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Analysis of T-Cell Subpopulations in T-Cell Non-Hodgkin's Lymphoma of Angioimmunoblastic Lymphadenopathy with Dysproteinemia Type by Single Target Gene Amplification of T Cell Receptor-β Gene Rearrangements

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Angioimmunoblastic lymphadenopathy with dysproteinemia (AILD) is defined in the current lymphoma classifications as a T-cell non-Hodgkin's lymphoma. However, in approximately one third of the cases of this lymphoproliferative disease rearrangements of T-cell receptor (TCR) genes indicating clonal expansion of T cells are not detectable. It is currently believed that these cases may represent early stages of a lymphoma with a minor oligoclonal T-cell population. In the present study, 18 lymph nodes with the characteristic histology of AILD were investigated for clonal T-cell receptor gene rearrangements by analysis of DNA extracted from whole tissue sections. Dominant T-cell clones were detected in 12 of these cases. Single $CD4^+$ and $CD8^+$ T cells and proliferating $Ki67^+$ cells of seven cases were micromanipulated from frozen tissue sections. TCRβ gene rearrangements were amplified from these cells by polymerase chain reaction and sequenced. In all informative cases, the clonal gene rearrangements were only detected among $CD4^+$, and not among $CD8^+$ T cells, indicating that the tumor clones in AILD usually derive from $CD4^+$ T cells. Minor clonal T-cell populations in those cases in which no clone was found by whole-tissue DNA analysis were not detectable even at single cell resolution. T-cell clones in 4 of 10 cases were found to express similar TCRβ chains, indicating a potential role of (super) antigen triggering in at least some cases of AILD. ***(Am J Pathol 2001, 158:1851–1857)***

Angioimmunoblastic lymphadenopathy with dysproteinemia (AILD) was initially described as a benign hyperimmune reaction[1] and also named immunoblastic lymphadenopathy or lymphogranulomatosis X.[2,3] These entities are now summarized as AILD, as they share clinical and histopathological features. Histologically, AILD may resemble Hodgkin's disease showing a polymorphous infiltrate composed of lymphocytes, plasma cells, immunoblasts, eosinophils, and histiocytes. Typical Hodgkin or Reed/Sternberg cells, however, are usually not found in AILD tissue.[1,3] Characteristically arborising blood vessels with high endothelia, an amorphous eosinophilic intercellular material, and irregular networks of follicular dendritic cells are found in effaced lymph nodes.[2,4] AILD was identified as a neoplastic disorder and is listed in the current lymphoma classifications as a T-cell non-Hodgkin's lymphoma.[5,6]

The classification of AILD as a neoplastic lymphoproliferative disease was supported by the finding of clonal antigen-receptor gene rearrangements in DNA extracted from tumor tissue.[4,7–10] Southern blot hybridization experiments revealed clonal T-cell receptor (TCR)β gene rearrangements in ~70% of the cases investigated and clonal immunoglobulin heavy chain (IgH) gene rearrangements in few cases.[9,11] Polymerase chain reaction (PCR) techniques for the amplification of TCR and IgH gene rearrangements yielded similar results.[12,13] In the remaining 30% of cases no dominant gene rearrangements were found. It remains unclear whether these cases lack clonal lymphocytic infiltrates, or whether such infiltrates escape detection because of limited sensitivity of the Southern blot and PCR techniques applied.

With the development of single target amplification of lymphocyte receptor gene rearrangements from single

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cells[14] analysis of even minor lymphocyte populations in lymph node tissue has become feasible. Recently, single target amplification of TCRβ gene rearrangements from individual T cells micromanipulated from tissue sections was established.[15] In the present study these techniques have been applied in seven cases of AILD to further characterize this tumor. This approach allows addressing the question of the phenotype of the tumor cells directly by analysis of single $CD4^+$ and $CD8^+$ T cells separately. Proliferating cells were investigated to detect minor lymphocyte proliferations in those cases with no obvious clone.

Materials and Methods

Tissue Samples

Lymph node specimens diagnostic of AILD were collected from the files of the Institutes of Pathology of the Universities of Kiel and Frankfurt, Germany. The diagnosis was based on the histological criteria as detailed above. In 12 cases frozen tissues and in six cases paraffin-embedded tissues were available.

Whole-Tissue DNA Analysis

Sections (10-μm thick) of lymph node tissue were cut and digested with proteinase K. Whole DNA was extracted using the Qiagen DNA extraction kit as recommended by the manufacturer (Qiagen, Hilden, Germany). For PCR analysis, 100 ng of DNA from frozen tissue and 500 ng of DNA from paraffin-embedded tissue were used for each reaction. TCRβ gene rearrangements were amplified and sequenced using the same conditions and primers as described below for second-round single-cell PCR. For DNA amplification from paraffin-embedded tissue an additional primer for the BJ2.4 segment was added to limit the length of PCR fragments to <450 bp (Jβ 2.4, Table 1).

Analysis of IgH and TCRγ chain complementarity-determining region III (CDR3) length distributions was performed as described elsewhere.[16,17] Briefly, IgH gene rearrangements were amplified by 40 cycles of PCR at an annealing temperature of 61°C in a standard reaction mixture containing 125 nmol/L of each VH and JH consensus primers (Table 1), 2 mmol/L $MgCl_2$ and 0.7 U *Taq* polymerase (Boehringer Mannheim, Mannheim, Germany). TCRγ gene rearrangements were amplified in two separate reactions for each DNA sample containing standard reagents, 400 nmol/L of each J and V primer, 1.5 mmol/L $MgCl_2$, and 0.7 U *Taq* polymerase (Boehringer Mannheim). A mixture of three J primers was used with either the Vγ 1-8 primer or a mixture of Vγ 9, Vγ 10, and Vγ 11 primers (Table 1) at an annealing temperature of 60°C for 40 cycles of amplification. VH consensus primer for IgH analysis and Jγ primers for TCRγ detection were fluorescence labeled with FAM6 (MWG, Ebersberg, Germany). PCR products were analyzed on an automated sequencer using the GeneScan software as recommended by the manufacturer (ABI377; Applied Biosystems, Weiterstadt, Germany).

Major histocompatibility complex class II (MHC II) gene polymorphism (DRB1, DQB1, DPB1) was typed by PCR amplification of genomic DNA extracted from paraffin-embedded tissue. HLA-DRB1 and HLA-DQB1 alleles were defined using sequence-specific oligonucleotides as recommended by the manufacturer (ELPHA HLA-DRB/HLA-DQB; Biotest, Dreieich, Germany). HLA-DPB1 typing was performed by reverse dot-blot hybridization (INNO-LiPA DPB; Innogenetics, Zwinjndrecht, Belgium).

Table 1. Sequences of Primers Used in This Study

VH con	5′-CTGTCGACACGGCCGTGTATTACTG-3′
JH con	5′-AACTGCTGAGGAGACGGTGACC-3′
Vγ 1–8	5′-ACCAGGAGGGGAAGGCCCCACAG-3′
Vγ 9	5′-GGAAAGGAATCTGGCATTCCG-3′
Vγ 10	5′-AATCCGCAGCTCGACGCAGCA-3′
Vγ 11	5′-GCTCAAGATTGCTCAGGTGGG-3′
Jγ 1/2	5′-ACCTGTGACACCAAGTGTTGTTC-3′
Jγ P1/2	5′-AGTTACTATGAGCT(CT)TAGTCCC-3′
Jγ P	5′-TGTAATGATAAGCTTTGTTCC-3′
Vβ 1/5	5′-ACAGCAAGTGAC⟨TAG⟩CTGAGATGCTC-3′
Vβ 2	5′-GAGTGCCGTTCCCTGGACTTTCAG-3′
Vβ 3	5′-GTAACCCAGAGCTCGAGATATCTA-3′
Vβ 4	5′-TCCAGTGTCAAGTCGATAGCCAAGTC-3′
Vβ 6.a	5′-ATGTAACT⟨CT⟩TCAGGTGTGATCCAA-3′
Vβ 6.b	5′-GTGTGATCCAATTTCAGGTCATAC-3′
Vβ 7	5′-TACGCAGACACCAA⟨GA⟩ACACCTGGTCA-3′
Vβ 8	5′-GGTGACAGAGATGGGACAAGAAGT-3′
Vβ 9	5′-CCCAGACTCCAAAATACCTGGTCA-3′
Vβ 10	5′-AAGGTCACCCAGAGACCTAGACTT-3′
Vβ 11	5′-GATCACTCTGGAATGTTCTCAAACC-3′
Vβ 12	5′-CCAAGACACAAGGTCACAGAGACA-3′
Vβ 13	5′-GTGTCACTCAGACCCCAAAATTCC-3′
Vβ 14	5′-GTGACCCAGAACCCAAGATACCTC-3′
Vβ 15	5′-GTTACCCAGACCCCAAGGAATAGG-3′
Vβ 16	5′-ATAGAAGCTGGAGTTACTCAGTTC-3′
Vβ 17	5′-CACTCAGTCCCCAAAGTACCTGTT-3′
Vβ 18	5′-TGCAGAACCCAAGACACCTGGTCA-3′
Vβ 19	5′-ACAAAGATGGATTGTACCCCCGAA-3′
Vβ 20	5′-GTCAGATCTCAGACTATTCATCAATGG-3′
Vβ 21	5′-CAGTCTCCCAGATATAAGATTA⟨TC⟩AGAG-3′
Vβ 22	5′-GGTCACACAGATGGGACAGGAAGT-3′
Vβ 23	5′-CTGATCAAAGAAAAGAGGGAAACAGCC-3′
Vβ 24	5′-CAAGATACCAGGTTACCCAGTTTG-3′
Vβ 25	5′-GACAGAAAGCAAAATTATATTGTGCC-3′
3′Jβ 1.2	5′-TACAACGGTTAACCTGGTCCCCGA-3′
5′Jβ 1.2	5′-TAACCTGGTCCCCGAACCGAAGG-3′
3′Jβ 1.3	5′-CACCTACAACAGTGAGCCAACTT-3′
5′Jβ 1.3	5′-GCCAACTTCCCTCTCCAAAATATATGG-3′
3′Jβ 1.5	5′-CCAACTTACCTAGGATGGAGAGTCGA-3′
5′Jβ 1.5	5′-GATGGAGAGTCGAGTCCCATCAC-3′
3′Jβ 1.6	5′-CCTGGTCCCATTCCCAAAGTGGA-3′
5′Jβ 1.6	5′-CCCATTCCCAAAGTGGAGGGGTG-3′
3′Jβ 2.2	5′-CCTTACCCAGTACGGTCAGCCTA-3′
5′Jβ 2.2	5′-AGTACGGTCAGCCTAGAGCCTTCT-3′
3′Jβ 2.6	5′-CAGCCGCCGCCTTCCACCTGAAT-3′
5′Jβ 2.6	5′-CGGCCCCGAAAGTCAGGACGTT-3′
3′Jβ 2.7	5′-TCCATCGTTCACCTTCTCTCTAAACA-3′
5′Jβ 2.7	5′-GCCCGAATCTCACCTGTGACCGTG-3′
Jβ 2.4	5′-CCAGCTTACCCAGCACTGAGAGC-3′

⟨ ⟩ denotes a mixture of nucleotides at that position.

Vβ primers 7, 9, and 18 expectedly cross-react with members of all of these three Vβ families and are, therefore, used in a mix at a concentration of 14 nmol/L and 50 nmol/L each for first round and second round amplifications, respectively.

Table 2. Results of Whole-Tissue DNA Analysis and MHC Class II Typing in AILD

Case	TCRβ PCR whole tissue	TCRγ† whole tissue	IgH† whole tissue	MHC II		
				DRB1*	DQB1*	DPB1*
1	Clonal	Clonal	Polyclonal	03, 07	02, 0302	0101, 0401
2	Clonal	Clonal	Polyclonal	03, 13	02, 06	0301, 0401
3	Clonal	Clonal	Polyclonal	11, 16	0301, 05	0201, 0401
4	Polyclonal	Polyclonal	Polyclonal	01, 07	05, 02	nd
5	Polyclonal	Clonal	Polyclonal	10, 13	05, 06	0201, 0402
6	Polyclonal	Polyclonal	Polyclonal	13, 13	06, 06	nd
7	Polyclonal	Polyclonal	Polyclonal	15, –	06, –	nd
8	Clonal	nd	nd	nd	nd	nd
9	Polyclonal	nd	nd	nd	nd	nd
10	Clonal	nd	nd	nd	nd	nd
11	Clonal	nd	nd	nd	nd	nd
12	Polyclonal	nd	nd	nd	nd	nd
13	Polyclonal	Clonal	Polyclonal	03, 11	02, 0301	nd
14	Clonal	Clonal	Polyclonal	14, –	15, –	nd
15	Clonal	Clonal	Clonal	11, 13	06, 0301	nd
16	Clonal	Clonal	Polyclonal	11, 15	06, 0301	0401, 0402
17	Polyclonal	Polyclonal	Polyclonal	11, –	02, 0301	nd
18	Clonal	Clonal	Polyclonal	01, 13	05, 06	0401, 0402

†CDR3 length distribution analysis of IgH and TCRγ gene rearrangements.

Immunostaining and Micromanipulation of Cells from Frozen Tissue Sections

Seven frozen lymph-node specimens showing the typical morphology of AILD were chosen for micromanipulation. Immunostaining of frozen tissue sections was performed as described,[14] using monoclonal antibodies against CD4 (MT310), CD8 (DK25), and the proliferation marker Ki67 (all antibodies by DAKO, Glostrup, Denmark). Alkaline phosphatase was developed using Fast Red TR (DAKO). $CD4^+$, $CD8^+$, and $Ki67^+$ cells were isolated from adjacent sections by micromanipulation and transferred into PCR tubes containing 20 μl of PCR buffer as described.[14] Samples of the buffer covering the sections during the micromanipulation procedure and tubes containing PCR buffer but no cell served as negative controls.

Amplification of TCR Vβ Gene Rearrangements from Single Cells

Amplification of rearranged TCR Vβ genes was performed according to a recently established protocol.[15] Briefly, micromanipulated cells were incubated with proteinase K. A first round of PCR was performed in the same tube using a mix of 25 Vβ family- and 7 Jβ-specific primers (Table 1) in a 50-μl volume containing standard reagents, 42 nmol/L of each primer, 2 mmol/L $MgCl_2$, and 2.5 U of Expand HF polymerase mix (Boehringer Mannheim) for 35 cycles of amplification at an annealing temperature of 61°C. A second round of amplification was performed in 96-well plates, adding 1 μl of the first round reaction to eight separate reaction mixtures, each containing a mixture of internal Jβ primers (Table 1) and 2 to 5 of the 25 Vβ primers in the following combinations: Vβ 2, 3, 22; Vβ 4, 6a, 14; Vβ 6b, 8, 21; Vβ 1/5, 11, 12; Vβ 13, 15; Vβ 7/9/18, 17, 20; Vβ 23, 24, 25; and Vβ 10, 16, 19. Second round amplification was performed in a 50-μl volume containing standard reagents, 150 nmol/L of each primer, 2 mmol/L $MgCl_2$, and 0.7 U *Taq* polymerase (Boehringer Mannheim) for 44 cycles at an annealing temperature of 61°C.

PCR products were gel-purified and directly sequenced using the Ready Reaction dRhodamine cycle sequencing kit (Perkin Elmer, Foster City, CA) and an automatic sequencer (ABI377) as recommended by the manufacturer. Sequences were deposited in the European Molecular Biology Laboratory database under accession numbers AJ301370 to AJ301551.

Results

Whole-Tissue DNA Analysis

Whole tissue DNA of 18 lymph nodes displaying the characteristic histology of AILD was extracted. TCRβ gene rearrangements were amplified in eight separate PCR reactions using Vβ family-specific primers. Ten cases harboring a dominant TCRβ gene rearrangement were identified (Table 2). Clonality was confirmed by direct sequencing of the PCR product. For case 8 only a nonfunctional gene rearrangement was obtained. In case 11, two in-frame gene rearrangements were amplified indicating the presence of either two clones in the lymph node tissue or one clone with in-frame rearrangements on both alleles. The eight other cases showed smeared bands for each of the eight PCR reactions, indicating a polyclonal T-cell population.

The dominant T-cell clones identified by TCRβ PCR were also detected by analysis of CDR3 length distributions of TCRγ gene rearrangements. This technique revealed the presence of a dominant clone in two further cases that had not been detected by PCR analysis of the TCRβ gene locus (Table 2). Analysis of CDR3 length distributions of IgH gene rearrangements revealed a clonal B-cell population in addition to a T-cell clone in one of the cases (case 15, Table 2).

Table 3. Single-Cell PCR Analysis in AILD

Case	Single CD4$^+$ cells			Single CD8$^+$ cells			Single Ki67$^+$ cells		
	Number of specific PCR products/ number of cells	Functional/ nonfunctional sequences	Clonal*/ unique sequences	Number of specific PCR products/ number of cells	Functional/ nonfunctional sequences	Clonal*/ unique sequences	Number of specific PCR products/ number of cells	Functional/ nonfunctional sequences	Clonal*/ unique sequences
1	9/32	9/0	7/2	12/32	10/2	0/10	3/32	3/0	3/0
2	2/32	2/0	2/0	9/52	6/3	0/7	3/48	3/0	3/0
3	11/32	9/2	6/3	19/84	13/6	2/11	6/32	6/0	5/1
4	16/48	15/1	0/15	12/32	8/4	0/8	19/48	17/2	0/17
5	8/48	8/0	5/3	7/48	5/1†	0/5	11/44	11/0	7/4
6	23/72	20/3	0/20	10/72	8/1†	0/8	4/72	4/0	0/4
7	11/54	11/0	0/11	13/36	12/†	0/12	5/54	3/†	0/3
Controls‡	0/106			0/118			0/110		

*In each of the four cases harboring clones, the same clonal rearrangements were detected in the CD4$^+$ and Ki67$^+$ cells. Only potentially functional sequences were considered.

†The reading frame of a few sequences could not be unequivocally determined.

‡Controls include aliquots of the buffer covering the sections during micromanipulation and PCR buffer.

Micromanipulation and Single-Cell PCR

Two hundred thirteen specific PCR products were obtained from 1,004 cells micromanipulated from frozen tissue sections of seven cases of AILD (Table 3). This PCR efficiency of 21% fits with our previous results.[15,18] No specific PCR product was obtained from any of the 334 negative control samples (Table 3). In four of these cases a dominant clone had been found by whole-tissue DNA analysis. In these cases the clonal gene rearrangement was repeatedly obtained from CD4$^+$ cells and the respective CD4$^+$ T-cell clone seemed to dominate the population of CD4$^+$ T cells. The clonal rearrangements were also detected in single Ki67$^+$ cells micromanipulated from adjacent sections. Thus, a proliferating CD4$^+$ T-cell clone was present in each of these cases. Only two clonally related T cells were identified among the CD8$^+$ T cells of one case (case 3), which were unrelated to the clone identified by whole-tissue DNA analysis and only unrelated rearrangements were obtained from CD4$^+$ and CD8$^+$ T cells of the remaining three cases that had been negative for clonal expansions in the initial analysis of whole-tissue DNA. All sequences of rearranged TCR Vβ gene segments obtained in this study were completely identical to the respective germline sequences, supporting the view that TCR-V genes are generally not subject to somatic hypermutation.[19]

TCRβ Gene Segment Usage

Table 4 displays the gene segments used in functional rearrangements of dominant T-cell expansions. In four of 10 cases in which a functional TCRβ gene rearrangement of an expanded T-cell clone was detected, this rearrangement used Vβ13S1. The overrepresentation of this gene segment raised the question whether the CD4$^+$ T-cell clones of different cases of AILD possibly shared common antigen specificity. Comparison of deduced CDR3 amino acid sequences supported this hypothesis to some degree as the CD4$^+$ clones identified in cases 2 and 3 not only shared the Vβ13S1 gene segment but also the length of the CDR3 and the Jβ element (Jβ1.5, Figure 1). However, MHC II-typing did not reveal a MHC II allele common to all of the cases that used the Vβ13S1 gene segment (Table 2).

Discussion

The neoplastic nature of AILD has been a matter of debate since this disease was first described.[1–3] Still some authors distinguish between AILD as a nonneoplastic hyperimmune reaction and AILD-like T-cell lymphoma because some patients experience regression even without treatment.[20] Most patients affected by AILD, however, have a poor outcome, even with polychemotherapy, and frequently die of fatal infections in a state of immunodeficiency or progress to immunoblastic lymphoma.[21] The malignant clinical behavior and the detection of clonal TCR gene rearrangements in ~70% of the cases support the concept that AILD is a T-cell lymphoma. No histological, immunohistochemical, or molecular biological parameter was found that could distinguish between patients with a favorable outcome and those with a severe prognosis.[12] Nature, origin, and biological rele-

Table 4. Gene Segment Usage of Functional TCRβ Rearrangements in T-Cell Clones of AILD

Case	Vβ segment	Jβ segment
1	2S1	1.6
2	13S1	1.5
3	13S1	1.5
5	13S1	1.1
10	15S1	2.7
11*	17S1/18S1	2.6/1.1
14	9S1	2.2
15	5S1	1.1
16	13S1	1.2
18	2S1	1.6

*Two in-frame gene rearrangements were amplified in case 11 (Vβ17S1Jβ2.6/Vβ18S1Jβ1.1).

Case	Vβ13S1-segment	N	Dβ1	N	Jβ-segment
2	C A S S S	G G P G		N Q P Q H F G	
	TGT GCC AGC AGT T	CC GGG GGA	CCG G	GC AAT CAG CCC CAG CAT TTT GGT	Jβ1.5
3	C A S S S	V P G P	R	Q P Q H F G	
	TGT GCC AGC AGT T	CA GTT CCG GGG CCC	AGG	CAG CCC CAG CAT TTT GGT	Jβ1.5
5	C A S S F	T G G A	G	N T E A F F G	
	TGT GCC AGC AGT T	TT ACA GGG GGC	GCA GG	G AAC ACT GAA GCT TTC TTT GGA	Jβ1.1
16	C A S S Y S	Q G A	N	Y G Y T F G	
	TGT GCC AGC AGT TAC TC	T CAG GGG GCG		AAC TAT GGC TAC ACC TTC GGT	Jβ1.2

Figure 1. CDR3 comparison of clonal TCR Vβ13S1 gene rearrangements in four cases of AILD. Segments are aligned according to the conserved motifs CAS of the Vβ and FG of the Jβ segment. The end of the Vβ and the beginning of the Jβ segment are marked by an empty space. Nucleotides encoded by the Dβ1 segment are underlined.

vance of the clonal lymphocyte populations are until now only poorly understood.

In the present study, in 12 of 18 cases of AILD a dominant T-cell clone was detected in DNA extracted from whole tissue specimens. We investigated $CD4^+$, $CD8^+$, and proliferating ($Ki67^+$) cells isolated from histological sections of seven of these cases. In four of these seven cases a dominant T-cell clone had been found. In one of these cases (case 5), the clone was only detected by single-cell PCR and analysis of TCRγ CDR3 length distribution. In this case many unique T cells were found besides those of the clone (Table 3). Hence, the clone was not detected by TCRβ PCR in whole tissue DNA because it was most likely below the detection limit of this technique. This might also apply for case 13 of which only paraffin-embedded tissue was investigated (Table 2). In the four cases in which a dominant clone had been found, the clonal TCRβ gene rearrangements could be assigned to $CD4^+$ and $Ki67^+$ cells by single cell amplification. These clones of proliferating $CD4^+$ cells seemed to account for the majority of T-helper cells in the tumor tissue. Only two clonally related cells were identified among the $CD8^+$ T cells of one case. Whereas clonal expansions of $CD8^+$ T cells have been described even in healthy individuals,[22,23] the presence of a dominant $CD4^+$ T-cell clone is uncommon in normal lymphoid tissue. Therefore, it seems likely that the dominant clones of proliferating $CD4^+$ T cells found in the four cases of AILD represent the tumor cell population. Previous investigations that tried to identify the lineage of the neoplastic T cells by double-immunohistochemical stainings with proliferation markers and T-cell markers failed to unequivocally determine the tumor cell phenotype. Some identified $CD4^+$ cells, others reported $CD8^+$ cells as the major proliferating T-cell population.[4,24–26] Furthermore, by double-immunohistochemical stainings alone it is not possible to make a statement regarding the clonality of cells stained. Our data support the view that the neoplastic T-cell clones in AILD are of $CD4^+$ phenotype.

The peculiar histology of AILD, which displays a polymorphous cellular infiltrate of reactive cells as outlined above, is thought to be caused by an abnormal production of cytokines. Enhanced expression of tumor necrosis factor-α, lymphotoxin, interleukin-6, and interleukin-1β transcripts has been reported in AILD tissue.[27–29] These findings fit well with a $CD4^+$ T-cell derivation of the neoplastic cells in AILD. Presuming that the reactive cells in the lymph node tissue are attracted by cytokines produced by the neoplastic cells this expression pattern would resemble that of inflammatory $CD4^+$ T_H1 cells.[30] The etiological factor that causes the abnormal proliferation of these cells, however, has yet to be established.

In three cases of the single cell analysis no dominant T-cell clone could be detected. By analysis of proliferating cells and T-cell subsets only sequences of unrelated TCR gene rearrangements were obtained. One might speculate that the neoplastic T cells do not necessarily dominate the $CD4^+$ T-cell population at all stages of the disease and hence, the clone was not detected because of the limited number of cells investigated. By aberrant cytokine expression even a minor tumor cell infiltrate could account for the peculiar histology of AILD, as it is also seen in Hodgkin's disease. It also has to be considered that the oligonucleotide primers applied for amplification of TCRβ gene rearrangements cover all but two Vβ gene segments.[15] T-cell clones using one of those two Vβ-gene segments in their respective gene rearrangements are thus not detected and, therefore, would have been missed in the single cell investigation. Furthermore, in a complex mixture of oligonucleotides as applied for amplification of TCRβ gene rearrangements in this study, PCR failure because of degradation of single primers cannot be totally ruled out.

At least in two cases of the present study, however, a different explanation seems more likely. In the single cell analysis, the fraction of micromanipulated proliferating cells that yielded a PCR product was very low in comparison to the $CD4^+$ and $CD8^+$ cells, especially in cases

6 and 7, suggesting that most of these cells were not T cells. Immunoblastic B-cell lymphomas developing in patients suffering from AILD have been reported frequently.[21,31–34] In these cases the AILD tumor clone may have been of B-cell origin. Indeed, in cases 4 and 6 of the present study dominant clonal B-cell proliferations were detected by single cell amplification of Ig gene rearrangements (manuscript in preparation, Tilmann Spieker, Andreas Bräuninger, personal communication).

Tumor clones in 4 of 10 cases used the Vβ13S1 gene segment. Given the limited number of cases studied, this overrepresentation might merely be coincidental, but in comparison to a control population of T cells from the peripheral blood where the Vβ13S1 segment is only found in 3.7% of the gene rearrangements[18] the usage of this segment is surprisingly high. The overexpression of a particular V gene segment raises the question whether the neoplastic $CD4^+$ T-cell clones of different patients shared a common antigen specificity. CDR3 similarities between two of the rearrangements using Vβ13S1 supported this hypothesis to some degree. Alternatively, this overrepresentation could have been caused by stimulation of these clones by a superantigen. There was, however, no MHC II allele common to all of the cases with Vβ13S1-expressing tumor clones. Restricted usage of certain Vβ gene segments in clones of AILD has also been reported by Smith and colleagues.[13] In their panel of cases the Vβ2S1 gene segment, which was also found in 2 of 10 cases of the present study, was repeatedly found. Clones using the Vβ13S1 gene segment in their rearrangements were not reported. However, the Vβ13S1 gene segment may not have been efficiently amplified by Smith and colleagues[13] given that their consensus primer carried several mismatches to the Vβ13S1 gene segment. Both Vβ2 and Vβ13 gene segments have been found to be predominantly expressed by $CD4^+$ T cells in lesions of Sjögren's syndrome,[35,36] an autoimmune disorder associated with AILD.[37–39] One might speculate on basis of this data that an unknown type of antigen or superantigen triggering of the tumor cells or their precursors could be involved in the pathogenesis of at least some cases of AILD.

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