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Angioimmunoblastic lymphadenopathy type of T-cell lymphoma and angioimmunoblastic lymphadenopathy: a clinicopathological and molecular biological study of 13 Chinese patients using polymerase chain reaction and paraffin-embedded tissues

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Abstract The morphological classification of angioimmunoblastic lymphadenopathy (AILD) or T-cell lymphoma of AILD-type (AILD-TCL) is still a subject of considerable difficulty and controversy. The aim of the current study was to examine the value of clinical, morphological, immunohistochemical variables in paraffin-embedded tissues in predicting the clonality of the respective lesion. Fifteen lymph node biopsies derived from 13 patients from Chengdu, China, were diagnosed as AILD or AILD-TCL and included in this study. The specimes were examined using a panel of monoclonal antibodies and a scoring system of morphological features. Clonality of the paraffin-embedded material was investigated using a novel polymerase chain reaction-technique to amplify rearranged T-cell receptor (TCR)-γ sequences. Additional experiments were carried out to investigate the presence of clonal rearrangements of the immunoglobulin heavy chain (IgH) locus. We found clonal rearrangements of the TCR-γ locus in 9 out of 15 lymph node biopsies. In 3 patients, the predominant cell clones carried clonal IgH and TCR-γ rearrangements whereas 1 patient with polyclonal TCR-γ pattern displayed IgH-monoclonality. The statistical evaluation of morphological and immunohistochemical data indicated that no single variable was able significantly to predict the clonality of the lesion. Furthermore, demonstrable clonality for the TCR-γ or the IgH loci of a lesion did not correlate with a bad clinical course. Our data correlate with findings of other studies investigating AILD-TCL in Caucasian populations.

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Introduction

Since its primary description by Frizzera et al. [10], the histological diagnosis and classification of "angioimmunoblastic lymphadenopathy with dysproteinaemia" (AILD) has been an area of considerable difficulty and controversy. Different designations have been employed to accommodate a spectrum of apparently related lymph node lesions. Lukes and Tindle [22] used the term "immunoblastic lymphadenopathy" (IBL) and Radaszkiewicz and Lennert [29a] advocated the use of "lymphogranulomatosis X" because of similarities to Hodgkin´s disease. Whereas it was first regarded as a reactive or prelymphomatous B-cell disorder, Shimoyama et al. [29b] described cases of similar morphology with clinical courses of peripheral T-cell lymphomas (TCL) which they called "IBL-like TCL". Morphologically, there exists a continuous spectrum of lymph node lesions. Several groups have devised schemes to subtype the varying appearance of AILD-like lesions. These subdivisions pay attention to the variable content of clear cells, germinal centres and immunoblasts. Again, different nomenclatures exist for the subdivision of AILD: AILD type A, B or C [3, 26] or IBL, IBL-like TCL and AILD [34].

Clinical and molecular studies have revealed that the majority of cases indistinguishable from AILD are in fact clonal and, therefore, can be regarded as neoplastic T-cell proliferations [12, 14, 32]. Interestingly, molecular studies have also discovered clonal rearrangements of the immunoglobulin genes in relatively high frequencies [8]. However, in approximately 30% of the cases no predominant T-cell clone can be detected by molecular studies [23, 35]. These cases might in fact constitute true hyperimmune reactions, the context in which Frizzera originally regarded AILD.

Since the discovery of Epstein-Barr virus (EBV) genomic material in AILD [4], numerous studies have investigated the incidence and possible implication of EBV infection in the pathogenesis of the disease [2, 18, 28, 36]. Using in-situ hybridisation and polymerase chain reaction (PCR), these studies have discovered a significant association of EBV infection with AILD or AILD-TCL – a situation reminiscent of Hodgkin's disease. Even if nasopharyngeal lymphomas are not taken into account, EBV DNA can be detected in Chinese cases of peripheral TCL more frequently than in cases occurring in the Western populations [37]. Because both monoclonal and polyclonal EBV genomes are known to occur in AILD and AILD-TCL, it is still a matter of controversy whether the high prevalence of EBV reflects the immune deficiency of patients or whether EBV acts as one necessary initiator of the disease.

In the present study, we have investigated the morphology, immunohistochemistry and rearrangements of all four functional variable (V)-γ-gene families of the T-cell receptor-γ (TCR-γ) as well as the immunoglobulin heavy chain (IgH) genes together with clinical data of 13 patients from West China. The aim of our study was to identify morphological features that show a correlation with clonality and prognosis and to compare cases from this region to other published series.

Material and methods

Formalin-fixed and paraffin-embedded material were taken from the files of the Department of Pathology, West China University of Medical Sciences, Chengdu. From a selection of 34 specimens collected during the period from 1985 to 1992 with features suggestive of AILD-TCL, paraffin blocks were reembedded and sections of 4 µm thickness were cut at the Department of Pathology, University of Cologne. The specimens were re-assessed according to the updated Kiel classificiation [30] and the diagnostic criteria for AILD published by Frizzera et al. [11] were meticulously applied. In 15 lymph node biopsies derived from 13 patients sufficient material was available for molecular biological studies. In these cases the following morphological features were evaluated: obliteration of lymph node architecture, involvement of capsular and pericapsular tissue, patency of sinuses, alteration of germinal follicles, extracellular deposition of amorphous periodic acid-Schiff (PAS)-positive substances, small vessel proliferation, presence and distribution of pale cells as well as basophilic immunoblasts and mitotic figures per ten high power fields (HPF).

Clinical data were available for seven patients. Medical records were reviewed for the clinical history, duration of symptoms at initial presentation, clinical and laboratory investigations and treatment.

Immunohistochemistry was performed with a panel of monoclonal antibodies (Table 1) using the streptavidin-biotin method [15]. Biotinylated rabbit-anti mouse monoclonal antibodies and streptavidin-biotin-complex were obtained from Dako (Hamburg, Germany). New fuchsin served as a chromogen and sections were counterstained with haematoxylin.

To amplify rearranged TCR-γ sequences sections of 10 µm thickness were cut using a new microtome blade for each case. They were dewaxed in xylene and following ethanol precipitation, the material was desiccated in a SpeedVac centrifuge and incubated with PCR-buffer (Gibco, Eggenstein, Germany) and proteinase K at a concentration of 1 mg/ml for 12–24 h. The proteinase K was inactivated by heating to 96° C for 10 min and samples were stored at 4° C until further investigation. DNA was extracted from these samples by phenol-chloroform extraction and precipitation with absolute ethanol. The quality of the DNA and the level of degradation was tested by agarose gel electrophoresis.

Amplification was performed as previously described [21]. Briefly, primers specific for the four functional V-gene families [16] and a consensus 3'-primer for the junctional (J1)- and J2-genes of the TCR-γ [33] were used. According to their sequence homologies, the V-γ-genes can be grouped into four families. Whereas family I comprises of 8 different V-γ-genes, the V-γ-families II to IV are made up of a single gene each. During rearrangement, a specific V-γ-gene is brought into proximity to a J-γ-gene (Fig. 1). Random nucleotides are inserted into the N-segments. These rearranged sequences can be amplified by PCR using primers for the V-γ- and J-γ-genes. A stretch of high homology between the different genes 120 bp upstream of the J-γ-gene was chosen to place

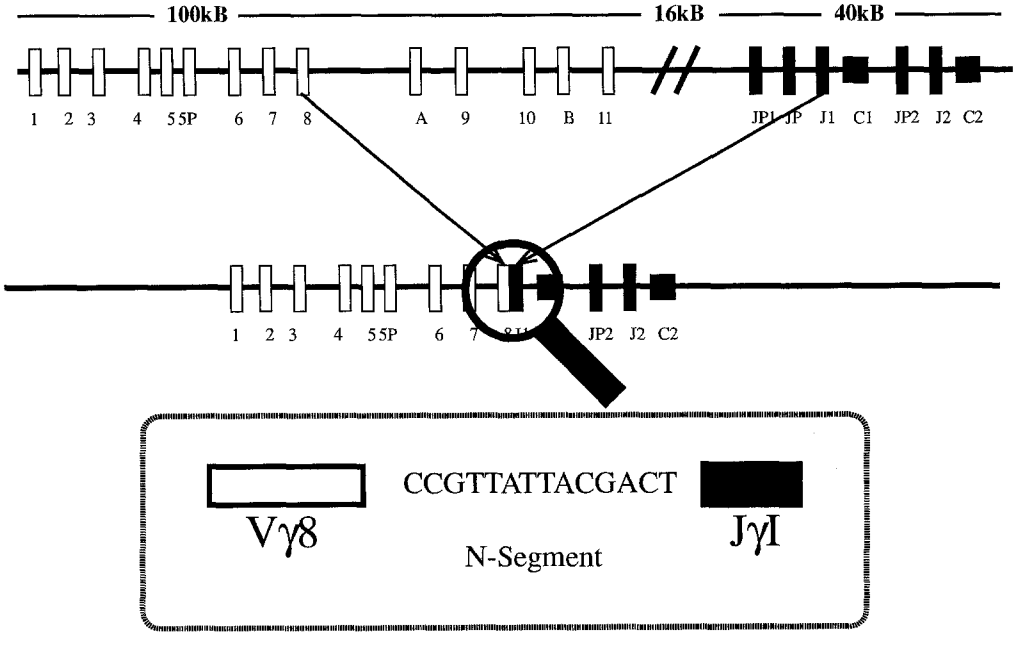

Fig. 1 Rearrangement of the T-cell receptor (TCR)-γ locus. In germ line configuration, theTCR-γ locus comprises of the four gene families variable (V)γI (Vγ-gene 1–8), VγII (Vγ9), VγIII (Vγ10) and VγIV (Vγ11) together with the pseudogenes Vγ 5P, A and B. These genes are separated by at least 16 kb from the closest joining (J)γ-gene, JP1. During T-cell development, these genes are rearranged so that one Vγ-gene is brought into the proximity of one Jγ-gene. Between these gene sequences, random nucleotides are inserted to form N-regions, as indicated in the *insert*. Using primers for Vγ- and Jγ-genes, polymerase chain reaction (PCR)-amplification can only take place after rearrangement. The length of the amplification products varies between of the variable length of the respective N-regions.

Table 1 Source and specificity of monoclonal antibodies

Antibody	CD	Specificity	Source
UCHL-1	CD45RO	T-cells	Dako
MT-1	CD43	T-cells, histiocytes	Dako
L26	CD20	B-cells	Dako
Ki-B5		B-cells	Prof. Parwaresch, Kiel
Ki-M4p		Follicular dendritic cells	Prof. Parwaresch, Kiel
BerH-2	CD30	Activated B- and T-cells, Hodgkin- and Reed-Sternberg-cells	Dako

Table 2 Sequences of oligonucleotide primers used in this study

Name	Sequence	Reference
Vγ I 260	5′-ACTACTACTGAACCTTATACATCCACTGGTACCT-3′	1
Vγ II 260	5′-CAACATCTGTATATTGGTATCG-3′	2
Vγ III 260	5′-CAGATGTCATTCACTGGTACCG-3′	2
Vγ IV 260	5′-GTAAAATCATACACTGGTACTG-3′	2
Vγ Ia 120	5′-GCCTTAAATTTATACTGGAAAAT-3′	2
Vγ Ib 120	5′-GCTGGATATTGA(GT)ACTGCAAAAT-3′	2
Vγ II 120	5′-CATCCACTCTCACCATTCACAAT-3′	2
Vγ III 120	5′-CTCTCACTTCAATCCTTACCAT-3′	2
Vγ IV 120	5′-CTTCCACTTCCACTTTGAAATA-3′	2
Jγ I/II	5′-GTGGAACAACACTTGTTGGATCCGAATTCCATCAT-3′	1
VH1	5′-CCTCAGTGAAGT(CT)TCCTGCAAGGC-3′	3
VH2	5′-GTCCTGCGCTGGTGAAA(GC)CCACACA-3′	3
VH3	5′-GGGGTCCCTGAGACTCTCCTGTGCAG-3′	3
VH4	5′-GACCCTGTCCCTCACCTGC(AG)CTGTC-3′	3
VH5	5′-AAAAAGCCCGGGGAGTCTCTGA(AG)GA-3′	3
VH6	5′-ACCTGTGCCATCTCCGGGGACAGTG-3′	3
JH1–5	5′-GGTGACCAGGGT(TGC)CC(TC)TGGCCCCAG-3′	3
JH6	5′-GGTGACCGTGGTCCCTTGCCCCCAG-3′	3
PGK 5′	5′-TCCTTAGAGCCAGTTGCTGTAGAACT-3′	3
PGK 3′	5′-TTCCCTTCTTCCTCCACATGAAAGCG-3′	3

References: 1 Volkenandt et al., 1991; 2 Lorenzen et al. 1994; 3 Küppers et al. 1993

the primers. The sequences of the respective primers are detailed in Table 2. For the V-γ-I family, we used the oligonucleotide primer described by Volkenandt [33]. Forty amplification rounds were performed on a Perkin-Elmer thermocycler with an annealing temperature of 55° C and a magnesium concentration of 2.5 mM. The amplified products were separated on 5%-MetaPhor agarose (Biozym, Hessisch Oldendorf, Germany) and visualized by ethidum bromide stain.

Negative controls consisted of samples without DNA and DNA isolated from the B-cell line U226. As a positive control with known rearrangement of the V-γ-I and V-γ-IV gene families, DNA isolated from the T-cell line Jurkat was employed. Apart from these controls, we used primers specific for the phosphoglycerate kinase gene [25].

Rearranged genes of the IgH locus were detected as previously described [19]. Briefly, DNA was extracted from the paraffin-embedded material as detailed above and subjected to PCR using primers specific for the V-genes of the IgH locus together with primers directed against the JH-genes. After a first round of amplification using a cocktail of oligonucleotides, family-specific amplification with nested VH-primers was performed. The sequences of the oligonucleotides are given in Table 2. Amplification products were separated on 5% NuSieve gels and evaluated after ethidium bromide stain.

Significance of clinical characteristics and histological findings were tested using Fischer's exact test. Cross correlations of all variables were calculated using software for a Macintosh personal computer.

Results

The clinical findings of the 13 patients are summarized in Table 3. The age of the youngest patient was 35 years, the eldest patient was 71 years old. Patients frequently presented with fever, generalized lymphadenopathy, hepatosplenomegaly and weight loss. The drug history was negative in all patients investigated. Male patients predominated with a male to female ratio of 2.25:1.

In 7 patients, follow-up data were available. The therapy of these patients differed and included chemotherapy (CHOP and COP), radiation, corticosteroids and traditional Chinese drugs. No complete remission was observed in this group. Except for 1 patient, who is still alive 10 months after initial diagnosis, all patients died of cachexia, cardiac failure or overwhelming infections within 8–16 months after diagnosis. Their mean survival was 12.1 months.

Table 4 summarizes the histological findings of the 15 lymph node biopsies with AILD-TCL. Obliteration of normal lymph node architecture with prominent proliferation of postcapillary venules (Fig. 2) was observed in 6 specimens. The marginal sinuses were partially patent. Whereas activated lymphoid follicles were absent, in 8

Table 3 Clinical features of patients investigated: clinical records of 13 patients included in this study were searched for symptoms at presentation (*M* male, *F*, female)

Age	Mean 52.5 years	
Sex	M:F=2.25:1	
Generalized lymphadenopathy	12/13	(92.3%)
Fever	9/13	(69.2%)
Hepatosplenomegaly	7/13	(53.8%)
Weight loss	3/13	(23.1%)
Skin rash	2/13	(15.4%)
Anaemia	2/13	(15.4%)
Hypoglobulinaemia	1/13	(7.7%)
Rheumatoid factor	1/13	(7.7%)

Table 4 Morphological features of lymph node biopsies (*PAS* periodic acid-Schiff, *HPF* high power field, *H* Hodgkin, *RS* Reed-Sternberg)

Increase of postcapillary vessels	15/15	(100%)
Burned out germinal centres	8/15	(53.3%)
Capsular involvement	8/15	(53.3%)
Partially patent sinus	6/15	(40.0%)
PAS-positive amorphous deposits	6/15	(40.0%)
Clear cells:		
patchy	6	(40.0%)
diffuse	4	(26.7%)
inconspicuous	4	(26.7%)
Mitotic figures>5.0/HPF	4	(26.7%)
	(Range: 1.1–8.9/HPF)	
H-RS-like cells	7	(46.7%)
Many plasma cells and precursors	6	(40.0%)
Many epitheloid cells	7	(46.7%)
Many eosinophils	10	(66.7%)

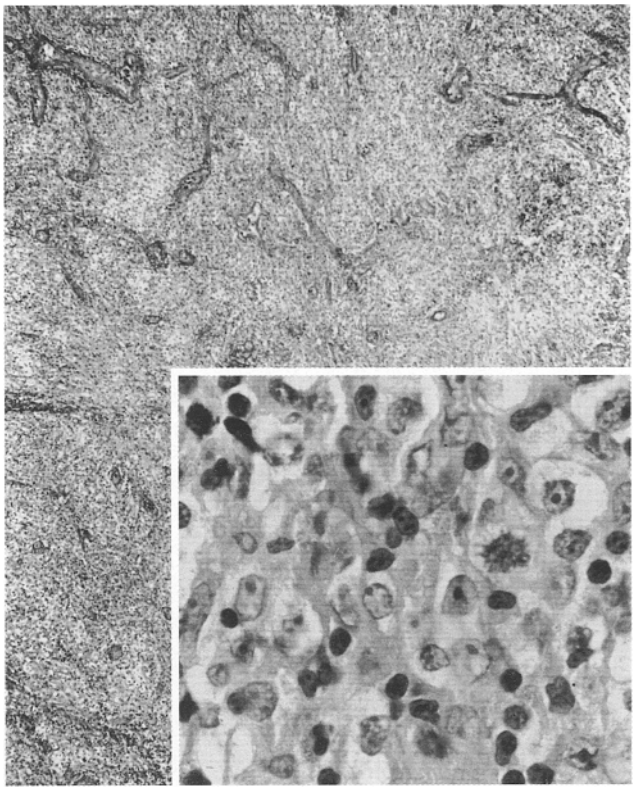

Fig. 2 Obliterated architecture in angioimmunoblastic lymphadenopathy. In this micrograph, the obliterated lymph node architecture and prominent proliferation of small blood vessels can be appreciated (haematoxylin and eosin, × 5). Note nests and sheets of clear cells in the vincinity of epithelioid venules (*inset*, × 100)

cases fibrotic remnants or burnt out germinal centres could be observed. PAS-positive amorphous intercellular deposits were predominantly located in burnt out germinal centres and were appreciable in 5 cases. Postcapillary venules were thickened by PAS-positive material or disrupted in 6 cases.

Cytologically, there was a mixed cellular infiltration consisting of small lymphoid cells, medium- to large-sized pale cells, immunoblasts and plasma cells. The contents of pale cells and atypia varied from case to case. In 6 lymph nodes there were no or only scant perivascular foci of pale cells. In 5 specimens medium-sized pale cells formed patches or clusters together with proliferating postcapillary venules suggesting a compartmentalization. In 4 other cases, the medium- to large-sized pale cells were arranged in diffuse sheet-like or nodular infiltrates. Medium-sized pale cells displayed fairly regular round or oval nuclei, fine chromatin with small nucleoli and moderately lightly stained cytoplasm. The large pale cells showed rounded or slightly irregular nuclei, predominant eosinophilic nucleoli and abundant lightly stained 'water-like' cytoplasm without PAS-positivity – all features described for T-immunoblasts.

Another characteristic change was the proliferation of plasma cells and their precursors, plasmablasts and B-immunoblasts; this was observed in 6 of our cases. They

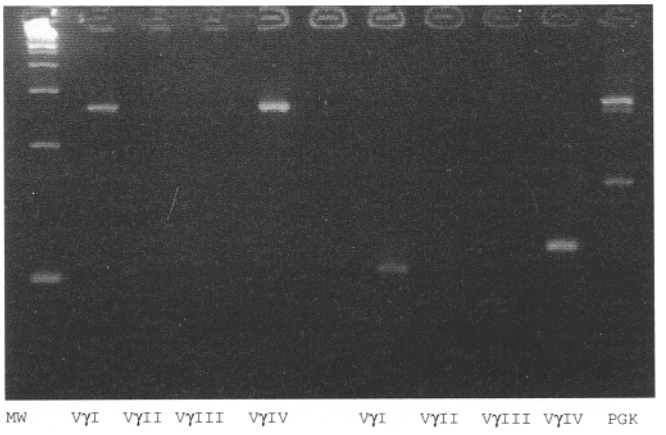

Fig. 3 Amplification of Jurkat-DNA. Rearranged sequences of the TCR-γ were amplified using family-specific primers. This cell line has rearranged both V-γ-family I and IV. On the left hand side, the outer set of primers amplify 260 bp sequences of the TCR-γ. On the right hand side, oligonucleotide primers for the 120 bp products have been employed. Because of N-regions of different lengths, the amplification products for Vγ IV carry a slightly higher molecular weight. As a positive control, a part of the phosphoglycerate kinase (*PGK*) gene was amplified

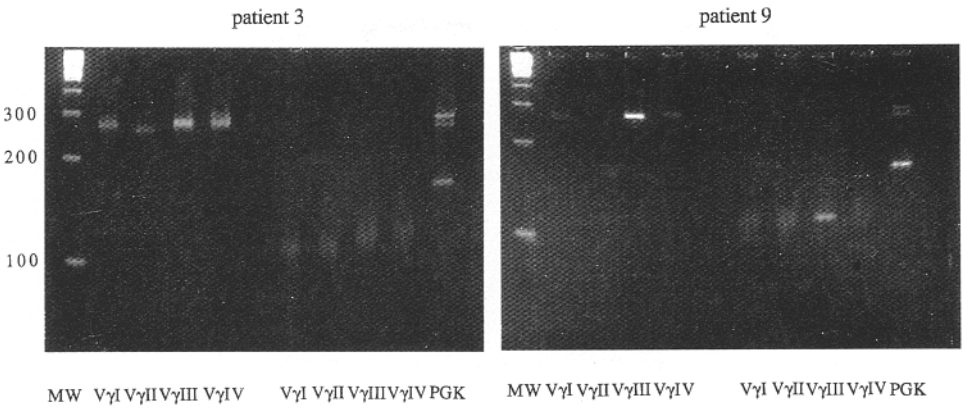

Fig. 4 Detection of a monoclonal T-cell population by PCR-technique. Rearranged sequences of the TCR-γ were amplified using family-specific primers. Gel electrophoresis was performed using high resolution 5% MetaPhor agarose. For comparison, a patient with polyclonal rearrangement pattern (patient 3) is shown together with the result of patient 9, who displayed a clonal pattern. Whereas for patient 3 smeared bands can be seen for each V-γ-family, a predominant clonal population with rearrangement in VγIII is detected for patient 9. Both 260 bp (*left*) and 120 bp (*right*) amplification products of all four V-γ-families are shown

were situated predominantly in perivascular areas or were intermingled with other cells. Occasional cells resembling Reed-Sternberg- or Hodgkin-cells were noticed in 7 specimens. Epithelioid cell clusters or single scattered histiocytes were detected in 7 cases. Eosinophilia was noted in 10 cases. Mitotic counts in cases with inconspicuous pale cells and B-immunoblastic proliferation were lower than 5.0 per HPF. However, in 4 cases with marked proliferation of pale cells and B-immunoblasts, more than 5.0 mitoses were observed per HPF. In these cases, abnormal mitotic figures together with cytologic features of higher cell atypia were observed. These cases displayed features of transformation into high-grade TCL (immunoblastic lymphoma).

Serial lymph node biopsies were available from two patients. In one case initial biopsies showed a nodular proliferation of pale cells, which disappeared after 6 months and prednisone treatment.

In all 15 lymph nodes the majority of cells including small- to medium-sized pleomorphic lymphoid cells, medium and large pale cells as well as some of the immunoblasts showed strong positive reactions with the T-cell markers UCHL-1 and MT-1. Only some residual cell islands or scattered individual cells reacted with the B-cell markers Ki-B5 and L26. The immunoblastic cells displayed partial reactivity with the latter antibodies in 6 cases. BerH-2 positivity of immunoblasts and Reed-Sternberg-like cells was observed in 5 cases. Follicular dendritic cells (FDC) were present in all lymph node biopsies. Markedly increased numbers of FDC were appreciated in 6 cases, where they were localized not only in burnt out germinal centres but also extended into T-cell areas.

In the amplification of TCR-γ genes we analysed DNA from the cell line Jurkat for rearranged TCR-γ se-

Table 5 Amplification of rearranged T-cell receptor (TCR)-γ genes using family-specific primers: DNA was extracted from paraffin-embedded material and subjected to polymerase chain reaction (PCR) amplification using oligonucleotide primers specific for the four functional Vγ-families. Discrete sharp bands are indicated by a (+). In parallel reactions, rearranged immunoglobulin heavy chain (IgH) genes were amplified in a family-specific manner. Band patterns were used to determine clonality of the lesion. (*M* male, *F* female)

Patient	Age (years)	Sex	Clear cells (%)	I_K	II_K	III_K	IV_K	TCR clonal	IgH clonal	Survival (months)
1	56	M	30	+	*	*	*	Yes	Yes	16
1	56	M	<5	*	+	*	*	Yes	Yes	16
2	64	M	<5	+	*	*	*	Yes	No	8
2	64	M	<5	*	*	+	*	Yes	No	8
3	35	F	<5	*	*	*	*	No	No	8
4	42	M	10	+	*	*	–	Yes	No	8
5	48	F	30	–	+	*	–	Yes	No	
6	48	M	<5	*	*	+	*	Yes	No	
7	64	M	10	*	*	*	–	No	No	
8	64	F	40	*	*	+	–	Yes	?	10
9	56	M	<5	*	*	+	*	Yes	Yes	13
10	71	M	5	*	*	*	*	No	No	
11	38	M	15	*	*	*	*	No	No	
12	44	F	<5	*	*	*	*	No	Yes	
13	53	M	5	*	*	*	*	No	Yes	>10

– No band, + sharp band, * smeared band

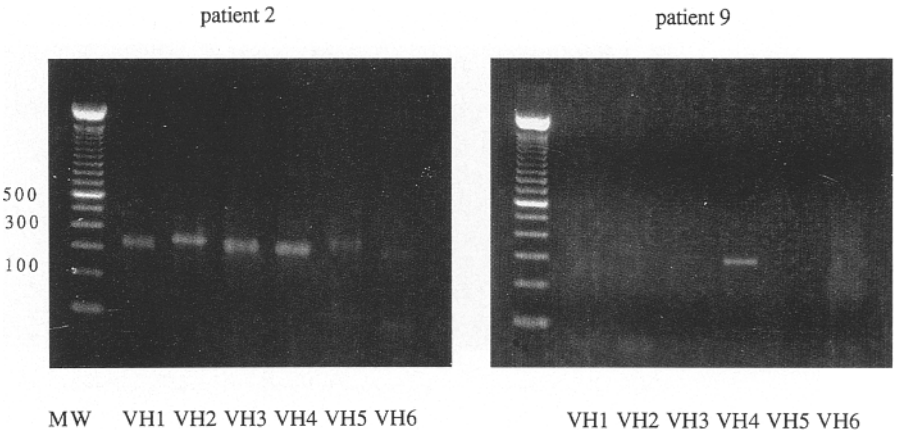

Fig. 5 Detection of clonal rearrangements of the immunoglobulin heavy chain (IgH)-locus. In parallel experiments, rearranged IgH-genes were amplified by PCR. After gel electrophoresis (2% LMP-agarose) of the reaction products, the clonal composition is reflected in the relative strength of the family-specific bands as well as in the acuity of the respective bands. For comparison, the results of patient 2 (polyclonal) and patient 9 (monoclonal) are shown

Table 6 Statistical analysis of investigated parameters: given are the correlations between age, sex, number and distribution of clear cells, clonality and survival of the 13 patients investigated. Number and distribution refer to the presence of clear cells and their distribution (inconspicuous, patch or diffuse)

Correlation	Age	Sex	Number	Distribution	B-clonality	T-clonality	Survival
Age	1						
Sex	–0.293	1					
Number	–0.329	0.333	1				
Distribution	0.286	0.417	–0.056	1			
B-clonality	–0.335	0.577	–0.289	0.145	1		
T-clonality	–0.048	–0.149	0	–0.311	–0.258	1	
Survival	0.451	–0.464	0.111	0.005	0.357	–0.289	1

quences using family specific primers as a preliminary control. This cell line has rearranged the Vγ-8 gene to Jγ1.2 and on the other chromosome Vγ-11 to Jγ1.2 [9]. As shown in Figure 3, the primer sets for the V-families I and IV specifically amplified the respective gene sequences.

The quality and quantity of the DNA extracted from our paraffin-embedded material was sufficient to amplify sequences of the phosphoglycerate kinase gene and TCR-γ sequences in all cases. The results are summarized in Table 5. Using the relative strength of the PCR products as well as the sharpness of the bands as measure of clonality, we classified 9 out of 15 reactions belonging to 9 patients as indicative of clonal cell proliferations. Six specimens gave a polyclonal pattern. As an example, the ethidium bromide stained PCR-products of 2 biopsies are demonstrated in Figure 4.

Using similar criteria as for TCR-γ genes, amplified IgH-rearrangements were evaluated after gel electrophoresis. The results are indicated in Table 5. As shown in Figure 5, the clonal composition of the investigated material is reflected in the band pattern after gel electrophoresis. Clonal IgH-rearrangements could be detected in 5 out of 15 lymph node biopsies (4 out of 13 patients). In 1 case no definitive result could be obtained. Three patients demonstrated monoclonal rearrangements of both TCR-γ and IgH gene loci, whereas clonal IgH rearrangements could be detected in 1 patient with polyclonal TCR-γ patterns.

The cross correlation of the variables investigated is given in Table 6. No single variable was found that predicted clonality of the lesion. Furthermore, the histological and molecular biologic criteria did not correlate with survival of the patient.

Discussion

We have investigated the clinical course, morphology and immunohistochemical data in 13 cases of AILD and AILD-TCL using a new approach based on the PCR to detect clonal TCR-γ rearrangements in paraffin-embedded material. Additional studies were done to investigate rearrangements of the IgH-locus.

Our findings are well in keeping with the recent literature on clonality in AILD and related disorders [11, 31, 32]. We were unable to identify morphological criteria that predicted the clonality of the respective lesion. The investigation of our cases with a PCR-based method revealed the presence of predominant cell clones carrying rearranged TCR-γ genes in 9 out of 15 biopsies (9 out of 13 patients). Clonal rearrangements of the V-γ-family IV, which consists only of the V-γ 11 gene, were not found among the cases investigated, even though control experiments performed on other TCL did not indicate a lower sensitivity of our method in the detection of rearranged sequences of this particular family (data not shown).

The detection of prevalent B- and T-cell clones by Southern blotting has become a well established technique and aids in typing problematic lymph node lesions [5]. The sensitivity of PCR-based methods for the detec-

tion of clonal immune gene rearrangements equals the sensitivity of Southern blotting. PCR-based techniques offer some major advantages because of their speed and because they can be applied to formalin-fixed and paraffin-embedded tissue samples. Several investigators have described PCR-methods to detect clonal B-cell proliferations [1, 6, 7, 19, 23] or T-cell lesions [13, 24]. These methods rely on sufficiently different N-segment lengths, so that polyclonal lesions produce smeared bands after gel electrophoresis. In contrast to other techniques previously described, we employed oligonucleotides that reliably amplify all four known families of functional V-γ-genes [21]. By amplifying each familiy in parallel reactions instead of using concensus primers or multiplex-PCR, information about the relative abundance of the rearranged V-γ-family in the starting material is preserved. After gel electrophoresis, the clonal composition is reflected not only by band sharpness but also by the relative amount of the amplified family-specific products. A similar approach for the detection of clonal rearrangements of the IgH-locus has been entertained by Küppers et al. [19]. Because the relative variance of product length is greater the shorter the amplified DNA stretch is, differences can be better appreciated in shorter PCR products. Hence, we routinely use primers for a 120 bp product and reserve the primers designed to amplify a 260 bp stretch of rearranged TCR-γ sequences for optional semi-nested PCR, if the amount of DNA in the material investigated is small.

According to the recent literature on clonality in AILD and related disorders, it appears that in approximately 30% of all lesions monoclonality cannot be detected. Our data based on the novel PCR-method to investigate TCR-γ rearrangements confirm this percentage. Technical and biological reasons may explain this observation. For instance, in both Southern blotting and PCR amplifiation the demonstration of clonal rearrangements usually fails if the clonal cell population represents less than 5% of all cells within the sample investigated. Some non-Hodgkin lymphomas display immune genes in germline configuation. Finally, abnormal rearrangements are sometimes present in malignant lymphomas and might escape detection by PCR amplification.

In 5 of 15 lymph node biopsies or in 4 of 13 patients, the amplification of IgH genes indicated the presence of clonal populations. The presence of both TCR-γ and IgH rearrangements in approximately every third case of AILD-TCL has first been described by Feller and coworkers [8]. This group has subdivided AILD-TCL according to the rearrangement patterns and found a correlation of clinical and immunohistochemical data. In our hands, this correlation could not be confirmed – possibly due to small case number. The experience with Southern blotting has shown that rearrangements of the TCR-γ or IgH locus are not always lineage-specific. For example, a high percentage of B-ALL carry both rearrangements of the IgH and the TCR-γ locus. Hence, lineage promiscuity is not a phenomenon restricted to immunophenotype, but is also reflected at the genomic level.

Whereas in most cases the malignant cell clone demonstrated IgH rearrangements together with TCR-γ rearrangements, a clonal proliferation was identified in 1 patient only because of its IgH rearrangement. This might represent the evolvement of a B-cell lymphoma, especially as B-cell neoplasms arising in patients with AILD or AILD-TCL have been described in the literature [20].

AILD has been subdivided morphologically into three groups based on the presence or absence of clear cells and immunoblast-like cells as well as nuclear atypia. In a study involving 56 cases of AILD, this distinction has been shown to correlate with the number of proliferating cells and the incidence of monoclonality [26]. In our hands, however, there was no significant association between the presence and distribution of clear cells and the detection of a predominant T-cell clone.

Our sample was too small to reveal significant trends correlating the clonality of AILD with clinical outcome or performance. This is in keeping with data published by Nakamura and Suchi [26]. However, the observation that some cases with no proven clonality show a malignant clinical course and vice versa raises questions still unresolved [11, 17, 27, 31, 35). On the data presently available, it seems justified to diagnose AILD-TCL or AILD on morphological and immunohistological grounds. Whether clonality has a significant impact on clinical course can only be detected in a multi-institutional study of this rare lymph node lesion. The PCR method for the detection of clonal T-cell populations by use of family-specific primers described in this paper facilitates retrospective studies on paraffin-embedded material which is available worldwide and easily distributed.

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