patients

A total of 172 mature NK cell leukemia/lymphomas (150 ENKLs and 22 ANKLs) were included in this study. Detailed clinicopathologic characteristics of 22 ANKLs were described previously [18]. This study was conducted by the NK-cell Tumor Study Group and approved by the institutional review board of participating institutions. Initial diagnosis was made at each institution and was revised when the original histologic material was available. Specimens were reviewed by two expert hematopathologists (SN and JS) and clinical data were reviewed by the Diagnostic Committee (RS, KK and KO) as described previously [23].

histologic and immunophenotypic examination

Histopathologic examination was conducted on formalin-fixed and paraffin-embedded sections of tissue after staining with hematoxylin–eosin. Immunohistochemical studies for various antigens and *in situ* hybridization for EBV-encoded RNA was carried out as described [24]. Briefly, the expression of antigens in the paraffin-embedded sections was examined by using the avidin–biotin complex peroxidase method. The antibodies comprised CD3 (Dako; Santa Fe, CA), CD56 (Novocastra Laboratories; Newcastle, UK), L26/CD20 (Dako), CD79a (Dako), UCHL1/CD45RO (Dako), MT1/CD43 (Bio-Science Products; Emmenbrucke, Switzerland), CD4 (Novocastra), CD8 (Dako), E29/EMA (Coulter Immunology; Hialeah, FL), Leu7/CD57 (Becton-Dickinson; Sunnyvale, CA), LMP-1 (Dako), DO-7/p53 (Dako), bcl-2 (Dako), TIA-1 (Coulter Immunology) and granzyme B (Monosan; Uden, The Netherlands). Flow cytometric immunophenotyping including cytoplasmic CD3 (cyCD3) was carried out, as described previously [25]. For CD16 antibody, Leu11 (Becton-Dickinson; Franklin Lakes, NJ) and ION16 (Beckman Coulter; Fullerton, CA) were used.

statistical analysis

The LDH index was calculated at each of the institutes from a patient's serum LDH level divided by the upper limit of serum LDH. IPI and prognostic index for T-cell lymphoma (PIT) scores were calculated as previously described [26, 27]. The treatment response was assessed according to standard response criteria [28]. Overall survival (OS) was measured from the date of diagnosis to the date of death or the last follow-up. Correlations between the two groups were examined with the χ^2 test, Fisher's exact test and the Mann–Whitney U test. Patient survival data were analyzed with the method of Kaplan and Meier and were compared by means of the log-rank test. Univariate and multivariate analyses were carried out using the Cox proportional hazards regression model, and variables were selected with the stepwise method. Data were analyzed with STATA version 9 (Stata Corporation, College Station, TX) and Fisher (Nakayama-Shoten; Tokyo, Japan) statistical software.

results

patient characteristics

The characteristics of 172 patients are listed in Table 1. Of the 150 ENKLs, 123 presented with nasal and/or paranasal lesions, which were categorized as nasal NK cell lymphoma in the following analyses. Remaining 27 did not show any nasal/paranasal involvements by physical examination and computer tomography and were categorized as extranasal NK cell lymphoma. The origin of these cases was the skin in 16 subjects, the liver and/or spleen in 10, and the intestine in 1. ANKL showed significantly younger age onset than ENKL (median: 42 versus 53 years, $P = 0.04$). Nasal ENKL showed male predominance (male : female, 81 : 42), but extranasal ENKL (12 : 15) and ANKL (7 : 15) did not ($P = 0.003$). Among ENKL cases, those originating from the nasal region showed a higher percentage of localized disease (stage I) than those from extranasal sites (45% versus 22%, $P = 0.03$). The former

Table 3. Therapy and response

	Number of patients					CR rate (%)	Response rate (%)
	CR	PR	NR	UE	Total		
Stage I	44	5	9	3	61	73	82
Nasal: chemotherapy alone	11	2	3	1	17	65	76
Nasal: radiotherapy alone	5	0	1	0	6	83	83
Nasal: chemotherapy followed by radiotherapy	13	1	4	0	18	72	78
Nasal: radiotherapy followed by chemotherapy	9	1	1	0	11	82	91
Nasal: concurrent chemoradiotherapy	1	0	0	0	1	100	100
Extranasal	5	1	0	0	6	83	100
Stage II	13	11	9	0	33	39	73
Nasal	11	9	9	0	29	38	69
Extranasal	2	2	0	0	4	50	100
Stage III	5	1	2	1	9	56	67
Nasal	4	1	2	1	8	50	63
Extranasal	1	0	0	0	1	100	100
Stage IV	7	10	25	5	47	15	36
Nasal	5	8	16	2	31	16	42
Extranasal	2	2	9	3	16	13	25
Aggressive NK cell leukemia	4	3	12	3	22	18	32

Two of the nasal ENKLs with stage I disease did not receive any therapy due to a poor condition.
CR, complete response; PR, partial response; NR, no response; UE, unevaluable.

showed a significantly lower distribution of the clinical stage than the latter ($P = 0.002$). The presence of B symptoms was high in extranasal ENKL and ANKL but low in nasal ENKL ($P = 0.004$). The performance status of patients with extranasal origin was significantly worse than that of patients with nasal origin ($P = 0.01$). All but one case showed extranodal involvement at initial presentation. Of the 123 cases of nasal NK cell lymphoma, 111 presented with involvement of the nasal/paranasal sinus, 28 with the pharynx/oral cavity and 16 with both. Only one case presented with systemic lymph node involvement. Distributions of all three prognostic indexes were significantly lower for the nasal ENKL group.

immunophenotype

Results of immunophenotyping are summarized in Table 2. Most cases of each type were positive for CD2, CD56, human leukocyte antigen-DR, TIA-1 and granzyme B, indicating their NK cell origin. CD7, CD8, CD16, CD57 and cyCD3 were positive in varying degrees. Those examined were proved to be uniformly negative for T- (CD1, CD3, CD4, CD5 and TCRs), B- (CD10, CD19, CD20 and CD79) and myelomonocytic markers (CD13, CD14, CD15 and CD33), as well as for CD25 and CD34. The expression of cyCD3 was significantly higher for ENKL (82% versus 43%, $P = 0.009$) but that of CD16 was lower (22% versus 75%, $P < 0.001$) than for ANKL. Epstein–Barr virus was also detected in most of the cases.

therapy and clinical course

Of 55 patients with stage I nasal ENKL, 17 received radiotherapy first and 35 chemotherapy first. Eleven of the former were further treated with supplemental chemotherapy, and 18 of the latter received additional radiotherapy. One patient was treated with simultaneous chemoradiotherapy, but two could not be treated due to their poor condition. Twenty-five patients received hematopoietic stem-cell transplantations (HSCTs; 17 autografts and 8 allografts), which were described previously [29, 30]. For stage I patients, complete response (CR) rate was 73%, and the response rate was 82% (Table 3). Although no statistical superiority was found, the CR rate exceeded 80% for patients who received radiotherapy first. In contrast, the CR rates for patients treated with chemotherapy alone and those with chemotherapy followed by radiotherapy were 65% and 72%, respectively. All but three patients with stage II disease received chemotherapy. Two of the patients received radiotherapy, but one could not receive any therapy due to the poor condition. Each two of the patients with stage III and IV disease could not also receive any therapy, but the others were treated with various types of combination chemotherapy. The CR rate was significantly different by the clinical stage (39% in stage II, 56% in stage III and 15% in stage IV). No significant differences in response rates were found between nasal and extranasal ENKLs when stratified by clinical stage. The CR and response rates of stage IV patients were comparable with those of ANKL.

difference between ANKL and ENKL

The OS curves of nasal and extranasal ENKL and ANKL are shown in Figure 1A. Prognosis was significantly different

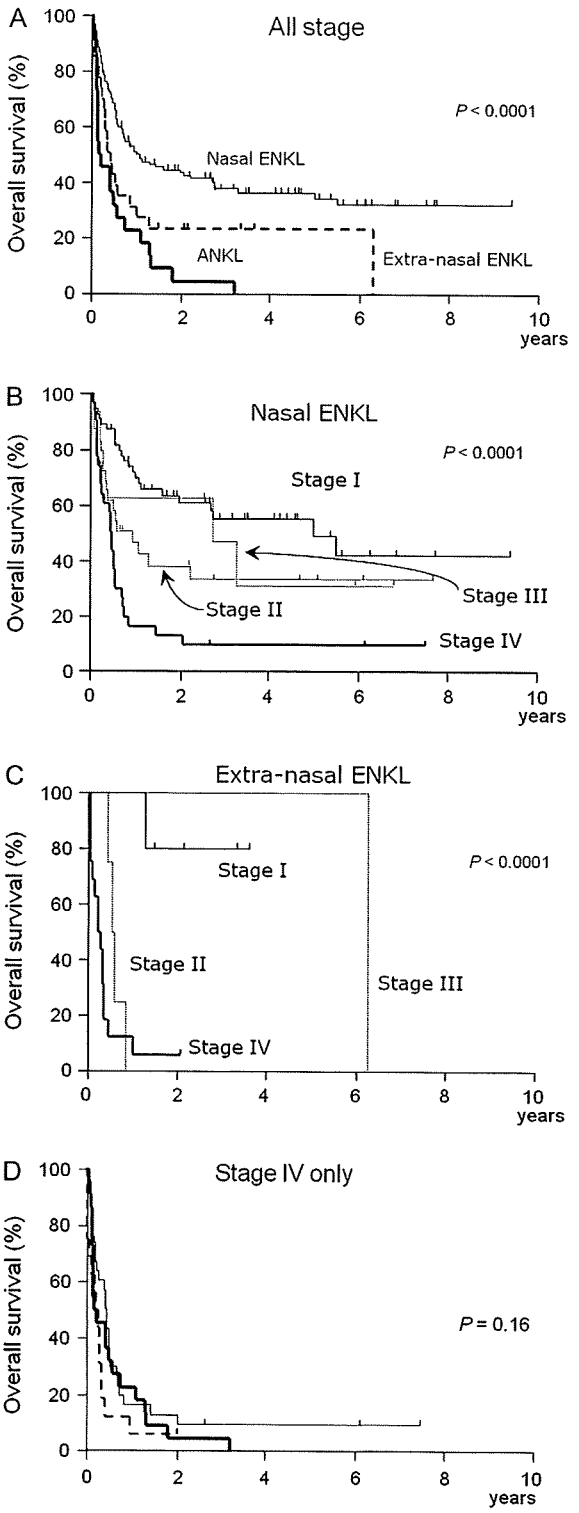

Figure 1. Overall survival (OS) of patients with aggressive NK cell leukemia (ANKL) and extranodal NK cell lymphoma (ENKL) according to clinical stage. (**A**) Prognosis was different among ANKL (thick line), nasal ENKL (thin line) and extranasal ENKL (broken line). Four-year OS was 36% for nasal ENKL and 23% for extranasal ENKL. (**B**) Nasal ENKL shows different prognosis according to the clinical stage ($P < 0.0001$). Four-year OS was 55% for stage I, 33% for stage II, 31% for stage III and 10% for stage IV patients. (**C**) Extranasal ENKL also shows different prognosis according to the clinical stage ($P = 0.001$). (**D**) If restricted to stage IV patients, no significant difference in OS was found ($P = 0.16$).

Table 4. Prognostic factors affecting overall survival

Variables	Unfavorable factors	Univariate		Multivariate[a]	
		Hazard ratio (CI)	*P*	Hazard ratio (CI)	*P*
Age (years)	>60	1.1 (0.8–1.6)	0.59	–	
Stage	III/IV	3.2 (2.2–4.7)	<0.000001	1.7 (1.0–2.8)	0.04
PS	2–4	3.0 (2.0–4.4)	0.000001	1.9 (1.2–2.9)	0.003
Extranodal disease	More than one site	3.0 (2.1–4.5)	<0.000001	1.8 (1.1–3.1)	0.03
LDH	Above normal	2.1 (1.5–3.1)	0.00008	–	
B symptom	Present	2.2 (1.5–3.3)	0.00002	–	
Bone marrow	Involved	2.7 (1.8–4.0)	0.000001	–	
Regional LN	Involved	1.5 (1.1–2.3)	0.03	–	
Disease type	Extranasal/aggressive	2.3 (1.6–3.4)	0.00002	1.6 (1.1–2.5)	0.02
WBC count	>10 000/mm^3	1.8 (1.0–3.3)	0.05	–	
IPI category	H-I/H	3.6 (2.5–5.2)	<0.000001		
PIT category	Group 3/4	2.7 (1.9–4.0)	0.000001		
Korean index	Group 3/4	2.9 (2.0–4.3)	0.000001		

[a]Final model.

CI, confidence interval; PS, performance status; LDH, lactate dehydrogenase; LN, lymph node; WBC, white blood cell; IPI, International Prognostic Index; H-I, high–intermediate; H, high; PIT, prognostic index for T-cell lymphoma.

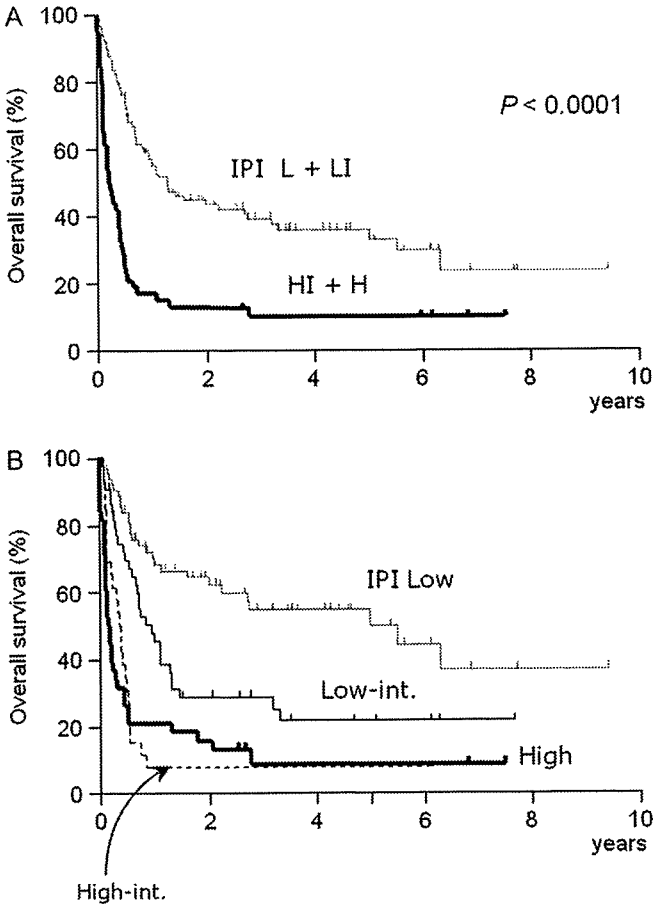

Figure 2. Overall survival of natural killer (NK) cell malignancies according to the International Prognostic Index (IPI). (**A**) Patients with high–intermediate/high IPI score showed significantly lower survival ($P < 0.0001$). (**B**) However, prognosis was almost the same for those with high–intermediate and high-risk categories.

among the three disease categories ($P < 0.0001$). The OS curves of nasal and extranasal ENKL with different clinical stages are shown in Figure 1B and C, respectively. Prognosis was significantly different according to the clinical stage ($P < 0.0001$), but stage III group showed better prognosis than stage II group. If restricted to patients with stage IV diseases, the prognosis was equally poor for all disease subtypes (Figure 1D, $P = 0.16$). Due to this result and the similar CR rates between ANKL and stage IV ENKL noted above, we compared the clinicopathologic characteristics of ANKL and stage IV ENKL (supplemental Table S1, available at *Annals of Oncology* online). Although differences in age of onset, presence of B symptoms, performance status and IPI vanished when restricted to stage IV cases, sex distribution and phenotypic markers (CD16 and cyCD3) were still significantly different. For stage IV ENKL cases, male : female ratio was 31 : 16, and CD16 and cyCD3 were positive in 5 of 18 (28%) and 27 of 33 cases (82%), respectively.

prognostic factors and model

Although patient age was not a significant prognostic factor, univariate Cox analysis identified the following prognostic factors: clinical stage, performance status, number of extranodal involvements, serum LDH index, presence of B symptoms, BM involvement, regional lymph node involvement, disease type and white blood cell count (Table 4 and supplemental Figure S1, available at *Annals of Oncology* online). IPI category, PIT and Korean index were also highly prognostic (Figures 2 and 3). Multivariate analysis revealed four factors, advanced stage (III or IV), poor Eastern Cooperative Oncology Group performance status (2–4), extranodal involvement (more than one) and disease type (extranasal/aggressive), to be significant and independent prognostic factors (Table 4). All patients were then scored according to these four factors. Fifty-eight patients did not have any of the factors, 29 had one factor, 37 had two factors, 29 had

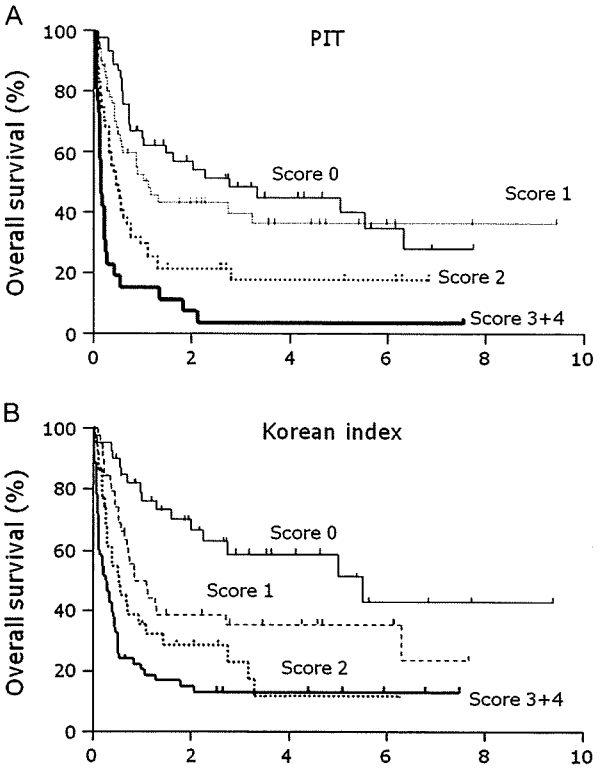

Figure 3. Overall survival of natural killer cell malignancies according to the prognostic index for T-cell lymphoma (PIT) and the Korean prognostic index. (**A**) Although the PIT score could successfully identify patients with poor prognosis, the differentiation between those with score 0 and score 1 was not clear enough. (**B**) Although the Korean index could successfully identify patients with poor prognosis, the distinction between those with score 2 and score 3 + 4 was not intelligible.

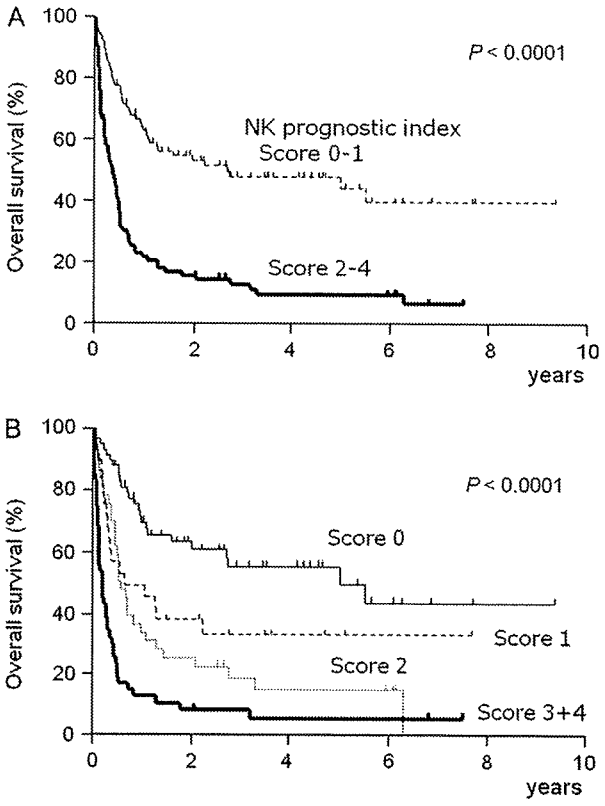

Figure 4. Overall survival of natural killer (NK) cell malignancies according to new NK prognostic index. Patients are successfully stratified according to the new prognostic index comprising clinical stage, performance status, number of extranodal involvements and disease type, either two (**A**) or four subgroups (**B**).

three factors and 18 had all four factors. The OS curves according to the new NK cell tumor prognostic index are shown in Figure 4. The new index categorized four groups with significantly different prognoses. Four-year OS rate was 48% for patients with score 0 or 1 and 11% for those with score 2–4 (Figure 4A, $P < 0.0001$). Four-year OS rates were 55%, 33%, 15% and 6% for patients with score 0, score 1, score 2 and score 3 or 4, respectively (Figure 4B, $P < 0.0001$).

discussion

Since the recognition of ENKL and ANKL 20 years ago, precise comparisons have not yet been satisfactorily conducted. Although the clinicopathologic characteristics of nasal NK cell lymphoma and ANKL are very different, the immunophenotypic profiles, genotype (germline TCR genes) and the close association with EBV are quite similar. Particularly, ENKL of extranasal origin shows high incidence of BM involvement and aggressive clinical course [31–36]. On the other hand, ANKL is characterized by a predilection to hepatosplenic involvement, indicating the existence of a spectrum between these two diseases [37]. However, our analysis showed discrete differences for the age of onset and expression of CD16. Although difference in cyCD3 expression was recognized, only a few numbers of cases were examined. The difference of cyCD3 expression should be examined in a large number of cases. For extranasal NK cell lymphomas, the skin was the most common site of involvement but not for ANKL. Considered together with the difference in genomic gain/loss profiles [38, 39], ANKL and ENKL are concluded to be different in several disease features. However, no specific genes or regions have been identified to separate these two diseases. We tentatively set the boundary of ANKL and ENKL as 30% of BM/PB involvement because this is the most objective criterion [18]. If this differentiation is not generalized, the boundary of ANKL and ENKL can be ambiguous. Further investigations are needed to identify the biological differences between the two diseases. For the consideration of clinical management, therapeutic responses and prognoses of ANKL and stage IV ENKL are similarly poor, indicating that both diseases should be treated equally.

Long-term survival of stage I cases from our series was ~40%. This is consistent with other studies for NK cell lymphoma in the literature [40–43] but lower than that of other lymphoma subtypes [44]. One reason for the poor prognosis of this lymphoma is the expression of P-glycoprotein, which mediates multidrug resistance [45, 46]. Therefore, radiotherapy plays a key role in the treatment of this lymphoma. We compared response rates and prognoses with the initial therapeutic strategy. Patients who were treated with radiotherapy followed by chemotherapy showed the most

favorable response and prognosis, but the differences were not statistically significant. Currently, involved-field radiotherapy followed by chemotherapy is still regarded as a standard treatment of early-stage diseases [47]. Simultaneous chemoradiotherapy is now under evaluation by a Japanese group [48].

For advanced-stage cases, although long-term survival after high-dose chemotherapy and HSCT has been reported [29, 49–51], no standard chemotherapy is currently available [52]. Because L-asparaginase has been reported to be effective in several patients with NK/T-cell lymphoma [53–55], we recently conducted a phase I study of combination chemotherapy including L-asparaginase [56]. The SMILE regimen comprises a steroid (dexamethasone), methotrexate, ifosfamide, L-asparaginase and etoposide. Methotrexate, ifosfamide and L-asparaginase are multidrug resistance-unrelated agents and etoposide shows both *in vitro* and *in vivo* efficacy for EBV-associated lymphoproliferative disorders. Level 1 SMILE was feasible and the overall response rate was 67% [56]. The SMILE regimen is a promising combination chemotherapy for advanced stage of ENKL and ANKL.

The current study identified that the IPI category was also prognostic for extranodal NK/T-cell lymphoma, which is consistent with other studies in the literature [19–21]. However, regarding the IPI components, only age was not prognostic, in contrast to other factors (clinical stage, serum LDH level, performance status and number of extranodal sites). Absence of age as a prognostic factor is consistent with the Korean study, which included the largest number of patients [21]. The Korean study also identified a novel prognostic index including B symptoms, clinical stage, serum LDH level and regional lymph node involvement. This Korean index [21], as well as the PIT category [27], was also prognostic for our series of patients, but multivariate analysis identified another combination of factors that was the most prognostic for patients of the current study. Another Korean group pointed out LTI as a significant prognostic factor [20]. In our patients, however, LTI was only recognized in a limited population, and therefore, LTI was not a significant prognostic factor. Notably, in our series, extranasal onset of disease has been identified as one of the significant prognostic factors. For ENKL, the difference between nasal and extranasal origin is a focus of interest in the recently published result of International Peripheral T/NK cell Lymphoma Project [57, 58]. Further investigations are needed to identify the appropriate prognostic model for extranodal NK cell lymphoma and leukemia.

In conclusion, stage IV ENKL and ANKL are different in several clinicopathologic features but show similar therapeutic response and prognosis. Our novel NK prognostic index is useful for improving treatment choices and designing clinical trials to evaluate new treatment strategies.

funding

Ministry of Health and Welfare, Japan, to Second-Term Comprehensive 10-year Strategy for Cancer Control; Ministry of Education, Science and Culture, Japan, to Science on Primary Areas (Cancer Research).

acknowledgements

The authors wish to thank the following collaborating institutions and their staffs for providing the patient data and specimens: Japanese Red Cross Asahikawa Hospital, National Sapporo Hospital, Japanese Red Cross Ashikaga Hospital, Saitama Medical University Medical Center, Tokyo University School of Medicine, Tokyo Women's Medical University, Showa University Fujigaoka Hospital, Nihon University Itabashi Hospital, Jikei University Aoto Hospital, Keio University School of Medicine, Chiba Cancer Center, Yokohama City University School of Medicine, Kanazawa University School of Medicine, Fukui University School of Medicine, Okazaki Municipal Hospital, National Nagoya Hospital, Kyoto University School of Medicine, Kyoto Prefectural Medical University, Japanese Red Cross Osaka Hospital, Kure Kyosai Hospital, Tokushima University School of Medicine and Kyushu University School of Medicine.

disclosure

KO is currently an employee of Eisai Pharmaceutical Company.

references

1. Jaffe ES. Classification of natural killer (NK) cell and NK-like T-cell malignancies. Blood 1996; 87: 1207–1210.
2. Oshimi K. Leukemia and lymphoma of natural killer lineage cells. Int J Hematol 2003; 78: 18–23.
3. Suzuki R, Takeuchi K, Ohshima K et al. Extranodal NK/T-cell lymphoma: diagnosis and treatment cues. Hematol Oncol 2008; 26: 66–72.
4. Chan JKC, Jaffe ES, Ralfkiaer E. Extranodal NK/T-cell lymphoma, nasal type. In Jaffe ES, Harris NL, Stein H, Vardiman JW (eds): World Health Organization Classification of Tumors. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press 2001; 204–207.
5. Chan JKC, Wong KF, Jaffe ES et al. Aggressive NK-cell leukemia. In Jaffe ES, Harris NL, Stein H, Vardiman JW (eds), World Health Organization Classification of Tumors. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press 2001; 198–200.
6. Lymphoma Study Group of Japanese Pathologists. The World Health Organization classification of malignant lymphomas in Japan: incidence of recently recognized entities. Pathol Int 2000; 50: 696–702.
7. Au WY, Ma SY, Chim CS et al. Clinicopathologic features and treatment outcome of mature T-cell and natural killer cell lymphomas diagnosed according to the World Health Organization classification scheme: a single center experience of 10 years. Ann Oncol 2005; 16: 206–214.
8. Ko YH, Kim CW, Park CS et al. REAL classification of malignant lymphomas in the Republic of Korea: incidence of recently recognized entities and changes in clinicopathologic features. Cancer 1998; 83: 806–812.
9. Chen CY, Yao M, Tang JL et al. Chromosomal abnormalities of 200 Chinese patients with non-Hodgkin's lymphoma in Taiwan: with special reference to T-cell lymphoma. Ann Oncol 2004; 15: 1091–1096.
10. Yamaguchi M, Ohno T, Oka K et al. Discordant reaction of Leu4 and rabbit anti-human CD3 epsilon in sinonasal 'T'-cell lymphoma. Int J Hematol 1993; 59: 25–30.
11. Suzumiya J, Takeshita M, Kimura N et al. Expression of adult and fetal natural killer cell markers in sinonasal lymphomas. Blood 1994; 83: 2255–2260.
12. Chan JK, Tsang WY, Pau MY. Discordant CD3 expression in lymphomas when studied on frozen and paraffin sections. Hum Pathol 1995; 26: 1139–1143.
13. Emile JF, Boulland ML, Haioun C et al. CD5-CD56+ T-cell receptor silent peripheral T-cell lymphomas are natural killer cell lymphomas. Blood 1996; 87: 1466–1473.

14. Harabuchi Y, Yamanaka N, Kataura A et al. Epstein-Barr virus in nasal T-cell lymphomas in patients with lethal midline granuloma. Lancet 1990; 335: 128–130.
15. Jaffe ES, Chan JK, Su IJ et al. Report of the Workshop on Nasal and Related Extranodal Angiocentric T/Natural Killer Cell Lymphomas. Definitions, differential diagnosis, and epidemiology. Am J Surg Pathol 1996; 20: 103–111.
16. Kawa-Ha K, Ishihara S, Ninomiya T et al. CD3-negative lymphoproliferative disease of granular lymphocytes containing Epstein-Barr viral DNA. J Clin Invest 1989; 84: 51–55.
17. Oshimi K, Yamada O, Kaneko T et al. Laboratory findings and clinical courses of 33 patients with granular lymphocyte-proliferative disorders. Leukemia 1993; 7: 782–788.
18. Suzuki R, Suzumiya J, Nakamura S et al. Aggressive natural killer (NK)-cell leukemia revisited: large granular lymphocyte leukemia of cytotoxic NK cells. Leukemia 2004; 18: 763–770.
19. Chim CS, Ma SY, Au WY et al. Primary nasal natural killer cell lymphoma: long-term treatment outcome and relationship with the international prognostic index. Blood 2004; 103: 216–221.
20. Kim TM, Park YH, Lee SY et al. Local tumor invasiveness is more predictive of survival than International Prognostic Index in stage I_E/II_E extranodal NK/T-cell lymphoma, nasal type. Blood 2005; 106: 3785–3790.
21. Lee J, Suh C, Park YH et al. Extranodal natural killer T-cell lymphoma, nasal-type: a prognostic model from a retrospective multicenter study. J Clin Oncol 2006; 24: 612–618.
22. Suzuki R, Nakamura S, Suzumiya J et al. Blastic natural killer cell lymphoma/leukemia (CD56-positive blastic tumor): prognostication and categorization according to anatomic sites of involvement. Cancer 2005; 104: 1022–1031.
23. Oshimi K, Kawa K, Nakamura S et al. NK-cell neoplasms in Japan. Hematology 2005; 10: 237–245.
24. Suzuki R, Kagami Y, Takeuchi K et al. Prognostic significance of CD56 expression for ALK-positive and ALK-negative anaplastic large cell lymphoma of T/null cell phenotype. Blood 2000; 96: 2993–3000.
25. Suzuki R, Yamamoto K, Seto M et al. $CD7^+$ and $CD56^+$ myeloid/natural killer cell precursor acute leukemia: a distinct hematolymphoid disease entity. Blood 1997; 90: 2417–2428.
26. The International Non-Hodgkin's Lymphoma Prognostic Factors Project: a predictive model for aggressive non-Hodgkin's lymphoma. N Engl J Med 1993; 329: 987–994.
27. Gallamini A, Stelitano C, Calvi R et al. Peripheral T-cell lymphoma unspecified (PTCL-U): a new prognostic model from a retrospective multicentric clinical study. Blood 2004; 103: 2474–2479.
28. Cheson BD, Horning SJ, Coiffier B et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphoma. J Clin Oncol 1999; 17: 1244–1253.
29. Suzuki R, Suzumiya J, Nakamura S et al. Hematopoietic stem cell transplantation for natural killer-cell lineage neoplasms. Bone Marrow Transplant 2006; 37: 425–431.
30. Lee J, Au WY, Park MJ et al. Autologous hematopoietic stem cell transplantation in extranodal NK/T-cell lymphoma: a multinational, multicenter, matched controlled study. Biol Blood Marrow Transplant 2008; 14: 1356–1364.
31. Chan JKC, Sin VC, Wong KF et al. Nonnasal lymphoma expressing the natural killer cell marker CD56: a clinicopathologic study of 49 cases of an uncommon aggressive neoplasm. Blood 1997; 89: 4501–4513.
32. Rodriguez J, Romaguera JE, Manning J et al. Nasal-type T/NK lymphomas: a clinicopathologic study of 13 cases. Leuk Lymphoma 2000; 39: 139–144.
33. Mraz-Gernhard S, Natkunam Y, Hoppe RT et al. Natural killer/natural killer-like T-cell lymphoma, CD56+, presenting in the skin: an increasingly recognized entity with an aggressive course. J Clin Oncol 2001; 19: 2179–2188.
34. Ng SB, Lai KW, Murugaya S et al. Nasal-type extranodal natural killer/T-cell lymphomas: a clinicopathologic and genotypic study of 42 cases in Singapore. Mod Pathol 2004; 17: 1097–1107.
35. Ko YH, Cho EY, Kim JE et al. NK and NK-like T-cell lymphoma in extranasal sites: a comparative clinicopathological study according to site and EBV status. Histopathology 2004; 44: 480–489.
36. Lee J, Kim WS, Park YH et al. Nasal-type NK/T cell lymphoma: clinical features and treatment outcome. Br J Cancer 2005; 92: 1226–1230.
37. Quintanilla-Martinez L, Jaffe ES. Aggressive NK cell lymphomas: insights into the spectrum of NK cell derived malignancies. Histopathology 2000; 37: 372–374.
38. Siu LL, Wong KF, Chan JK, Kwong YL. Comparative genomic hybridization analysis of natural killer cell lymphoma/leukemia. Recognition of consistent patterns of genetic alterations. Am J Pathol 1999; 155: 1419–1425.
39. Nakashima Y, Tagawa H, Suzuki R et al. Genome-wide array-based comparative genomic hybridization of natural killer cell lymphoma/leukemia: different genomic alteration patterns of aggressive NK-cell leukemia and extranodal NK/T-cell lymphoma, nasal type. Genes Chromosomes Cancer 2005; 44: 247–255.
40. Kim WS, Song SY, Ahn YC et al. CHOP followed by involved field radiation: is it optimal for localized nasal natural killer/T-cell lymphoma? Ann Oncol 2001; 12: 349–352.
41. Kim GE, Cho JH, Yang WI et al. Angiocentric lymphoma of the head and neck: patterns of systemic failure after radiation treatment. J Clin Oncol 2000; 18: 54–63.
42. Li CC, Tien HF, Tang JL et al. Treatment outcome and pattern of failure in 77 patients with sinonasal natural killer/T-cell or T-cell lymphoma. Cancer 2004; 100: 366–375.
43. Isobe K, Uno T, Tamaru J et al. Extranodal natural killer/T-cell lymphoma, nasal type: the significance of radiotherapeutic parameters. Cancer 2006; 106: 609–615.
44. Miller TP, Dahlberg S, Cassady JR et al. Chemotherapy alone compared with chemotherapy plus radiotherapy for localized intermediate- and high-grade non-Hodgkin's lymphoma. N Engl J Med 1998; 339: 21–26.
45. Yamaguchi M, Kita K, Miwa H et al. Frequent expression of P-glycoprotein/MDR1 by nasal T-cell lymphoma cells. Cancer 1995; 76: 2351–2356.
46. Egashira M, Kawamata N, Sugimoto K et al. P-glycoprotein expression on normal and abnormally expanded natural killer cells and inhibition of P-glycoprotein function by cyclosporin A and its analogue, PSC833. Blood 1999; 93: 599–606.
47. Suzuki R. Leukemia and lymphoma of natural killer cells. J Clin Exp Hematop 2005; 45: 51–70.
48. Yamaguchi M, Oguchi M, Tobinai K et al. Phase I/II study of concurrent chemoradiotherapy for newly-diagnosed, localized nasal NK/T-cell lymphoma: results of a phase I portion of JCOG0211-DI. Blood 2005; 106: 754a (Abstr 2685).
49. Au WY, Lie AK, Liang R et al. Autologous stem cell transplantation for nasal NK/T-cell lymphoma: a progress report on its value. Ann Oncol 2003; 14: 1673–1676.
50. Murashige N, Kami M, Kishi Y et al. Allogeneic haematopoietic stem cell transplantation as a promising treatment for natural killer-cell neoplasms. Br J Haematol 2005; 130: 561–567.
51. Kim HJ, Bang SM, Lee J et al. High-dose chemotherapy with autologous stem cell transplantation in extranodal NK/T-cell lymphoma: a retrospective comparison with non-transplantation cases. Bone Marrow Transplant 2006; 37: 819–824.
52. Oshimi K. Progress in understanding and managing natural killer-cell malignancies. Br J Haematol 2007; 139: 532–544.
53. Yong W, Zheng W, Zhang Y et al. L-asparaginase-based regimen in the treatment of refractory midline nasal/nasal-type T/NK-cell lymphoma. Int J Hematol 2003; 78: 163–167.
54. Obama K, Tara M, Niina K. L-asparaginase-based induction therapy for advanced extranodal NK/T-cell lymphoma. Int J Hematol 2003; 78: 248–250.
55. Matsumoto Y, Nomura K, Kanda-Akano Y et al. Successful treatment with Erwinia L-asparaginase for recurrent natural killer/T cell lymphoma. Leuk Lymphoma 2003; 44: 879–882.
56. Yamaguchi M, Suzuki R, Kwong YL et al. Phase I study of dexamethasone, methotrexate, ifosfamide, l-asparaginase, and etoposide (SMILE) chemotherapy for advanced-stage, relapsed or refractory extranodal natural killer (NK)/T-cell lymphoma and leukemia. Cancer Sci 2008; 99: 1016–1020.
57. Au WY, Weisenburger DD, Intragumtornchai T et al. Clinical differences between nasal and extranasal natural killer/T-cell lymphoma: a study of 136 cases from the International Peripheral T-Cell Lymphoma Project. Blood 2009; 113: 3931–3937.
58. Suzuki R, Suzumiya J, Oshimi K. Differences between nasal and extranasal NK/T-cell lymphoma. Blood 2009; 113: 6261–6262.

Int J Hematol
DOI 10.1007/s12185-010-0492-1

ORIGINAL ARTICLE

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

Ritsuro Suzuki · Shigeki Ohtake · Jin Takeuchi · Masami Nagai · Yoshihisa Kodera · Motohiro Hamaguchi · Shuichi Miyawaki · Takahiro Karasuno · Shigetaka Shimodaira · Ryuzo Ohno · Shigeo Nakamura · Tomoki Naoe

Received: 2 June 2009 / Revised: 22 December 2009 / Accepted: 4 January 2010

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

R. Suzuki (✉)
Department of HSCT Data Management,
Nagoya University School of Medicine,
1-1-20 Daiko-minami, Higashi-ku,
Nagoya 461-0047, Japan
e-mail: r-suzuki@med.nagoya-u.ac.jp

S. Ohtake
Third Department of Internal Medicine,
Kanazawa University School of Medicine,
Kanazawa, Japan

J. Takeuchi
Department of Internal Medicine,
Nihon University School of Medicine,
Tokyo, Japan

M. Nagai
First Department of Internal Medicine,
Kagawa Medical University, Takamatsu, Japan

Y. Kodera
Department of Internal Medicine,
Japanese Red Cross Nagoya First Hospital,
Nagoya, Japan

M. Hamaguchi
Department of Hematology,
National Hospital Organization,
Nagoya Medical Center, Nagoya, Japan

S. Miyawaki
Department of Internal Medicine,
Saiseikai Maebashi Hospital, Maebashi, Japan

T. Karasuno
Department of Internal Medicine,
Osaka Medical Center for Cancer
and Cardiovascular Diseases, Osaka, Japan

S. Shimodaira
Department of Internal Medicine,
Japanese Red Cross Nagano Hospital,
Nagano, Japan

R. Ohno
Aichi Cancer Center, Nagoya, Japan

S. Nakamura
Department of Pathology,
Nagoya University School of Medicine, Nagoya, Japan

T. Naoe
Department of Hematology and Oncology,
Nagoya University School of Medicine, Nagoya, Japan

Published online: 29 January 2010

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

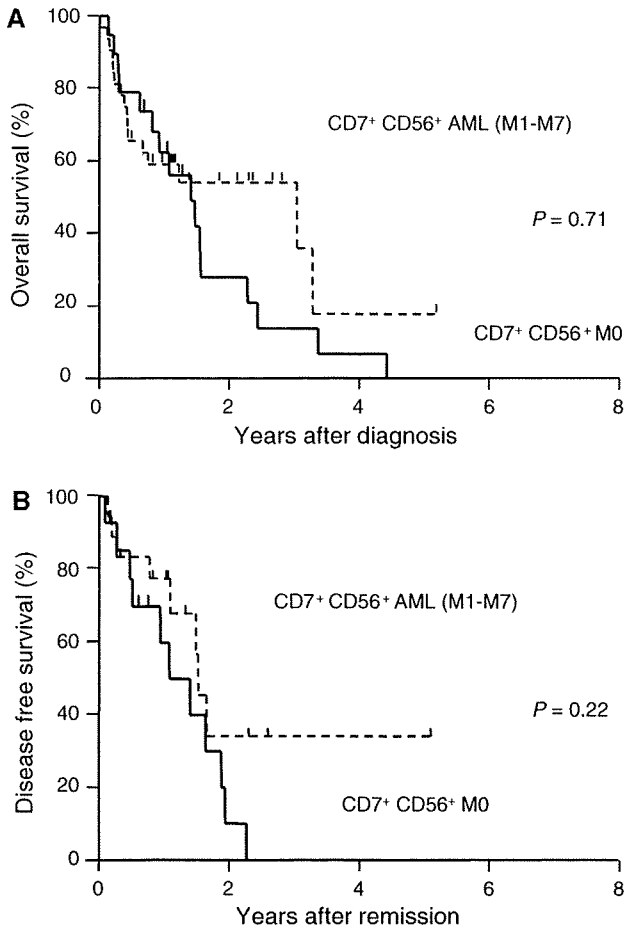

Fig. 1 Overall survival (**a**) and disease-free survival (**b**) curves of $CD7^+$ $CD56^+$ AML patients. *Thick lines* indicate survival curves of $CD7^+$ $CD56^+$ M0 and *broken lines* indicate those of $CD7^+$ $CD56^+$ AML M1–M7. No statistical differences were found between the two groups

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.

Acknowledgments This work was supported in part by a Grant-in-Aid for the Second-Term Comprehensive 10-year Strategy for Cancer Control from the Ministry of Health, Labour, and Welfare; a Grant-in-Aid for Science on Primary Areas (Cancer Research); and a Grant-in-Aid for Encouragement of Young Scientists from the Ministry of Education, Science, and Culture, Japan.

Appendix

The authors thank the collaborators from the following institutions for providing the patients' data and specimens: Akita University School of Medicine; Ohta Nishinouchi Hospital; Jikei University School of Medicine; University of Tokyo; Toranomon Hospital; Tokyo Metropolitan Komagome Hospital; Tokyo Medical University; Yaizu Municipal Hospital; Aichi Cancer Center; Nagoya City University School of Medicine; Social Insurance Kyoto Hospital; Nara Medical University; Kansai Medical University; Hyogo College of Medicine; Kochi University School of Medicine; Nagasaki University School of Medicine; Oita Prefectural Hospital.

References

1. Bennett JM, Catovsky D, Daniel M-T, et al. Proposals for the classification of the acute leukaemias. French–American–British (FAB) co-operative group. Br J Haematol. 1976;33:451–8.
2. Jaffe ES, Harris NL, Stein H, Vardiman JW. World Health Organization classification of tumours. Pathology & genetics:

tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2001.
3. Bene MC, Castoldi G, Knapp W, et al. Proposals for the immunological classification of acute leukemias. Leukemia. 1995; 9:1783–6.
4. Jennings CD, Foon KA. Recent advances in flow cytometry: application to the diagnosis of hematologic malignancy. Blood. 1997;90:2863–92.
5. Haferlach T, Kern W, Schnittger S, Schoch C. Modern diagnostics in acute leukemias. Crit Rev Oncol Hematol. 2005; 56:223–34.
6. Syrjala M, Anttila VJ, Ruutu T, Jansson SE. Flow cytometric detection of residual disease in acute leukemia by assaying blasts co-expressing myeloid and lymphatic antigens. Leukemia. 1994;8:1564–70.
7. San Miguel JF, Martinez A, Macedo A, et al. Immunophenotyping investigation of minimal residual disease is a useful approach for predicting relapse in acute myeloid leukemia patients. Blood. 1997;90:2465–70.
8. Bradstock K, Matthews J, Benson E, Page F, Bishop J. Prognostic value of immunophenotyping in acute myeloid leukemia. Australian Leukaemia Study Group. Blood. 1994;84:1220–5.
9. Legrand O, Perrot JY, Baudard M, et al. The immunophenotype of 177 adults with acute myeloid leukemia: proposal of a prognostic score. Blood. 2000;96:870–7.
10. Chang H, Salma F, Yi QL, Patterson B, Brien B, Minden MD. Prognostic relevance of immunophenotyping in 379 patients with acute myeloid leukemia. Leuk Res. 2004;28:43–8.
11. Suzuki R, Yamamoto K, Seto M, et al. $CD7^+$ and $CD56^+$ myeloid/natural killer cell precursor acute leukemia: a distinct hematolymphoid disease entity. Blood. 1997;90:2417–28.
12. Sanchez MJ, Muench MO, Roncarolo MG, Lanier LL, Phillips JH. Identification of a common T/natural killer cell progenitor in human fetal thymus. J Exp Med. 1994;180:569–76.
13. Shibuya A, Nagayoshi K, Nakamura K, Nakauchi H. Lymphokine requirement for the generation of natural killer cells from CD34+ hematopoietic progenitor cells. Blood. 1995;85:3538–46.
14. Perez SA, Sotiropoulou PA, Gkika DG, et al. A novel myeloid-like NK cell progenitor in human umbilical cord blood. Blood. 2003;101:3444–50.
15. Suzuki R, Nakamura S. Malignancies of natural killer (NK) cell precursor: myeloid/NK cell precursor acute leukemia and blastic NK cell lymphoma/leukemia. Leuk Res. 1999;23:615–24.
16. Suzuki R, Murata M, Kami M, et al. Prognostic significance of $CD7^+$ $CD56^+$ phenotype and chromosome 5 abnormalities for acute myeloid leukemia M0. Int J Hematol. 2003;77:482–9.
17. Bennett JM, Catovsky D, Daniel M-T, et al. Proposal for the recognition of minimally differentiated acute myeloid leukemia (AML-M0). Br J Haematol. 1991;78:325–9.
18. Jaffe ES, Harris NL, Stein H, et al. Tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2001.
19. ISCN 2005: an International system for Human Cytogenetic Nomenclature. In: Shaffer LG, Tommerup N, editors. Basal: Karger; 2005.
20. Seymour JF, Pierce SA, Kantarjian HM, Keating MI, Estey EH. Investigation of karyotypic, morphologic and clinical features in patients with acute myeloid leukemia blast cells expressing the neural cell adhesion molecule (CD56). Leukemia. 1994;8:823–6.
21. Thomas X, Vila L, Campos L, Sabido O, Archimbaud E. Expression N-CAM (CD56) on acute leukemia cells: relationship with disease characteristics and outcome. Leuk Lymphoma. 1995;19:295–300.
22. Ikushima S, Yoshihara T, Matsumura T, et al. Expression of CD56/NCAM on hematopoietic malignant cells. A useful marker for acute monocytic and megakaryocytic leukemias. Int J Hematol. 1991;54:395–403.
23. Tallman MS, Neuberg D, Bennett JM, et al. Acute megakaryocytic leukemia: the Eastern Cooperative Oncology Group experience. Blood. 2000;96:2405–11.
24. Scott AA, Head DR, Kopecky KJ, et al. HLA-DR−, CD33+, CD56+ CD16− myeloid/natural killer cell acute leukemia: a previously unrecognized form of acute leukemia potentially misdiagnosed as French–American–British acute myeloid leukemia-M3. Blood. 1994;84:244–55.
25. Girodon F, Carli P-M, Favre B, et al. Acute myeloid leukemia with hypergranular cytoplasm: a differential diagnosis of acute promyelocytic leukemia. Leuk Res. 2000;24:979–82.
26. Baer MR, Stewart CC, Lawrence D, et al. Expression of the neural cell adhesion molecule CD56 is associated with short remission duration and survival in acute myeloid leukemia with t(8;21)(q22;q22). Blood. 1997;90:1643–8.
27. Murray CK, Estey E, Paietta E, et al. CD56 expression in acute promyelocytic leukemia: a possible indicator of poor treatment outcome? J Clin Oncol. 1999;17:293–7.
28. Ferrara F, Morabito F, Martino B, et al. CD56 expression is an indicator of poor clinical outcome in patients with acute promyelocytic leukemia treated with simultaneous all-*trans*-retinoic acid and chemotherapy. J Clin Oncol. 2000;18:1295–300.
29. Ito S, Ishida Y, Oyake T, et al. Clinical and biological significance of CD56 antigen expression in acute promyelocytic leukemia. Leuk Lymphoma. 2004;45:1783–9.
30. Raspadori D, Damiani D, Lenoci M, et al. CD56 antigenic expression in acute myeloid leukemia identifies patients with poor clinical prognosis. Leukemia. 2001;15:1161–4.
31. Di Bona E, Sartori R, Zambello R, Guercini N, Madeo D, Rodeghiero F. Prognostic significance of CD56 antigen expression in acute myeloid leukemia. Haematologica. 2002;87:250–6.
32. Raspadori D, Damiani D, Michieli M, et al. CD56 and PGP expression in acute myeloid leukemia: impact on clinical outcome. Haematologica. 2002;87:1135–40.
33. Suzuki R. Leukemia and lymphoma of natural killer cells. J Clin Exp Hematopathol. 2005;45:51–70.
34. Oshimi K. Progress in understanding and managing natural killer-cell malignancies. Br J Haematol. 2007;139:532–44.
35. Suzuki R, Takeuchi K, Ohshima K, Nakamura S. Extranodal NK/T-cell lymphoma: diagnosis and treatment cues. Hematol Oncol. 2008;26:66–72.
36. Suzuki R, Kagami Y, Takeuchi K, et al. Prognostic significance of CD56 expression for ALK-positive and ALK-negative anaplastic large cell lymphoma of T/null cell phenotype. Blood. 2000;96:2993–3000.
37. Asano N, Suzuki R, Kagami Y, et al. Clinicopathologic and prognostic significance of cytotoxic molecule expression in nodal peripheral T-cell lymphoma, unspecified. Am J Surg Pathol. 2005;29:1284–93.
38. Suzuki R, Suzumiya J, Nakamura S, et al. Hematopoietic stem cell transplantation for natural killer-cell lineage neoplasms. Bone Marrow Transplant. 2006;37:425–31.
39. Tassone P, Gozzini A, Goldmacher V, et al. In vitro and in vivo activity of the maytansinoid immunoconjugate huN901–N2′-deacetyl-N2′-(3-mercapto-1-oxopropyl)-maytansine against CD56+ multiple myeloma cells. Cancer Res. 2004;64:4629–36.
40. Ishitsuka K, Jimi S, Goldmacher VS, et al. Targeting CD56 by the maytansinoid immunoconjugate IMGN901 (huN901-DM1): a potential therapeutic modality implication against natural killer/T cell malignancy. Br J Haematol. 2008;141:129–31.

review

Annals of Oncology 20: 1472–1482, 2009
doi:10.1093/annonc/mdp064
Published online 10 June 2009

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

J. I. Cohen[1], H. Kimura[2], S. Nakamura[3], Y.-H. Ko[4] & E. S. Jaffe[5]*

[1]*Medical Virology Section, Laboratory of Clinical Infectious Diseases, National Institutes of Health, Bethesda, MD, USA;* [2]*Department of Virology, Nagoya University Graduate School of Medicine, Nagoya;* [3]*Department of Pathology and Clinical Laboratories, Nagoya University Hospital, Showa-ku, Japan;* [4]*Department of Pathology, Samsung Medical Center, Seoul, Korea and* [5]*Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA*

Received 22 December 2008; revised 16 February 2009; accepted 18 February 2009

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.

Correspondence to: Dr E. S. Jaffe, Hematopathology Section and Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, Building 10/Room 2B 42, 10 Center Drive MSC-1500, Bethesda, MD 20892-1500, USA.
Tel: +1-301-496-0183; Fax: +1-301-402-2415; E-mail: elainejaffe@nih.gov

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

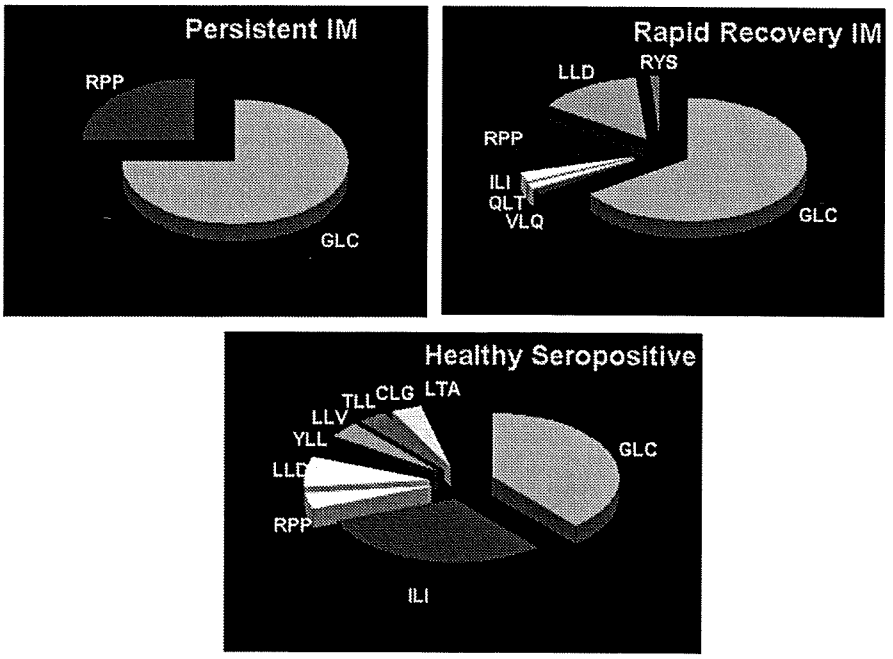

Figure 1. A healthy Epstein–Barr virus (EBV) seropositive individual and a patient rapidly recovering from infectious mononucleosis have diverse CD8+ T cells that recognize a variety of EBV peptides, while a patient with persistent infectious mononucleosis has a limited diversity of CD8+ T cells that 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

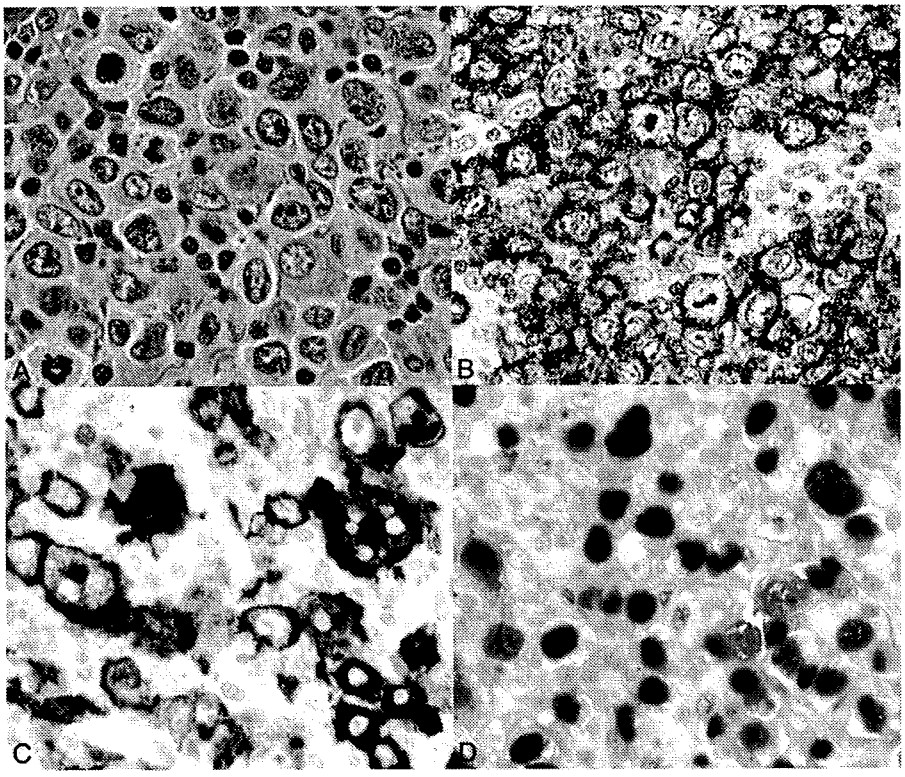

Figure 2. Epstein–Barr virus (EBV)-positive large B-cell lymphoma of the elderly. (A) Large lymphoid cells predominate and in (B) express CD20. (C) Some cells have more pleomorphic features, and CD30 is often positive. (D) EBV encoded RNA (EBER) highlights atypical cells.

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.

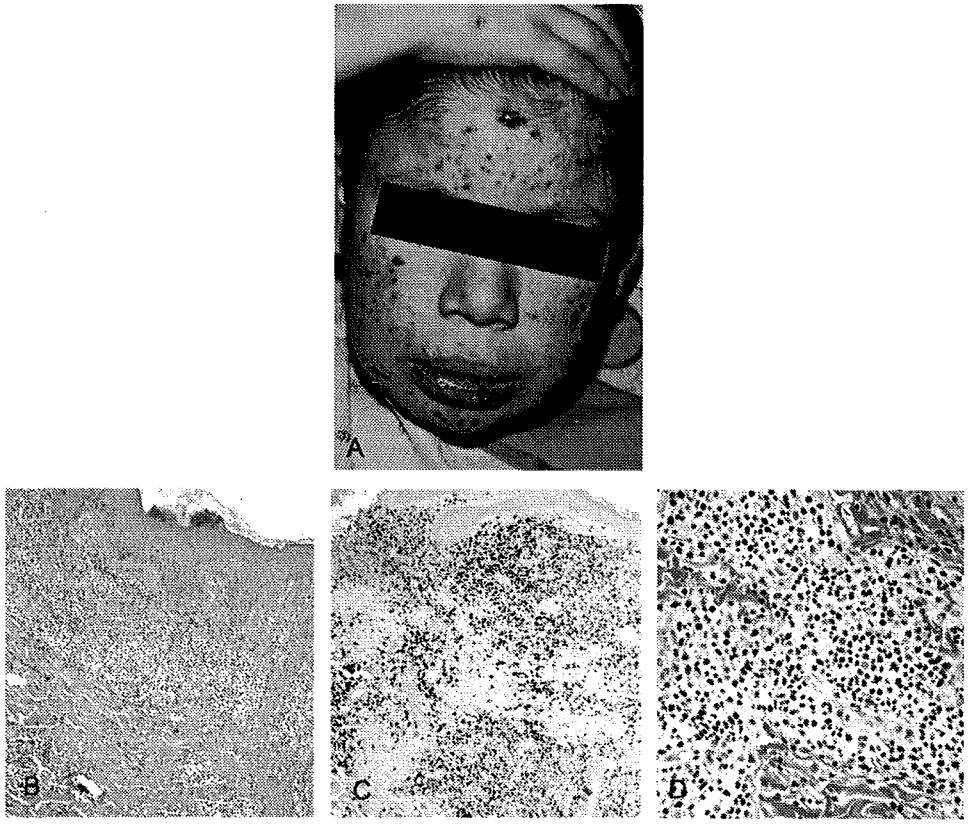

Figure 3. Hydroa vacciniforme-like lymphoma. (A) Sun-exposed areas of the skin exhibit a papulovesicular eruption, with ulceration and crusting. (B) The infiltrate is present in the superficial dermis. (C) Lymphoid cells are positive with EBER *in situ* hybridization. (D) Lymphoid cells are small to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