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Genetic changes in atypical hyperplasia and lymphoma with angioimmunoblastic lymphadenopathy and dysproteinaemia in the same patients

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Abstract The transition between atypical hyperplasia and lymphoma with angioimmunoblastic lymphadenopathy and dysproteinaemia (AILD) was studied in serial lymp node biopsy specimens from five patients using DNA analysis with Southern blot analysis, polymerase chain reaction, chromosomal analysis, and immunophenotyping. The chromosomal analysis showed additional abnormalities as the disease progressed to those present initially, and immunological staining showed a corresponding increase in the numbers of CD4- and Ki67-positive cells. In the first biopsy from each patient a diagnosis of atypical hyperplasia with AILD was made and lymphoma excluding by the finding of only a few atypical lymphoid cells and the preservation of follicles with germinal centres. DNA analysis of lymph nodes at this stage showed either germ lines or oligoclonal rearrangements of the T-cell receptor (TCR) and immunoglobulin heavy chain genes. In the final biopsy, when a diagnosis of lymphoma with AILD was made, either a monoclonal rearrangement of the TCR was observed or one of the rearranged bands had increased in density. These results suggest selective proliferation of a clone of abnormal cells may account for the progression of atypical hyperplasia to lymphoma with AILD.

Key words Angioimmunoblastic lymphadenopathy with dysproteinaemia · Polymerase chain reaction
T-cell receptor gene

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Introduction

Angioimmunoblastic lymphadenopathy with dysproteinaemia (AILD) is a systemic disease of uncertain aetiology in which the major pathological findings are in lymph nodes [5, 6]. Lymph node biopsy specimens typically show effacement of the normal architecture by a mixed cellular infiltrate, in which immunoblasts and plasma cells are prominent. This infiltrate is accompanied by a striking "arborizing" proliferation of blood vessels and often by deposition of acidophilic material [5, 6, 19, 24].

Nathwani and colleagues [24], reporting immunoperoxidase studies on paraffin sections in 32 cases of AILD, were unable to demonstrate monoclonal B cell populations for light and heavy chains. Shimoyama et al. [33] described both immunoblasts, including plasmacytoid forms, and pale cells possessing T-cell markers. By DNA analysis, many cases of AILD showed monoclonal rearrangements of T cell receptor (TCR) genes, although in some the rearrangements were polyclonal. A few also showed rearrangement of heavy and light chain immunoglobulin (Ig) genes, as well as the co-existing rearrangements of TCR and Ig genes [8, 18, 27, 28, 36, 41].

O'Connor and colleagues [27] proposed two hypotheses for the aetiology of AILD based on Southern blotting: (1) that a monoclonal proliferation of T-cells is always present (but not always detected since current techniques can only determine a population of over 5%) and that the characteristic pronounced polyclonal proliferation of T- and B-cells is secondary to this underlying monoclonal population; or (2) that AILD is initially a polyclonal hyperreactive disorder, affecting both T- and B-cells, and that monoclonal T-cell proliferation may arise as a secondary event.

The polymerase chain reaction (PCR), a molecular technique with sufficient sensitivity to detect the presence of minute amounts of DNA, as well as the clonality of a small cell population, has been used recently to detect the V-D-J recombination of the Ig [20] and TCR [21].

To improve understanding of the aetiology of AILD and the relationship between atypical hyperplasia and lymphoma, a comprehensive study was made in serial lymph node biopsies from five patients in whom the initial histological findings were of atypical hyperplasia, later developing into lymphoma with AILD. These studies comprised DNA investigation with PCR and Southern blotting, chromosomal examinations and immunophenotyping.

Materials and methods

Tissue specimens from files in the Department of Pathology, Fukuoka University were used for the analysis. Lymph nodes had been fixed in either buffered formalin or a B5 solution, embedded in paraffin wax, and had been stained with haematoxylin-eosin, Giemsa, periodic acidic-Schiff, and Gomori's silver impregnation. Immunostaining was performed using L26 for B-cells (Dakopatts, Glostrup, Denmark), UCHL-1 for T-cells (Dakopatts), as well as Leu-M1 (Becton-Dickinson; Mountain View, Calif.), Ber-H2 (Dakopatts), EMA (Dakopatts), S100 (Dakopatts) and DRC (Dakopatts). A portion of each lymph node had been kept in the deep-freeze at −80° C and cryostat sections of these specimens were examined using monoclonal antibodies for T-cell (CD2, CD3, CD4, CD8), B-cells (CD20, CD22) (Ortho, Becton-Dickinson, or Dakopatts), and activated cells, etc. (CD25, CD30, Ki67, Ia) (Dakopatts).

For Southern blotting part of the frozen material was used for DNA isolation and gene analysis, as previously reported [42]. The TCR $C\beta$, $J\gamma$, the immunoglobulin heavy chain (JH) gene, and the proviral DNA of HTLV-1 (full length; gag, pol, env, pX, LTR) were used as probes.

For PCR, isolated DNA was used. The consensus primers used for the amplification of the immuno-globulin heavy chain were primer VH: CTGTCGACGGCCGTGTTATTCT and primer JH: AACTGCAGAGGAGACGGTGAC, which were synthesized to correspond to the V and J regions [20]. The primers for amplification of the TCR γ chain were primer Vγ: TCTGG-(G/A)GTCTATTACTGTGC, mixed with CTCACACTC(C/T)-CACTT, primer Jγ: CAAGTGTTGTTCCACTGCC, and primer Jp: GTTACTATGAGC(T/C)TAGTCC, and were synthesized on the basis of a previously published report [21]. The primers for amplification of the TCR β1 chain were primer Dβ1: CCCGAGT-CAAGAGTGGAGCC, and primer Jβ1: CCCGAGTCAAGA-GTGGAGCC, while the primers for the TCR β2 chain were primer Dβ2: GTATCATGGTGTAACATTGTGGGG, and Jβ2: AA-GGTGGGGAGACGCCCGAAT; they were synthesized to correspond to the D and J regions [38]. Amplification was performed with the GeneAmp DNA amplification reagent kit and a DNA Thermal Cycler (Perkin-Elmer Cetus, Norwalk, Conn.). To exclude any non-specific reactions, perfect match polymerase enhancer was added (Stratagene, La Jolla, Calif.). After 30 cycles of PCR amplification, one-tenth of the reaction mixture (10 µl) was analysed by Southern blotting, using the JH, TCR Jγ, Jβ1, and Jβ2 probes. DNA from lymph nodes showing nonspecific lymphadenitis were used as controls. Chromosomal analyses were performed using freshly suspended lymph nodes.

Results

Clinical data (Table 1)

Lymph nodes were obtained from four men and one woman aged between 50 and 61 years. These patients had been followed for between 13 and 44 months; one patient died of pneumonia but the others are still alive. Generalized lymphadenopathy at presentation was observed in only one patient, but appeared later in the other four. Lactate dehydrogenase (LDH) was abnormally elevated in only three patients at the time of the first biopsy, but was eventually elevated in all. A skin rash was present in three and polyclonal hypergammaglobulinaemia in two patients. Mild leucocytosis with or without occasional atypical lymphocytes was seen in all patients. A diagnosis of atypical hyperplasia with AILD was made on the first biopsy in all five patients and all except case 4 received no treatment or steroid therapy alone. One patient (case 4) received chemotherapy be-

Table 1 Clinical data 1a, 1b, 2a, et al. are patient numbers, and the same number is same patient (*Aty ly* Atypical lymphocytes, *LDH* lactate dehydrogenase, *γglb* γ globulin, *VEPA* vincristine, Endoxan Adriamycin, prednisone, *VEMP* vincristine Endoxan, procarbazine, prednisone, *CHOP* Endoxan, Adriamicin, oncovin, prednisone, *R* radiation, *INF* interferon, *VP16* etoposide, *ABEP* aclacinomycin, Bihinoid ara C, etoposide, prednisone, *BCNU* bischloroethyl nitrosourea, *PR* partial remission, *NC* no change, *NE* no effect, *CR* complete remission, *M* months from the last biopsy)

Patient no.	Age (years)	Sex	Date	Biopsy site	Stage	Skin lesion	WBC (/mm^3) (Aty ly)	LDH γ-glb	Therapy	Effect	Prognosis
1a	54	M	15/10/88	Neck	I	+	6400 (0%)	→ →	None	NC	
1b			31/1/89	Neck	III	+	7100 (0%)	↑ →	VEMP, CHOP	NE	Dead (9M)
2a	50	M	18/4/86	Neck	I	−	7100 (3%)	→ →	None	NC	
2b			9/12/89	Neck	II	−	6200 (0%)	↑ →	VEPA, VEMP R(40GY)	CR	Alive (24M)
3a	55	M	13/8/85	Neck	II	−	5000 (0%)	↑ →	None	NC	
3b			14/7/86	Neck	II	−	9600 (0%)	↑ →	VEPA	NE	
3c			22/8/86	Neck	III	−	8600 (0%)	↑ →	VP16,ACNC	CR	Alive (26M)
4a	64	M	29/1/87	Neck	III	+	9200(2%)	↑ ↑	VEPA	CR	
4b			5/9/90	Inguinal	III	−	6200 (0%)	↑ →	VEPA	CR	Alive (8M)
5a	61	F	7/10/88	Neck	II	+	9700 (0%)	↑ →	Steroid	NE	
5b			29/11/88	Axilla	II	+	7500 (0%)	↑ →	INFα	CR	
5c			27/10/89	Neck	III	+	8000 (0%)	↑ ↑	INFα,VP16 ABEP	CR	
5d			15/12/90	Axilla	IV	+	7600 (0%)	↑ ↑	BCNU	CR	Alive (5M)

Table 2 Histological pattern (modified from a previous report [23])

	Histology	Nuclear atypia	No. of clear cells	No. of immunoblasts	Germinal centres
I	Atypical hyperblasia with AILD-type features	No	No	Very small or small	Preserved
IIA	Lymphoma with AILD-type features	Mild	No or minimal	Small	Diminished
IIB	Lymphoma with AILD-type features	Moderate	No or small	Moderate	Diminished
IIC	Lymphoma with AILD-type features	Severe	Moderate or large	Large	Diminished

Table 3 Chromosomal pattern (modified from a previous report [33])

Step	Cytogenetic findings
I	Normal karyotype
II	Increased number of single cell abberations
III	Establishment of aberrant clones
IV	Monoclonal proliferation of cells with +3 or +5
V	Secondary chromosomal aberrations

Table 4 Data of histology, immunology and chromosomal analysis (/:not done)

Patient no.	Histological pattern	T:B (CD3:20)	CD4:CD8	Ki67 (%)	Cytogenetic pattern
1a	I	2:1	3:2	5	I
b	IIB	10:1	5:1	30	V
2a	I	3:1	2:1	5	II
b	IIC	10:1	4:1	40	III
3a	I	2:1	3:2	2	/
b	IIA	6:1	3:1	20	IV
c	IIC	10:1	5:1	30	/
4a	I	3:1	2:1	5	/
b	IIB	8:1	4:1	20	/
5a	I	2:1	3:2	2	I
b	I	3:1	3:2	5	II
c	IIB	8:1	4:1	40	IV
d	IIC	10:1	5:1	50	IV

cause of his poor condition, generalized lymphadenopathy, and high serum LDH, as well as the demonstration of a rearrangement of TCR. During the course of their disease all patients received chemotherapy and/or radiotherapy, which produced a partial or complete remission for over 4 months, although in all the symptoms and lymphadenopathy eventually recurred.

Histopathology (Tables 2, 4; Fig. 1)

In the first biopsy specimens the nodal architecture was preserved and follicles with germinal centres were noted. There was mild vascular proliferation and an obvious polymorphic cellular infiltration of reactive cells, including a few immunoblasts, but no atypical or clear cells. A diagnosis of atypical hyperplasia with AILD was made [15], lymphoma being excluded on the basis of germinal centre preservation [17]. In the final biopsy specimens from each of the five patients, the histological diagnosis was changed from atypical hyperplasia to lymphoma with AILD. This diagnosis was based on observation of the normal nodal architecture, with loss of germinal centres and the presence of numerous high endothelial venules. The nodes were infiltrated by medium-sized lymphocytes, immunoblasts with large prominent nuclei and abundant amphophilic cytoplasm and medium-sized atypical lymphoid cells with a moderate amount of clear cytoplasm (so-called clear cells). Based on the number of clear cells and immunoblasts and the degree of nuclear atypia, this lymphoma can be graded A, B, or C (see Table 2) [22].

Immunopathology (Table 4)

In all the biopsies examined, plasma cells were polyclonal in that both kappa and lambda light chain were demonstrated. T-cells increased in numbers as the disease progressed and in biopsies were studied with frozen sections. Most of the T-cells had a CD4 phenotype (inducer/helper). In serial biopsies the numbers of CD4-positive cells increased while the CD8-positive cells decreased; B-cells were present in small numbers and their proportion remained unchanged. Most of the proliferating cells detected by Ki67 were clear cells and their numbers increased with time. Some T-cells also showed activated antigens of Ia (HLA-DR) and CD25.

Southern blot analysis (Table 5, Fig. 2)

One lymph node (from case 5) showed rearrangements of both Ig heavy chain genes and TCR. At some stage in the disease process, specimens from all five patients exhibited rearrangement of TCR. The initial lymph node biopsies from patient 1, 2 and 5 showed germ lines of TCR β, but the final biopsies from all five patients showed a monoclonal rearranged band of TCR β and/or γ, in which the density increased during the observation period. In patient 5, DNA analysis showed oligoclonal rearranged bands for TCR and Ig. The rearranged band for TCR-β, using the restriction enzyme EcoRI, increased in density from the second biopsy and the germline band was deleted. However, using *Hin*dIII the different rearranged bands appeared at the second biopsy and disappeared in the third and final specimens. These findings indicate that only the clone of the rearranged band using EcoRI actually increased with time. No specimens

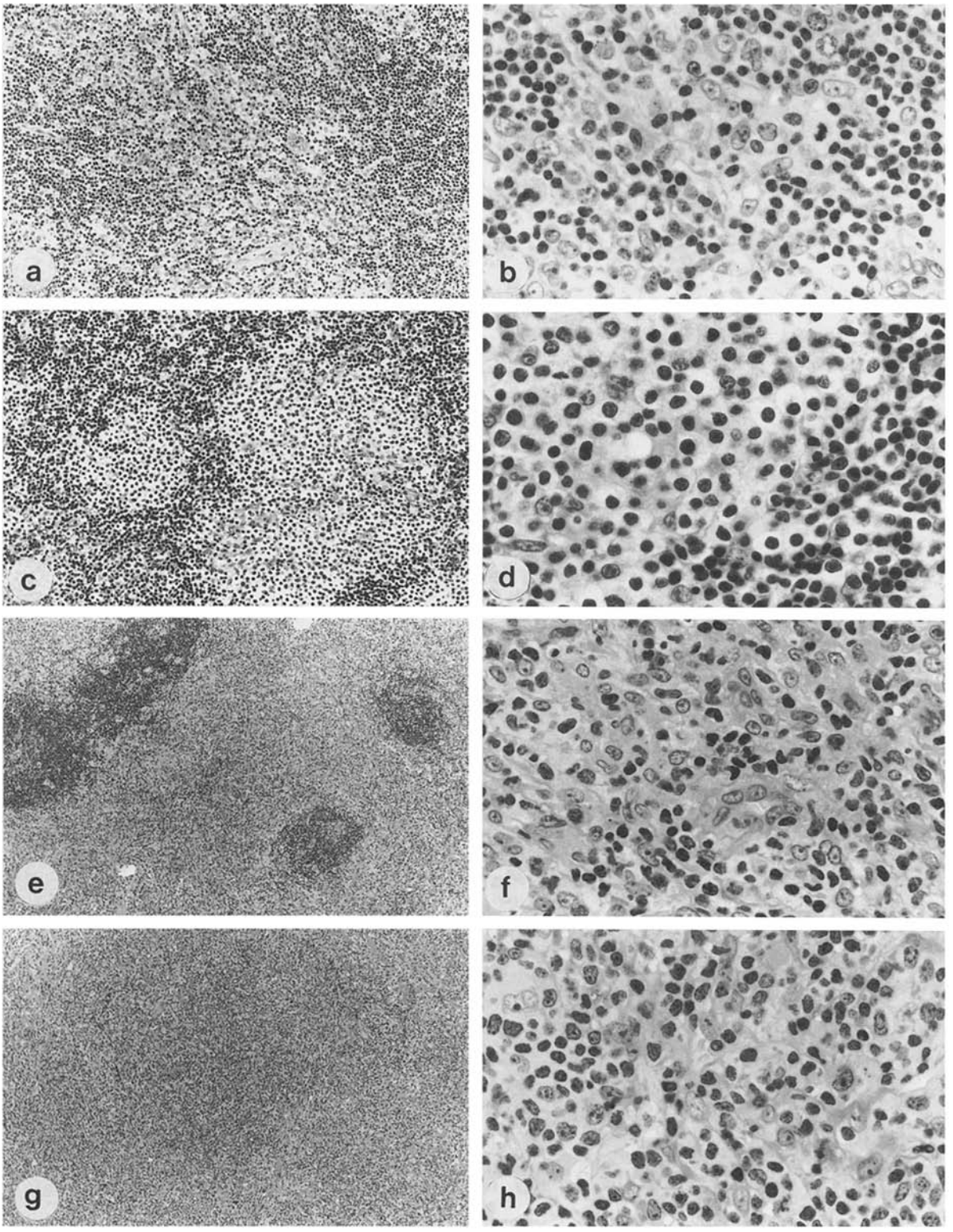

Fig. 1 a, b Case 3 a, **c, d** case 3 c, **e, f** case 5 a, **g, h** case 5 d (cases 3 a, c, d and cases 5 a and d are the patient numbers in Table 1). **a** Decreased lymph follicles with germinal centres with an expanded T-zone. **b** Scattered immunoblasts. **c, d** Foci of clear cells. **e** Lymph follicles with germinal centre and an expanded T-zone. **f** A few immunoblasts near the vessels. **g** Diminished lymph follicles. **h** Some clear cells and large lymphoid cells with nuclear atypia

showed any proviral HTLV-1 DNA integration. The 15 control lymph node biopsies showing non-specific lympadenitis exhibited no changes in either TCR or Ig.

PCR analysis (Table 5, Fig. 3)

One patient (case 5) demonstrated two clonal recombinated bands of Ig VH/JH in the first biopsy, but the others

Table 5 DNA analysis (*G* germ line, *R* rearrangement, large population, *r* rearrangement, small population, *number* indicates number of rearranged bands, *s* smear band, *number* indicates number of PCR produced bands), [a,b,c] show a different band length

Patient no.	Southern blot				PCR				
	TCR Cβ		Jγ	JH	TCR				Ig
	EcoRI	HindIII	EcoRI	BamHI	Dβ1/Jβ1	Dβ2/Jβ2	Vγ/Jγ	Vγ/Jp	VH/JH
1a	G	G	r	G	5	0	1	0	s
b	G	r	R+r	G	1	3	1	1	s
2a	G	G	r	G	5	3	1	1	s
b	G	G	r	G	5	2	1	1	s
3a	G	G	G	G	0	1	0	0	s
b	R	R	R	G	3	1	1	0	s
c	R	R	R	G	0	1	1	0	s
4a	G	r	R	G	3	3	1	1	s
b	G	R	R	G	3	2	1	1	s
5a	r	G	2r	r	4	3	0	1[a]	2
b	R	r	2r	G	2	3	0	1[b]	s
c	R	r	2r	G	2	1	0	1[c]	s
d	R	G	R	G	1	1	0	1[c]	s

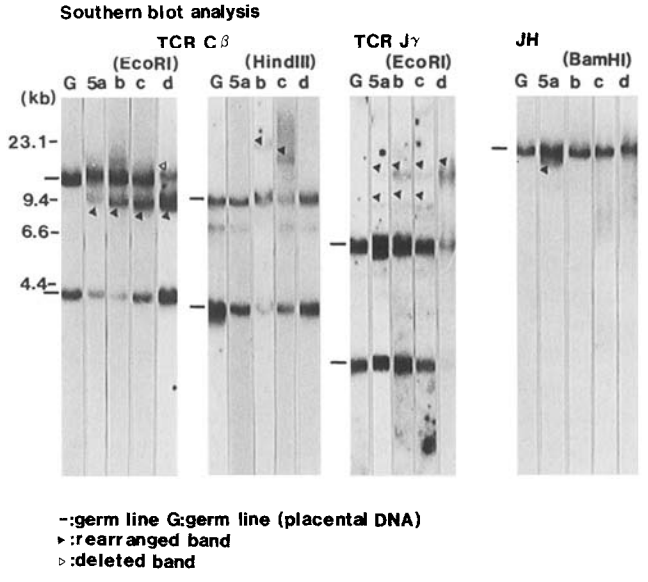

Fig 2 Southern blotting analysis of cases 5 a, b, c, and d. Oligoclonal rearranged bands for T-cell receptor (TCR) and Ig (JH) genes were seen during the observation period. An increased density of the rearranged band of TCR-β was noted using the restriction enzyme EcoRI in serial biopsy for this patient

showed smear bands, which probably reflect a polyclonal population of B-lymphocytes. Specimens from all patients (except patient 3), showed monoclonal recombinated bands of TCR Vγ/Jγ and/or Vγ/Jp during the observation period. All the initial biopsy, specimens showed oligoclonal recombined bands of TCR Dβ1/Jβ1 and/or Dβ2/Jβ2, but in the final biopsy the number of the recombined bands decreased. In patient 5, Dβ1/Jβ1 and Dβ2/Jβ2 showed four and three clonal bands initially, but these were reduced to monoclonal bands in the final biopsy. The preserved band increased in density during the observation period. This probably indicates that one population was selected from initial oligoclonal populations during the development of the disease, reflecting the similar changes observed by Southern blot analysis. Dβ1/Jβ1 and Dβ2/Jβ2 showed oligoclonal bands, while Vγ/Jp showed different monoclonal bands during the observation period, and these findings may be due to the detection of only major clones.

Chromosome analysis (Tables 3, 4)

The chromosomal abnormalities were divided into five groups, as described by Schlegelberger et al. [32]. Four patients were analysed, and only one (case 5) showed normal cells in the initial biopsy, the other patients exhibiting non-clonal abnormalities. In patients 1 and 5, in whom chromosome analyses were performed more than twice, +3 and +18 abnormalities were constantly present in all specimens, and other additional abnormalities occurred in the later specimens.

Discussion

Angioimmunoblastic lymphadenopathy with dysproteinaemia (AILD) is a systemic disease of uncertain aetiology, originally defined as a non-neoplastic lymphoproliferative process [5, 6]. Similar conditions have been reported under the names of immunoblastic lymphadenopathy and lymphogranulomatosis X [16, 19]. AILD is characterized by minor nuclear atypicalities and reactive features such as prominent vascularity, cellular infiltrates, and residual germinal centres. The appearances indicate a hyperplastic/dysplastic state of T-cell [35], from which lymphoma could develop and this process is illustrated by evolution from hyperplasia to lymphoma described here. However, it is also possible that the reactive features are themselves evoked by the development of the lymphoma.

Many immunophenotypic studies of lesions with morphological features of AILD have been performed, mostly on paraffin-embedded tissue. Some of these have indicated a primary T-cell [13, 31, 34], and others a B-cell proliferation [1, 2, 25]. With the application of mono-

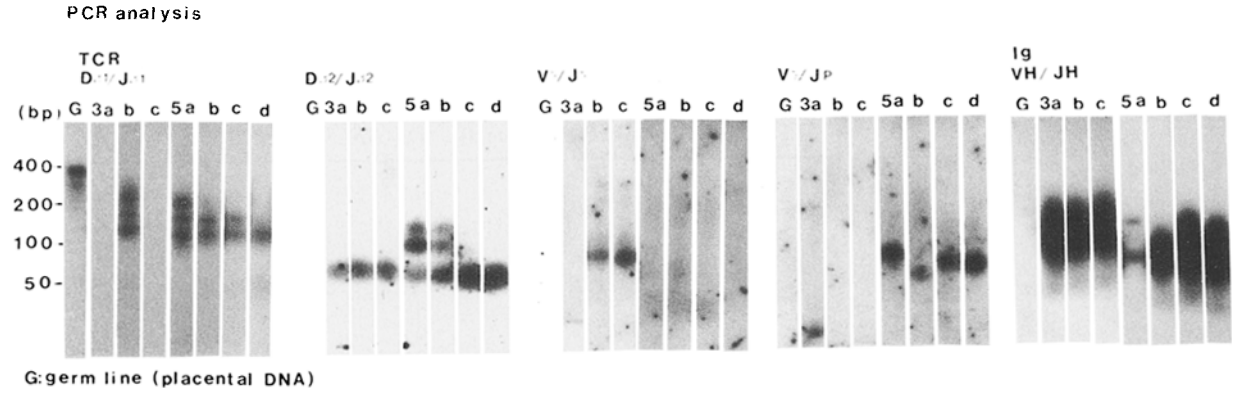

Fig 3 Polymerase chain reaction (PCR) analysis of cases 3 a, b, c, cases 5 a, b, c, and d. Oligoclonal recombinated bands for TCR Dβ1/Jβ1 and/or Dβ2/Jβ2 were seen. Two clonal bands for Ig VH/JH were detected in the initial biopsy, but subsequently only smear bands were observed

clonal antibodies to frozen sections, the general consensus is that AILD represents a predominantly T-cell proliferation [10, 23, 28, 40, 41], and most reports describe a distinct predominance of the CD4 (helper/inducer) phenotype [10] or increased ratios of CD4 to CD8 (cytotoxic/suppressor) phenotypes [28, 41]. All our patients eventually showed a CD4 phenotype and the CD4-positive cells increased in number with time.

Previously, double immunoenzymatic staining to determine the surface phenotype of proliferating lymphocytes in AILD [23, 37] has shown that most are of the CD4 phenotype. In this study, immunological staining showed an increase of CD4- and Ki67-positive cells during the course of the disease, indicating that the proliferating cells were probably of the CD4 phenotype. Proliferation of CD4-positive cells also correlated with a clonal TCR rearrangement, as previously reported [37].

Previous chromosomal analyses of AILD has demonstrated an unstable coexistence of normal mitotic cells and small clonal and/or nonclonal abnormal cells [11, 12, 32]. Such heterogeneity corresponds well with other data previously provided by DNA hybridization studies [8, 27, 36]. Our data also show an oligoclonal population, so that lymphoma with AILD need not necessarily be monoclonal.

In recent DNA studies the cell lineage of clonal populations in AILD has been determined in tissue biopsies obtained either at presentation or later in the course of the disease [8, 18, 27–29, 41]. Frizzera and co-workers [7] summarized previous studies using Southern blotting and showed that in 85% there is a rearrangement of the TCR-β gene. In addition, 8% had rearrangements of both the Ig heavy chain and one light chain gene, or of the lambda chain gene only, indicating the presence of B-cell clones. Also, 7% had co-existing rearrangements of the TCR-β and Ig heavy or light chain genes, indicating the presence of either two different clonal populations or one clone of indefinite lineage, carrying both TCR-β and Ig gene rearrangements. It is significant that in AILD, as in other atypical lymphoproliferative disorders [9, 30], the rearrangement bands have been noted to be multiple and "significantly lighter in intensity" than normal bands [18, 41]. In addition, in one study, multiple different clones appeared and disappeared with time in the same patient [18]. Weiss and co-workers [41] provided evidence that a "prelymphomatous" lesion may exist, and in one patient an initial lymph node biopsy specimen interpreted as AILD showed no evidence of immunoglobulin or TCR-β gene rearrangement. One year later, a splenectomy specimen showed histological and immunological features supportive of lymphoma as well as immunogenotypic evidence of a monoclonal T-cell lymphoma. O'Connor et al. [27] advocated two hypotheses concerning the aetiology of AILD. If a monoclonal proliferation of T-cells is always present, the pronounced polyclonal proliferation of T- and B-cells characteristic of this disorder may merely reflect a reaction to the underlying monoclonal population. However, an alternative hypothesis is that AILD is initially a polyclonal hyperreactive disorder affecting both T- and B-cells, and that T-cell clones arise as a secondary event. Those hypotheses are based primarily on the gene rearrangements seen by Southern blotting, which cannot detect a monoclonal population of either B- or T-cells when they form less than 5% of the cells present in tissue samples [3, 26]. However, PCR can detect smaller monoclonal populations of under 2% [39]. Our PCR data support the selective proliferation of one clone of T-cells among several clones, rather than an initial monoclonal proliferation of T-cells.

Using genetic, phenotypic, and morphological features, the classification of AILD into three different types has been proposed by Frizzera et al. [7] as follows: (1) those examples without evidence of clonality by immunophenotypic, immunogenotypic, or cytogenetic parameters, for which the term AILD alone might be reserved; (2) those with evidence of clonality by all parameters, to be called AILD-type lymphoma; and (3) those with clonality by one or two, but not all three parameters, for which the term AILD-like dysplasia has been proposed. This intermediate group seems to be composed of unstable lymphoproliferative conditions, in which a predominant component of normal cells co-exists with one or more clonal populations that may either

disappear with time or selectively proliferate and develop into frank lymphoma. In our study, on initial biopsy, all the patients were diagnosed as atypical hyperplasia with AILD. By genetic criteria, patient 3 might be categorized as AILD, patients 1, 2 and 5 as AILD-like dysplasia, and patient 4 as AILD-type lymphoma. On the final biopsies, all were categorized as AILD-type lymphoma. During the observation period, PCR, Southern blotting and chromosomal analysis all indicated that a monoclonal population had been selected from original oligoclonal populations of T-and/or B-lymphocytes. In Burkitt's lymphoma, it is suggested that the lymphoma arises in the setting of chronic Epstein-Barr virus stimulation, in which B-cells that undergo repeated cycles of cell division ultimately undergo chromosomal translocations involving the c-myc oncogene and one of the immunoglobulin genes [4, 14]. Similarly, as a disorder of cellular immunoregulation in which lymphoid cells may escape normal growth controls, AILD provides a model of selective proliferation of a malignant clone.

There appears to be some correlation between the percentage of normal mitotic cells and survival in AILD [12], and there remains a significant difference in the survival curves between the groups with and without TCR-β rearrangement [37]. Thus, the finding of either oligoclonal or monoclonal T-cells by PCR may be useful in diagnosis and in determining prognosis. However, it is noteworthy that Schlegelberger et al. [32] reported a patient with clonal chromosomal abnormalities who survived without treatment.

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