Clonal Rearrangement of the T Cell Receptor β Gene in the Circulating Lymphocytes of Erythrodermic Follicular Mucinosis

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Follicular mucinosis is a condition characterized by the abnormal accumulation of acidic mucopolysaccharides in hair follicles. It is classically described as occurring idiopathically in young persons and within the infiltrates of mycosis fungoides in older individuals. We report a 12-year-old girl who had erythrodermic follicular mucinosis, hypereosinophilia, circulating Sezary cells, and both immunophenotypic and genotypic evidence of T cell neoplasia. Erythrodermic follicular mucinosis may represent an unusual variant of the Sezary syndrome, which to date has not been described in children or adolescents.

FOLLICULAR MUCINOSIS is a histologic process whose hallmark is the accumulation of acid mucopolysaccharides within hair follicle epithelium.[1] Follicular mucinosis has been reported in four clinical settings.[1-7] In the first group of cases, follicular mucinosis occurs as slightly raised and boggy plaques on the face and scalp, usually of children, adolescents, or young adults. A second group has, in addition to lesions on the head, involvement of the trunk and extremities, usually as plaques, but occasionally as widespread follicular papules; a variety of systemic processes have been seen in association with this type of presentation. In either setting, areas of hair loss due to follicular mucinosis are referred to as alopecia mucinosa, and such hair loss is often the presenting complaint. In a third group of cases, follicular mucinosis occurs within the plaques of cutaneous T cell lymphoma, most typically of the mycosis fungoides type and rarely, Sezary's syndrome.[8] A fourth group comprises a wide variety of cutaneous neoplasms and inflammatory conditions within which follicular mucinosis has been reported as an incidental microscopic finding, affecting only a few hair follicles.[1]

An unusual and distinct presentation has recently been reported as erythrodermic follicular mucinosis.[9] The patient who was the subject of that report had widespread follicular papules over most of the skin, as well as alopecia. On hematologic examination there was relative and absolute eosinophilia, and increased numbers of "activated" circulating T cells.

We recently encountered a 12-year-old girl who presented with erythrodermic follicular mucinosis whose peripheral blood eosinophilia was accompanied by massive eosinophilic infiltrates in the lungs. The patient's blood was found to contain a preponderance of morphologically and immunophenotypically abnormal T lymphocytes with a clonal pattern of rearrangement of the T cell receptor (TCR) β gene. Our data strongly suggest that a T cell neoplasm is the underlying process in this unusual condition.

MATERIALS AND METHODS

Case report. The patient was a 12-year-old white girl who presented in early 1986 with a cutaneous eruption of 10 months duration. Her skin findings included areas of alopecia that had a boggy quality, erythema and scaling over the face, thinning of the eyebrows, and innumerable follicular papules over the trunk and extremities with areas of confluent erythema (Fig 1). Laboratory studies showed a leukocytosis (25.7 kg/μL; normal range, 4.5 to 15.5 kg/μL) with marked eosinophilia (6.19 kg/μL; normal, 0 to 1.012 kg/μL). IgE was also elevated (840 U/mL; normal, <177 U/mL). Bilateral axillary, inguinal, and cervical lymph nodes were enlarged. A chest x-ray showed an interstitial infiltrate. Oral prednisone (60 mg/d orally) was started.

In July 1986, there was an episode in which the skin infiltration became more marked, there were fevers to 39.4°C, and the pulmonary infiltration progressed to require oxygen for 1 week. An open lung biopsy performed at that time showed large aggregates of eosinophils and mononuclear phagocytes.

Because of the patient's inability to tolerate prednisone dosages below 30 mg/d orally, methotrexate (20 mg/m²/wk) was added to her regimen. Following completion of the studies described below, her condition was reevaluated, she was classified as having a T cell lymphoproliferative condition, and was placed on an extracorporeal photopheresis protocol.

Histopathologic, immunophenotypic, and genotypic studies. Multiple skin biopsies from the scalp and trunk were processed routinely. Two other biopsies (from the palm and buttock) were split, with one half fixed in paraformaldehyde, embedded in glycol methacrylate, and stained with hematoxylin and eosin, as well as by an enzymatic method for α-naphthyl acetate esterase and with anti–Leu-4 (CD3; Becton Dickinson, Sunnyvale, CA) using avidin-biotin immunoperoxidase. The other halves, and a third biopsy from the occipital scalp, were snap frozen in isopentane, sectioned on a cryostat, and stained by the avidin-biotin immunoperoxidase method using a panel of antisera to lymphoid determinants, including Leu 1 through 5 (CD2 through 5 and 8), Leu 8 and 9 (CD7; Becton Dickinson).

The peripheral blood mononuclear cell layer was separated using Ficoll hypaque. Samples were embedded in glycol methacrylate, sectioned at 2 μm, and stained by an enzymatic method for α-naphthyl acetate esterase. Sezary cells were enumerated as described elsewhere.[10] The peripheral blood mononuclear cell layer

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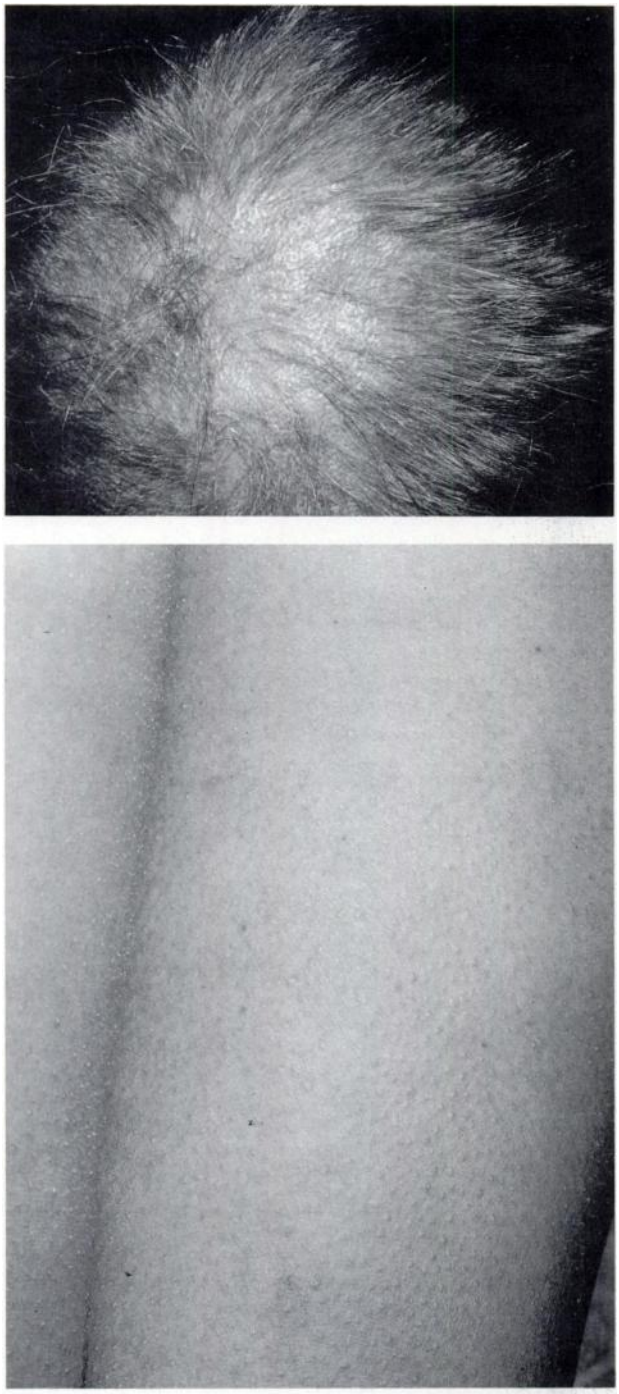

Fig 1. The clinical presentation induced boggy erythematous plaques with alopecia on the scalp (A) as well as innumerable follicular papules on the trunk and extremities (B).

was also analyzed on a FACS (Becton-Dickinson) flow cytometer using double labeling with fluoroscein and phycoerythrin-conjugated antisera, including Leu 1 through 5 (CD2 through 5 and 8), Leu 8, Leu 9 (CD7), Leu 12 (CD 19) (Becton Dickinson), and BE2 (Dr Carol Berger, Columbia University).

DNA was extracted from peripheral blood mononuclear cells, digested with restriction enzymes *Bam*HI, *Hind*III, and *Eco*RI, size fractionated on 0.8% agarose gels, and probed with radiolabeled cDNA probe YTJ-2, devised from the Jurkat-2 T cell lymphoma lines, which hybridizes with both the constant and variable regions of TCRβ. The probe was provided by Dr Mak Tak, University of Toronto. Digests of *Bam*HI and *Bgl*II were also assayed with radiolabeled genomic DNA probes specific for the J regions of the TCR β-chain gene.

RESULTS

Biopsies of lesional skin from the scalp and trunk showed a moderately dense infiltrate in the perifollicular adventitial dermis and follicular epithelium, principally composed of lymphocytes, but also containing scattered eosinophils (Fig 2). The follicular epithelium contained varying quantities of acid mucopolysaccharides, evidenced by reactivity with colloidal iron stains. These histologic features do not satisfy criteria for the diagnosis of cutaneous T cell lymphoma. There were no markedly atypical lymphocytes, nor did the infiltrate involve the epidermis or papillary dermis between the affected hair follicles, a histologic feature reported in almost all cases of Sezary's syndrome.[11]

Frozen sections stained with a battery of antisera to lymphocyte antigens, including Leu 1 through 5, 8, and 9, showed that most of the infiltrate marked as T helper cells, with a normal distribution of other T cell determinants, except for a mild deficiency of Leu-8 antigen (20% to 40% positive) in one biopsy, a nonspecific finding.

On three occasions, the plastic-embedded Sezary preparation showed small Sezary cells in the peripheral blood (10 to 12 μm), comprising 41% to 45% of circulating lymphocytes (Fig 3); following a course of systemic corticosteroid therapy this value declined transiently to 25%.

Flow cytometry with fluorescein and phycoerythrin-labeled antisera was performed on peripheral blood mononuclear cells, using a large panel of antisera to hematopoietic determinants (Table 1). Significant findings included predominant expression of the helper-cell antigen Leu 3 (89%) as well as the sheep erythrocyte-receptor antigen Leu-5 (96%). Abnormal findings included diminished expression of the pan T cell antigens Leu 1 (25%) and Leu 4 (23%), as well as diminished expression of the majority T cell antigen Leu 9 (37%). Markedly increased numbers (49%) of cells binding an antibody raised against neoplastic T cells (BE2+) were also seen.

DNA extracted from peripheral blood mononuclear cells and assayed by the Southern blot method demonstrated rearranged bands, following analysis with radiolabeled cDNA to the TCR β gene using the restriction enzymes *Eco*RI, *Bam*HI, and *Hind*III (Fig 4), and using genomic DNA probes to $J_{\beta 1}$ and $J_{\beta 2}$, and the enzymes *Bam*HI and *Bgl*II (data not shown). As discussed elsewhere,[12] detection of rearranged TCR bands, in conjunction with immunophenotypic studies, provides evidence of a predominant clonal population of T lymphocytes in the peripheral blood.

DISCUSSION

Although follicular mucinosis can be found within lesions of mycosis fungoides in older patients, its occurrence in young persons has been regarded as benign. Indeed, some

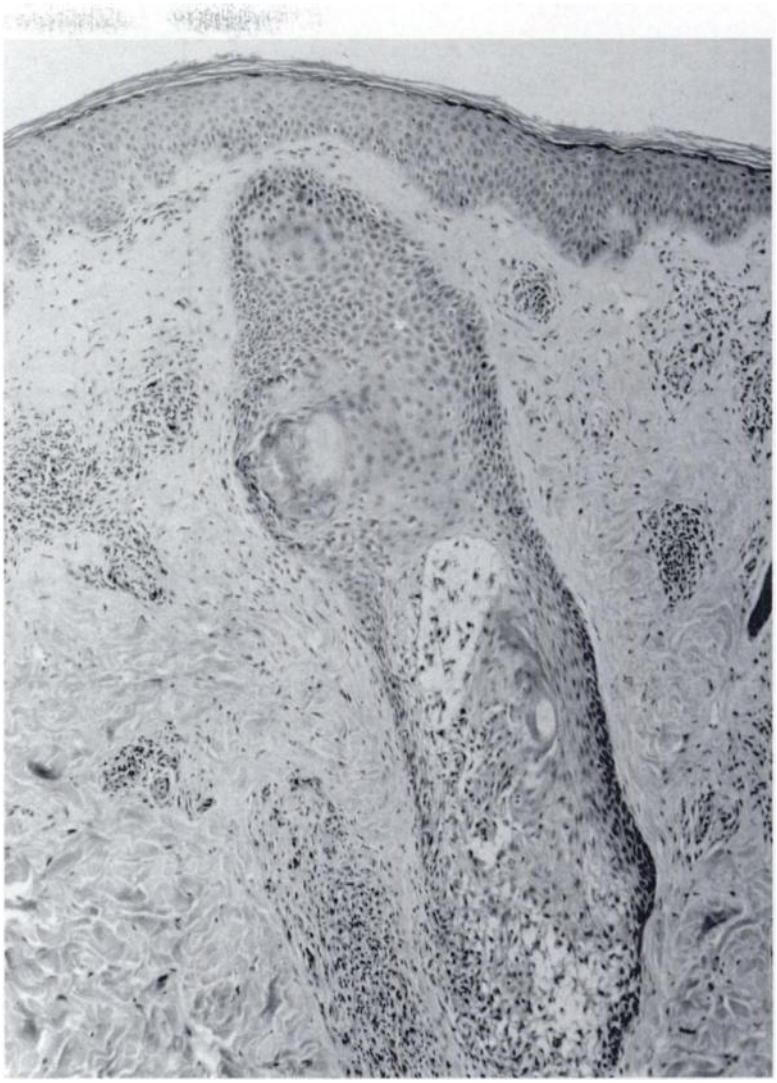

Fig 2. Biopsies of both the alopecic plaques and follicular papules demonstrated peri- and intrafollicular infiltrates of small lymphocytes and eosinophils, and accumulation of acid mucin within the hair follicle epithelium.

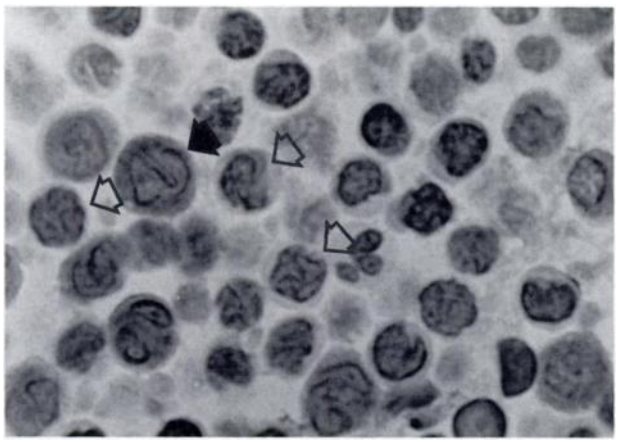

Fig 3. Four-micron section of a glycol methacrylate-embedded mononuclear cell suspension stained with an enzymatic method for nonspecific esterase demonstrates that the majority of the strikingly cerebriform cells do not contain cytoplasmic esterase (open arrows), and hence represent lymphocytes. Monocytes (closed arrow) appear as having convoluted nuclei, but demonstrate cytoplasmic reaction product with this technique.

investigators have stated that follicular mucinosis in the absence of a specific infiltrate of mycosis fungoides is a wholly benign condition and does not necessitate a search for lymphoma.[1] Our patient presented with an unusual erythyodermic form of follicular mucinosis, accompanied by elevated numbers of circulating eosinophils and lymphocytes whose morphology was similar to those of the Sezary's syndrome. Numerous skin biopsies failed to demonstrate features that would satisfy criteria for cutaneous lymphoma of any kind.[11]

Nevertheless, the peripheral blood picture provided evidence of a T cell neoplasm. On repeated occasions, large numbers of hyperconvoluted lymphocytes were noted, exceeding the numbers (up to 30%) of such cells that we have seen to date in the peripheral blood of patients with reactive erythrodermic conditions. The immunophenotype of these circulating lymphocytes was strikingly abnormal with many cells failing to bind various anti-T cell antisera. High-grade T cell neoplasms are frequently characterized by the absence of antigens normally present on mature peripheral blood T cells.[13] In contrast, low-grade T cell neoplasms such as patch or early plaque stage mycosis fungoides and the circulating

Table 1. Peripheral Blood Immunophenotype by Two-Color FACS Analysis

Antibody or Antibody Pair*	Cells Reactive (%)
Leu 1+ 5−	0
Leu 1+ 5+	25
Leu 1− 5+	71
Leu 4+ 9−	6
Leu 4+ 9+	17
Leu 4− 9+	20
Leu 3+ 8+	87
Leu 3+ 8−	2
Leu 3− 8+	0
Leu 3+ BE2−	41
Leu 3+ BE2+	49
Leu 3− BE2+	1
Leu 2+	7
Leu 12+	1

*When antibody pairs were given, the first was conjugated to fluorescein isothiocyanate and the second to phycoerythrin; otherwise, the antibodies were conjugated to fluorescein.

cells of Sezary syndrome usually show a mature T (usually helper) cell phenotype. Thus, the peripheral blood immunophenotype in this case is most unusual in that it would be more consistent with a high-grade T cell neoplasm. It should be noted, however, that multiple antigen "deficiencies" can be seen in several cutaneous lymphoproliferative conditions that are usually non-progressive or slowly progressive, such as lymphomatoid papulosis, pagetoid reticulosis, and regressing atypical histiocytosis. Whether these antigen "deficiencies" represent genetic mutations at the DNA level, failure to transcribe DNA, or reflect modulation of cell surface proteins remains to be determined.

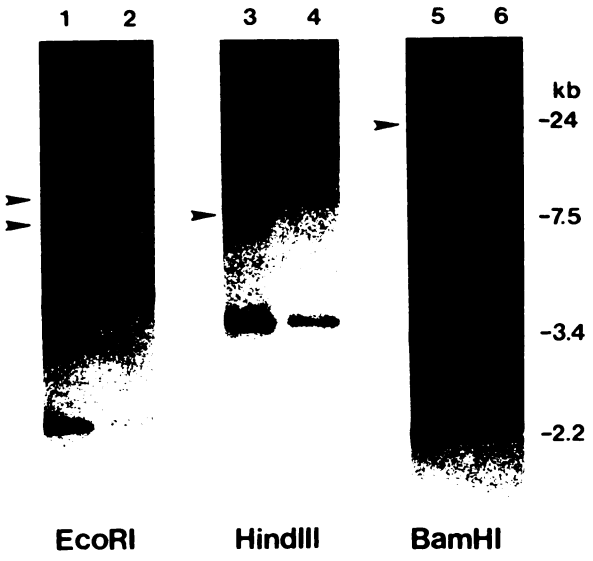

Fig 4. Southern blot analysis of peripheral blood mononuclear cell-derived DNA revealed anomalous bands (arrows) with the three restriction enzymes shown above and a radiolabeled cDNA probe to the T cell receptor β gene. Patient DNA is in lanes 1, 3, and 5; control DNA is in lanes 2, 4, and 6. In addition, anomalous bands were detected using genomic DNA probes to the $J_{\beta 1}$ and $J_{\beta 2}$ regions of the receptor, following digests with the enzymes *Bam*HI and *Bgl*II.

Another finding favoring the diagnosis of a T cell neoplasm was the large number of cells co-expressing the helper cell antigen Leu-3 and an antigen frequently expressed by the cells of cutaneous T cell lymphomas, BE2. While BE2 has been reported in the tissue infiltrates of some benign dermatoses, high levels of expression in peripheral blood in settings other than CTCL have not been reported to date.[14,15]

In contrast, the infiltrate around the mucinous hair follicles did not demonstrate the anomalous phenotype seen in the peripheral blood, indicating that the perifollicular T cells were not a spillover of the circulating abnormal cells. This finding indicates that, in this case, follicular mucinosis represents a reactive condition involving hair follicle epithelium rather than being part of the T cell neoplasm per se.

Using two different genetic probes to the TCR β gene, and the Southern blot method, we demonstrated non-germline or clonal bands in digests from a total of four enzymes. While restriction site polymorphisms can result in such bands, they are so rare that it is extremely unlikely for more than one to be present in an individual.

Our finding that this patient's peripheral blood lymphocytes harbor a predominant clone is indicative of a lymphoproliferative disorder but is not pathognomonic of malignancy. Clonal proliferation of T cells has been seen in a number of conditions universally accepted as malignancies, including peripheral lymph node-based T cell lymphoma, mycosis fungoides, Sezary syndrome, adult T cell leukemia/lymphoma, T cell chronic lymphocytic leukemia, and lymphoblastic lymphoma.[12] Clonal T cell proliferation has also been noted in a number of diseases whose nosology is less certain, such as lymphomatoid papulosis,[16] regressing atypical histiocytosis,[17] granulomatous slack skin,[18] T gamma lymphocytosis,[19] angioimmunoblastic lymphadenopathy,[20] and pityriasis lichenoides et varioliformis acuta.[21] Some of the latter group of disorders show a high rate of transformation to aggressive disseminated lymphomas, while others such as pityriasis lichenoides are almost always biologically benign. However, given the unremitting course of disease in our patient, it seems likely that she has a biologically malignant condition.

Although erythrodermic follicular mucinosis seems likely to be closely related to the Sezary syndrome, several features mark it as a distinct subset of that condition or a distinct condition. In neither reported case was there involvement of interfollicular epidermis or a band-like infiltrate in the papillary dermis as is uniformally found in skin biopsies from patients with Sezary's syndrome. Our case was marked by an unusual immunophenotype that included deletions of multiple pan T cell antigens, a finding more characteristic of high-grade T cell neoplasms than of Sezary's syndrome. Both cases of erythrodermic follicular mucinosis have been associated with eosinophilia. Although eosinophilia occasionally accompanies Sezary syndrome, the degree of eosinophilia seen here is most unusual, as is the occurrence of eosinophilic

pulmonary infiltrates. In both of the reported cases, the patients have been much younger than the mean age for Sezary syndrome; if our patient does indeed have Sezary syndrome, she would be the only patient in the pediatric age group ever reported with that disorder. However, it should be noted that mycosis fungoides, a closely related disorder, can begin in childhood.[22] Whether erythrodermic follicular mucinosis represents a variant of Sezary syndrome or a unique lymphoproliferative disorder awaits follow-up and the reports of other cases.

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