Analysis of Clonality of Atypical Cutaneous Lymphoid Infiltrates Associated With Drug Therapy By PCR/DGGE

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Atypical lymphocytic infiltrates that mimic cutaneous lymphoma (ie, pseudolymphoma) are often observed in skin biopsy specimens from patients with altered immune function. The latter may reflect systemic immune dysregulatory states such as collagen vascular disease or human immunodeficiency virus infection. Among the iatrogenic causes are drug therapy with agents that abrogate lymphocyte function. These drugs encompass the anticonvulsants, antidepressants, phenothiazines, calcium channel blockers, and angiotensin-converting enzyme inhibitors. The appellation of lymphomatoid hypersensitivity reaction has been applied to cases of drug-associated pseudolymphoma. Pathologically and clinically, the distinction of such cases from cutaneous lymphoma is difficult. We employed the polymerase chain reaction (PCR) on archival material of proven drug-associated lymphomatoid hypersensitivity reactions both to explore its utility as an adjunct in diagnosis and to investigate the genotypic aberrations induced by drug therapy. Formalin-fixed, paraffin-embedded biopsy specimens from seven cutaneous T-cell lymphomas (CTCL), one nodal T-cell lymphoma, two cutaneous B-cell lymphomas, three typical hypersensitivity reactions, one tonsil, and 14 lymphomatoid hypersensitivity reactions were studied. Control cases for which DNA derived from fresh tissue was used include the Jurkat T-cell tumor line, placenta, one nodal B-cell lymphoma, and one case of reactive lymph node hyperplasia. DNA was obtained and purified by standard methods, then amplified with oligonucleotide primers specific for the T-cell receptor gamma locus and the immunoglobulin heavy chain genes. T-cell amplicons were analyzed by denaturing gradient gel electrophoresis (DGGE) and B-cell amplicons by either nondenaturing polyacrylamide or agarose gel electrophoresis. The nodal and Jurkat T-cell lymphomas, six of seven CTCL, one cutaneous B-cell lymphoma, and 2 of 14 lymphomatoid hypersensitivity reactions showed dominant ("monoclonal") T-cell gene rearrangement patterns, and the remainder of cases were polyclonal. A causal relationship between drug therapy and skin eruption was ascertained in the two patients showing T-cell rearrangements, and both experienced complete and sustained lesional resolution on discontinuation of the implicated drug. The only immunoglobulin heavy chain gene rearrangements detected by PCR were in two of the three B-cell lymphomas. We conclude that PCR/DGGE is a powerful method for assaying T-cell clonality in archival tissue and can aid in the discrimination of reactive from malignant cutaneous infiltrates with appropriate clinicopathologic correlation. Recognition that a monoclonal TCRγ rearrangement can be observed in cases of drug-associated lymphomatoid hypersensitivity may help in avoiding a misdiagnosis of malignant lymphoma. HUM PATHOL 30:130-136. Copyright © 1999 by W.B. Saunders Company

Key words: **Clonality, T-cell receptor, PCR/DGGE, cutaneous pseudolymphoma, immunodysregulation.**

Abbreviations: **DGGE, denaturing gradient gel electrophoresis; IgH, Immunoglobulin heavy chain; PCR, polymerase chain reaction; TCR, T cell receptor.**

Atypical cutaneous lymphoid infiltrates that clinically and light microscopically resemble lymphoma are problematic for the clinician and pathologist. Such cases fall under the broad designation of pseudolymphoma. It appears that the predisposed populace are those with underlying perturbations in immune function.[1,2] Systemic causes include autoimmune disease, atopy, and hematologic malignancy, while therapy with drugs known to alter lymphocyte function is a common iatrogenic cause. Several commonly prescribed agents, including angiotensin-converting enzyme inhibitors, calcium channel blockers, histamine antagonists, antidepressants, lipid-lowering agents, anticonvulsants, benzodiazepines, and phenothiazines,[1-3] can exert such effects. Among their effects are depression of T suppresser function or promotion of lymphocyte mitogenesis. It has been hypothesized that the aberrant immune response in such cases either may be directed against the immune-dysregulating drug itself or may be triggered by an unrelated antigen.[1-3] In either scenario, the immune response triggered by the antigenic stimulus is abnormal. On histomorphologic grounds alone, the differentiation of these atypical lesions from cutaneous lymphoma often poses difficulty. We explored the utility of gene rearrangement studies as an aid in diagnosis. Such information also may facilitate our comprehension at a molecular level of lesions of lymphomatoid hypersensitivity. The T-cell receptor gamma (TCRγ) locus was chosen for analysis because of both its relatively simple organization (Fig 1) and the fact that gamma rearrangements occur early in T lymphocyte ontogeny and persist after rearrangements of other TCR loci.[4,5] Rearrangements involving the immunoglobulin heavy chain genes were studied to assess B-cell clonality.

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MATERIALS AND METHODS

Selection of Cases/Criteria Used for Diagnosis of Lymphomatoid Hypersensitivity

The cases were provided by one of the authors (C.M.M.) and included three cases (cases 1, 4, 14) previously reported by Magro and Crowson.[1] In all of the cases, a diagnosis of drug-associated lymphomatoid hypersensitivity was made based

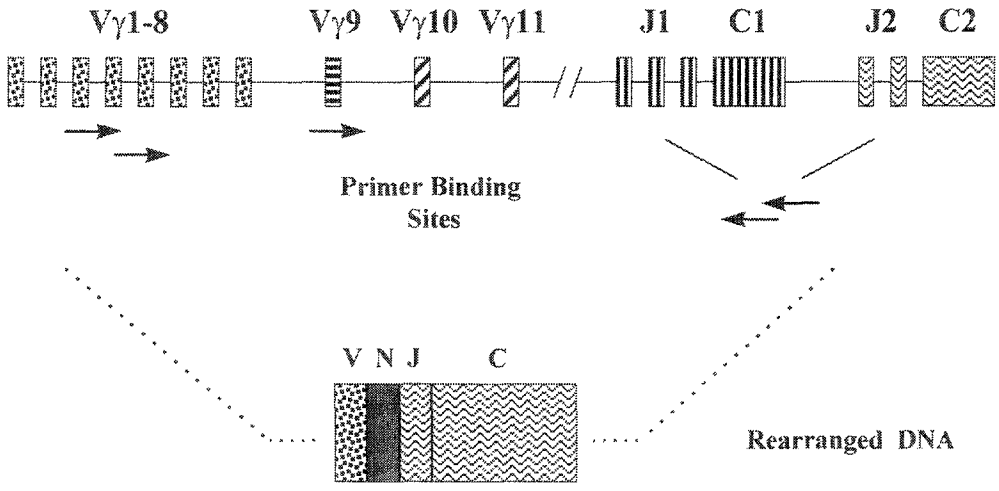

FIGURE 1. The relative simplicity of the TCRγ locus and its high frequency of conserved sequences permit the design of just a few primer pairs capable of detecting most T-cell gene rearrangements. N depicts the variable number of nucleotides randomly inserted or deleted during V and J joining. Arrows indicate primer binding sites for both Vγ1-8 (nested PCR) and Vγ9 (seminested PCR) genes.

on ingestion of drugs with potential immune dysregulating properties before the onset of the eruption, characteristic light microscopic findings alluded to below, lesional resolution in those cases in which there was cessation of one or more of the implicated drugs or response to other treatment modalities, and isolation of a definite antigenic trigger in the biopsy specimen (ie, an infective agent). By light microscopy, lymphomatoid hypersensitivity can be categorized as (1 and 2) interface dermatitis with variable epitheliotropism versus a psoriasiform and eczematous dermatitis with lymphoid atypia, hence recapitulating the morphology of patch/plaque stage mycosis fungoides, (3) nodular lymphoid hyperplasia producing a morphology resembling B-cell lymphoma, (4) angiocentric atypical lymphocytic infiltrates (described as lymphomatoid vascular reactions) resembling angiocentric T-cell lymphoma, and (5) lymphomatoid follicular mucinosis whereby the outer root sheath of the follicular epithelium is permeated by atypical lymphocytes and there is attendant mucinosis. Such cases raise diagnostic consideration to folliculocentric mycosis fungoides. Positive controls included one nodal T-cell lymphoma, seven cutaneous T-cell lymphomas inclusive of mycosis fungoides, and the Jurkat T-cell tumor line. Negative controls consisted of three typical cutaneous hypersensitivity reactions, one tonsil, one reactive lymph node, and placenta. Light microscopic features of typical cutaneous responses include vacuolar interface dermatitis, perivascular lymphocytic infiltrates with tissue eosinophilia, and eczematous dermatitis. Three B-cell lymphomas (two cutaneous) also were examined. All cases in the series were formalin fixed and paraffin embedded except for the Jurkat clone, nodal B-cell lymphoma, lymph node hyperplasia, and placenta, for which DNA derived from fresh-frozen tissue was available. Figure 2 illustrates the histopathology of representative cases.

Analysis of T- and B-Cell Clonality

DNA Extraction

Five 20-μm unbaked unstained sections were prepared from each block and the epidermis microdissected using a fine needle to enrich for lymphocyte-derived DNA. These thick sections were preceded and followed by standard 5-μm hematoxylin and eosin sections to ensure lesional persistence through the leveling process. The tissue sections were deparaffinized in xylene, washed with ethanol, and subjected to prolonged proteinase K digestion (5 to 6 days), and the subsequently released DNA was purified by phenol-chloroform extraction as recently described[6] (Fig 3). Finally, the DNA was ethanol precipitated and resuspended in 40 μL Tris-ethylenediaminetetra-acetic acid buffer before use in the polymerase chain reaction (PCR).

TCRγ Amplification and Criteria for DGGE Interpretation

Previously published nested consensus primers recognizing Vγ1-8 genes and semi-nested consensus primers recognizing the Vγ9 gene were employed to detect T-cell gene rearrangements.[7] A two-round amplification strategy was employed, with a "nested" approach used for Vγ1-8 targets and a "semi-nested" for Vγ9 (Fig 1). Reaction and thermocycling conditions as described by Wood et al[7] were followed, with the exception that 30 first-round cycles succeeded by 20 cycles in the second round was found to be optimal for DNA extracted from paraffin-embedded tissues. PCR amplification was performed using a Perkin-Elmer 9600 thermocycler (Norwalk, CT), and all runs included a previously established positive control and tubes to which no DNA was added. One-half microgram DNA isolated from fresh or frozen tissues was used as template in the first reaction round, and DNA derived from archival tissues was not quantitated (5 μL of each Tris-ethylenediaminetetra-acetic acid resuspended sample was used). The presence of amplification product in the expected size range was verified by agarose gel electrophoresis (Fig 4), and then the amplicons were separated by denaturing gradient gel electrophoresis as previously described.[7,8] All cases were amplified in duplicate using both nondiluted and 1/20 diluted first-round DNA as template in the second round. Gels were stained 15 minutes with ethidium bromide and photographed under ultraviolet illumination.

Denaturing gradient gel electrophoresis (DGGE) patterns were interpreted as polyclonal if electrophoresis resulted in either a diffuse smear or in multiple weak bands, and as clonally rearranged if a dominant banding pattern was achieved. Bands were classified as dominant if they were discrete, sharp, and persisted or intensified after dilution of the first-round product. Up to four such bands were permitted because of the possibilities of bi-allelic rearrangements and

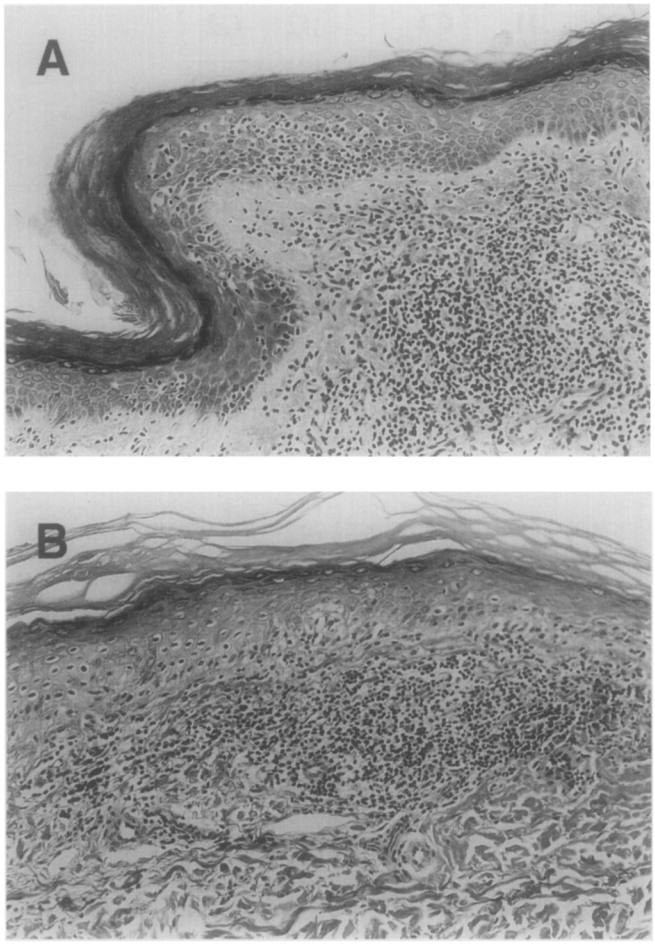

FIGURE 2. (A) Plaque-stage mycosis fungoides with hyperkeratosis, extensive epidermal colonization by atypical lymphocytes, and dermal fibrosis. (H&E, original magnification ×125 (same case as illustrated in Fig 5B, lanes 13-14).) (B) Case of lymphomatoid lichenoid hypersensitivity with striking similarity to the lymphoma depicted in A (patient no. 4). (H&E, original magnification ×125.) Note the hyperkeratosis and papillary dermal fibrosis, as well as focal epidermal permeation by small atypical lymphocytes. This single-plaque lesion was associated with a histamine antagonist and showed monoclonal TCRγ rearrangement by PCR/DGGE (Fig 5C, lane 17). Complete resolution occurred within several weeks of discontinuing the medication, and the patient remains free of disease 2 years later.

heteroduplex formation, but usually only one to two bands were present. Weakly staining bands were not infrequently noted, especially after amplification with the Vγ9 primers, and tended to disappear or lose intensity after template dilution—such bands could not be considered representative of a clonal expansion.

Immunoglobulin Heavy Chain Amplification

Primers recognizing consensus immunoglobulin H (IgH) variable region framework (FR) I-IV and III-IV sequences and a consensus IgH joining region primer were used to study B-cell gene rearrangements as described by Segal et al.[9,10] The B-cell amplicons were separated by either 3% agarose (FR I-IV) or 12% nondenaturing polyacrylamide (FR III-IV) gel electrophoresis. The gels were stained and photographed in the same manner as for the T-cell studies. In contrast to TCRγ, rearrangements involving the IgH locus result in products of widely variable size, such that clones generate unique banding patterns detectable by nondenaturing electrophoresis.

RESULTS

Clinical Features

The clinical information is summarized in Table 1.

Histology

Case 1 (MF-like)

The biopsy specimen was remarkable for a superficial infiltrate, which focally assumed a band-like pattern of infiltration within the epidermis. There was striking epitheliotropism with localization to the rete and acrosyringium. The infiltrate had a polymorphous composition comprising small, intermediate, and transformed hyperchromatic and irregularly contoured lymphocytes.

Case 2 (MF-like)

The biopsy specimen displayed a superficial band-like lymphocytic infiltrate. The epidermis showed variable hyperplasia. The lymphocytes were in the 7- to 9-μm range. A few scattered cells with a Sezary morphology were identified.

Case 3 (MF-like)

The biopsy specimen showed a band-like lymphocytic infiltrate with obscuration of the dermoepidermal junction. There was prominent infiltration of the epidermis by atypical lymphocytes with foci of frank architectural ablation of the epidermis. The cells within the dermis were dominated by small mature lymphocytes with minimal atypia, whereas those cells within the epidermis were significantly atypical and included cells with a Sezary morphology.

Case 4 (MF-like)

A band-like lymphocytic infiltrate in close apposition to the epidermis was present, with colonization of the basal and parabasilar epidermis by small lymphocytes showing significant nuclear contour irregularity (Fig 2B). A coarse sclerosing dermal response within the papillary and superficial reticular dermis was additionally noted.

Case 5 (B-Cell Lymphoma Cutis-like Pattern)

The biopsy specimen was remarkable for a pandermal dense nodular nonepitheliotropic lymphocytic infiltrate. Centrally the dominant composition of the infiltrate was one of transformed lymphocytes, and the periphery showed small mature lymphocytes with admixed eosinophils.

Case 6 (Lymphomatoid Vascular Reaction)

The biopsy specimen was remarkable for dense superficial and deep angiocentric lymphocytic infil-

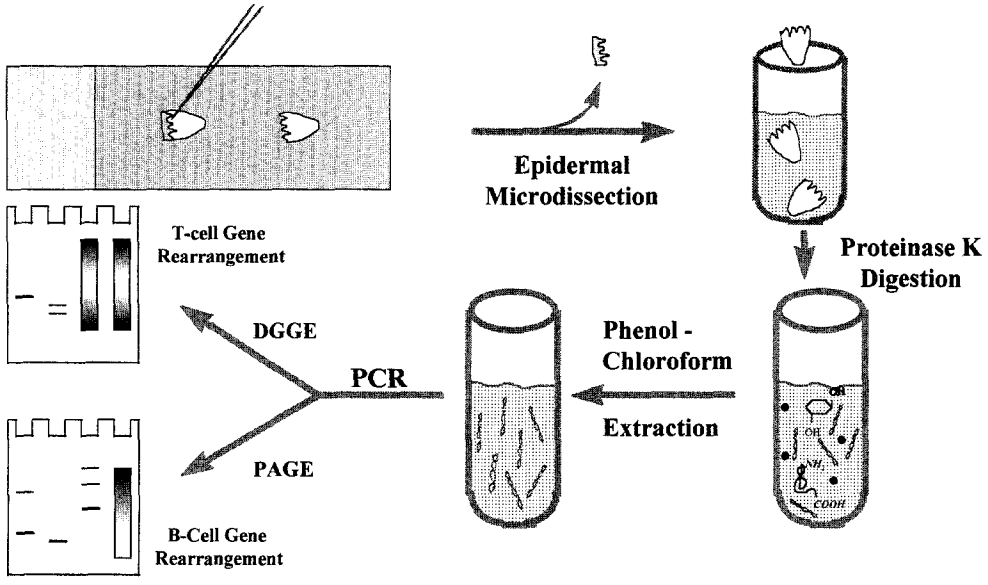

FIGURE 3. Schematic illustration of DNA extraction process from archival tissue with subsequent amplification and product analysis.

trates predominated by immunoblasts with an admixture of other inflammatory cell elements, including small mature lymphocytes, plasma cells, and eosinophils. Scabetic mites were identified within the stratum corneum.

Case 7 (Lymphomatoid Vascular Reaction)

The biopsy specimen showed a striking vascular reaction involving the entire sampled cutaneous vasculature. The infiltrates comprised a monomorphic populace of small and intermediate-sized lymphocytes with significant nuclear contour irregularity. A concomitant diffuse interstitial granulomatous component also was present.

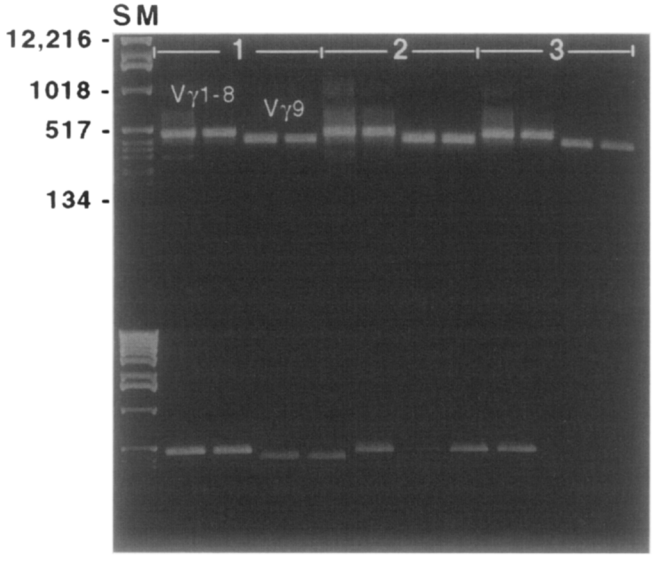

FIGURE 4. Agarose gel with typical second-round PCR products. Each sample was amplified in duplicate with both Vγ1-8 and Vγ9 primers, accounting for the four lanes/sample (representative samples indicated by white numbers within gel). The staggered appearance results from the larger product obtained after Vγ1-8 amplification (~420 bp) versus Vγ9 (~380 bp).

Case 8 (Folliculotropic MF-like and Lymphoma Cutis-like)

A dense lymphohistiocytic infiltrate involving the mid and deep dermis with extension into the subcutaneous fat was present, as well as marked infiltration of sampled hair follicles by this infiltrate. One of the inflamed follicles showed extensive permeation by fungal forms consistent with an endothrix infection.

Case 9 (Folliculotropic MF-like)

A striking reaction involving sampled hair follicles characterized by dense perifollicular and intrafollicular lymphohistiocytic infiltrates with attendant follicular mucinosis was present, as were nodular mononuclear cell infiltrates surrounding and permeating perifollicular vessels.

Case 10 (Lymphomatoid Vascular Reaction)

The biopsy specimen was remarkable for nodular angiocentric mononuclear cell infiltrates involving the superficial and mid-dermal vasculature, with attendant luminal and mural fibrin deposition and red cell extravasation. The cytomorphology of the mononuclear cells encompassed intermediate and transformed lymphocytes with mild nuclear contour irregularity.

Case 11 (Folliculotropic MF-like and Lymphoma Cutis-like)

The biopsy specimen was remarkable for a dense nodular lymphohistiocytic infiltrate, which had as its epicenter sampled follicles. Concomitant cytopathic alterations of the follicles were noted consistent with molluscum contagiosum infection.

TABLE 1. Summary of Clinical Data and DGGE Results

Patient No.	Age (yr)/Sex	Clinical Presentation	Nature of Immunodysregulation	Therapy and Current Status	DGGE Pattern
1	44F	Chest papule	H2A	Lesion Excised/NED	P
2	63F	Plaque on left leg	CCB, SS	CCB discontinued/NED	P
3	66M	Plaque on palm	H2A	H2A discontinued/NED	M
4	48F	Plaque on thigh	H1A	H1A discontinued/NED	M
5	59F	Nodule on tip of nose	SS	Treated with x-ray therapy/NED	P
6	86F	Nodules on back and left arm (scabetic)	ACE, H1A × 2; 2 others	Continued drug therapy, lesions resolved with treatment of scabetic infestation/NED	P
7	70M	Maculopapular rash on extremities	Prilosec	Lesions resolved with steroid therapy/NED	P
8	52F	Papules and plaques on face associated with Endothrix	CCB, Anticonv ×2	Lesions resolved with anti-fungal therapy/NED	P
9	48M	Papules on leg and torso	AD, Anticonv	AD discontinued/NED	P
10	39F	Multiple papules on distal extremities	ACE, AD ×2	ACE discontinued/NED	P
11	18F	Papule on cheek	SS; Atopic diathesis	Excision of lesion/NED	P
12	54F	Plaques on face, neck, and forearm	CCB	CCB discontinued/NED	P
13	43F	Generalized papules	H1A; 1 other	H1A discontinued/NED	P
14	65M	Patches & plaques on trunk & extremities	BB, ACE	Drugs discontinued/NED	P

Abbreviations: NED, no evidence or recurrence of disease after discontinuation of implicated drug or other therapy; AD, antidepressants (ie, Amitriptyline [case no. 9], Prozac [case no. 10], Klonopin [case no. 10]); Li, Lithium; ACE, angiotensin-converting enzyme inhibitor; CCB, calcium channel blocker; Bzp, benzodiazepine; LiLA, lipid-lowering agent; H2A, histamine 2A-receptor antagonist; H1A, histamine 1A receptor antagonist; SS, sex steroids (ie, estrogen and progesterone); anticonv, anticonvulsants (ie, carbamazepine, dilantin, phenobarbital); BB, beta-blocker; other, Nitroprusside, Dyazide, Flexeril, Relafan; ×, number of drugs of that class that the patient was receiving; P, polyclonal; M, monoclonal.

Case 12 (MF-like)

The biopsy specimen showed a vacuolar and lichenoid interface dermatitis with epitheliotropism.

Case 13 (MF-like)

Two biopsy specimens were available and appeared morphologically similar. In each a superficial interstitial and perivascular lymphocytic infiltrate was observed. There was haphazard infiltration of the epidermis by lymphocytes with lymphoid forms extending into the upper layers of the epidermis. The cytomorphology comprised small and intermediate-sized lymphocytes with nuclear contour irregularity.

Case 14 (MF-like)

The biopsy specimen was remarkable for a superficial perivascular and interstitial mononuclear cell infiltrate. There was slight exocytosis of lymphocytes. The vessels amidst the infiltrate showed injurious alterations as indicated by mural and luminal fibrin deposition. The infiltrate was dominated by intermediate and transformed atypical lymphocytes inclusive of cells with a Sezary morphology.

Molecular Studies

The nodal and Jurkat T-cell lymphomas, six of seven cutaneous T-cell lymphomas (86%), and 2 of 14 of the study cases (14%) displayed dominant TCRγ gene rearrangement patterns, whereas the remainder of cases were polyclonal (Fig 5). The only immunoglobulin heavy chain gene rearrangements detected by PCR were in two of the three B-cell lymphomas, including the nodal and one of the two cutaneous lymphomas (Fig 6). The latter also showed an apparent TCRγ rearrangement. Although rearrangements discordant with immunophenotype have been described,[11] it is more likely in this particular case that amplification of DNA from relatively rare infiltrating T lymphocytes resulted in a pseudoclonal pattern.

When the DGGE results were compared with tissue reaction pattern, a correlation between detectable clonal

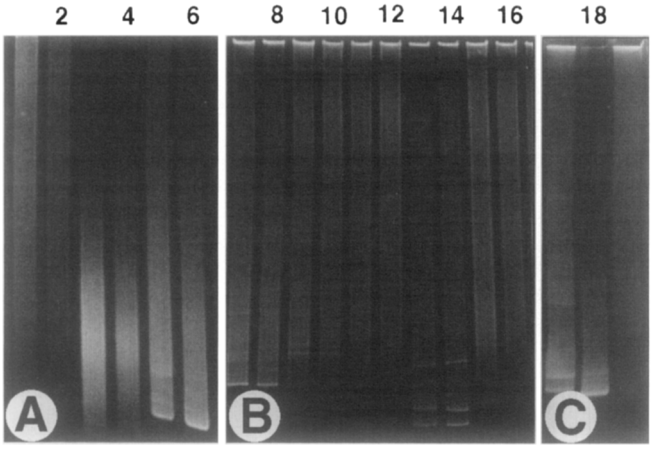

FIGURE 5. DGGE Results. (A) Lymph node hyperplasia shows a diffuse smear indicative of its polyclonal nature (lanes 1-2), whereas the Jurkat T-cell tumor line shows a Vγ1-8 rearrangement (lanes 5-6; results of Jurkat amplification with Vγ9 primers are seen in lanes 3-4). (B) Nodal T-cell lymphoma with monoclonal pattern (lanes 7-8), two cases of polyclonal LyHR (lanes 9-10 (patient no. 2) and 11-12 (patient no. 10)), MF showing a dominant banding pattern diagnostic of rearrangement (lanes 13-14, same case as in Fig 2A), and polyclonal case of typical hypersensitivity (lanes 15-16); (C) Monoclonal LyHR (lane 17, patient no. 4), monoclonal MF (lane 18), and polyclonal tonsil (lane 19).

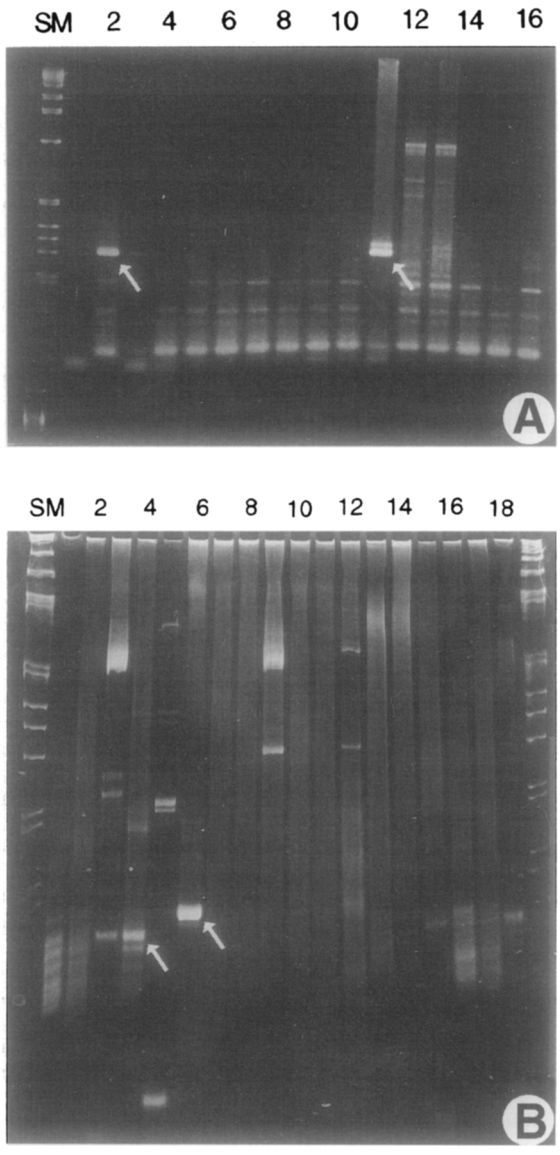

FIGURE 6. (A) FR I-IV, Multiplex PCR: 3% agarose gel shows IgH chain gene rearrangement in case of paraffin-embedded cutaneous B-cell lymphoma (arrow, lane 2) and fresh-frozen nodal B-cell lymphoma (arrow, lane 11). (B) FR III-IV, semi-nested PCR: 12% nondenaturing polyacrylamide gel with first- and second-round PCR products. Arrows point to clonal gene rearrangement detected after first (lane 6) and second (lane 4) amplification rounds. Same case as depicted in lane 2 of (A).

expansion and a dense band-like (ie, lichenoid) morphology was apparent. Only 4 of 14 cases displayed this pattern (patients 1 through 4), yet they included both monoclonal cases. The nonlichenoid patterns encompassed (1) a lower-density epitheliotropic, eczematous, and psoriasiform morphology, (2) lymphomatoid vasculitis, (3) follicular mucinosis, and (4) lymphocytoma cutis, none of which manifested any T-cell clonal rearrangement.

DISCUSSION

We have investigated T-cell clonality of drug-associated lymphomatoid hypersensitivity reactions by PCR/DGGE using formalin-fixed, paraffin-embedded tissues. Although many investigators employing similar techniques to study malignant and reactive lymphocytic infiltrates of skin have relied on fresh or frozen material,[12-14] there are increasing reports in the literature regarding the use of such archival tissue for TCRγ analysis.[15-18] Our 86% detection of TCRγ rearrangements in our cutaneous T-cell lymphoma controls (89% detection of positives if the nodal lymphoma and Jurkat samples are included) is in close agreement with that published by others.[7,12,16] It also reaffirms PCR as a powerful method for assaying T-cell clonality, a characteristic hallmark of malignant T-cell lymphoma. Regarding B-cell clonality, none of our study cases showed IgH chain rearrangement. This contrasts with the findings of Ritter et al,[19] who not uncommonly identified such rearrangements in mixed B and T as well as predominantly T-cell cutaneous lymphoid infiltrates.[19]

The current series represents a collection of atypical reactive lymphocytic infiltrates that raised cutaneous lymphoma as a diagnostic possibility. The appellation of lymphomatoid hypersensitivity reaction has been applied to such cases.[1] When the antigenic trigger is isolated, the terminology is modified accordingly. Hence, in the setting of an abnormal immune response to a contact, the term *lymphomatoid contact dermatitis* is used.[20] Although previous studies of reactive cutaneous infiltrates have shown clonal rearrangements ranging from 0%[12-14,17,18] to 6%,[7] detectable rearrangements were present in 2 of 14 of our lymphomatoid hypersensitivity cases (14%). This finding is similar to that of Staib and Sterry[16] who also found dominant rearrangements in 14% of "pseudolymphoma." The association of atypical lymphoid hyperplasia with a variety of therapeutic agents has previously been reported by Magro and Crowson,[1-3] who postulated that blocking or stimulation of receptor-mediated lymphocyte function may result in aberrant immune responses to a variety of antigenic triggers. A similar theory of systemic or iatrogenic immune dysregulation leading to atypical lymphoid hyperplasia has been postulated by other authors.[21,22] In one of the reports, they isolated herpes as the antigenic trigger; the underlying immune dysregulatory state was malignant lymphoma.[21] The role of an infective antigenic trigger in the propagation of the infiltrate was similarly documented in three of our cases. It is possible that emergence of a dominant clone or clones is facilitated by dysregulated T-cell function or by direct mitogenic effects of pharmacological agents on lymphocytes, and that this accounts for our increased finding of monoclonality.

When the implicated drugs were discontinued in the two patients showing clonal rearrangement, both experienced complete resolution of their lesions and remain free of disease up to 2 years later (patients 3 and 4). Although both drug-associated pseudolymphoma progressing to lymphoma[23] and drug-associated lymphoma are described,[24] a diagnosis of malignant lymphoma is unwarranted in our two cases in light of the spontaneous and sustained resolution of lesions after drug withdrawal. It is increasingly recognized that

clonality in itself does not equate with malignancy, and examples of clinically indolent disorders displaying gene rearrangement include pityriasis lichenoides et varioliformis acuta, lymphomatoid papulosis, cutaneous B-cell pseudolymphomas,[25,26] and lymphoepithelial lesions associated with both Sjögren's disease and chronic *Helicobacter pylori* gastritis.[27] It must be acknowledged, however, that these disorders are associated with an increased incidence of true lymphoid malignancies such as Hodgkin's disease and disseminated lymphoma of mucosa-associated lymphoid type. The factors involved in such transformation are currently incompletely understood but may relate in part to underlying immune dysregulation or reflect the increased likelihood of a clone acquiring genetic mutations during prolonged stimulation driven by either defined or undefined antigen triggers. We regard our drug-related clonal processes to be similar and believe it likely that previously described drug-induced lymphomas[23,24] proceeded through a clinically benign stage (ie, one capable of regression on withdrawal of the initiating/promoting stimulus) in which detectable clonal expansion had already occurred before malignant transformation. It is possible that the detection of clonality in such cases isolates those cases of pseudolymphoma with a greater risk of malignant transformation. Proof of this hypothesis requires the long-term follow-up of patients with clonally rearranged drug-associated atypical lymphoid infiltrates that follow a persistent course in the setting of continued drug therapy.

We conclude that although drug-associated lymphomatoid hypersensitivity reactions usually manifest polyclonal infiltrates, a monoclonal TCRγ rearrangement can be seen. Recognition of this may aid in avoiding misdiagnosis of malignant lymphoma in the appropriate clinical setting and underscores the importance of histopathologic and clinical correlation in the interpretation of molecular studies. It is recommended a thorough drug history be procured for all patients who develop an atypical lymphoid infiltrate, and a drug-associated pseudolymphoma should be considered in patients taking drugs known to alter lymphocyte function.

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REFERENCES

1. Magro CM, Crowson AN: Drug-induced immune dysregulation as a cause of atypical cutaneous lymphoid infiltrates: A hypothesis. HUM PATHOL 27:125-132, 1996

2. Magro CM, Crowson AN: Drugs with anti-histaminic properties as a cause of atypical lymphoid hyperplasia. J Am Acad Dermatol 32:419-428, 1995

3. Crowson AN, Magro CM: Antidepressant therapy: A possible cause of atypical cutaneous lymphoid hyperplasia. Arch Dermatol 131:925-929, 1995

4. Wood GS: Cutaneous lymphoproliferative disorders: Strategies for molecular biological analysis and their major findings. Springer Semin Immunopathol 13:387-399, 1992

5. Wood GS, Haeffner A, Dummer R, et al: Molecular biology techniques for the diagnosis of cutaneous T-cell lymphoma. Dermatol Clin 12:231-241, 1994

6. Diaz-Cano SJ, Brady SP: DNA extraction from formalin-fixed paraffin-embedded tissues: Protein digestion as a limiting step for retrieval of high quality DNA. Diagn Mol Pathol 6:342-346, 1997

7. Wood GS, Tung RM, Haeffner AC, et al: Detection of clonal T-cell receptor γ gene rearrangements in early mycosis fungoides/Sezary syndrome by polymerase chain reaction and denaturing gradient gel electrophoresis (PCR/DGGE). J Invest Dermatol 103:34-41, 1994

8. Borguin A, Tung R, Galili N, et al: Rapid, non-radioactive detection of T-cell receptor gene rearrangements in lymphoid neoplasms. Proc Natl Acad Sci USA 87:8536-8540, 1990

9. Segal GH, Jorgensen T, Masih AS, et al: Optimal primer selection for clonality assessment by polymerase chain reaction analysis: I. Low grade B-cell lymphoproliferative disorders of nonfollicular center cell type. HUM PATHOL 25:1269-1275, 1994

10. Segal GH, Jorgensen T, Scott M, et al: Optimal primer selection for clonality assessment by polymerase chain reaction analysis: II. Follicular lymphomas. HUM PATHOL 25:1276-1282, 1994

11. Sklar J: Molecular genetic methods of diagnosis for lymphoid neoplasia, in Colvin RB, Bhan AK, McCluskey RT (eds): Diagnostic Immunopathology (ed 2). New York, NY, Raven, 1995, pp 773-796

12. Algara P, Soria C, Martinez P, et al: Value of PCR detection of TCRγ gene rearrangement in the diagnosis of cutaneous lymphocytic infiltrates. Diagn Mol Pathol 3:275-282, 1994

13. Theodorou I, Delfau-Larue M, Bigorgne C, et al: Cutaneous T-cell infiltrates: Analysis of T-cell receptor γ gene rearrangement by polymerase chain reaction and denaturing gradient gel electrophoresis. Blood 86:305-310, 1995

14. Bachelez H, Bioul L, Flageul B, et al: Detection of clonal T-cell receptor γ gene rearrangements with the use of the polymerase chain reaction in cutaneous lesions of mycosis fungoides and Sezary syndrome. Arch Dermatol 131:1027-1031, 1995

15. Volkenandt M, Wienecke R, Koch OM, et al: Conformation polymorphisms of cRNA of T-cell-receptor genes as a clone-specific molecular marker for cutaneous lymphoma. J Invest Dermatol 101:514-516, 1993

16. Staib G, Sterry W: Use of polymerase chain reaction in the detection of clones in lymphoproliferative diseases of the skin. Recent Results in Cancer Research 139:239-247, 1995

17. Graf A, Kaudewitz P, Simon M, et al: Detection of clonality of cutaneous T-cell lymphomas using polymerase chain reaction (German). Pathologe 17:446-450, 1996

18. Ashton-Key M, Diss TC, Du MQ, et al: The value of polymerase chain reaction in the diagnosis of cutaneous T-cell infiltrates. Am J Surg Pathol 21:743-747, 1997

19. Ritter JH, Wick MR, Adesokan PN, et al: Assessment of clonality in cutaneous lymphoid infiltrates by polymerase chain reaction analysis of immunoglobulin heavy chain gene rearrangement. Am J Clin Pathol 108:60-67, 1997

20. Orbaneja JG, Diez L, Lorano JLS, et al: Lymphomatoid contact dermatitis: A syndrome produced by epicutaneous hypersensitivity with clinical features and a histopathologic picture similar to that of mycosis fungoides. Contact Dermatitis 2:130-143, 1976

21. Hassell MH, Lescher JL Jr.: Herpes simplex mimicking leukemia cutis. J Am Acad Dermatol 23:401-410, 1990

22. Rijlaarsdam U, Bakels W, van Oostveen JW, et al: Demonstration of clonal immunoglobulin gene rearrangement in cutaneous B cell lymphomas and pseudolymphomas: Differential diagnostic and pathogenetic aspects. J Invest Dermatol 99:749-754, 1992

23. Gams RA, Neal JA, Conrad FG: Hydantoin-induced pseudopseudolymphoma. Ann Intern Med 69:557-568, 1968

24. Li FP, Willard DR, Goodman R, et al: Malignant lymphoma after diphenylhydantoin (Dilantin) therapy. Cancer 36:1359-1362, 1975

25. Weiss LM, Wood GS, Ellison LW, et al: Clonal T-cell populations in pityriasis lichenoides et varioliformis acuta (Mucha-Haberman disease). Am J Pathol 126:417-421, 1987

26. Weiss LM, Wood GS, Trela, M, et al: Clonal T cell populations in lymphomatoid papulosis: Evidence for a lymphoproliferative etiology in a clinically benign disease. N Engl J Med 315:475-479, 1986

27. Quintana PG, Kapadia SB, Bahler DW, et al: Salivary gland lymphoid infiltrates associated with lymphoepithelial lesions: A clinicopathologic, immunophenotypic, and genotypic study. HUM PATHOL 28:850-861, 1997