The Same Dominant T Cell Clone Is Present in Multiple Regressing Skin Lesions and Associated T Cell Lymphomas of Patients with Lymphomatoid Papulosis

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This study was undertaken to determine the clonality of lymphomatoid papulosis (LyP), its clonal relationship to lymphomas, which occur at high frequency in LyP patients, and to define the cell lineage of Reed-Sternberg-like cells in type A lesions of LyP. Punch biopsies of skin of 11 adult patients with LyP were analyzed for morphologic subtype of LyP, surface antigens, and clonal T-cell receptor (TCR) gene rearrangements. Clonal rearrangements were identified by semiquantitative polymerase chain reaction amplification and sequencing of TCR-β chain genes in nine patients and TCR-γ chain genes in two patients. A single dominant clone was detected in multiple separate LyP lesions, often of different histologies, in nine patients. The same clone was detected in LyP lesions and the anaplastic large cell lymphoma (ALCL) of 2 patients and the mycosis fungoides (MF) of 2 other patients. No dominant clone could be detected in one patient with LyP uncomplicated by lymphoma or in a second patient with LyP and MF. A T-cell lineage was evident for RS-like cells in cell culture and in type A lesions. These results show that multiple regressing skin lesions and associated T cell lymphomas (MF and ALCL) are clonally related in most LyP patients, which suggests that the disease in these patients was initiated by a non-random genetic event. *Key words: mycosis fungoides. J Invest Dermatol 106:696–700, 1996*

The clonality of separate lymphomatoid papulosis (LyP) lesions and their relationship to clinically associated lymphomas have not been studied extensively. Using the Southern blot technique, Weiss *et al* (1986) found one or more rearranged bands, representing clonally expanded T cells, in individual lesions of five of six LyP patients, three of whom had an associated lymphoma. In the only patient in whom several LyP lesions could be studied, different rearrangements were detected, suggesting that separate LyP lesions might contain different T cell clones. We also found clonal T cells in LyP lesions of four LyP patients, and in one patient the same rearrangement was detected in separate lesions removed 11 mo apart (Kadin *et al*, 1987). Similarly, Whittaker *et al* (1991) found identical rearrangements in separate LyP lesions of type B or mixed type A and B histology, but none in patients with pure type A lesions, which suggests that type A lesions might arise from a non-T, non-B cell type.

The disparity in results of these studies could be due to the low sensitivity of the Southern blot method to analyze clonal T cells in LyP lesions which contain many inflammatory cells and only a low number of atypical lymphoid cells. Moreover, the Southern blot method has limitations due to incomplete digestion of DNA, restriction fragment polymorphisms, and limited resolution of clonal populations in the gel. To overcome these difficulties and to clarify the clonality of LyP and its relationship to malignant lymphoma, we used a more sensitive, specific approach based on polymerase chain reaction (PCR) amplification and sequencing of T-cell receptor (TCR) genes from multiple LyP lesions of individual patients and associated lymphomas. The results offer strong evidence that LyP is a clonal lymphoproliferative disorder and indicate that regressing lesions of LyP are clonally related to the malignant lymphoma of most LyP patients.

MATERIALS AND METHODS

Patients Eleven adults, eight female and three male, with clinical and pathologic features of LyP were studied. All patients had recurrent spontaneously regressing papular or papulonecrotic skin lesions, 2–12 mm in diameter, for 1–38 years, median 13 years. Five patients had additional evidence of malignant lymphoma, either nodular progressive lesions of anaplastic large cell lymphoma (ALCL) (patients 7 and 8), or patch/plaque lesions of mycosis fungoides (MF) (patients 9 to 11).

Tissues Punch biopsies (4–6 mm) of skin were divided and fixed in B5 or 10% formalin for histology, fixed in 4% paraformaldehyde-lysin-periodate, and snap-frozen for immunopathology (Muramoto and Kadin, 1987), or snap-frozen directly for cryostat sections and extraction of RNA and DNA.

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Abbreviations: ALCL, anaplastic large-cell lymphoma; LyP, lymphomatoid papulosis; MF, mycosis fungoides; PCR, polymerase chain reaction; RS, Reed-Sternberg; TCR, T-cell receptor.

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Histopathology Subtyping of LyP as type A, type B, or mixed type A and B (A/B) was done according to the criteria of Willemze (Willemze *et al*, 1982). A diagnosis of ALCL was made for non-regressing lesions with sheets of large atypical $CD30^+$ cells that extended into the subcutis (Paulli *et al*, 1995). A diagnosis of MF was made for stable lesions containing epidermotropic atypical lymphocytes with convoluted nuclei.

Immunophenotyping Paraffin sections were labeled with monoclonal antibodies against lymphocyte activation antigens Ber-H2 (CD30) and Leu-M1 (CD15), B-cell antigen L26 (CD20), and leukocyte common antigen (CD45). Frozen sections were labeled with monoclonal antibodies against CD30, CD15, CD20, IL2-R α chain (CD25) and T-cell antigens Leu-1 (CD5), Leu-2 (CD8), Leu-3 (CD4), Leu-4 (CD3), UCHL-1 (CD45RO), and TCR framework antigen (βf1), with a 3-step immunoperoxidase technique utilizing streptavidin-horseradish peroxidase (Carton and Pedersen, 1989).

Semi-quantitative PCR Amplification of TCR-β Chains For analyses of TCR-β chains in 9 patients, 10-μm frozen sections from each lesion were examined for atypical lymphocytes. After confirmation of the presence of atypical lymphocytes, RNA was extracted using RNAzol-B (TM Cinna Scientific, Friendswood, TX) and cDNA was synthesized using oligo-dT and MMTV reverse transcriptase (GIBCO-BRL, Grand Island, NY). Use of different Vβ genes was then determined by a semiquantitative PCR method using a panel of oligonucleotide primers for each of 24 human Vβ families and a Cβ antisense primer (5′GCCTTTTCCCTGTGGGAGAT3′). The primers for Vβ 1-24 have been described previously (Blumberg *et al*, 1993; Gross *et al*, 1994). Twenty-microliter reactions contained 10 pmol each of a Vβ primer and the Cβ primer, 0.1 mg/ml BSA, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, and 0.5 unit Taq polymerase. Each cycle was 94°C for 20 s, 55°C for 30 s and 72°C for 60 s. Aliquots of 5 μl were withdrawn after 30 cycles and dot blotted with a ^{32}P-labeled internal Cβ antisense probe (5′GGCTCAAACACAGCGACCT3′). Quantitation was performed on a Molecular Dynamics Phosphorimager (Molecular Dynamics, Sunnyvale, CA).

Analysis of PCR-amplified TCR-β Chains by Polyacrylamide Gel Electrophoresis PCR amplified TCRs were reamplified using a nested ^{32}P end-labeled antisense Cβ primer (5′GGCTCAAACACAGCGACCT3′). Each 20-μl reaction contained 0.5 μl of amplified TCR from the quantitative PCR reactions, 50 ng of labeled Cβ primer, enzyme, nucleotides, and buffer. Amplification was for 22 cycles. PCR products were heat denatured and separated on 6% DNA-sequencing gels. The presence of dominant bands was confirmed by repeating analyses at least three times using separate aliquots of cDNA.

Direct Sequencing of PCR-amplified TCR-β Chains PCR products corresponding to dominant bands were excised from the gels, eluted by overnight incubation in 0.5 M ammonium acetate, 0.1% SDS, 0.1 mM EDTA, and ethanol-precipitated. After reamplification with the particular Vβ and Cβ primer, direct sequencing was carried out on an Applied Biosystems 373A DNA sequencer, using Taq DNA polymerase and flourescent dideoxynucleotides (Applied Biosystems, Foster City, CA).

Genomic DNA Extraction and PCR Amplification of TCR-γ Chains For analysis of TCR-γ chains in two patients, genomic DNA was extracted from 10-μm sections of formalin-fixed, paraffin-embedded tissues using standard protocols (Wright and Manos, 1990). For PCR amplification of rearranged TCR-γ segments, a two-step semi-nested protocol was designed. For primary amplification an external primer for each of the two Vγ subgroups (VγI and VγII) was used with a Jγ primer, followed by amplification with an internal primer for each of the two Vγ subgroups and the same Jγ primer. Primers for VγI contained the sequences 5′ TTCCCATTGCAGCCAGTCAGA 3′ and 5′ GACTGGGTCATCTGCTGA(T)AATCAC 3′ (corresponding to conserved external and internal Vγ1–8 sequences, respectively), and for VγII 5′ TTGGTTTATTTACTCCCTCCCAT 3′ and 5′ GGGGTACCTAGAGCAACCTCAAATTTCCA 3′ (corresponding to external and internal Vγ9 sequences, respectively). Jγ primer sequences were 5′ GTAATGATAAGCTTTGGTTCCGGGA 3′, 5′ GTGACAACAAGTGTTGTTCCACTG 3′, and 5′ GAAGTTACTATGAGCTTAGTCCCT 3′ for JγP, Jγ1.2, and JγP1.P2, respectively. Primary amplification was performed with external primers VγI or VγII and pooled Jγ primers. The 100-μl reaction mixtures contained 200 ng of each primer and reagents at the same concentrations as described for TCR-β PCR. Five cycles were at 94°C for 1 min, 55°C for 1 min, and 72°C for 5 min, followed by 20 cycles of 94°C for 20 s, 55°C for 30 s, and 72°C for 1 min, with a final 7-min extension at 72°C. Semi-nested PCR amplification was performed with each of the internal VγI or VγII primers and each of ^{32}P end-labeled Jγ primers (JγP, Jγ1.2, and JγP1.P2) in a 20-μl reaction mixture containing 1-μl of the initial PCR product and the same concentration of other reagents. Thirty-five cycles at 94°C for 20 s, 55°C for 30 s, and 72°C for 1 min were followed by a 7-min extension at 72°C. PCR products were heat-denatured and separated on 6% DNA-sequencing gels.

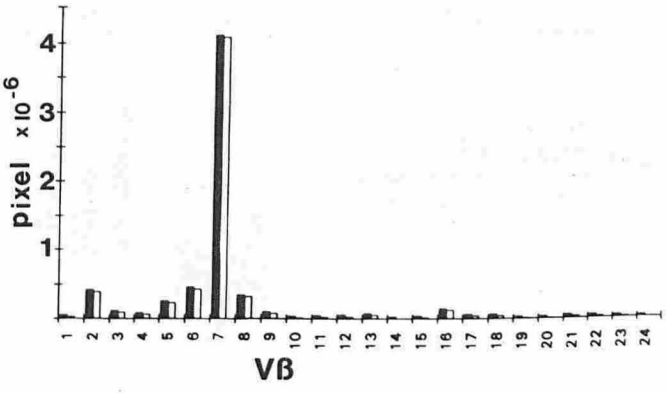

Figure 1. Vβ7 is used by the majority of T cells from a LyP type A lesion of patient 1. Semiquantitative PCR amplification of cDNA. *x*-axis, Vβ 1-24; *y*-axis, pixel value $\times 10^{-5}$, proportional to cpm.

Cloning and Sequencing of TCR-γ Chains PCR products were blunt-ended with T4 DNA polymerase, digested with *Kpn*I and ligated into *Kpn*I and *Sma*I sites of pBluescript II KS +/− (Stratagene, La Jolla, CA). Colonies were screened with a ^{32}P-labeled internal VγI probe (5′ TCTATTACTGTGCCACCT 3′), which hybridizes to conserved sequences of Vγ1–8, or with an internal VγII probe (5′ GTAGAGAAACAGGACATAGC 3′). Sequencing of multiple isolates was carried out using the Sequenase kit (United States Biochemicals, Cleveland, OH).

RESULTS

LyP Cells Are Activated T Cells Resembling Tumor Cells of HD or MF Six patients (patients 1–6) had LyP uncomplicated by lymphoma. Three patients had only type A lesions, two had type A, type B, and mixed type A/B lesions, and one had type A and mixed type A/B lesions. Patient 7 with ALCL had coexistent and prior lesions of LyP type A and patch lesions consistent with LyP type B or early MF. Patient 8 had an ALCL with sheets of Reed-Sternberg (RS)-like cells followed by LyP lesions of mixed type A/B histology. Patients 9–11 had MF lesions as well as LyP type A.

The immunophenotype of atypical LyP cells was consistent with that of activated T lymphocytes (Kadin *et al*, 1985). The atypical cells expressed CD4 in all but one case (patient 11) in whom a CD8 phenotype was found. A somewhat different immunophenotype was detected for the atypical cells in separate LyP lesions of five patients (1, 2, 3, 6, 7), although these lesions were subsequently found to be clonally related (see below). All lesions of LyP type A or ALCL had $CD30^+$, $CD25^+$ large atypical cells; in four patients one or more LyP type A lesions also contained large atypical $CD15^+$ cells. Lesions of LyP type B or MF had few or no $CD30^+$ or $CD25^+$ cells, and no $CD15^+$ cells. Lesions of LyP mixed type A/B contained moderate numbers of $CD30^+$, $CD25^+$ cells, and no $CD15^+$ cells.

Multiple Regressing Lesions of Lymphomatoid Papulosis Are Clonally Related Frozen sections from lesions of patients 1–5 were used to extract RNA and prepare cDNAs. Semiquantitative PCR amplification analysis indicated that Vβ7 was the predominant Vβ expressed by T cells in an LyP lesion of patient 1 **(Fig 1)**. To compare the Vβ7 transcripts of this and other lesions, the precise length of the Vβ7 transcripts was determined on DNA sequencing gels. This analysis revealed a single Vβ7 transcript of identical size in each lesion, suggesting that the lesions were monoclonal (**Fig 2**, *lanes 1–3*). To confirm that the lesions were monoclonal, the dominant band from each lesion was excised, reamplified, and directly sequenced **(Table I)**.

The cDNA from patients 2–5 were studied similarly. The results indicate that all lesions from patients 2, 3, and 4 contained a single dominant T cell clone **(Table I)**. In contrast, a dominant clone was

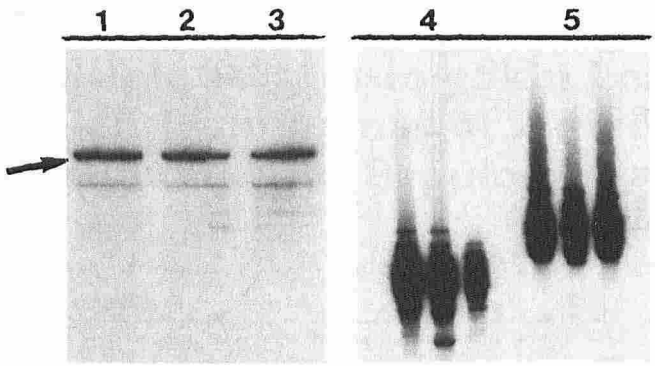

Figure 2. The same dominant T-cell clone using Vβ7 is found in three LyP lesions from patient 1 (*lanes 1–3*), whereas two LyP lesions of patient 5 are polyclonal (*lane 4*, Vβ5; *lane 5*, Vβ3). TCR-β transcripts were separated by size on polyacrylamide gels. All experiments were done in triplicate using three separate aliquots of cDNA.

not detected in patient 5. Semiquantitative PCR amplification analysis from this patient did not reveal any dominant Vβs (data not shown). Analysis of TCR N-region lengths in each lesion using several Vβs failed to detect the presence of a clonal population (**Fig 2**, *lanes 4, 5*; data not shown).

Four formalin-fixed, paraffin-embedded LyP lesions were available from patient 6. Genomic DNA from these tissues was amplified with primers specific for the TCR-γ chain. One primer combination (VγI and Jγ1,2) generated a prominent band at exactly the same position in each of three anatomically separated lesions. Cloning and sequencing confirmed that all three lesions contained the same dominant clonal T cell population using Vγ8-TTCT-Jγ1,2. DNA extracted from the fourth biopsy obtained in 1966 did not amplify with the primer set VγI and Jγ1,2. Successful amplification of a smaller DNA fragment was achieved by using a primer located further 3′ in Vγ8 (5′ TACTGGAAAATCTAATTGAA-CGTGAC 3′). To exclude the possibility of contamination, a new Jγ1,2 primer located 3′ of the previous Jγ1,2 primer was also used (5′ TTGGAAATGTTGAATTCTTCCGATAC 3′). Sequencing of this PCR product confirmed that it was identical to the Vγ identified in later lesions **(Table I)**. This finding demonstrated that all LyP lesions of patient 6, separated by as much as 26 years, were derived from a common T cell clone. Thus, the anatomically and temporally separate lesions of five of six patients with LyP uncomplicated by lymphoma were monoclonal.

Lesions of Lymphomatoid Papulosis Are Clonally Related to Anaplastic Large Cell Lymphoma Two patients had LyP and ALCL. In patient 7, a cell culture of atypical RS-like cells was derived from the ALCL biopsy. These cells were aneuploid and had marker chromosomes consistent with a monoclonal origin (in preparation). Semiquantitative PCR amplification with a panel of Vβ primers revealed that the cultured cells all used Vβ2. Analysis of frozen sections from the biopsy used to generate this cell line showed that the dominant Vβ chain used was also Vβ2. The lengths of the Vβ2 using TCR-β chains from the cultured cells and the biopsy were compared and evidence for a single identical clone *in vitro* and *in vivo* was obtained **(Fig 3)** and confirmed by DNA sequencing **(Table I)**.

To determine whether this clone was present in archival LyP lesions dating back 6 years, PCR reactions were carried out with a ^{32}P-labeled clone-specific primer. This primer, based in the VDJ junction, together with a Vβ2 primer, was used to amplify DNA extracted from prior LyP lesions. **Figure 4** shows that PCR

Table I. DNA Nucleotide Sequences of Skin Lesions from Patients with LyP and Associated Lymphomas*

Patient No.	Specimens Diagnosis/site/date	Vβ/γ	N	Jβ/γ
1	1. LyP A, thigh, 1991	Vβ7	CAAGTTGATATGGGGCTAGCGGGATTC	Jβ2.1
	2. LyP A/B, thigh, 1991	Vβ7	CAAGTTGATATGGGGCTAGCGGGATTC	Jβ2.1
	3. LyP B, wrist, 1991	Vβ7	CAAGTTGATATGGGGCTAGCGGGATTC	Jβ2.1
2	1. LyP A, back, 1991	Vβ5	GGAGGTGGAGAT	Jβ1.5
	2. LyP B, back, 1991	Vβ5	GGAGGTGGAGAT	Jβ1.5
	3. LyP B, wrist, 1991	Vβ5	GGAGGTGGAGAT	Jβ1.5
	4. LyP A/B, thumb, 1991	Vβ5	GGAGGTGGAGAT	Jβ1.5
3	1. LyP A, leg, 1984	Vβ5	AGACCCAGGACAGGGGCTACTTCG	Jβ2.7
	2. LyP A/B, trunk, 1985	Vβ5	AGACCCAGGACAGGGGCTACTTCG	Jβ2.7
4	1. LyP A, left forearm, 1988	Vβ8	CCTGAAGGAAGG	Jβ2.7
	2. LyP A, right thigh, 1988	Vβ8	CCTGAAGGAAGG	Jβ2.7
	3. LyP A, right thigh, 1988	Vβ8	CCTGAAGGAAGG	Jβ2.7
	4. LyP A, left knee, 1988	Vβ8	CCTGAAGGAAGG	Jβ2.7
	5. LyP A, 1990	Vβ8	CCTGAAGGAAGG	Jβ2.7
6	1. LyP A, back, 1992	Vγ8	TTCT	Jγ1.2
	2. LyP A, abdomen, 1992	Vγ8	TTCT	Jγ1.2
	3. LyP A, left thigh, 1992	Vγ8	TTCT	Jγ1.2
	4. LyP A, left arm, 1966	Vγ8	TTCT	Jγ1.2
7	1. LyP A, right thigh, 1988	Vβ2	ACTAGCGGGAGAAGATATTTTAAC	Jβ2.1
	2. LyP B, left forearm, 1988	Vβ2	ACTAGCGGGAGAAGATATTTTAAC	Jβ2.1
	3. LyP A, right forearm, 1989	Vβ2	ACTAGCGGGAGAAGATATTTTAAC	Jβ2.1
	4. LyP A, right forearm, 1992	Vβ2	ACTAGCGGGAGAAGATATTTTAAC	Jβ2.1
	5. ALCL, right forearm, 4/1993	Vβ2	ACTAGCGGGAGAAGATATTTTAAC	Jβ2.1
	6. ALCL, right forearm, 11/1993	Vβ2	ACTAGCGGGAGAAGATATTTTAAC	Jβ2.1
8	1. ALCL, abdomen, 1990	Vβ5	TTACAGGCACAC	Jβ2.7
	2. LyP A/B, abdomen, 1991	Vβ5	TTACAGGCACAC	Jβ2.7
9	1. LyP A, back, 1984	Vβ6	TTAGTTAGCGGGGGGTCGGTACCT	Jβ2.4
	2. LyP A, chest, 1987	Vβ6	TTAGTTAGCGGGGGGTCGGTACCT	Jβ2.4
	3. MF, right lat. trunk, 1993	Vβ6	TTAGTTAGCGGGGGGTCGGTACCT	Jβ2.4
11	1. LyP A, right leg, 1984	Vγ2	TT	JγP2
	2. LyP A, lower back, 1984	Vγ2	TT	JγP2
	3. MF, buttock, 1984	Vγ2	TT	JγP2

* Data on patients 5 and 10 are not included. Because of the polyclonal nature of LyP lesions in patient 5, no clearly readable sequences were obtained by direct sequencing

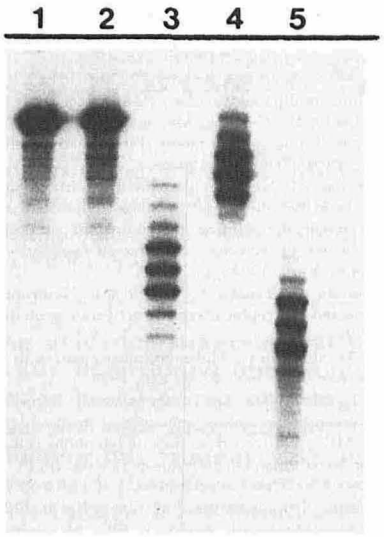

Figure 3. The ALCL biopsy from patient 7 (*lane 1*) and the cell culture generated thereof (*lane 2*) contain the same dominant T-cell clone using Vβ2. In contrast, amplifications of the ALCL biopsy cDNA with Vβ3 (*lane 3*), Vβ8 (*lane 4*), and Vβ13 (*lane 5*) generated multiple bands of similar intensity, suggestive of a polyclonal T cell population.

products of precisely the correct size were amplified from each LyP lesion, indicating that the ALCL clone was present in these samples. No product could be detected in peripheral blood lymphocytes from this patient or a series of negative control samples. As a further control, an additional Vβ2 primer located further 5′ was used together with the primer in the VDJ junction. These primers similarly amplified the TCR of the ALCL clone specifically from the archival LyP lesions. These results demonstrate that the dominant T cell clone in the ALCL was present in the LyP lesions for 6 years and was represented by RS-like cells rather than tumor-infiltrating lymphocytes.

Similar results were obtained in patient 8, who had LyP lesions one year after experiencing an ALCL on the abdomen. An identical dominant T-cell clone was identified in the ALCL and an LyP lesion from this patient **(Table I)**.

Lesions of Lymphomatoid Papulosis Are Clonally Related to Mycosis Fungoides Frozen tissues were available from the

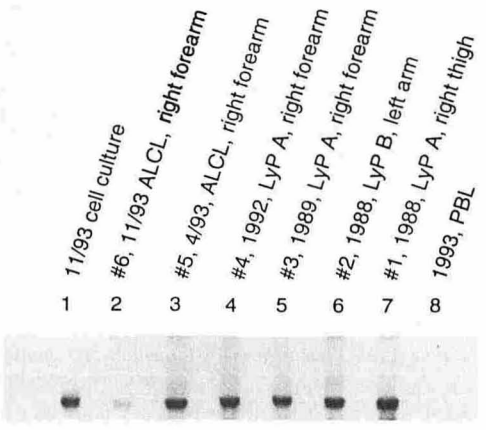

Figure 4. The same dominant T cell clone is present in archival paraffin-embedded ALCL and LyP lesions of patient 7. Genomic DNA extracted from multiple lesions (*lanes 3–7*) or cDNA (*lanes 1, 2, 8*) was amplified with a Vβ2 primer and a ^{32}P-labeled clone-specific primer and the products loaded onto polyacrylamide gel.

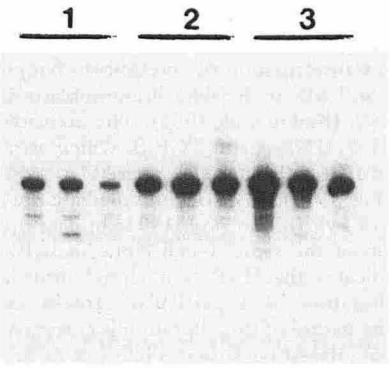

Figure 5. The same dominant T-cell clone is present in LyP lesions and MF of patient 9. Three separate cDNA aliquots each of two LyP type A lesions (*lane 1, 2*) and an MF lesion (*lane 3*) were amplified with a Vβ6 primer and a ^{32}P-labeled internal Cβ primer. The PCR products were loaded onto polyacrylamide gel.

MF and LyP lesions of patients 9 and 10 and Vβ gene use was analyzed. A common dominant clone using Vβ6 was detected in both the MF and LyP lesions of patient 9 **(Fig 5, Table I)**; however, a dominant T-cell clone could not be detected consistently in the MF or LyP lesions of patient 10. Patient 11 had only paraffin-embedded tissue available for analysis. To compare the clonal relationship between LyP and MF in this patient, TCR-γ chains from the MF lesion were PCR-amplified. A single dominant clone using Vγ2 and JγP2 was identified and sequenced **(Table I)**. A clone-specific primer together with a Vγ2 primer showed that the two available LyP lesions from this patient contained the same TCR-γ rearrangement as the MF lesion.

DISCUSSION

Southern blot analyses in LyP have demonstrated clonal rearrangements of TCR genes in approximately 65% of cases (Weiss *et al*, 1986; Kadin *et al*, 1987; Whittaker *et al*, 1991; Zelickson *et al*, 1991; Parks *et al*, 1992). A recent PCR-based study showed clonal TCR-γ rearrangements in isolated lesions from 7 of 10 patients with LyP type A (Theodorou *et al*, 1995). Three studies using PCR amplification and sequencing of TCR-γ genes showed a common clonal origin of LyP skin lesions and either CD30$^+$ large cell lymphoma or early patch/plaque MF in four patients (Volkenandt *et al*, 1993; Bottaro *et al*, 1994; Wood *et al*, 1995).

This study examined a series of patients with multiple lesions and demonstrates that in most cases (9 of 11 or 82%) LyP is a monoclonal disorder arising from a single transformed T cell. Moreover, the failure to detect a dominant clone in two cases does not necessarily reflect the absence of such clones. The panel of primers will not amplify all Vβ genes and may amplify some clones inefficiently. Such clones would be missed because the Vβ they use would appear to be expressed at very low frequency or the N-region length analysis would not show clear evidence of a dominant band. Other genetic events, such as deletion of the rearranged TCR or clonal expansion prior to TCR gene rearrangement (Davis *et al*, 1992), could also explain the absence of a dominant TCR. It remains possible, however, that a small subset of LyP cases have a distinct etiology and are truly polyclonal.

This study indicates that the RS-like cells in LyP are commonly derived from T lymphocytes, a finding that had been difficult to discern in previous studies using less sensitive methods (Whittaker *et al*, 1991). The T-cell lineage of RS-like cells was particularly clear in case 7, in which RS-like cells in culture were shown to be derived from a dominant T cell clone in a series of LyP and ALCL skin lesions. In several patients, the same clone was found in lesions of different histologies and immunophenotypes. These variable histologies and immunophenotypes may be due to differences in local cytokine production. IL-7 produced by keratinocytes (Heufler

et al, 1993) and IL-2 by tumor-infiltrating lymphocytes (Reinhold *et al*, 1991) could act synergistically to activate the LyP clone, resulting in the transformation of cerebriform lymphocytes in LyP type B lesions and MF to RS-like immunoblasts in LyP type A lesions and ALCL (Kadin *et al*, 1984). The activated T-cell clone can elaborate IL-8, TNF-α and TGF-β, which attract neutrophils characteristic of type A lesions (Leonhard *et al*, 1991; Ming *et al*, 1987; Reiban *et al*, 1991). TGF-β could mediate the regression and fibrosis typical of LyP (Kadin *et al*, 1994a,b; data not shown).

The detection of the same T-cell clone in LyP and associated lymphomas indicates that LyP is a clonal disorder presumably initiated by alteration of a particular protein in a T lineage lymphocyte. The nature of this alteration is currently unknown but is clearly of great interest because it is likely to be involved in some cases of Hodgkin's disease and non-Hodgkin's lymphoma. The development of lymphoma in only a minority of LyP patients further indicates that subsequent genetic events cause progression to malignant lymphoma (Nowell, 1986; Ludwig, 1994). One such event apparently is a mutation of the type II receptor for TGF-β, which facilitates growth of the LyP clone (Kadin *et al*, 1994; Knaus *et al*, submitted). It is likely that additional genetic alterations will be identified, since we and others have demonstrated multiple chromosomal abnormalities in LyP and LyP-associated lymphomas (Espinoza *et al*, 1985; Parks *et al*, 1992; Peters *et al*, 1995).

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