 and CD56 antigens on leukemic cells. The poor prognosis of $CD7^+$ $CD56^+$ M1–M7 suggests that this phenotype may act as a prognostic factor for AML, but this should be confirmed in further studies.

Keywords Acute myeloid leukemia · Immunophenotyping · CD7 · CD56

1 Introduction

Acute myeloid leukemia (AML) comprises a heterogeneous group of diseases that differ in their etiology, pathogenesis, and prognosis. It was first classified by its morphology and cytochemical reactions in the French–American–British (FAB) classification [1] and the World Health Organization (WHO) classification [2]. In the past two decades, the immunological classification of AML has developed on the basis of progress on the use of monoclonal antibodies and flow-cytometric analyses [3–5]. Several phenotypic markers have been demonstrated to have clinical significance other than for diagnosis including detection of minimal residual disease [6, 7] and prognostication [8–10].

We previously identified an immunophenotypically novel AML with the $CD7^+$ $CD56^+$ myeloid $antigen^+$ phenotype and termed it "myeloid/natural killer (NK) cell precursor acute leukemia" [11]. Myeloid/NK cell precursor acute leukemia presents a similar phenotype to its normal counterpart (precursor NK cells with myeloid antigens) [12–14], but shows distinct clinicopathologic features [11, 15, 16]. Tumor cells of myeloid/NK cell precursor acute leukemia show immature blastic morphology and are positive for myeloid antigens, but are negative for the cytochemical myeloperoxidase (MPO) reaction, suggesting that this leukemia falls within the category of AML M0 according to the FAB classification. However, apart from its $CD7^+$ $CD56^+$ phenotype, its clinical presentation is quite different from those of other M0 leukemias [16]. Patients with myeloid/NK cell precursor acute leukemia frequently exhibit extramedullary involvement and lymphadenopathy with or without a mediastinal mass. Although they are responsive to AML-type chemotherapy, the prognosis is extremely poor, even for younger patients [11, 16]. In this context, it is necessary to understand whether the $CD7^+$ $CD56^+$ phenotype is responsible for these particular characteristics of myeloid/NK cell precursor acute leukemia. To clarify this issue, we collected data from patients with $CD7^+$ $CD56^+$ AML other than M0 (M1–M7), and compared their clinical characteristics with those of patients with myeloid/NK cell precursor acute leukemia [16].

2 Patients and methods

2.1 Patients

A total of 32 patients with $CD7^+$ $CD56^+$ AML other than M0 (M1–M7) were identified in the collaborating institutes of the Japan Adult Leukemia Study Group and the Japan Clinical Oncology Study Group. Data were collected with a survey form in participating institutions separately from prospective studies. The diagnosis of AML was based on the FAB and WHO classification [1, 17, 18]. Cases with extramedullary leukemia were included in this study, even though less than 20% of their bone marrow cells were leukemic [16]. The patients' records and clinical data were reviewed retrospectively. As for chemotherapeutic regimens, those containing high dose cytosine arabinoside (Ara-C) or those involving Ara-C for at least five consecutive days accompanied by anthracyclines for at least 3 days were categorized as AML-type chemotherapy. The clinical characteristics of patients with $CD7^+$ $CD56^+$ AML (M1–M7) were compared with those of patents with myeloid/NK cell precursor acute leukemia ($CD7^+$ $CD56^+$ AML M0) as previously described [16]. This study was approved by the Ethical Committee as a part of retrospective survey for NK cell-related tumors (approval #625-3).

2.2 Immunophenotyping

Flow-cytometric analyses were performed as previously described [11]. The reactivity for the following markers was analyzed: CD1, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD13, CD14, CD15, CD16, CD19, CD20, CD25, CD33, CD34, CD38, CD41, CD56, CD57, CD71, CD117, CD122, HLA-DR, T cell receptor (TCR) $\alpha\beta$, TCR $\gamma\delta$, IgA, IgG, IgM, IgD, kappa, lambda, cytoplasmic CD3 (cyCD3), cyCD22, cyCD33, cyIgM, cyMPO, and terminal deoxynucleotidyl transferase (TdT). Cytoplasmic antigens and TdT were analyzed as previously described with fixation in 50% ethanol with 1% paraformaldehyde. Leukemic cells were judged as positive for each antigen when more than 20% of the gated cell reacted with the antibody.

2.3 Cytogenetic analysis

Leukemic cells were cultured, and the chromosomes were banded. Cytogenetic abnormalities were determined according to the International System for Human Cytogenetic Nomenclature [19].

2.4 Statistical analysis

The χ^2 test and Fisher's exact test were used to examine relationships between two factors, and the Mann–Whitney

U test was used to compare graded factors. Survival curves were estimated with the Kaplan–Meier method and compared by means of the log-rank test. Data were analyzed with STATA version 9 (College Station, TX) and Fisher (Nakayama-Shoten, Tokyo, Japan) statistical software.

3 Results

3.1 Patient characteristics

A total of 49 AML patients with the CD7+ CD56+ phenotype were identified in the collaborating institutes. Of these, 17 M0 patients had previously been reported as having myeloid/NK cell precursor acute leukemia. The clinical features of the 32 patients with CD7+ CD56+ AML (M1–M7) are listed in Table 1. The CD7+ CD56+ phenotype was recognized in all FAB subtypes except M6. Notably, 6 patients were found with AML M7. No recurrent structural abnormalities were identified by chromosome examinations. One patient showed t(15;17), but none presented with t(8;21), inv(16), or 11q23 translocations. Trisomy 4 was found in 3 patients. Total or partial deletions in chromosome 7 were seen in 4 patients. A comparison of the patients' characteristics with those of patients suffering from CD7+ CD56+ M0 is shown in Table 2. The median age of the patients was 49 years, and their age distribution was not statistically different from that of the CD7+ CD56+ AML

Table 1 Patient characteristics of the CD7+ CD56+ AML (M1–M7) group

No.	Age	Sex	FAB	WBC	Blast (%)	RBC	Plt	BM blast (%)	MPO (%)	Extra-medulla	LN	Others
1	21	M	M1	91,100	86.0	298	5.8	85.5	99.9	N	–	
2	21	M	M1	17,000	97.0	250	6.2	89.2	99.9	N	–	
3	36	M	M1	39,200	77.5	480	21.0	91.6	3.0	N	–	
4	45	F	M1	43,800	98.0	278	0.6	83.5	90.0	N	–	
5	47	M	M1	4,600	56.0	325	0.8	Dry tap	99.9	N	–	
6	50	M	M1	35,800	82.0	454	3.9	89.2	5.0	Y	+	
7	53	F	M1	149,300	94.0	377	2.8	85.0	90.0	N	–	
8	54	M	M1	203,600	93.0	240	3.5	86.6	28.0	N	–	
9	70	M	M1	3,000	0	369	23.8	4.0	11.0[a]	Y	+	Spleen
10	26	F	M2	2,800	0	348	13.0	0.4	95.0[a]	Y	+	Tonsil
11	29	F	M2	31,400	75.4	439	2.0	61.9	99.2	N	–	
12	32	F	M2	1,700	0	309	23.7	55.7	61.5	N	–	
13	49	F	M2	10,100	13.0	369	1.6	53.6	50.0	N	–	
14	63	F	M2	3,600	54.0	367	3.9	48.8	100.0	N	–	
15	63	M	M2	13,200	93.5	359	1.7	87.9	90.0	Y	+	
16	70	M	M2	22,200	90.0	170	0.6	49.0	98.0	Y	–	Skin
17	74	F	M2	3,600	54.0	203	2.1	85.2	96.0	N	–	
18	76	F	M2	1,800	13.0	109	2.2	43.2	41.0	Y	–	Spleen
19	37	F	M3	4,300	90.0	213	0.4	50.0	80.0	N	–	
20	34	F	M4	3,000	14.0	104	1.7	87.6	99.0	N	–	
21	59	M	M4	55,290	39.0	246	5.7	67.4	81.5	Y	–	Skin
22	86	F	M4	211,900	60.0	264	4.2	79.0	80.0	N	–	
23	21	F	M5a	269,700	96.0	248	5.7	98.8	90.0	Y	+	Gingiva
24	23	F	M5a	540,000	98.0	210	4.4	99.0	10.0	Y	–	Meningeal
25	53	M	M5a	13,320	79.0	376	7.0	89.0	80.0	N	–	
26	17	M	M5b	3,900	20.0	409	18.8	83.5	0	N	–	
27	31	M	M7	600	20.0	344	22.2	83.0	0	N	–	
28	42	M	M7	2,100	24.0	239	11.6	81.5	18.0	N	–	
29	48	F	M7	17,000	92.0	215	37.8	70.5	2.0	N	–	
30	68	F	M7	2,800	69.0	350	2.6	54.0	10.0	N	–	
31	70	M	M7	2,600	1.5	229	9.9	20.0	0	N	–	
32	74	F	M7	3,600	32.0	400	6.0	32.4	0	N	–	

[a] Examined at the time of recurrence/progression

Table 2 Comparison of $CD7^+$ $CD56^+$ AML patient characteristics (M0 versus M1–M7)

	$CD7^+$ $CD56^+$ AML M1–M7 ($n = 32$)	$CD7^+$ $CD56^+$ AML M0 ($n = 17$)	*P* value
Age (years), median (range)	49 (17–86)	46 (15–81)	0.32
Sex (male/female)	15/17	15/2	0.002
Peripheral blood count			
WBC (/μl), median (range)	11,650 (600–540,000)	4,500 (1000–51,000)	0.04
PB blast (%), median (range)	64.5 (0–98.0)	5.0 (0–95.0)	0.0006
Hb (g/dl), median (range)	9.7 (4.1–14.2)	13.1 (5.5–17.0)	0.004
PLT ($\times 10^4$/μl), median (range)	4.3 (0.4–37.8)	12.8 (3.9–38.5)	0.002
Sites of involvement			
Bone marrow			
Median blast (%)	81.5%	80.0%	0.59
No marrow involvement	2	5	0.04
Extramedullary	9 (28%)	14 (82%)	0.0004
Lymph node	5	12	0.0002
Mediastinum	1	4	0.04
Liver and/or spleen	2	2	0.43
Skin	2	1	0.73
Others	3	3	0.34

WBC white blood cell, *PB* peripheral blood, *PLT* platelets

M0 patients. There was almost an equal sex distribution for $CD7^+$ $CD56^+$ M1–M7 (male:female = 15:17), and the male:female ratio was significantly different from that of $CD7^+$ $CD56^+$ M0 ($P = 0.006$). The peripheral blood cell count at diagnosis showed a significantly higher white blood cell count ($P = 0.04$) and higher leukemic cell percentage ($P = 0.0006$) in the $CD7^+$ $CD56^+$ M1–M7 patients than in the $CD7^+$ $CD56^+$ M0 patients. In addition, the red blood cell and platelet counts for the former were significantly lower than those for the latter (Table 2). Overall, the $CD7^+$ $CD56^+$ M1–M7 patients showed many peripheral blood count abnormalities, which is comparable to standard AML.

Two $CD7^+$ $CD56^+$ M1–M7 patients did not show bone marrow (BM) involvement at the initial diagnosis, but the other cases showed a high percentage of BM leukemic cells. However, both of the 2 cases without BM involvement at the initial presentation progressed predominantly in the BM with manifestations of acute leukemia. Extramedullary involvement was recognized in 9 patients of the $CD7^+$ $CD56^+$ M1–M7 group (28%), which was significantly lower than the number in the $CD7^+$ $CD56^+$ M0 group ($P = 0.0004$). Although lymph node involvement was the most common manifestation of the extramedullary diseases of the $CD7^+$ $CD56^+$ M1–M7 group, the absolute incidence was significantly lower than that in the $CD7^+$ $CD56^+$ M0 group ($P = 0.0002$), as was the incidence of mediastinal involvement ($P = 0.04$).

In summary, no clinical manifestations of "myeloid/NK cell precursor acute leukemia" were recognized in the $CD7^+$ $CD56^+$ M1–M7 group.

3.2 Immunophenotyping

The immunophenotypic characteristics of the patients are summarized in Table 3. By definition, all patients were positive for both CD7 and CD56 antigens. Most of the cases were positive for CD13, CD33, CD34, CD117, and HLA-DR, while all were negative for lymphoid-specific markers including CD16 and CD57. CD41 was expressed in all 6 cases of megakaryoblastic leukemia (AML M7). Several lymphoid markers that are known to be also expressed in AML, such as CD2, CD4, CD5, CD10, and TdT were expressed in some of the patients. The incidence was higher in the M1 and M7 cases.

3.3 Therapeutic response and prognosis

In the $CD7^+$ $CD56^+$ AML M1–M7 group, 20 of the 29 patients that were initially treated with AML-type chemotherapy attained complete remission (CR), whereas none of the two cases treated with CHOP chemotherapy did (Table 4). Because of the low numbers of patients, the difference was not statistically significant. Another patient could not receive any chemotherapy due to their poor condition. The CR rate was 67% (6 of 9) for M1, 56% (5 of 9) for M2, 100% (1 of 1) for M3, 67% (2 of 3) for M4, 75% (3 of 4) for M5, and 50% (3 of 6) for M7. Of the 20 patients who achieved CR, two received allogeneic hematopoietic stem cell transplantation in first CR, and both are alive without disease. Eight of the 20 patients experienced disease recurrence.

Table 3 Phenotypic characteristics of CD7$^+$ CD56$^+$ AML patients (M1–M7)

FAB	M1 ($n = 9$)	M2 ($n = 9$)	M3 ($n = 1$)	M4 ($n = 3$)	M5 ($n = 4$)	M7 ($n = 6$)	Total ($n = 32$)	%
CD1	0/3	0/2	ND	0/2	0/1	0/3	0/12	0
CD2	0/8	0/9	1/1	0/3	0/3	1/6	2/30	7
CD3	0/9	0/8	0/1	0/3	0/4	0/6	0/31	0
CD4	0/9	0/5	0/1	0/3	0/3	2/6	2/27	7
CD5	2/9	0/7	0/1	0/3	0/4	1/6	3/30	10
CD7	9/9	9/9	1/1	3/3	4/4	6/6	32/32	100
CD8	0/9	0/5	0/1	0/3	0/3	0/5	0/26	0
CD10	2/9	0/8	1/1	0/3	0/4	1/6	4/31	13
CD11b	1/7	0/2	1/1	1/2	1/2	4/4	8/18	44
CD13	7/9	9/9	1/1	2/3	4/4	3/6	26/32	81
CD14	0/9	0/8	1/1	1/3	0/4	1/6	3/31	10
CD15	1/5	1/3	0/1	1/2	1/2	0/4	4/17	24
CD16	0/1	0/4	ND	0/1	ND	0/5	0/11	0
CD19	0/9	0/9	0/1	0/3	0/4	0/6	0/32	0
CD20	0/9	0/9	0/1	0/3	0/4	0/6	0/32	0
CD25	0/6	0/2	0/1	0/2	0/3	0/4	0/18	0
CD33	8/9	9/9	1/1	3/3	4/4	6/6	31/32	97
CD34	9/9	7/7	1/1	2/3	4/4	4/6	27/30	90
CD41	0/7	0/4	0/1	0/3	0/2	6/6	6/17	35
CD56	9/9	9/9	1/1	3/3	4/4	6/6	32/32	100
CD57	0/3	0/2	ND	ND	ND	0/3	0/8	0
CD117	2/2	1/1	ND	ND	1/1	1/2	5/6	83
HLA-DR	7/9	8/8	1/1	2/3	4/4	4/6	26/31	84
TdT	1/3	ND	ND	ND	ND	0/2	1/5	20

ND not determined

Table 4 Therapy and response

	CD7$^+$ CD56$^+$ AML (M1–M7)	CD7$^+$ CD56$^+$ AML M0
CR rate		
AML chemotherapy	20/29 (68%)	7/9 (78%)
NHL chemotherapy	0/2[a] (0%)	0/5 (0%)
P value	0.12	0.02

CR complete remission, *AML* acute myeloid leukemia, *NHL* non-Hodgkin's lymphoma

[a] Both patients presented with extramedullary myeloid leukemia

The overall survival (OS) and disease-free survival (DFS) curves are shown in Fig. 1a. The prognosis of the CD7$^+$ CD56$^+$ M1–M7 patients was also poor, and no statistical difference was found from that of the CD7$^+$ CD56$^+$ M0 (myeloid/NK cell precursor acute leukemia) group.

4 Discussion

In this study, we demonstrated that CD7$^+$ CD56$^+$ AML M1–M7 does not show extramedullary leukemic involvement, which is a typical manifestation of myeloid/NK cell precursor acute leukemia, but does have a poor prognosis. The reason for the peculiar clinical manifestation of myeloid/NK cell precursor acute leukemia remains unclear, but our current results suggest that it is not caused by the expression of two key molecules, CD7 and CD56.

The low incidence of extramedullary involvement in our CD7$^+$ CD56$^+$ AML M1–M7 cases is consistent with the findings of previous large-scale studies that investigated CD56 expression in AML [20, 21]. We could not identify any specific features for the CD7$^+$ CD56$^+$ AML M1–M7 group except for a preference for FAB M7 (6 of 32 cases). The association of CD56 expression and megakaryoblastic leukemia has been documented in a study with a small number of the cases [22], but was not examined in a recent, larger study [23]. Although several similarities exist between AML M0 and M7, such as male predominance, negativity for the cytochemical MPO reaction, myeloid antigen expression, and poor prognosis [23], other clinical characteristics were different between the AML M0 and M7 CD7$^+$ CD56$^+$ phenotypes. This is particularly important for the correct diagnosis of myeloid/NK cell precursor acute leukemia. In the CD7$^+$ CD56$^+$ M1–M7 group, we

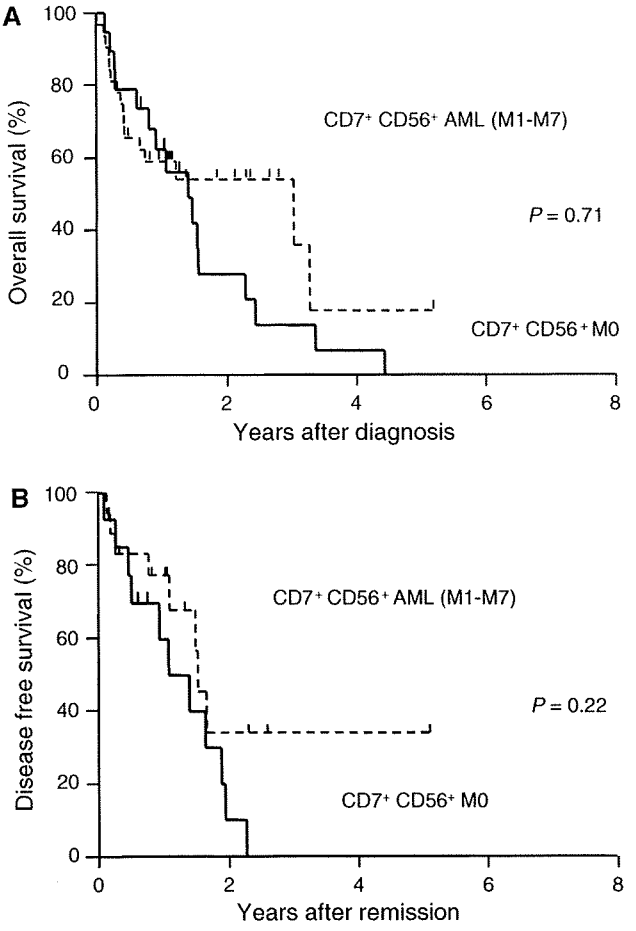

Fig. 1 Overall survival (**a**) and disease-free survival (**b**) curves of $CD7^+$ $CD56^+$ AML patients. *Thick lines* indicate survival curves of $CD7^+$ $CD56^+$ M0 and *broken lines* indicate those of $CD7^+$ $CD56^+$ AML M1–M7. No statistical differences were found between the two groups

identified one case with AML M3. This case showed the t(15;17) karyotype and responded to therapy with all-*trans* retinoic acid, indicating that the patient did not have myeloid/NK cell acute leukemia [24, 25] but typical M3.

The reason for the difference in extramedullary involvement between myeloid/NK cell precursor acute leukemia and $CD7^+$ $CD56^+$ AML M1–M7 remains unclear. Because CD56 was expressed in every case by definition, the extramedullary tumorigenesis does not directly derive from the hemophilic adhesion by CD56. Other adhesion molecules or chemokine/chemokine receptor might be responsible for this difference, which needs further investigations. Another hypothesis is that differentiation status of these leukemias is different. Since the origin of myeloid/NK cell precursor acute leukemia has been speculated as myeloid antigen-positive T/NK bi-potential progenitor [12, 13], the leukemic cell may retain affinity to lymph node or mediastinum.

The appropriate therapeutic approach for $CD7^+$ $CD56^+$ M1–M7 patients remains unknown. Expression of CD56 has been documented in various types of AML [20, 21], including specific subtypes, i.e., AML M2 with t(8;21) [26], AML M3 [27–29]. It is currently accepted as a marker of poor prognosis in AML [30–32]. Furthermore, the prognosis for NK cell malignancies, which are generally positive for CD56, is mostly poor [33–35], as is that for anaplastic large cell lymphoma [36], but not for those of peripheral T cell lymphoma, unspecified [37] or diffuse large B cell lymphoma. In this context, CD56 does not seem to cause the poor prognosis, but is rather a surrogate marker of poor prognosis. Hematopoietic stem cell transplantation, which was performed in several of our cases, is a treatment option [38], but this approach needs to be examined further in prospective studies. New agents such as CD56 monoclonal antibody conjugated with toxin or radio isotope are also good candidates [39, 40].

In summary, we found that $CD7^+$ $CD56^+$ M1–M7 shows a low incidence of extramedullary involvement, which is different from $CD7^+$ $CD56^+$ M0 or myeloid/NK cell precursor acute leukemia, but it still has a poor prognosis.

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Appendix

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review

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Epstein–Barr virus-associated lymphoproliferative disease in non-immunocompromised hosts: a status report and summary of an international meeting, 8–9 September 2008

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Background: Recently novel Epstein–Barr virus (EBV) lymphoproliferative diseases (LPDs) have been identified in non-immunocompromised hosts, both in Asia and Western countries. These include aggressive T-cell and NK-cell LPDs often subsumed under the heading of chronic active Epstein–Barr virus (CAEBV) infection and EBV-driven B-cell LPDs mainly affecting the elderly.

Design: To better define the pathogenesis, classification, and treatment of these disorders, participants from Asia, The Americas, Europe, and Australia presented clinical and experimental data at an international meeting.

Results: The term systemic EBV-positive T-cell LPD, as adopted by the WHO classification, is preferred as a pathological classification over CAEBV (the favored clinical term) for those cases that are clonal. The disease has an aggressive clinical course, but may arise in the background of CAEBV. Hydroa vacciniforme (HV) and HV-like lymphoma represent a spectrum of clonal EBV-positive T-cell LPDs, which have a more protracted clinical course; spontaneous regression may occur in adult life. Severe mosquito bite allergy is a related syndrome usually of NK cell origin. Immune senescence in the elderly is associated with both reactive and neoplastic EBV-driven LPDs, including EBV-positive diffuse large B-cell lymphomas.

Conclusion: The participants proposed an international consortium to facilitate further clinical and biological studies of novel EBV-driven LPDs.

Key words: chronic active EBV infection, diffuse large B-cell lymphoma, hemophagocytic syndrome, hydroa vacciniforme, immune senescence, senile EBV-positive lymphoproliferative disease, systemic EBV-positive lymphoproliferative disease

introduction

Over 90% of humans are infected with the Epstein–Barr virus (EBV) and the infection persists for life. Most persons have a chronic asymptomatic infection with EBV, but the virus has been associated with a number of malignancies and can infect B cells, T cells, NK cells, and epithelial cells. Patients with iatrogenic, congenital, or acquired immunodeficiency are at increased risk for EBV-associated lymphomas, which are in nearly all instances of B-cell lineage.

Chronic active Epstein–Barr virus (CAEBV) disease has been defined as a systemic EBV-positive lymphoproliferative disease (LPD) characterized by fever, lymphadenopathy, and splenomegaly developing after primary virus infection in patients without known immunodeficiency [1]. Affected patients have high levels of EBV DNA in the blood, histological evidence of organ disease, and elevated levels of EBV RNA or viral proteins in affected tissues. While initially proposed as a progressive EBV infection of B cells as the primary target, the term as used in the recent literature refers to an aggressive EBV-positive T-cell, NK cell, or B-cell LPD, mainly affecting persons of Asian origin [2].

EBV-associated hemophagocytic syndrome (HPS), which can appear with CAEBV, or as a complication of other EBV-associated LPD, is due to excessive macrophage activation and hemophagocytosis [3, 4]. Patients present with fever, lymphadenopathy, pancytopenia, and hepatosplenomegaly, and have marked elevation of cytokines including tumor necrosis factor-α (TNF-α) and interferon (IFN)-γ [5, 6]. The disease is often fatal, despite therapy directed at the virus-infected T cells.

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In addition to corticosteroids or cyclosporine to inhibit cytokine production, bone marrow or hematopoietic stem-cell transplant may be effective in some cases.

In recent years, it has been appreciated that otherwise healthy adults of advanced age are at risk for EBV-associated B-cell lymphomas, initially reported as 'senile EBV-associated B-cell LPD' [7]. Clinical series have identified a high risk of treatment failure [8, 9]. This process was incorporated in the fourth edition of the World Health Organization (WHO) classification of lymphomas as EBV-positive diffuse large B-cell lymphoma (DLBCL) of the elderly [10].

To better define the pathogenesis, classification, and treatment of EBV LPDs in non-immunocompromised hosts, an international meeting was organized at the National Institutes of Health in Bethesda, MD, on 8 and 9 September 2008. Virologists, immunologists, pathologists, infectious disease specialists, hematologists, and oncologists presented topics on a wide variety of EBV-associated diseases and discussed the pathogenesis, classification, and treatment of these diseases. This report presents highlights from that meeting and a consensus document regarding the classification of these diseases (Table 1).

biology of EBV and EBV-associated B-cell LPD

A variety of EBV viral proteins are expressed in EBV-associated LPD, and these influence the nature and effectiveness of the immune response and the potential risk for lymphomagenesis. As reviewed by E. Kieff (Boston), EBV nuclear antigen-1 (EBNA-1) maintains the EBV episome in these cells when the cells divide. EBNA-2 interacts with a variety of host cell proteins, including RBP-JK, p300/CBP, and p100 to upregulate expression of viral and cellular genes including c-myc, CD21, and CD23. EBNA-3A, B, and C also upregulate expression of cellular genes. EBV nuclear antigen-leader protein activates transcription in concert with EBNA-2 by reducing levels of histone deacetylase 4 in the nucleus. Latent membrane protein 1 (LMP1) is a functional homolog of CD40 and upregulates expression of

Table 1. EBV-associated lymphoproliferative diseases in non-immunocompromised hosts: clinicopathological and biological features

Disease	Lineage clonality	Primary age group	Epidemiological features	Clinical features	Related entities and comments
CAEBV B-cell type	B cells, polyclonal/ monoclonal	Children, young adults	More common in Western countries, very rare	Fever, systemic symptoms with organ involvement e.g. pneumonitis, uveitis, hepatitis; splenomegaly, adenopathy, hypogammaglobulinemia	Chronic/persistent infectious mononucleosis with organ involvement
EBV+ large B-cell lymphoma of the elderly (senile EBV LPD)	B cells, monoclonal	Adults, >60 years	No ethnic or geographic predilection	Usually extranodal: skin, GI tract, lung; aggressive clinical course	EBV-positive lymphoid hyperplasia in the elderly
Lymphomatoid granulomatosis	B cells, oligoclonal/ monoclonal	Adults, median ~ 40 years	More common in Western countries	Extranodal: predominantly lung, also kidney, liver, CNS, skin	May also occur with immunodeficiency disorders
CAEBV T-cell/NK cell types (an umbrella term that encompasses specific diseases below)	T cells, NK cells, monoclonal > oligo/polyclonal	Children/less often young adults	Asians: mainly Japan, Taiwan, Korea; Native Americans in Mexico, Central, South America	Fever, hepatosplenomegaly, thrombocytopenia, lymphadenopathy; also HV, severe mosquito bite allergy, systemic EBV-positive T-cell LPD of childhood	T-cell disease has poorer long-term prognosis than NK cell disease
HV	T cells, oligoclonal/ polyclonal	Children/less often young adults	As above for T/NK cell CAEBV	Papulovesicular rash with ulceration; sun-exposed or unexposed areas of skin; may regress in adulthood or progress to systemic disease with hepatic failure and lymphoma	Some cases of severe HV may be monoclonal and overlap with HV-like lymphoma
HV-like lymphoma	Monoclonal				
Severe mosquito bite allergy	NK cells, clonality undetermined	Children/less often young adults	As above for T/NK cell CAEBV	Hypersensitivity to mosquito bites with ulcers and necrosis, high IgE	More indolent than HV
Systemic EBV+ T-cell LPD of childhood	T cells, monoclonal	Children/less often young adults	As above for T/NK cell CAEBV	Fever, lymphadenopathy, hepatosplenomegaly, HPS, DIC, hepatic failure; aggressive course	Severe CAEBV—75% of cases of systemic CAEBV are clonal and overlap with systemic EBV+ T LPD of childhood

EBV, Epstein–Barr virus; CAEBV, chronic active Epstein–Barr virus infection; LPD, lymphoproliferative disease; GI, gastrointestinal; CNS, central nervous system; NK, natural killer; HPS, hemophagocytic syndrome; DIC, disseminated intravascular coagulation; HV, hydroa vacciniforme.

a wide variety of cellular genes including nuclear factor-κB (NF-κB), c-Jun, AP1, AP2, and p38.

the host immune response to EBV—insights into effective immunosurveillance

As discussed by R. Khanna (Brisbane), both the quantity and quality of the CD8+ T-cell response to EBV are critical to control infection. CD8+ T cells isolated from healthy seropositive individuals or from individuals who rapidly recover from infectious mononucleosis recognize a wide variety of EBV epitopes [11]. In contrast, CD8+ T cells from persons with persistent infectious mononucleosis recognize only a few EBV epitopes (Figure 1). The diversity of the T-cell repertoire (as defined by Vβ T-cell receptors) is expanded in persons infected with EBV who are asymptomatic, while the repertoire is much narrower in persons with infectious mononucleosis [12]. A similar reduction in diversity of the T-cell receptor repertoire was seen in some symptomatic transplant recipients, in contrast to a broader diversity observed in asymptomatic transplant recipients. No correlation was noted between the level of the EBV DNA load in the blood in transplant recipients and the diversity of the T-cell receptor repertoire.

the cytokine/chemokine response to EBV—insights into EBV-related syndromes

A variety of potent cytokines and chemokines are induced by EBV infection of both B and T lymphocytes. These mediators are produced by the EBV-infected lymphoid cells directly, as well as bystander immune populations. As discussed by G. Tosato (Bethesda), vIL-10 (BCRF1) is structurally homologous to hIL-10 and is a paradigm for viral piracy of a cellular gene [13]. Viral IL-10 stimulates B-cell growth, inhibits antigen presentation and T-cell growth, protects T cells from death, and suppresses IFN-γ secretion. EBV also promotes the expression of cellular cytokines in infected cells, including IL-6, IL-10, and EBI3. These lead to autocrine and paracrine stimulation of EBV-infected B-cell growth and likely promote the development of B-cell lymphomas.

EBV also stimulates secretion of a variety of cytokines and chemokines by noninfected cells, and these have an impact on the clinical features of acute and CAEBV infection. The LMP1-induced chemokines interferon-gamma-inducible protein-10 and the monokine induced by interferon-gamma mediate vascular damage, resulting in tissue necrosis [14]. High levels of IFN-γ, soluble IL-2 receptor, TNF-α, and MIP-1α have been implicated in the pathogenesis of EBV-associated HPS, associated with CAEBV and T-cell and NK-cell lymphomas [5, 6, 15, 16].

acute and chronic EBV syndromes of B cells

CAEBV infection was first identified by Straus [1] as a disease related to chronic or persistent EBV infection of B cells. It was defined as a severe illness greater than 6-month duration that (i) begins as a primary EBV infection or is associated with markedly abnormal EBV antibody titers (e.g. anti-EBV viral capsid antigen IgG ≥ 5120, anti-EBV early-antigen IgG ≥ 640, or anti-EBNA < 2); (ii) histological evidence of major organ

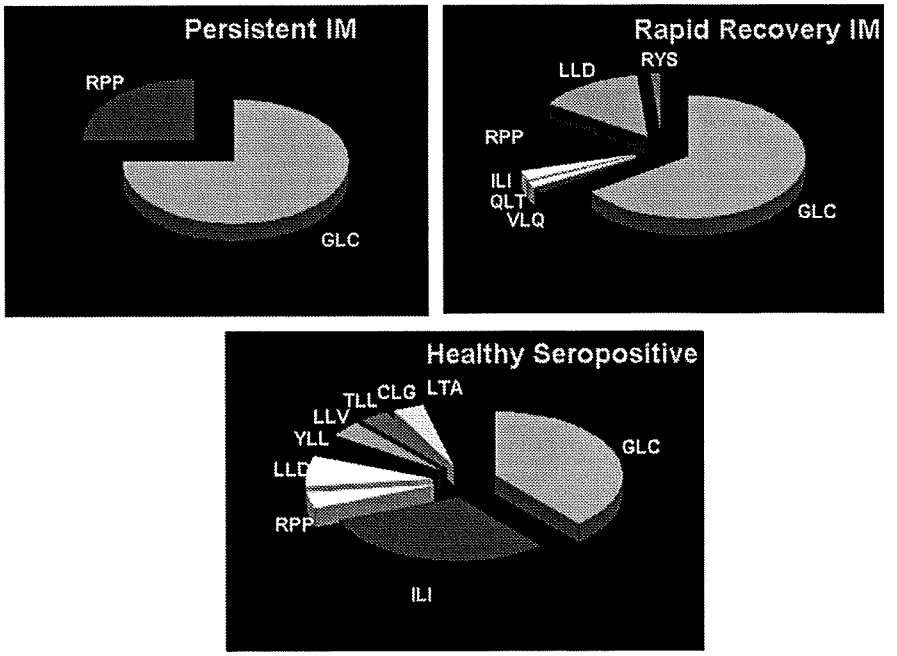

Figure 1. A healthy Epstein–Barr virus (EBV) seropositive individual and a patient rapidly recovering from infectious mononucleosis have diverse CD8+ T cells that recognize a variety of EBV peptides, while a patient with persistent infectious mononucleosis has a limited diversity of CD8+ T cells that recognize few viral peptides. Labels on pie charts indicate individual EBV peptides recognized by CD8+ T cells [11].

involvement such as interstitial pneumonia, hypoplasia of the bone marrow, uveitis, lymphadenitis, persistent hepatitis, or splenomegaly; and (iii) increased EBV RNA or proteins in affected tissues. Kimura et al. [2] revised the diagnostic criteria of CAEBV, so that patients could have either increased EBV RNA or proteins in infected tissues or increased levels of EBV in the peripheral blood, in addition to the other criteria. As reviewed by JIC (Bethesda), patients who have CAEBV of B cells usually develop a progressive cellular and humoral immunodeficiency and other complications including HPS and B-cell LPD. The etiology of B-cell CAEBV remains uncertain, although mutations in perforin were implicated in one patient, whose T cells were impaired for killing of Fas-deficient target cells [17].

adult—late-onset EBV-associated B-cell LPD

In recent years, it has been appreciated that defective immune surveillance for EBV may develop late in life and be associated with the development of EBV-positive B-cell LPD in individuals who otherwise have no apparent immune deficiency. As reviewed by SN (Nagoya), aggressive EBV-positive B-cell lymphomas that occur in older individuals are often extranodal, frequently involving the skin, gastrointestinal tract, or lung [7, 18, 19]. Termed EBV-positive DLBCL of the elderly in the 2008 WHO classification, the disease is characterized by proliferation of atypical large B cells including immunoblasts and Reed–Sternberg-like cells (Figure 2). Some cases have a more varied cytological composition and resemble the EBV-positive B-cell lymphomas that occur in iatrogenically immunosuppressed patients. Necrosis is prominent. Patients with EBV-positive DLBCL have a worse prognosis than patients with EBV-negative DLBCL or EBV-positive classical Hodgkin's lymphoma (CHL) [9, 20]. Most cases occur after the age of 60 with a median age of 70–79 years, and the incidence continues to increase with age.

ESJ (Bethesda) described the spectrum of EBV-associated B-cell LPD observed in a Western population without known immunodeficiency [21]. As with the reports from Japan, most patients are of advanced age, generally >60 years; 116 cases were identified over a 7-year period and fell into five diagnostic categories: (i) lymph node-based reactive hyperplasia with increased EBV-positive B cells, (ii) EBV-positive nodal B-cell lymphoproliferations resembling post-transplant LPD (PTLD), (iii) EBV-positive extranodal B-cell lymphoproliferations resembling PTLD, (iv) EBV-positive diffuse DLBCLs, and (v) EBV-positive B-cell proliferations resembling CHL.

Twenty-eight patients had EBV-associated reactive lymphoid hyperplasia, with a median age of 67 years. The process was self-limited in most patients, with only one patient showing progression to a more aggressive lymphoproliferative process. All cases tested were polyclonal by IgH PCR. T-cell clonality or a restricted T-cell receptor gene rearrangement pattern was seen in three (11%) of the cases studied. This finding suggests a reduction in diversity of the T-cell receptor repertoire, as discussed by Khanna [11]. Features included preserved architecture and a broad spectrum in cell size of the

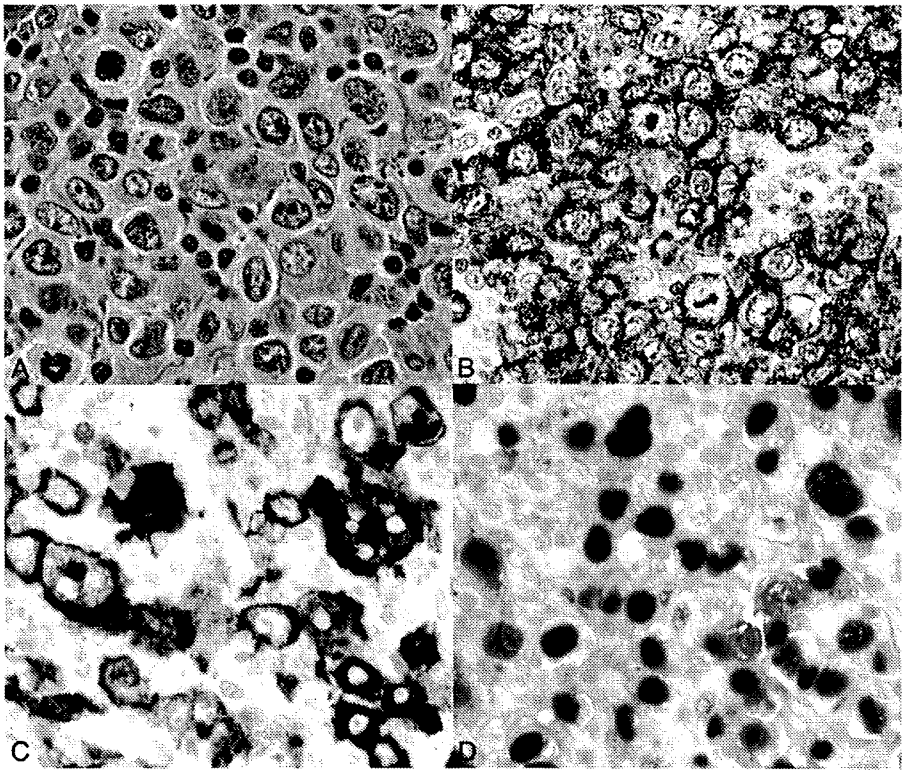

Figure 2. Epstein–Barr virus (EBV)-positive large B-cell lymphoma of the elderly. (A) Large lymphoid cells predominate and in (B) express CD20. (C) Some cells have more pleomorphic features, and CD30 is often positive. (D) EBV encoded RNA (EBER) highlights atypical cells.

EBV-positive cells with frequent localization to germinal centers. Kojima et al. [22] reported similar EBV-associated reactive hyperplasias in middle-aged or elderly patients.

The median age was highest in patients with EBV-positive DLBCL (77 years), which included 11 nodal DLBCL and four nodal or extranodal plasmablastic lymphomas. There were 73 polymorphic B-cell lymphomas approximately equally divided between nodal and extranodal sites. Median ages were 73 and 76 years, respectively. Seven cases, median age 79, histologically and phenotypically resembled CHL (CD30+; CD15+) but presented in sites unusual for CHL such as the oral cavity (palate, gingiva, tongue, lips) and adrenal glands. Supporting the concept that a restricted T-cell response to EBV may be associated with defective immune response, ~20% of patients with either polymorphic B-cell lymphoma or DLBCL had evidence of a restricted clonal or oligoclonal T-cell response.

Lymphomatoid granulomatosis (LYG) is an EBV-related B-cell LPD that can affect patients with known immunodeficiency, but also occurs in adults without any known predisposing risk factors [23]. As reviewed by K. Dunleavy (Bethesda) lung involvement is nearly constant (98% of patients); ~30% of patients have lesions in the kidneys, liver, skin, or central nervous system [24]. The number of EBV-infected B cells is relatively low, in proportion to the number of T cells identified within the lesions. However, there is evidence of defective immune surveillance, as the mean CD4 and CD8 T-cell counts are below normal in most patients at diagnosis. The EBV viral load in the blood is usually not elevated. A clinical trial at the National Cancer Institute involving 40 patients used dose-adjusted IFN-α for patients with grade 1 or 2 LYG, and dose-adjusted EPOCH with rituximab for patients with grade 3 disease [25]. Twenty-seven percent of the patients were previously untreated. Progression-free survival for patients receiving IFN-α was 56% with median follow-up of 5.1 years; nine patients progressed to grade 3 disease [26]. The overall complete response (the disappearance of all signs of cancer in response to treatment) rate with dose-adjusted chemotherapy was 68%. The overall survival in patients with grades 1–3 disease was 69% with median follow-up of 4.3 years. These preliminary results represent an improvement over older series; however, due to the rarity of this condition, most reports are limited to few patients [24, 27, 28].

The spectrum of EBV-associated B-cell lymphomas and LPDs is extremely broad and includes acute infectious mononucleosis, benign reactivation as may be seen in the elderly; CAEBV infection involving B cells, LYG, post-transplant and other iatrogenically associated LPDs (e.g. methotrexate-associated LPD); pyothorax-associated lymphoma (now defined as EBV-positive DLBCL associated with chronic inflammation) [10]; EBV-positive DLBCL of the elderly; Burkitt lymphoma (EBV more often associated with endemic than sporadic); plasmablastic lymphomas (most cases associated with EBV), and CHL (EBV mainly in mixed cellularity and lymphocyte depletion subtypes). As discussed by the participants, the clinical syndrome and pathology are influenced by the virus and viral genes and the host, including both intrinsic and iatrogenic factors [10].

acute and chronic EBV syndromes of T cells and NK cells

While CAEBV was first described as a persistent EBV infection targeting B cells, over the years the syndrome has been primarily associated with EBV infection of T cells and less often NK cells [2]. The minimal diagnostic criteria for CAEBV are summarized above, all of which must be met. It has a strong racial predisposition, with most cases occurring in Japan and Korea and some cases in Native American populations in the Western Hemisphere from Mexico, Peru, and Central America. It is rare in Caucasians and African-Americans. The term T/NK cell CAEBV has been used in the literature to encompass a very broad spectrum of diseases, including a systemic form which may be polyclonal, fulminant and systemic EBV-positive T-cell LPDs that are clonal, hydroa vacciniforme (HV) of T-cell derivation, and severe mosquito bite allergy (usually of NK cell origin). The 2008 WHO classification has recognized the following disease entities that are considered neoplasms: systemic EBV-positive T-cell LPD of childhood (a clonal T-cell LPD) and HV-like T-cell lymphoma [10].

HK (Nagoya) carried out a nation-wide survey of T/NK–CAEBV in Japan in 2001 and identified 82 cases (42 males and 40 females). The mean age of onset of the disease was 11.3 years with a range of 9 months to 53 years and all patients had elevated levels of EBV DNA in the blood [29, 30]. The majority of patients had evidence of systemic disease, presenting with fever (93% of patients), hepatomegaly (79%), splenomegaly (73%), thrombocytopenia (45%), anemia (44%), and lymphadenopathy (40%). Cutaneous manifestations were common and included hypersensitivity to mosquito bites (33%), skin rash (26%), and HV (10%). Patients with only cutaneous disease had a better prognosis, although the criteria to distinguish HV, which may be clonal, from HV-like T-cell lymphoma are not well delineated [31, 32]. It has been controversial as to whether CAEBV is a type of T-cell or NK cell malignancy or a progressive infectious disease; of the patients in whom clonality could be analyzed, the proliferation was monoclonal in 76%, oligoclonal in 13%, and polyclonal in 11% [30]. The EBV-infected cells were shown to be T cells in 46% of patients, NK cells in 33%, T/NK cells in 4%, B cells in 2%, and unclassified or not studied in the remaining 15%.

Patients with T-cell CAEBV often presented with high fever, lymphadenopathy, hepatosplenomegaly, high titer of EBV-specific antibodies, and had rapid progression of their disease. Patients with NK cell disease, in contrast, often had hypersensitivity to mosquito bites, rash, high levels of IgE, and did not necessarily have elevated EBV-specific antibody titers. The 5-year survival rate of patients with T-cell CAEBV was 59%, while that for NK cell disease was 87% [30]. However, uncomplicated HV (of clonal T-cell derivation) had a better prognosis. Life-threatening complications of T/NK cell CAEBV included HPS (24% of patients), disseminated intravascular coagulation (16%), hepatic failure (15%), peptic ulcer disease/perforation (11%), coronary artery aneurysms (9%), central nervous system complications (9%), myocarditis (6%), and interstitial pneumonitis (5%).

The pathogenesis of T-cell and NK cell CAEBV is uncertain. EBV-positive T/NK cells have been identified in the tonsils

and peripheral blood from patients with infectious mononucleosis [33], and the virus has been shown to infect NK cells *in vitro* [34]. NK cells can acquire the EBV receptor, CD21, by synaptic transfer from B cells [35], allowing EBV binding to NK cells. T and NK cells from patients with CAEBV often have latency 2 phenotype with expression of EBV EBNA-1, LMP1, and LMP2 [36]. There is evidence that defective T-cell and NK cell responses to EBV may play a role in the pathogenesis [29, 37].

While a number of therapies have been tried for CAEBV including antiviral agents (acyclovir, ganciclovir), immunomodulators (IFN-α, IL-2), chemotherapy (etoposide, corticosteroids), cyclosporine, and EBV-specific cytotoxic T cells (CTLs), recently more promising results have been obtained with hematopoietic cell transplantation [38, 39]. Since the first report of successful allogeneic bone marrow transplantation for the disease [40], many successful cases have been reported using related or unrelated bone marrow transplants, with myeloablative or nonmyeloablative transplantation or with cord blood transplantation [41]. Hematopoietic stem-cell transplantation can eliminate EBV-infected cells, reconstitute EBV-specific cellular immunity, and have a graft-versus-tumor effect. However, the procedure carries a high risk of transplantation-related complications and the 5-year survival rate was only 53% (Japanese Association for Research on EBV Study Group, unpublished data). Poor prognostic factors that argue for early intervention and transplant are (i) age at onset >8 years, (ii) platelet count <120 000/μl, and (iii) T-cell- rather than NK cell-associated disease [30]. Patients with HV have a better prognosis and may be followed conservatively, if there are no systemic symptoms. Measurement of the viral load after transplantation was helpful in determining the response to transplantation.

K. Oshima (Kurume) presented a proposed categorization of CAEBV from the CAEBV study group [42]. They divided cases into four categories: A1 (polymorphic and polyclonal), A2 (polymorphic and generally monoclonal), A3 (monomorphic and monoclonal proliferation of T-cell or NK cell origin, and B (monomorphic and monoclonal T-cell LPD with fulminant clinical course). The clinical course in groups A1–A3 was generally protracted with most patients surviving for several years. Group B was defined as equivalent to fulminant EBV-positive LPD of childhood [43]; patients were under the age of five, had a fulminant clinical course that emerged soon after EBV infection, and morphology and phenotype that overlapped with group A3. Patients with the clinical syndromes of mosquito bite allergy and HV were distributed in groups A2 and A3. All patients had very high viral loads at presentation. Anti-EBV antibody titers were highest in A1 (VCA IgG 2560) and lowest in B (VCA IgG 160). Interestingly, antibody titers to EBV also were reported to be low in fulminant EBV-positive LPD of childhood [43] (now designated 'systemic EBV-positive T-cell LPD of childhood' in the WHO classification of 2008 [10]). It will be of interest to apply this classification system prospectively to CAEBV cases to determine its applicability as a diagnostic and prognostic system.

The perspective of EBV-related T-cell and NK cell disease in Korea was presented by Y-HK (Seoul) [44]. Cases of systemic EBV-positive T-cell LPD and related entities were compared with more well-defined diseases such as extranodal NK/T-cell lymphoma and aggressive NK cell leukemia. Systemic EBV-positive T-cell LPD patients were mainly children and young adults and presented with acute illness with a fulminant clinical course, similar to aggressive NK cell leukemia, with death in a matter of weeks. These cases were comparable in behavior to those reported in the literature as 'fatal infectious mononucleosis' with HPS [45]. There was a subset of children and young adults with CAEBV and a somewhat more protracted clinical course. Some of these patients had cutaneous manifestations, such as HV, but the median survival was still <1 year. Y-HK also identified a subset of patients presenting in adult life, who were often coinfected with hepatitis B or C virus, leading to reactivation of EBV [46].

The perspective of systemic T-cell LPD of childhood (CAEBV) in the Western hemisphere was presented by L. Quintanilla-Martinez (Tübingen). There is evidence of a strong racial predisposition, as nearly all patients were of Native American ethnic origin from Mexico or Central America [43]. Previously healthy patients presented with acute onset of fever suggestive of an acute viral respiratory illness. Within a period of weeks patients developed hepatosplenomegaly and liver failure, sometimes accompanied by lymphadenopathy. Laboratory tests showed pancytopenia, abnormal liver function tests, and often an abnormal EBV serology with low or absent anti-VCA IgM antibodies. The disease was usually complicated by HPS, coagulopathy, multiorgan failure, and sepsis [43]. The clinical course was aggressive, with a median survival of <1 year. The value of morphological subtyping was felt to be questionable, as in most cases the EBV-positive T cells lacked cytological atypia. The immunophenotype was of cytotoxic T-cell origin, CD8 > CD4. All cases studied were monoclonal for *TCR* gamma genes, and on this basis as well as the poor clinical outcome, the process has been considered to represent a form of mature T-cell malignancy in the 2008 WHO classification [10].

X-linked lymphoproliferative disease (XLPD), which is caused by mutations in the *SAP* gene, and CAEBV share many clinical features in common. Acute infection usually results in a fulminant disease with infiltration of multiple organs by EBV-infected B cells and activated T cells with HPS and tissue necrosis. Survivors often have hypogammaglobulemia and may develop B-cell lymphomas. Other complications include aplastic anemia, necrotizing vasculitis, or LYG. Based on data derived from a *SAP* knockout mouse model, J. Sullivan (Worcester) suggested that patients with XLPD may have defective apoptosis of CD8 T cells that predisposes them to the HPS and fatal EBV infections [47].

The clinical spectrum of HV in Asia and the Western hemisphere was presented by X. Zhou (Beijing) and C. Barrionuevo (Lima). The median age of patients from China was 7 years (range 3–15 years), with an increased male-to-female ratio. All the patients presented with a papulovesicular rash, with ulceration and crusting, primarily affecting sun-exposed areas of the skin. Twenty-five percent (4 of 16) of the

patients also reported hypersensitivity to mosquito bites. EBV-positive cells were abundant in the lesions during periods of active disease (spring, summer); lesions often regressed during the autumn and winter. Most of the HV patients also had evidence of systemic disease. About 80% (13 of 16) of patients presented with high fever 38°C–40°C and 38% of patients had hepatosplenomegaly and/or lymphadenopathy. Follow-up data (mean 22 months; range 4–46 months) was available for 44% (7 of 16) of cases. Two patients died of liver or multiple organ failure, and five were still alive with a stable or smoldering disease.

C. Barrionuevo (Lima) presented cases with HV-like lesions from Peru, which were categorized as HV-like T-cell lymphoma in their series based on infiltrative growth pattern, often aberrant T-cell phenotype, clonal rearrangement of TCR genes, and poor clinical outcome [48]. The clinical and pathological features are very similar to those observed in Japan and Korea (Figure 3). The mean age of patients in Peru was 11 years (range 5–17 years). Lesions most commonly involved sun-exposed areas (face and upper limbs). Lesions often showed edema, papules, blisters, crusts, ulcers, and healed as vacciniforme scars. Some patients had hypersensitivity to insect bites. Systemic symptoms were common and lymphadenopathy was present in 30% of cases and hepatosplenomegaly in 10%. Less frequent were intercurrent infections, HPS, or visceral involvement. The 2-year survival rate was 43%. Patients receiving chemotherapy or chemotherapy and radiation therapy had partial response (a decrease in the size of a tumor, or in the extent of cancer in the body in response to treatment) rates of 30%. Deaths were due to sepsis, liver failure, malignancy, or HPS.

The criteria for the distinction of HV from HV-like T-cell lymphoma have not been clearly delineated in the literature. Based on the published experience and reports presented at the meeting, EBV and T-cell clonality were found in both types of cases. Some patients with HV have eventual resolution of their disease in adult life, whereas other patients develop progressive disease with worsening of cutaneous symptoms and eventual systemic dissemination [31, 32, 48, 49]. In addition, some patients with HV-like symptomatology have severe CAEBV early in the course of the disease. Cases of HV lacking clonal rearrangement of TCR genes appear to have a more benign clinical course [50]. The entity of HV-like lymphoma as included in the WHO classification stipulates an EBV-positive clonal proliferation [51]. However, it is not clear that T-cell clonality is always predictive of a progressive clinical course, as discussed by HK. A related issue is severe mosquito bite allergy, which is usually of NK cell derivation, but shows overlap with HV [52, 53]. Both HV and severe mosquito bite allergy are considered part of the spectrum of CAEBV, with a broad spectrum of clinical aggressiveness.

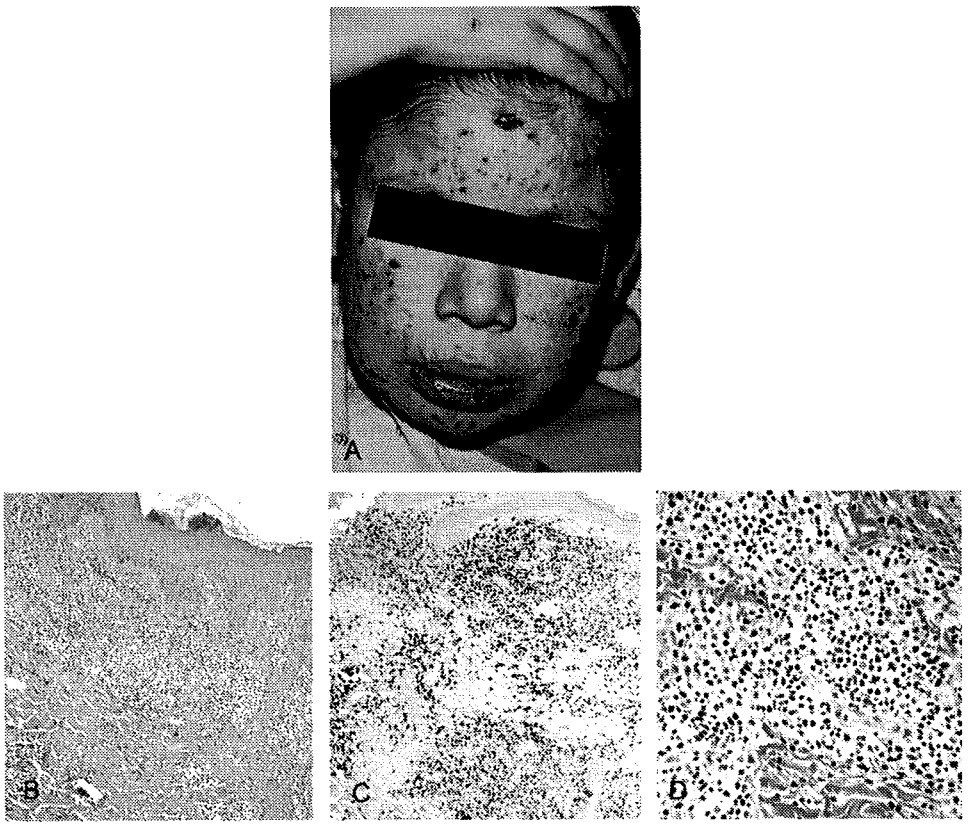

Figure 3. Hydroa vacciniforme-like lymphoma. (A) Sun-exposed areas of the skin exhibit a papulovesicular eruption, with ulceration and crusting. (B) The infiltrate is present in the superficial dermis. (C) Lymphoid cells are positive with EBER *in situ* hybridization. (D) Lymphoid cells are small to medium in size; clonal TCR gene rearrangement was shown by PCR studies.

novel therapeutic approaches for EBV-related LPD

C. Rooney (Houston) discussed EBV-specific T-cell therapy for patients with chronic and persistent EBV infection. In Western populations (e.g. United States, Europe) these patients usually have expansion of EBV-infected B cells, i.e. the B-cell type of CAEBV. Historical therapeutic approaches, before the use of bone marrow or stem-cell transplantation, have included high-dose immunoglobulin, IL-2, antiviral agents, IFN-α or IFN-γ, corticosteroids, and rituximab. Other than isolated case reports, these therapies generally have not been successful and relapses were common. More recently autologous EBV-specific T cells have been used in the therapy of persistent active EBV infection [54]. This therapy also has been successful in the post-transplant setting [55]. Incubation of the patient's peripheral blood mononuclear cells with their irradiated EBV-transformed B cells results in activation of EBV-specific T cells in all seropositive donors, including both healthy persons and those with EBV-associated LPDs. In some cases, however, generation of EBV-transformed B cells may be difficult or impossible if the patient received ritxumab within the prior 6 months or high doses of chemotherapy. The induced activated EBV-specific T cells recognize EBV EBNA-3A, 3B, and 3C and early-lytic antigens to a greater extent than T cells that recognize LMP1, LMP2, or EBNA-1.

A phase I study was reported using autologous EBV-specific T cells in patients with mild CAEBV defined as >6 months of symptoms (most often fever and fatigue) and either elevated peripheral blood EBV load or free EBV DNA in serum/cerebrospinal fluid or EBV VCA antibody titer >1 : 640 [54]. The study showed improvement or resolution of symptoms and the follow-up on these patients is now 2–6 years. Many of these patients have had normalization or reduction of their EBV VCA antibody titers, a decrease in the EBV DNA load, and an increase in circulating EBV-specific CTLs after therapy. Additional patients have now been enrolled with severe CAEBV involving B or T cells; while some of these patients have shown clear responses to therapy, other have required hematopoietic stem-cell transplantation. Newer techniques are being developed to target additional EBV proteins, particularly LMP1, LMP2, and EBNA-1. Adenovirus vectors have been used to infect both dendritic cells and EBV-transformed B cells to enrich for the frequency of these antigens as targets for the patient's peripheral blood mononuclear cells [56, 57] (Figure 4). Adenovirus expressing EBV LMP2 has been used to generate LMP2-specific autologous CTLs in a clinical trial in patients with EBV-positive lymphomas [58]. Most patients with relapsed disease had a complete response to the CTLs.

H. Heslop (Houston) discussed the use of hematopoietic stem-cell transplantation for treatment of CAEBV. This approach has been used in children with hemophagocytic lymphohistiocytosis (HLH), a hereditary disorder that shares many features with CAEBV [59]. As with CAEBV, most of the deaths are related to early complications of transplantation [38]. More recent studies in HLH using reduced intensity conditioning with fludarabine, melphalan, and alemtuzumab have resulted in improved survival rates of 75%–93% at 1 year [60], as compared with fully ablative transplantation.

Transplantation for CAEBV has resulted in survival rates of 50%–64%. Younger patients, those with lower viral loads and those with less intense conditioning regimens have had improved survival [30]. A number of strategies are being considered to reduce the risk of relapse including the use of additional boosts of EBV-specific T cells after transplant, infusions of T cells that recognize antigens on lymphoma cells (e.g. CD70), or enhancing alloreactivity through the use of antibodies that can link tumor cells and CTLs by binding both tumor cell antigens and antigen receptors on CTLs.

W. Wilson (Bethesda) discussed the use of novel therapies for EBV LPD. Antiviral therapy may have some activity, particularly at an early stage when virus replication is more prominent. A study of valganciclovir in 47 children with EBV infection after liver transplantation showed a reduction in EBV

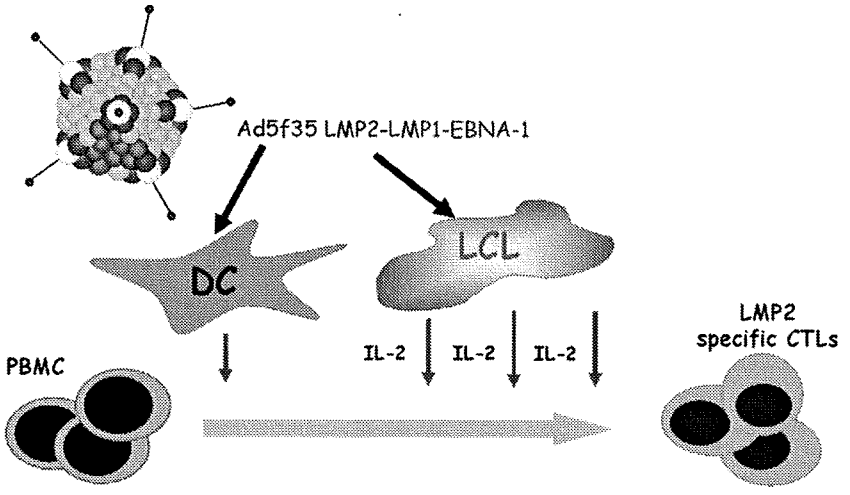

Figure 4. Generation of cytotoxic T cells that recognize EBV LMP2. Autologous dendritic cells and EBV-transformed B cells [lymphoblastoid cell line (LCL)] are infected with adenovirus expressing LMP2 (Ad5f35). These cells are used to present LMP2 to peripheral blood mononuclear cells (PBMCs). After stimulation of PBMCs with irradiated adenovirus-infected dendritic cells and LCLs in the presence of IL-2, LMP2-specific autologous cytotoxic T cells (CTLs) are obtained [58].

viral load and no new cases of post-transplant LPD [61]. Histone deacetylase inhibitors, such as sodium butyrate induce viral gene expression with lytic EBV replication. These agents induce expression of the viral thymidine kinase which can phosphorylate ganciclovir which is toxic to cells [62]. A recent clinical trial using arginine butyrate and ganciclovir resulted in improved survival [63]. High-dose sodium butyrate and ganciclovir can block the phosphotidylinositol-3-kinase kinase/Akt pathway to kill virus-infected cells. Valproic acid is a potent histone deacetylase inhibitor and induces lytic EBV gene expression. Combination therapy of valproic acid with gemcitabine kills EBV-transformed B cells *in vitro* and in SCID mice more effectively than chemotherapy alone [64, 65]. EBV-transformed B cells show activation of NF-κB, and bortezomib, a proteasome inhibitor, leads to increased levels of IκB kinase and inhibits activation of NF-κB. EBV LMP1 inhibits apoptosis to prevent death of transformed B cells. HA14-1 is a small molecule inhibitor of Bcl-2 that kills EBV-transformed B cells [66]. The combination of bortezomib and HA14-1 was synergistic for killing EBV-transformed B cells *in vitro*. Another NF-κB inhibitor, dehydroxymethylepoxyquinomicin induced apoptosis of EBV-transformed B cells [67]. The mammalian target of rapamycin pathway is another target for killing EBV-transformed B cells. Inhibition of this pathway affects multiple downstream signaling molecules required for protein synthesis and cell cycle progression. Rapamycin kills EBV-transformed B cells *in vitro* and in SCID mice [68]. Thus, the study of molecular pathways activated in EBV-transformed B cells has led to candidates for the treatment of EBV LPD.

conclusions

The lack of understanding of uncommon EBV LPDs affecting B cells, T cells, and NK cells is aggravated by confusion in the literature regarding terminology and diagnostic criteria for individual disease entities and clinical syndromes. Meeting participants concluded that the term CAEBV should be applied to systemic LPDs that are not frank lymphomas and that arise during primary infection and persist for over 6 months. In addition, the nature of the EBV-infected cell, B, T, or NK, should be specified in all instances. CAEBV of B-cell origin also has been referred to as chronic (or persistent) infectious mononucleosis, which is a distinct entity from the chronic fatigue syndrome. The term 'systemic EBV-positive T-cell LPD', as adopted by the WHO classification, is the preferred pathologic designation over CAEBV for those cases that are clearly clonal, as they are generally associated with an aggressive clinical course and require aggressive treatment. These cases may, however, arise in the background of CAEBV. HV, which may be clonal, has a chronic and protracted clinical course and may regress spontaneously in adult life. As it has distinctive clinical and pathological features, the term HV is preferred over CAEBV for patients with these features. Criteria for the distinction of HV and HV-like lymphoma remain to be defined. Severe mosquito bite allergy appears to be a related syndrome, but is usually of NK cell origin.

Participants proposed the establishment of an international consortium to collect further clinical outcome data on CAEBV and related disorders. In addition, a proposed biobank will allow studies of cellular immunity, gene expression profiling, and sequencing of candidate genes. It is hoped that these studies will identify new pathways involved in the pathogenesis of these diseases and lead to multicenter international clinical trials to evaluate novel therapies for these diseases.

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MAJOR ARTICLE

Identification of Epstein-Barr Virus (EBV)–Infected Lymphocyte Subtypes by Flow Cytometric In Situ Hybridization in EBV-Associated Lymphoproliferative Diseases

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To diagnose Epstein-Barr virus (EBV)–associated diseases and to explore the pathogenesis of EBV infection, not only must the EBV load be measured, but EBV-infected cells must also be identified. We established a novel flow cytometric in situ hybridization assay to detect EBV$^+$ suspension cells using a peptide nucleic acid probe specific for EBV-encoded small RNA (EBER). By enhancing fluorescence and photostability, we successfully stained EBER and surface antigens on the same cells. In 3 patients with hydroa vacciniforme–like lymphoproliferative disease, we demonstrated that 1.7%–25.9% of peripheral lymphocytes were infected with EBV and specifically identified these lymphocytes as $CD3^+CD4^-CD8^-$ $\gamma\delta$ T cell receptor–positive T cells. The results indicate that this novel and noninvasive assay is a direct and reliable method of characterizing EBV-infected lymphocytes that can be used not only to diagnose EBV infection but also to clarify the pathogenesis of EBV-associated diseases.

Epstein–Barr virus (EBV) is a ubiquitous virus and occasionally causes infectious mononucleosis in primary infection. In rare cases, chronic active EBV infection develops in apparently immunocompetent hosts [1–3]. EBV preferentially infects B cells through CD21 and HLA class II molecules and establishes latent infection in memory B cells [4]. Several types of B cell–origin lymphomas or lymphoproliferative diseases, including Burkitt lymphoma, Hodgkin lymphoma, primary central nervous system lymphoma, and opportunistic B cell lymphoproliferative disorders, are etiologically linked to EBV infection [2, 3, 5]. EBV also infects T cells and natural killer (NK) cells and is associated with T/NK lymphoproliferative diseases and lymphoma or leukemia, such as EBV-related hemophagocytic lymphohistiocytosis, systemic EBV$^+$ T cell lymphoproliferative disease of childhood, hydroa vacciniforme–like lymphoma, nasal NK cell lymphoma, and aggressive NK cell leukemia [2, 3, 5, 6].

Because EBV is a ubiquitous virus that latently infects various lymphocytes, simply detecting EBV is insufficient to diagnose EBV-associated diseases [7]. To diagnose EBV-associated diseases and to explore the pathogenesis of EBV infection, one must not only measure the EBV load, but one must also identify EBV-infected cells. In situ hybridization (ISH) with the EBV-encoded small RNA (EBER) is widely used to detect EBV-infected cells in tissue specimens [8, 9]. EBER is a good marker for EBV infection because it is detectable in virtually all EBV-infected cells and is expressed at very high levels, reaching 10^7 molecules per cell [5]. Therefore, EBER ISH is a specific and direct method of identifying EBV-infected cells in tissue specimens [9]. How-

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