RAPID COMMUNICATION

Clonal Composition of T Cells in Lymphomatoid Papulosis

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A cDNA of the Cβ2 gene of the T-cell receptor was used as a probe to investigate the clonal composition of T cells in skin lesions of 5 patients with lymphomatoid papulosis (LyP), a chronic recurrent eruption characterized by morphologically abnormal activated T cells in the cutaneous infiltrate. Clonal T-cell populations, as evidenced by rearranged DNA bands, were demonstrated in the skin lesions of four patients, one of whom has shown clinical progression toward lymphoma. Three of these patients had lesions of type A histology, a type previously shown to be associated with aneuploidy. The remaining patient with clonal lesions appeared to have the same gene rearrangement pattern in DNA obtained from separate lesions taken 11 months apart, providing evidence that the T cells in both sites were derived from the same clone. This patient had lesions of type B histology, which is not associated with aneuploidy. Absence of a rearranged band and deletion or near absence of the 10.8 kb band in Eco RI digests was interpreted as evidence of polyclonal T-cell hyperplasia, accounting for the skin infiltrate of a fifth patient who had a prolonged clinical course without progression to lymphoma. This patient had lesions of type A histology with frequent Ki-1-positive Reed-Sternberg-like cells. Our results show that gene rearrangement analysis provides information that is independent of histology in LyP and may in part explain the variable progression of LyP to lymphoma in 10-20% of patients. (Am J Pathol 1987, 126:13-17)

IN 1968, Macaulay introduced the term "lymphomatoid papulosis" (LyP) to describe patients with recurrent self-healing papulonodular skin lesions clinically resembling pityriasis lichenoides but with histologic features suggesting a malignant lymphoma.[1] Since then, the clinical spectrum has been expanded to include similar rhythmic paradoxical eruptions.[2] The clinical course of LyP is variable, with progression to disseminated malignant lymphoma in 10-20% of patients.[3,4] In particular, a histogenetic relationship among LyP, cutaneous T-cell lymphoma, and Hodgkin's disease has been suggested.[5,6] Pathologic review of LyP cases has revealed two major histologic types, A and B, with overlapping features, but no reliable criteria to predict progression to lymphoma.[7]

Until recently, evidence for the malignant potential of cells in LyP lesions has been limited to studies of DNA content by Feulgen cytophotometry,[8] flow cytometry,[9] and in one case cytogenetics.[10] Aneuploidy has been associated with lesions of type A histology which contain large atypical Reed-Sternberg-like cells, whereas type B lesions with cerebriform mononuclear cells are unassociated with aneuploidy.[8] Two patients with aneuploidy had evidence of clinical progression of their disease.[9]

This study was undertaken to determine the clonal composition of atypical cells in LyP lesions by the recently developed gene arrangement technique.[11] Because we and others have demonstrated that the large atypical cells in LyP have the immunophenotype of activated T cells,[12,13] a probe for the T-cell receptor beta chain gene was chosen as a means of detecting clonality. Our study confirms the high frequency of clonal T-cell populations in skin lesions of

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patients with LyP recently reported by Weiss et al.[14] However, in their report, 3 of 5 patients with clonal T-cell lesions had prior or coexistent lymphoma and therefore may not be representative of the most typical patients with LyP who have only a 10–20% risk of lymphoma development in previously reported series.[3,4] Additionally, gene rearrangement analysis of a skin infiltrate in one of our cases was interpreted as providing evidence for a polyclonal T-cell hyperplasia which may precede the clonal T-cell lesions of LyP by an as yet undefined period of time.

Materials and Methods

This study was approved by the Human Subjects Committee at Beth Israel Hospital. Four-millimeter punch or excisional skin biopsies were obtained from patients with the clinical diagnosis of lymphomatoid papulosis without evidence of coexistent mycosis fungoides. One-half of each bisected specimen was snap-frozen in liquid nitrogen for BNA analysis. The remainder was used for routine histologic and immunoperoxidase studies as previously described.[12] Specimens analyzed had the characteristic histology and immunophenotype of lymphomatoid papulosis and provided at least 20 μg of DNA for gene rearrangement analysis.

High-molecular-weight DNA was extracted from frozen samples by proteinase-K digestion and purified by standard procedures of phenol/chloroform extraction and ethanol precipitation.[15] Ten micrograms of DNA was digested by each of the restriction enzymes (Bam HI, Eco RI, Hind III) in accordance with the directions of the manufacturer (New England Biolabs, Beverly, Mass). Digested DNA was size-fractionated by electrophoresis in 0.8% agarose gels and transferred onto nitrocellulose filters as described by Southern.[16] Filters were hybridized 12–18 hours with a ^{32}P-labeled probe[17] specific for the human T-cell receptor beta chain gene (Cβ2). After hybridization the filters were washed thoroughly and exposed 24 hours at −70 C to Kodak X-Omat AR film with a single intensifying screen. DNA fragment size was determined by co-electrophoresis of patient samples and Hind III digests of lambda phage DNA. Human genomic DNA isolated from normal volunteers was used to establish germline patterns.

The probe was derived from a cDNA clone of the T-cell receptor Cβ2 gene from the T-cell line Jurkat (kindly provided by Dr. Tak Mak, Ontario Cancer Institute, Toronto, Canada).[18] The JUR-β2 probe consists of a 390 base-pair Bgl II - Bgl II fragment excised from the cDNA. This probe contains only the constant region sequences. It cross-hybridizes to the Cβ1 gene.

Sensitivity of the Southern blot hybridization technique in our laboratory was examined in preliminary experiments and determined to detect 1–5% of clonally derived leukemic T cells added to germline somatic cells.

Results

Table 1 gives the clinical characteristics and morphologic features of skin lesions in the 5 patients with LyP. All patients were adults who had spontaneously regressing papular or nodular skin lesions for 2.5–10 years. Four patients had type A lesions distinguished by large atypical cells with vesicular nuclei and prominent nucleoli. One patient had type B lesions characterized by a predominance of atypical cerebriform mononuclear cells, which infiltrated the epidermis.

Table 2 gives the immunologic phenotype of the atypical cells. As shown in previous studies,[12,13] the atypical cells in each case had the immunophenotype of activated T cells expressing Hodgkin's disease associated antigen Ki-1. The atypical cells were further defined as activated helper T cells ($T4^+$/$T11^+$ or $T4^+/T3^+$) in 4 of the 5 cases.

Four of the 5 patients (Table 2, Patients 1, 2, 4, and 5) had evidence of rearranged DNA bands in South-

Table 1 — Clinical Characteristics and Morphologic Features of Skin Lesions of 5 Patients With Lymphomatoid Papulosis

Patient	Age/ sex	Duration (years)	Clinical course	Specimen	Histologic* type	Site
1	58/M	10	Poor control on multiple therapies; lesions now form 4-cm ulcerated tumors	Nodule	A	Left anterior chest
2	51/F	5	Good control on low dose methotrexate	Papule	A	Left knee
3	69/F	10	Good control on low dose methotrexate	Papule	A	Left upper thigh
4	27/M	2.5	Partial response to tetracycline; complete response to 13-*cis*-retinoic acid	Nodule	A	Left flank
5	27/F	2.5	Few scattered papular lesions, no treatment	(1) Papule (2) Papule	B B	Right hip

*Described in greater detail by Willemze et al.[7]

Table 2—Immunophenotype and Gene Rearrangement Characteristics of Atypical Cells in Skin Lesions of 5 Patients With Lymphomatoid Papulosis

	Antibody											Restriction enzyme*		
Patient	Leu 1	3Al	T11	T3	T4	T8	T9	Tac	Ki-1	Ia	B-1	Hind III	Eco RI	Bam HI
1	0	0	+	+	+	0	0	+	+	+	0	R	G	R
2	0	0	+	0	0	0	0	+	+	+	0	ND	G	R
3	0	0	0	+	+	0	+	+	+	+	0	G	DI	ND
4	0	0	+	+	+	0	0	+	+	+	0	R	R	R
5	+	0	+	0	+	0	0	+	+	+	0	(1) R	DI	R
												(2) R	DI	R

*G, germline; R, rearranged; DI, decreased intensity 10.8-kb band; ND, not done.

ern blot autoradiograms (Figures 1 and 2). Patient 5, who had lesions of histologic type B, appeared to have the same gene rearrangement patterns in DNA from lesions obtained 11 months apart. This suggests that DNA obtained from both sites was derived from the same T-cell clone. In contrast, 1 patient (3) appeared to have a polyclonal T-cell population accounting for a skin infiltrate of histologic type A which contained numerous Ki-1 positive large atypical Reed–Sternberg-like cells (Figure 3). This patient had no rearrangements detected with either restriction enzyme Eco RI or Hind III. In this circumstance the deletion or absence of the 10.8-kb band in Eco RI digests is interpreted as evidence of a polyclonal T-cell population.[11]

Discussion

The purpose of this study was to investigate the clonal composition of atypical cellular infiltrates in skin lesions of patients with LyP. A probe for the T-cell receptor beta chain gene was used for this purpose because we and others had shown that the atypical infiltrate had the characteristics of activated T cells. The analysis showed that 4 of the 5 patients had evidence for a distinct clone of T cells within their skin infiltrates. One of these patients had histologic type B lesions, which previously had been shown to be unassociated with aneuploidy.[8] This patient appeared to have the same gene rearrangement pattern in DNA from 2 separate lesions taken 11 months apart, sug-

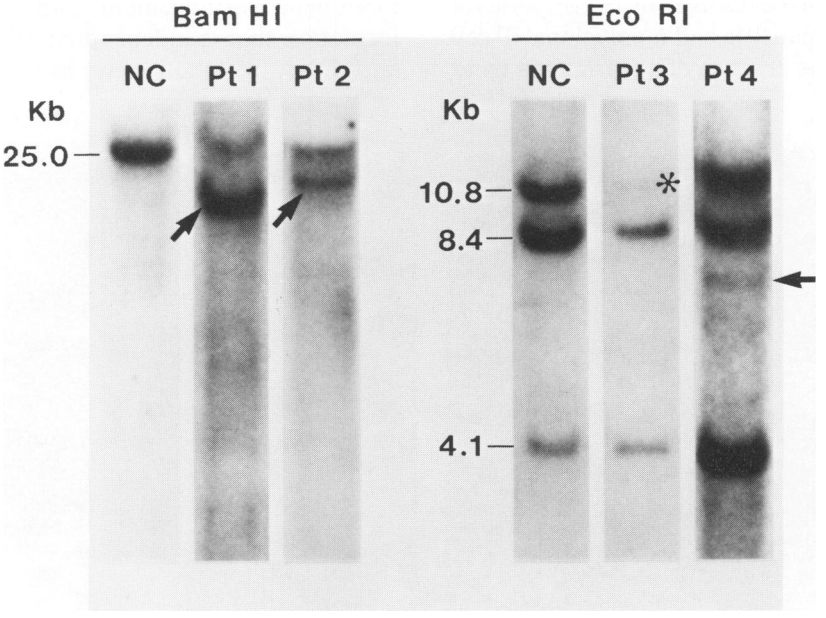

Figure 1—Autoradiograms obtained from DNA prepared from skin biopsy specimens of patients with lymphomatoid papulosis and normal controls *(NC)*. Analyses with Bam H I and Eco R I restriction enzymes are shown. *Numbers* at the top of lanes correspond to Patients 1, 2, 3, and 4 in Table 1. Germline bands are indicated by *dashes*. *Arrows* show rearranged bands detected by a probe specific for the Cβ2 gene of the T-cell receptor. In the lane labeled *Patient 3*, absence of a rearranged band and near absence of the 10.8-kb band (*) indicates a polyclonal population of T cells.[11] The 8.4-kb germline band in Eco RI digests of skin biopsies of patients and controls was not seen in similar preparations from blood, lymph node, or spleen and may result from differential digestion of nonlymphoid cell DNA in the region of the Cβ2 gene.

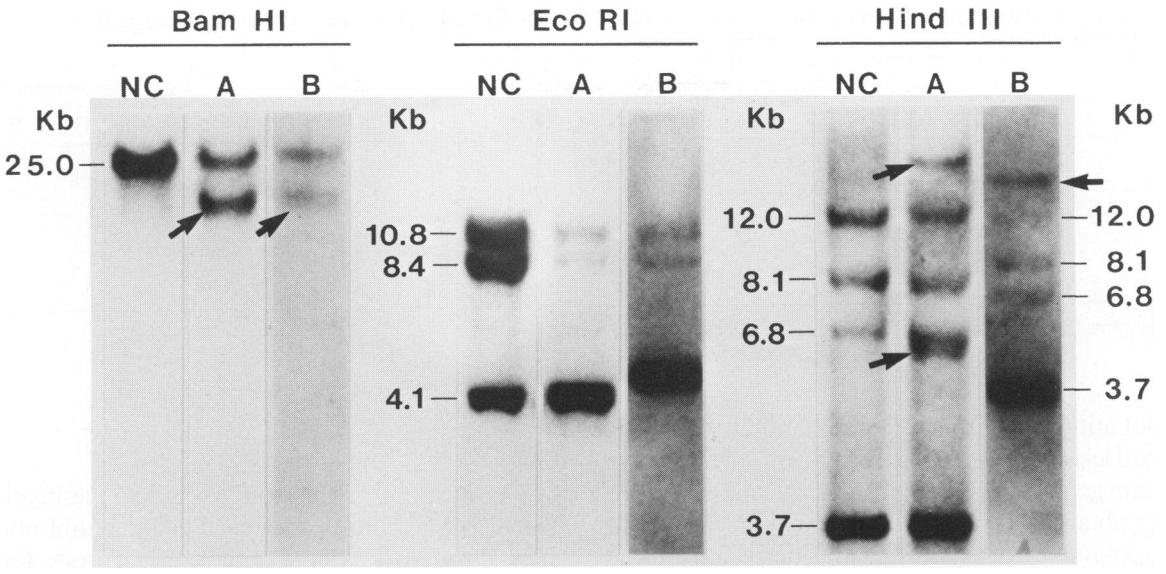

Figure 2— Southern analysis of a normal control and two separate lesions, A and B, from Patient 5 are shown. Rearranged bands *(arrows)* of similar molecular weight were found for each of the restriction enzymes Bam HI and Hind III. Specimens A and B were obtained 11 months apart and were not run on the same gel. With the restriction enzyme Hind III, a unique pattern of 23-kb rearranged bands is seen which has not been observed with LyP samples from other patients. In addition, specimen A contains a rearranged band of lower molecular weight than the 6.8-kb germline band. In the corresponding digest of specimen B, two distinct bands are not resolved in this region, presumably due to co-migration of the bands in a gel electrophoresed for a shorter period of time.

gesting that the T cells in both sites were derived from the same clone. This result is different from that of Weiss et al, who found varying patterns of gene arrangements in three separate specimens from one of their patients.[14] The frequency of single versus multiple separate T-cell clones accounting for separate LyP skin lesions will have to be resolved in a larger series of patients. One of our patients had a polyclonal T-cell infiltrate shown by the absence of a rearranged band and a near absence of the 10.8-kb Eco RI germline band in her Southern blot autoradiogram. This patient had histologic type A lesions of LyP with frequent Ki-1 positive large atypical Reed–Sternberg-like cells (Figure 3). Type A lesions are frequently associated with aneuploidy.[8] These results show that DNA gene rearrangement studies in LyP provide information that is independent of histology and perhaps DNA cytophotometry as well.

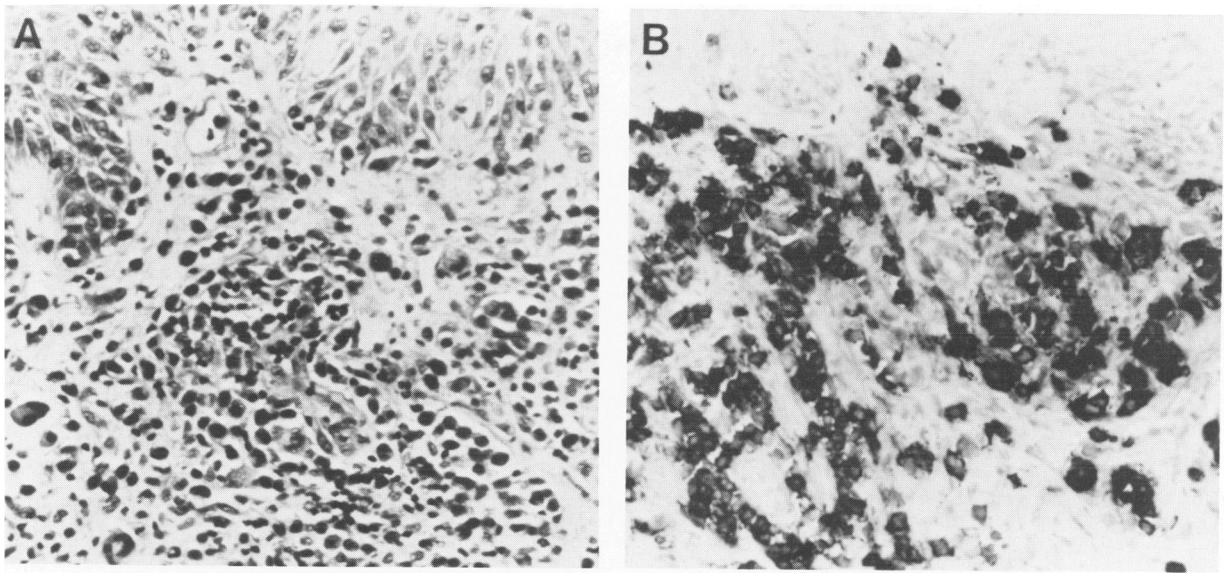

Figure 3— Infiltrate of large atypical Ki-1 positive cells in skin lesion of Patient 3 with a polyclonal T-cell population shown by gene rearrangement analysis. (**A**, H&E; **B**, Immunoperoxidase stain for Ki-1, ×200)

Lymphomatoid papulosis is a chronic recurrent papulonodular eruption that usually runs a protracted benign course. Individual skin lesions characteristically regress spontaneously. In our experience, low doses of methotrexate, 15–25 mg given every other week, were particularly useful to control LyP without adverse effects. However, after 10 years, the first patient in this series who had a clonal population of T cells by gene rearrangement studies has failed to respond adequately to multiple treatments, including multiagent systemic chemotherapy. The development of large relatively persistent tumors with ulceration in this patient suggests evolution into malignant lymphoma, which may occur in 10–20% of patients with LyP.[3,4] Systemic lymphoma was associated with lymphomatoid papulosis in 3 of the 6 patients reported by Weiss et al.[14] In our series, 3 of 4 patients with clonal populations of T cells in their skin lesions have run a chronic course without evidence of progression to lymphoma. The mechanism of regression of skin lesions in these patients is not yet known but may be of importance in guiding the future therapy of LyP and related lymphomas. We suggest that host immune factors may be important in the regression of skin lesions and containment of their disease.

One patient whose analyzed skin lesion was composed of polyclonal T cells has shown no progression to lymphoma over 10 years. It will be necessary to follow this patient and similar patients with subsequent biopsies over time to determine whether an evolution from polyclonal to clonal T-cell infiltrates occurs and is associated with a change in the character of the disease. Therefore, it is recommended that future studies of LyP include clonal analysis of skin lesions and surveillance tests of humoral and cellular immunity. Correlations between these tests and clinical course may predict which patients are likely to progress to systemic lymphoma.

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