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Potential macrophage-T progenitor origin of systemic anaplastic large cell lymphoma

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Abstract：Systemic anaplastic large cell lymphoma（ALCL）is considered a cytotoxic T-cell neoplasm. We characterized 21 systemic anaplastic lymphoma kinase（ALK）-positive and 11 ALK-negative ALCL cases by immunophenotyping, Southern blot（SBA）and BIOMED-2 PCR analysis of immunoassociated genes. ALK-positive cases expressed T-cell markers（TCR βF1, 19%; CD3, 14%; CD4, 71%; CD25, 86%）, T/NK-cell-related antigens（TIA1, 95%; granzyme B, 57%; CD122, 77%）and myelomonocyte markers（CD13, 28%; CD68, 27%）by immunostaining. ALK-negative cases expressed TCR βF1（40%）, CD3（27%）, CD4（73%）, CD25（91%）, TIA1（46%）, CD122（44%）and CD13（10%）. SBA indicated TCR β gene rearrangement in 4（31%）of 13 ALK-positive and 3（33%）of 9 ALK-negative cases. BIOMED-2 PCR analysis indicated clonal VDJ peaks of TCR β and γ genes in 6（67%）and 8（89%）of 9 ALK-positive cases, and in 3（38%）and 5（63%）of 8 ALK-negative cases respectively. All CD13- and/or CD68-positive lymphomas showed a germline configuration of TCR genes. T-cell related antigens, especially of TCR βF1 tended to express in systemic ALCL with rearrangement of TCR β gene by the SBA. BIOMED-2 PCR analysis suggested the presence of reactive T cell clone around the tumor cells in about one-third of ALCL cases. These data suggest that systemic ALCL has characteristics of T cells, NK cells and/or myelomonocytes. Therefore, systemic ALCL may be derived from a macrophage-T progenitor with potential differentiation to T, NK cells and histiomonocytes.

Key words：**Anaplastic large cell lymphoma, gene expression, immunohistochemistry**

Introduction

Anaplastic large cell lymphoma（ALCL）comprises distinct entities of lymphomas that express the lymphoid activating molecule CD30 and T cell markers, in particular CD4 and CD25（interleukin（IL）-2α receptor）.[1] The World Health Organization（WHO）classification distinguishes systemic ALCL and the primary cutaneous form based on the tumor site and the expression of anaplastic lymphoma kinase（ALK）.[2] Furthermore, ALK expression in systemic ALCL has a large influence on patient clinical behavior and prognosis.[3,4] The chromosomal abnormality t（2;5）（p23;q35）and its related chimeric gene nucleophosmin（NPM）/ALK are common in cases of systemic ALCL.[5]

It has been postulated that systemic ALCL originates from cytotoxic T cells or NK cells, since the tumor cells frequently express cytotoxic molecules, T-cell intercellular antigen（TIA1）, serine protease granzyme B, and the pore-forming protein perforin.[6,7] Genetically, systemic ALCL is mostly considered to be of cytotoxic T cell origin, because 70-90% of ALCL cases show rearrangement of

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T-cell receptor (TCR) genes by the polymerase chain reaction (PCR), including the BIOMED-2 assay.[6,8] However, many systemic ALCL cases also show a loss of T cell antigens such as TCR βF1, CD3 and CD3 zeta-associated protein ZAP70, as well as of the chemokine receptors CXCR3 and CCR4.[6,9,10] Southern blot analysis (SBA) is the gold standard for detection of the lineage and/or clonality of tumor cells.[11] A number of research groups have detected clonal rearrangement of TCR genes by SBA. In these reports, 8 (40%) out of 20 ALCL cases showed rearrangement of TCR genes and the TCR β gene was rearranged in 13 (59%) of 22 ALCL cases, or in 8 (42%) of 17 cases.[12-14]

However, questions have been raised concerning the cellular origins of ALCL. Le Deley et al.[15] demonstrated that bone marrow and mediastinal involvements were found in 17 (8%) and 81 (36%) of 225 pediatric ALCL cases, respectively. Although bone marrow has been considered to be a rare involved site, Damm-Welk et al.[16] reported that 38 of 80 ALK-positive ALCL cases (48%) showed NPM/ALK chimeric gene in bone marrow samples by reverse transcriptase (RT)-PCR. Using flow cytometry mainly of the nodal tumor tissues, CD7, a marker of precursor T cells and myelomonocytes, was detected in 8 (32%) of 25 ALCL cases, and CD13, a marker of myelomonocytes, was detected in 7 (78%) of 9 cases.[17] Since the CD13-positive lymphoma cells expressed very little CD3, it was pointed out that ALCL could possibly be misdiagnosed as a myeloid sarcoma.

Therefore, the precise cellular origin and characteristics of systemic ALCL are still uncertain. To further characterize systemic ALCL, we examined 32 cases of systemic ALK-positive and ALK-negative ALCL by immunohistochemistry and immunoassociated gene analysis using SBA and BIOMED-2 PCR assays. We found that over two-thirds of the systemic ALCL cases examined showed clonality of TCR genes in the BIOMED-2 assay, while about one-third of the cases showed rearrangement of TCR genes by SBA. Immunohistologically, ALK-positive and ALK-negative ALCL frequently stained positive for NK cell markers, NK receptors, and myelomonocyte markers. Based on these data, we suggest that systemic ALCL may be derived from macrophage-T progenitors, which have the potential to become T-cells, NK cells and histiomonocytes, proposed in mouse thymus.[18]

Materials and Methods

Patients

Approval for these studies was obtained from the institutional Review Board of the Faculty of Medicine, Fukuoka University. Thirty-two Japanese cases of primary systemic ALCL were selected for study; all patients were sero-negative for anti-human T-cell lymphotropic virus I (HTLV-1) antibodies. All tumors were positive for CD30 and negative for CD15, CD20 and CD79a. The cases were classified according to the proposed WHO classification and were subclassified into ALK-positive and ALK-negative groups.[2] Six primary cutaneous CD30-positive ALCL cases and 14 adult T cell leukemia/lymphoma (ATL/L) ALC type[19] were selected as the control group. All participants in the study gave their written informed consent for inclusion in the present study.

Statistical analysis

To confirm that the differences observed between ALK-positive and ALK-negative ALCL cases were statistically significant, univariate χ^2 analyses and Fisher's exact tests were performed. The cumulative survival time of the 24 cases was calculated by the Kaplan-Meier method and analyzed by the log-rank and generalized Wilcoxon tests.

Histology, immunohistochemistry and in situ hybridization (ISH)

Tissue specimens were fixed with 20% formalin, embedded in paraffin, and stained with hematoxylin/eosin solution. For immunohistochemistry, a battery of monoclonal antibodies (Table 1) was applied to the formalin-fixed and fresh frozen tumor samples using the Envision™ polymer method (Dako, Carpinteria, CA, USA), and the peroxidase reaction was developed using diaminobenzidine and hydrogen peroxide.

For ISH, deparaffinized tissue sections were digested with proteinase K and hybridized in a solution of 50% formamide containing digoxigenin/biotin-labeled EBV encoded RNA (EBER) oligonucleotides (Dako). A peroxidase-conjugated anti-digoxigenin antibody was applied to detect the hybridized probes and the reaction was developed by incubation with bromochloroindolylphosphate/nitroblue tetrazolium substrate.

Reverse transcriptase polymerase chain reaction (RT-PCR) analysis of NPM/ALK

Total RNA was isolated from the frozen cells of 8 ALK-

positive ALCL and 6 ALK-negative ALCL tumors using the Ultraspec RNA extraction kit (Biotecx, Houston, TX, USA). Reverse transcriptase was performed on 1 μg of total RNA using the Super Script II RT kit (Life Technologies, Paisley, UK) with random hexamer priming. The 5' NPM and 3' ALK junction oligonucleotides: 5'-TCCCTTGGGGGCTTTGAAATAAC ACC-3', 5'-CGAGGTGCGGAGCTTGCTCAGC-3', and 5'-AGCACTTAGTAGTGTACCGCCGGA-3' respectively, were used to test for expression of the NPM/ALK fusion gene.[20] PCR amplification was performed simultaneously with the above probes and procedures. The RT-PCR products were electrophoresed on 2% agarose gels and visualized with ethidium bromide staining.

Southern blot analysis (SBA) of immunoassociated genes

Frozen samples were examined by HE staining and by immunohistological analysis to confirm the genotypes. High molecular weight DNA was extracted from fresh tumor material of 22 ALCL patients and SBA was performed as described previously.[21] Briefly, 10 μg of DNA digested with EcoRI, Hind III and BamHI, was electrophoresed on 0.7% agarose gels, transferred to a nitrocellulose filter, and hybridized to ^{32}P-labeled DNA probes. All probes were labeled using a DNA random primer kit (Amersham, Buckinghamshire, UK). The JH (joining region of IgH) probe was a 3-kb germline EcoRI-HindIII fragment. The Cβ probe was a 720-bp fragment isolated from the HBVT 96 cDNA clone.[22] The TCR δ probes were a 1.0-kb germline PstI-EcoRI fragment containing the first Jδ region (Jδ 1) and a 1.5-kb Xbal genomic fragment containing the third Jδ region (Jδ 3).[23,24] DNA rearrangement was scored on the basis of loss of the germline band (deletion) or on the appearance of newly rearranged bands.

BIOMED-2 PCR analysis of immunoassociated genes

The same DNA from fresh tumor samples that was used for SBA was examined by BIOMED-2 PCR in 17 cases. To assess the quality of the DNA, the human β-globin gene was amplified with specific primers and all cases showed a single 268-bp band for β-globin gene products (data not shown). The BIOMED-2 multiplex PCR assays (InVivoScribe Technologies, San Diego, CA, USA) of IgH (bottles A-E; for analysis of V-J and D-J gene rearrangements), TCR β (bottles A-C; for analysis of V-J and D-J gene rearrangement), and TCR γ (bottles A, B;

Table 1. Antibodies used in this study

Antibody/clone	CD-no	Main target	Source
TCR β F1/8A3		T-cell β receptor	Endogen
CD3/PS1	3ε	T cells, NK cells	Novacastra
CD4 /1F6	4	T cells, NK cells	MBL
CD8/C81/44B	8	T cells, NK cells	Dako
B1/H299	20	B cells	Coulter
CD79a/JCB117	79a	B cells	Dako
Ki1 /M723	30	Activated T and NK cells, Hodgkin's cells, B cells	Dako
ALK protein /ALK1		NPM/ALK, 80 kD, 200 kD	Dako
CD15/Leu-M1	15	Neutrophils, Hodgkin's cells and Reed Sternberg cells	Dako
EMA/E29		Epithelial cells	Dako
CD56/1B6	56	NK cells, cytotoxic T cells, neural cells	Novocastra
TIA1/NS/1-AG4		Cytotoxic granule protein, neutrophils, NK cells, T cells	Coulter
Granzyme B/11F1		Neutral serine protease, NK cells, T cells	Novocastra
Clusterin/41D		Apoprotein J, antiapoptosis, anaplastic large cell lymphoma	Upstate
CD25/4C9	25	T cells, IL 2a receptor, T cells, rare NK cells	Novocastra
Mikβ1*/9A2(DU-2)	122	IL 2β and 15 receptors, NK cells, a subset of T cells	Nichirei
CD161*/HP-3G10	161	NK cell receptor protein 1, pre NK cells, IFNγ-producing T cells	Acris
CD94*/HP-3D9	94	Killer cell inhibitory receptor, NK cells	Sero tec
CD13/32C12	13	Myelocytes, monocytes, macrophages, their precursors	Novocastra
CD68/KP-1	68	Histiocytes, monocytes, macrophages	Dako

*, examined in fresh material.

for analysis of V-J gene rearrangement), were performed using standardized protocols and primers, as recently described.[10] Following amplification the Ig/TCR-PCR products were evaluated by GeneScan analysis. Clonality was defined as unequivocal clonal Ig/TCR peaks, and low level clonality was defined as the presence of equivocal, mostly weak Ig/TCR peaks within a background of polyclonality. Finally, clear polyclonality was defined as polyclonal patterns that appeared in all multiplex PCR reactions.

Results

Clinical features

The clinicopathological data of patients with systemic primary ALK-positive and ALK-negative ALCL is summarized in Table 2. For the ALK-positive 21 cases the median age at diagnosis was 16 years old. In 18 cases, the main lesions were located in the lymph nodes and there was one case each in which the main lesion was in the skin, nose or bone marrow. Histologically, these 18 cases consisted of the common type of ALCL (Figure 1) showing diffuse infiltration of lymphoid cells with large, giant round, or slightly indented nuclei and abundant pale cytoplasm. The other cases were of the lymphohistiocytic type (2 cases) or a sarcomatous variant (1 case). The 5-year survival of 15 of the cases was 75% as assessed by the Kaplan Meier method. For the 11 ALK-negative ALCL cases the median age at diagnosis was 60 years old. The main tumor site in 10 of the cases was the lymph nodes and in one case was the oral cavity. All of the cases were the common type of ALCL. The 5-year survival of 9 of the cases examined was 25%, and Log-rank analysis revealed that the ALK-negative cases were associated with a significantly ($p<0.05$) worse prognosis than the ALK-positive cases.

Immunohistological and EBER analysis of tumor samples

The results of immunohistological and EBER analyses of the ALK-positive and ALK-negative ALCL groups are summarized in Table 3. When assayed for the presence of T cell markers, out of 21 ALK-positive cases, 4 (19%) were positive for TCR β F1, 3 (14%) for CD3, 15 (71%) for CD4, 18 (86%) for CD25 (IL2αR), 19 (95%) for clusterin and 17 (81%) for EMA. With regards to cytotoxic proteins and NK cell markers, 20 (95%) cases were positive for the cytotoxic related protein TIA1, 12 (57%) for granzyme

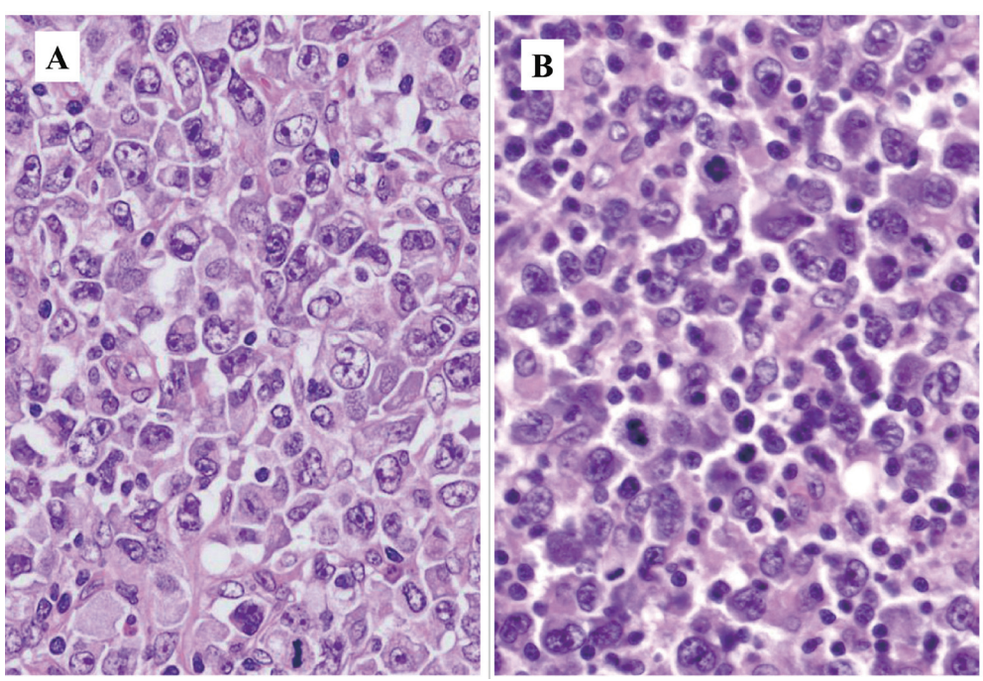

Figure 1. Similar histological findings in ALCL cases with, or without, TCR β gene rearrangement as assessed by SBA. Hematoxylin and eosin staining of ALK-positive ALCL, common type, with, (A) or without, (B) rearrangement of the TCR β gene as assessed by SBA and with clonal peaks of the TCR β gene as judged by PCR. Many intermingled small lymphocytes are observed only in Figure 1B. The images shown are at a magnification of 400X.

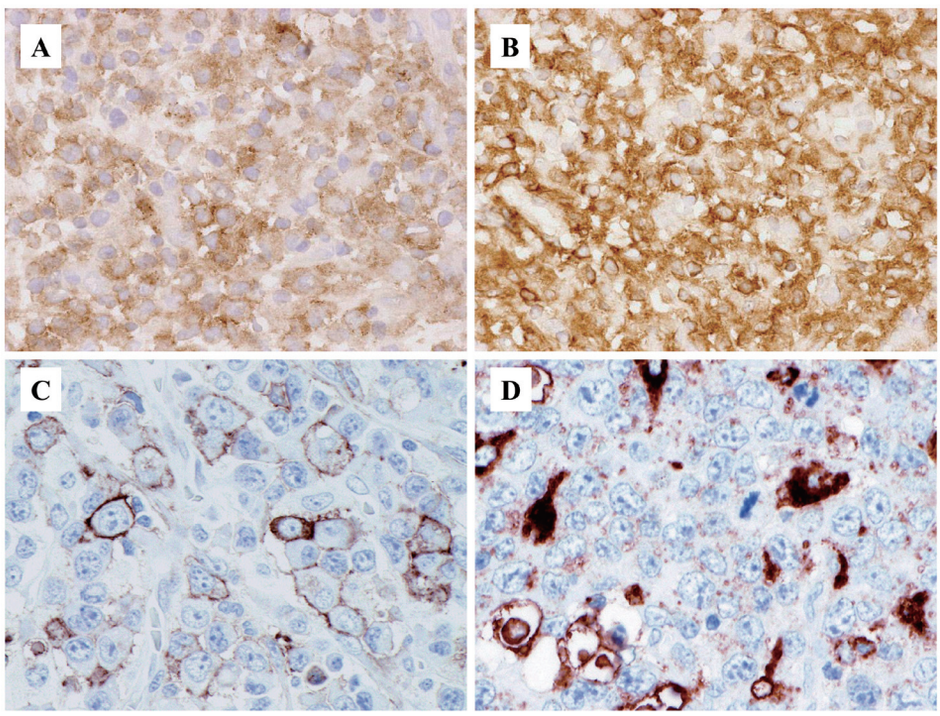

Figure 2. Expression of NK cell and myelomonocyte markers in ALK-positive and ALK-negative ALCL cases. ALCL cases were analyzed for cell markers by immunohistological analysis using the Envision™ polymer method. ALK-positive large lymphoma cells are diffusely positive for the NK cell markers (A) CD122 (IL2 β /IL15Rs, x200) and (B) CD94 (x200) and for the myelomonocyte marker CD13 (C, x400). ALK-negative lymphoma cells are positive for CD68 (D, x400). Frozen (A, B) and formalin fixed (C, D) tumor samples were analyzed with specific antibodies.

B, 10 (77%) for the T/NK cell receptor CD122 (IL2 β / IL15Rs) and 4 (40%) for the NK cell inhibitory receptor CD94, and all cases were negative for the other NK cell related protein CD161. The lymphoma cells were weakly positive for the myelomonocyte markers CD13 in 5 cases (28%) and CD68 in 4 (27%). Tumor cell expression of CD122, CD94, CD13 and CD68 is shown in Figure 2.

Out of 11 ALK-negative cases, 3 (27%) were positive for TCR βF1, 3 (27%) for CD3, 8 (73%) for CD4, 10 (91%) for CD25, 3 (27%) for clusterin and 4 (40%) for EMA. With regard to the other markers tested, 5 cases (46%) were positive for TIA1, 1 (9%) for granzyme B, 3 (27%) for CD56, 4 (44%) for CD122, 2 (22%) for CD94, 1 (10%) for CD13, and all were negative for CD68. Univariate analysis of the data indicated that the ALK-positive ALCL cases had a significantly higher expression of clusterin ($p = 0.0001$), EMA ($p = 0.05$), TIA1 ($p = 0.0029$) and granzyme B ($p = 0.01$) than the ALK-negative cases. Using ISH, EBER positive nuclear signals were detected in tumor cells of one case.

Presence of the NPM-ALK fusion gene

The expression of the NPM-ALK fusion gene in the tumors was examined by RT-PCR analysis using a specific oligonucleotide probe against the ALK-NPM junction region. The standard RT-PCR method detected the expected 177 bp NPM-ALK fusion gene in all of the 8 ALK-positive lymphoma cases examined but not in any of the 6 ALK-negative cases.

Analysis of tumor TCR- and Ig-gene rearrangements by southern blot; co-existence with immunophenotypic markers

Gene rearrangement in 22 tumor tissues was performed by SBA. Out of 13 ALK-positive ALCL cases examined, 4 (31%) showed rearrangement of the TCR β gene (Figure 3), and 1 showed a rearrangement of the TCR δ gene that suggested a Vδ1-Jδ1 pattern. When examined by histochemistry, 3 out of 4 of the ALK-positive cases with TCR gene rearrangements were positive for TCR βF1 expression. All 4 cases were negative for the myelomonocyte markers CD13 and CD68. Five cases that had a germline configuration of the TCR genes

Table 2. Clinical features and histology of 32 cases of systemic anaplastic large cell lymphoma

	ALK positive	ALK negative	total
Number of cases	21	11	32
Median (range)	16 yo (3-42)	60 yo (41-69)	31 yo (3-69)
Male : Female	14 : 7	8 : 3	22 : 10
Site of biopsy	LN: 18	LN: 10	LN: 28
	Nose: 1	Oral cavity: 1	Nose: 1
	BM: 1		Oral cavity: 1
	Skin: 1		BM: 1
			Skin: 1
Histological type			
Common	18	11	29
Lymphohistiocytic	2	0	2
Sarcomatoid	1	0	1
5-year survival*	75%	25%	57%

ALK, anaplastic lymphoma kinase; LN, lymph node; BM, bone marrow; *, significant ($p<0.05$) difference between 2 groups.

Table 3. Immunohistological findings, nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) and rearrangements of TCR and IgH genes in systemic anaplastic large cell lymphoma

	ALK-positive	ALK-negative	total	p†
No of cases	21	11	32	-
Clusterin	20 (95%)	3/10 (30%)	23/30 (77%)	.0003
EMA	17 (81%)	4/10 (40%)	21/31 (68%)	.05
TCR β F1	4 (19%)	3 (27%)	7 (22%)	.66
CD3	3 (14%)	3 (27%)	6 (19%)	.39
CD4	15 (71%)	8 (73%)	23 (72%)	-
CD8	3 (14%)	2 (18%)	5 (16%)	-
CD25 (IL2αR)	18 (86%)	10 (91%)	28 (88%)	-
TIA1	20 (95%)	5 (46%)	25 (78%)	.003
Granzyme B	12 (57%)	1 (9%)	13 (41%)	.01
CD122(IL2β /IL15Rs)	10/13 (77%)	4/9 (44%)	14/22 (64%)	.19
CD56	2 (10%)	3 (27%)	5 (16%)	.31
CD94	4/10 (40%)	2/9 (22%)	6/19 (32%)	.63
CD13	5/18 (28%)	1/10 (10%)	6/28 (21%)	.37
CD68	4 */15 (27%)	0/10	4/25 (16%)	.12
NPM-ALK gene (RT-PCR)	8/8 (100%)	0/6	8/14 (57%)	.0003
Southern blot analysis (SBA)				
TCR β	4/13 (31%)	3/9 (33%)	7/22 (32%)	-
TCRδ	1/13** (8%)	0/9**	1/22 (5%)	-
IgH	0/13	0/9	0/22	-
Polymerase chain reaction (PCR)				
TCR β VDJ (bottles A and B)	6/9 (67%)	3/8 (38%)	9/17 (53%)	.35
TCR β DJ	7/9 (78%)	4/8 (50%)	11/17 (65%)	.33
TCR γ VDJ (bottles A and B)	8/9 (89%)	5/8 (63%)	13/17 (77%)	.29
IgH	1/9 (11%)	1/8 (13%)	2/17 (12%)	-

† : ALK-positive versus ALK-negative. RT, reverse transcriptase; SBA: southern blot analysis; PCR, polymerase chain reaction. #/#, # of positive / total # of cases examined; *, tumor cells are weakly positive for CD68; **, deletion of TCR δ genes is seen in 1 ALK-positive and 3 ALK-negative cases.

were positive for CD13 and/or CD68. For the ALK-negative ALCL cases, 3 (33%) of the 9 cases examined showed a rearrangement of the TCR β gene. Two of these 3 cases were positive for TCR βF1 and CD3 by immunohistochemistry, and all 3 cases were negative for CD13 and CD68. One ALK-positive and 3 ALK-negative cases showed a deletion of the TCR δ gene. Germline configuration of the IgH gene was confirmed in all ALCL cases.

Clonality analysis of immunoassociated genes by BIOMED-2 PCR combined with histological analysis

Clonality analysis of Ig- and TCR-gene rearrangements was carried out by the BIOMED-2 PCR method on 17 cases of ALCL, and the results is shown in Table 4. Out of 9 ALK-positive cases examined, clonal VDJ peaks of the TCR β gene were found in 6 (67%, Figure 4), clonal DJ peaks of the TCR β in 7 (78%), and clonal VDJ peaks of the TCR γ in 8 (89%). One case showed a clonal VDJ peak of IgH, suggesting either a mixed lineage or the presence of reactive small clone. Out of 8 ALK-negative cases examined, clonal VDJ peaks of TCR β were found in 3 (38%), clonal DJ peaks of TCR β in 4 (50%) and clonal VDJ peaks of TCR γ in 5 (63%). One case showed clonal VDJ peaks of IgH. Two of 3 ALK-positive and 1 of 2 ALK-negative cases with germline configuration of TCR genes by SBA showed clonal VDJ peaks of the TCR β gene by PCR. Histological analysis of the immunophenotype of these cases indicated the presence of many scattered CD3- and TCR βF1-positive small reactive T cells among tumor cells (Figure 1B). Three ALK-positive cases with germline configuration of TCR genes by SBA and no clonal VDJ peaks of TCR β gene by PCR were positive for CD13 and/or CD68.

Immunohistological and genotypic characteristics of cutaneous CD30-positive ALCL and ATL/L, ALC type tumors

The 6 patients with cutaneous ALCL were all ALK-negative. The 5 cases (83%) were positive for TCR βF1, 1 (17%) for CD3, 5 (83%) for CD25 (IL2α R), and 1 (17%) for each of TIA1 and granzyme B. All cases were positive for CD4. By SBA, 3 of the cases (50%) showed a rearrangement of the TCR β gene, and another one (17%) showed a rearranged band of the TCR δ gene that suggested a Vδ1-Jδ1 pattern. In the BIOMED-2 assay, clonal VDJ peaks of TCR β and γ genes were detected in 4 (67%) and 6 cases (100%), respectively. In ATL/L, ALC type, 9 cases (64%) were positive for TCR βF1, 8 (57%) for CD3, 13 (86%) for CD4, and 14 (86%) for CD25 (IL2α R). By SBA, 12 of the cases (85%) showed rearrangement of the TCR β gene. In the BIOMED-2 assay, all 5 of the ATL/L cases examined showed clonal, complete, VDJ peaks of TCR β and γ genes. Two (40%) of the 5 ATL/L cases showed clonal DJ peaks of TCR β.

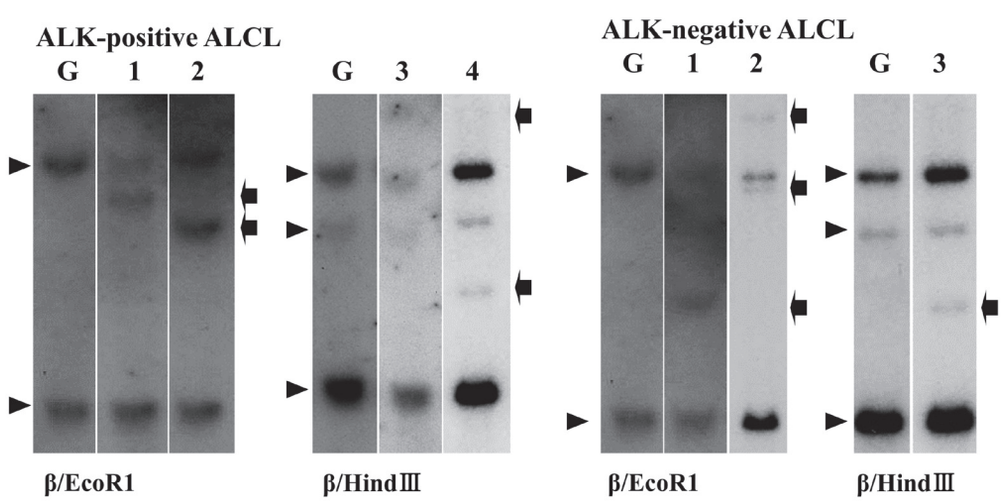

Figure 3. Southern blot analysis of the TCR β gene in systemic ALCL. High molecular weight DNA from fresh ALCL tumors (lanes 1-4), or control germline (placental DNA, G) was digested with EcoR1 or HindIII as indicated, and, following electrophoresis, was hybridized to a ^{32}P-labeled TCR C β probe. Rearranged bands, indicated by arrows, are detected in 4 ALK-positive cases (Nos. 1-4) and 3 ALK-negative cases (Nos. 1-3). Arrowheads indicate germline bands.

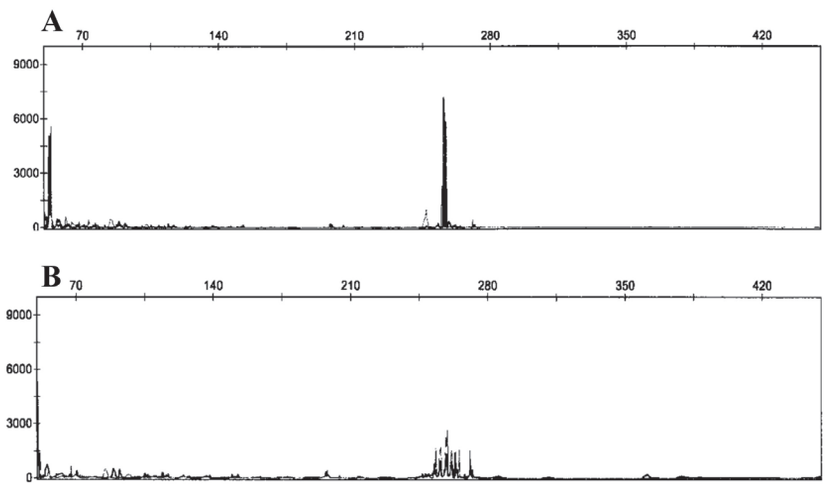

Figure 4. BIOMED-2 multiplex PCR GeneScan analysis of the TCR β VDJ gene in 2 ALK-positive cases. Clonality of 2 ALK-positive ALCL cases was analyzed by multiplex PCR GeneScan analysis using primers specific for IgH, TCR β and TCR γ gene rearrangements. Two closed, clear clonal peaks were detected at a size of 255 bp (A). Several small peaks were detected between 240 and 285 bp (B).

Discussion

Analysis of the rearrangement of TCR genes in ALCL samples is important to determine the exact origin of ALCL cells. Filippa et al.[12] reported that 5 (56%) of the 9 ALK-positive ALCL cases and 3 (60%) of the 5 ALK-negative cases showed a rearrangement of TCR genes by SBA. Other studies have also detected rearrangement of the TCR β gene by SBA, including rearrangements in 13 (59%) of 22 ALCL cases tested, [13] 8 (42%) of 17 cases tested[14] and rearrangement in the 3 ALCL cell lines.[25,26] In the present study, 4 (31%) of the ALK-positive and 3 (33%) of the ALK-negative ALCL cases showed rearrangement of the TCR β gene by SBA. Therefore, based on SBA, the combined data of the present and previous studies strongly suggest that approximately half, or more, of systemic ALCL cases are not derived from mature T cells. In contrast to the results of SBA, a higher number of cases, 19 (91%) of 21 ALCL cases, including 2 cutaneous cases, have been reported to show clonal rearrangement of the TCR β gene by PCR analysis.[6] It has also been demonstrated that among 43 cases of ALCL, 68% of the ALK-positive and 78% of the ALK-negative cases showed clonal VDJ peaks of the TCR β gene in the BIOMED-2 assay.[8] In the present study, 6 (67%) and 8 (89%) of ALK-positive ALCL cases showed clonal VDJ peaks of TCR β and δ genes, respectively, in the BIOMED-2 assay. Although the BIOMED-2 assay is an improved reliable method for detection of T or B cell clonality, 10% of reactive lymphoproliferations and 16% of B-cell lymphomas showed clonal VDJ or DJ peaks of TCR β genes, suggesting derivation from a mixed lineage or the presence of reactive small clone.[27,28] It is therefore strongly speculated that the BIOMED-2 analysis might be detecting infiltrating reactive T cells around the tumor cells in about one-third of the ALK-positive as well as the ALK-negative ALCL cases. The conclusion drawn from these results is that data derived from SBA is a better indicator of the origin of ALCL than BIOMED-2 analysis.

It has been reported that, out of 24 systemic ALK-positive ALCL cases examined, only 1 (4%) showed expression of TCR βF1, CD3, and CD3 ZAP70.[9] In ALK-negative ALCL, 9 (60%) of 15 cases examined showed expression of CD3, but only 1 case was positive for TCR βF1 and ZAP70. Among the 12 ALK-positive and 7 ALK-negative cases examined, 14 (74%) cases showed clonal VDJ or DJ peaks of TCR β and γ genes, respectively by BIOMED-2 analysis. It was suggested that mutations in the coding or the regulatory regions of their genes or a lack of TCR-specific transcription factors may underlie the absence of TCR-related proteins in systemic ALCL. In the present study, less than 30% of each ALK-positive and ALK-negative ALCL cases were positive for TCR βF1 and CD3. However, 4 (57%) and 3 (43%) of 7 ALCL cases with TCR β gene rearrangement as assessed by SBA were positive for the presence of TCR βF1 and CD3, respectively. In the control group, 9 (75%) out of 12 ATL/L ALC type cases with rearranged TCR β genes

Table 4. Analysis of TCR genes and the immunoglobulin gene by southern blot, BIOMED-2 PCR and immunohistochemistry in ALK-positive and ALK-negative systemic anaplastic large cell lymphomas

No of cases	Age	Sex	Southern blot analysis			Polymerase chain reaction				Immunophenotype				
			TCR β	δ	IgH	TCR β VDJ*	TCR β DJ	TCR γ VDJ	IgH	β F1	CD3	TIA1	CD13	CD68
ALK-positive anaplastic large cell lymphoma														
1	10	M	G/R	G	G	−/+	+	+	−	−	−	+	−	−
2	12	M	R/R	G	G	+/−	+	+	−	+	−	+	−	−
3	16	F	R/G	G	G	+/−	+	+	−	+	−	+	−	−
4	7	F	G/G	del	G	+/−	−	+	+	−	+	+	−	−
5	7	M	G/G	G	G	−/−	+	+	−	−	−	+	+	−
6	32	M	G/G	G	G	−/−	−	−	−	−	−	+	+	+
7	20	M	G/G	G	G	−/−	+	+	−	+	−	+	−	+
8	12	F	G/G	G	G	+/−	+	+	−	−	−	−	−	−
9	16	M	G/G	G	G	−/+	+	+	−	−	−	+	−	−
total			3 (33%)	0	0	4/2 (67%)	7 (78%)	8 (89%)	1 (11%)	3 (33%)	1 (11%)	8 (89%)	2 (22%)	2 (22%)
ALK-negative anaplastic large cell lymphoma														
1	48	M	R/R	G	G	+/+	+	+	−	+	+	−	−	−
2	64	M	R/R	del	G	+/−	−	+	−	−	−	−	−	−
3	73	F	R/R	G	G	−/−	+	+	−	+	+	−	−	−
4	53	M	G/G	del	G	+/−	+	+	−	−	−	−	−	−
5	67	M	G/G	del	G	−/−	+	+	−	−	−	−	−	−
6	72	M	G/G	G	G	−/−	−	−	−	−	−	+	−	−
7	50	M	G/G	G	G	−/−	−	−	−	−	−	+	−	−
8	57	M	G/G	G	G	−/−	−	−	+	−	−	+	−	−
total			3 (38%)	0	0	3 / 1 (38%)	4 (50%)	5 (63%)	1 (13%)	2 (25%)	2 (25%)	3 (38%)	0	0

R, rearrangement; G: germline; del, deletion of TCR δ gene; *, examined by bottles A and B primer sets.

as assessed by SBA were positive for TCR β F1, and 7 cases (58%) were positive for CD3. Infrequent expression of T-cell related proteins is a characteristics finding of systemic ALCL. Thus, T-cell related antigens, especially TCR β F1, tended to be expressed in systemic ALCL cases that showed rearrangement of the TCR β gene by SBA.

It was of interest to determine the expression of key signaling molecules involved in the development, regulation and function of NK and cytotoxic T cells in the ALCL cases in this study. The molecules tested included CD122 (IL2 β /IL15 receptor subunits),[29] and CD94, a major NK cell receptor that controls the inhibitory function of NK cells and CD8-positive T cells.[30] Nasal type CD56-positive NK cell lymphoma, aggressive NK cell and CD8-positive T-LGL leukemia all continue to express CD122 (IL2 β /IL15Rs).[31-33] Furthermore, during NK cell development, CD122 (IL2 β /IL15Rs)-, CD161-positive and CD94-negative precursor NK cells develop into CD94-positive immature and mature NK cells following the interaction of IL15.[34] It has been speculated that CD94 is a good marker both of normal NK cells and of NK-cell neoplasms.[30,35] In this study, 10 (77%) ALK-positive and 4 (44%) ALK-negative ALCL cases were positive for the expression of CD122 (IL2 β /IL15Rs), and one-third of the combined ALCL cases expressed CD94. All cases were negative for CD161. Therefore, systemic ALCL, especially the ALK-positive cases, expressed receptors and cytotoxic molecules that are characteristic of immature or mature NK cells.

In NK cell neoplasms, the nasal type of NK cell lymphoma predominantly shows an angiodestructive features, constant integration of EBV, a germline configuration of TCR genes and pursues a progressive clinical course.[36] Systemic ALK-positive ALCL, with or without rearrangement of TCR genes as assessed by SBA, were mostly positive for TIA1, showed no angiodestruction, rare EBV infection and mostly express clusterin, which functions in cell aggregation and controls apoptosis.[37] Clusterin is frequently expressed in systemic ALCL, but rarely in T-lymphoblastic leukemia, peripheral T/NK-cell lymphoma,[38] or in the ATL/L ALC cases that have been examined. It has been suggested that ALK-positive ALCL show local tumor cell aggregation that is mediated by clusterin as well as by other cell adhesion molecules,[39] and that it is weakly cytotoxic.

In the present study, the expression of the cytotoxic molecule granzyme B was absent in 9 (43%) of the ALK-positive cases. The characteristics of the tumor cells could potentially be influenced by clinical features of the patients such as a lower incidence of leukemic changes and an indolent clinical course. Although a few examined cases expressed CD56, ALK-positive ALCL had different clinicopathological findings from those of other mature T/NK cell neoplasms.

Both previous reports[4,5] and our results support the fact that ALK-negative and ALK-positive ALCL cases show different clinicopathological findings and have a different prognosis. The ALK-negative ALCL cases examined showed a significantly ($p<0.01$) lower expression of clusterin and the cytotoxic molecules TIA1 and granzyme B than the ALK-positive cases. Only one third of the ALC-negative cases examined showed a rearrangement of TCR genes by SBA leading to the speculation that ALCL cases that do not have T, NK cell or myelomonocyte characteristics were included within this group.

CD13 is a cell adhesion molecule that is expressed in myelomonocytes at every maturation stage, at the early stages of T and B cell development and occasionally in lymphoblastic leukemia cells.[40] It has been reported that expressions of CD7 and CD13 were reported in 8 of 25 cases (32%) and 7 of 9 cases (78%) of ALCL, respectively, by flow cytometry.[17] This study group concluded that there was aberrant, biphenotypic expression of CD13 in systemic ALK-positive ALCL. A second study reported a case of a CD30- and ALK-positive leukemic neoplasm that was positive for the myelomonocytic antigen CD33 as well as CD122 (IL2β/IL15Rs) and granzyme B, was negative for CD3 and TCR receptor proteins, and showed a germline configuration of TCR genes by SBA.[41] It was suggested that this case may have been derived from putative myeloid-NK precursor cells. In the present study, 7 of 19 cases (37%) of ALK-positive ALCL expressed the myelomonocyte markers CD13 and/or CD68, and 5 of the cases examined showed no rearrangement of the TCR gene by SBA. One (10%) of the ALK-negative ALCL cases also expressed CD13. It has been speculated that macrophage-T progenitors have the potential to become T cells, NK cells and dendritic cells after the potential to form B-cells has been shut down in the mouse thymus (Figure 5).[18] It has also been speculated that ALK-positive and ALK-negative ALCL has phenotypic and genotypic characteristics of macrophage-T progenitor cells. These cells have the potential to differentiate into peripheral T, NK cells and histiomonocytes. It has been confirmed that CD30-positive tumor cells from a classical Hodgkin lymphoma were derived from germinal center B cells. These cells showed clonality of the IgH gene by PCR using microdissection, but showed a germline configuration of the IgH gene by SBA. The cells also expressed low CD20 and immunoglobulin protein.[42,43] Thus, similar genotypic and phenotypic characteristics

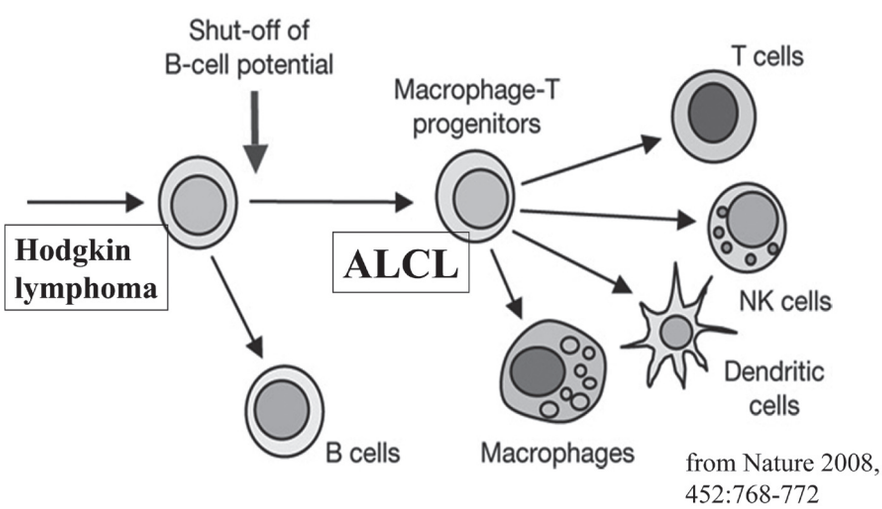

Figure 5. Proposed scheme of the cell lineage of anaplastic large cell lymphoma. This scheme is taken from Figure 4 of Wada H. et al., Nature 2008, 452:768-772, with the author's permission. ALK-positive and ALK-negative anaplastic large lymphoma cells may be derived from a macrophage-T progenitor, which has the potential to differentiate into mature T cells, NK cells, dendritic cells, and macrophages. Hodgkin lymphoma may also be derived from a myeloid-B cell progenitor.

that are representative of T and B cells have been observed in 2 different types of CD30-positive neoplasm. These data suggest that hematolymphoid progenitor macrophage-T or B cells migrated to the lymph nodes where they underwent neoplastic changes resulting in immature-type phenotypic and genotypic expressions. Cumulative detailed phenotypic and genetic studies are necessary to confirm the cellular characteristics and origins of systemic ALK-positive and ALK-negative ALCL.

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Disclosure/conflict of interest

There is no competing financial interest, and the authors have no conflicts of interest to disclose.

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