Cutaneous Involvement in Patients With Angioimmunoblastic Lymphadenopathy With Dysproteinemia

A Clinical, Immunohistological, and Molecular Analysis

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Objective: To determine whether cutaneous involvement in patients with angioimmunoblastic lymphadenopathy with dysproteinemia (AILD) is related to a clonal T-cell proliferation.

Design: Retrospective study.

Setting: University hospitals.

Patients: Ten patients with AILD and cutaneous involvement.

Main Outcome Measure: The T-cell receptor-γ (TCRG) gene rearrangement was studied with the use of polymerase chain reaction and denaturing gradient gel electrophoresis in blood, nodal, and skin samples. Skin and nodal samples were investigated also for the presence of Epstein-Barr virus (EBV) RNA by in situ hybridization.

Results: A transient morbilliform eruption of the trunk was seen most often. Other cutaneous features were infiltrated plaques and purpuric or urticarial lesions. A clonal TCRG gene rearrangement was detected in 7 skin samples, corresponding to a maculopapular eruption with a histological pattern of nonspecific mild lymphoid dermal infiltrate in 6 patients, and to erythematous plaques with histological findings of typical cutaneous lymphoma in 1 patient. In the 5 patients in whom a TCRG gene rearrangement was evidenced in skin and lymph node samples, identical clones were detected in both. Five patients died by the end of the study, with a mean survival of 33.2 months. Four of these 5 patients had a clonal infiltrate in skin and lymph nodes. The EBV RNA was detected in only 1 of 10 skin biopsy specimens and in 5 of 8 lymph nodes tested.

Conclusions: Cutaneous involvement is often related to a clonal T-cell proliferation in AILD, even when clinical and histological features are nonspecific. Cutaneous infiltrate seems to be clonally related to the nodal T-cell proliferation. The role of EBV infection in skin lesions was not evidenced.

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Angioimmunoblastic lymphadenopathy with dysproteinemia (AILD) is a clinicopathologic syndrome characterized by fever, night sweats, weight loss, generalized lymphadenopathy, hepatomegaly, and splenomegaly. Histological examination of lymph nodes typically shows an effacement of lymph node architecture, a polymorphous infiltrate including immunoblasts, small lymphocytes, plasma cells, eosinophils, epithelioid histiocytes, and a prominent arborizing postcapillary vascularity. Hematologic findings usually show anemia, leukocytosis, and polyclonal hypergammaglobulinemia. The overall clinical course of AILD is aggressive, with a mortality rate ranging from 50% to 72% and a median survival from 11 to 30 months. The major causes of mortality are infection and transformation to an aggressive lymphoma. The disease is considered to be a subtype of peripheral T-cell lymphoma. However, the pathogenesis of the disease remains unclear; some authors consider AILD to be a primary monoclonal T-cell disorder, whereas others suggest a primary polyclonal proliferation, sometimes triggered by drug administration or viral infection, leading to a secondary monoclonal T-cell proliferation.

Cutaneous features are present in nearly half of the cases and often consist of a nonspecific maculopapular eruption that mimics a viral exanthema. We herein describe the clinical and immunohistological features of a series of 10 patients with AILD presenting with cutaneous involvement. To determine whether skin events might be related to a clonal T-cell proliferation, we analyzed the T-cell receptor-γ (TCRG) gene rearrangement by the polymerase chain reaction and denaturing gradient gel electrophoresis in blood, nodal, and skin samples.

Acknowledgments appear at the end of the article. A complete list of the members of the French Study Group on Cutaneous Lymphomas appears in a box on page 886.

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PATIENTS AND METHODS

PATIENTS

Ten patients (3 men and 7 women; mean age, 60.9 years; range, 26-89 years) registered from January 1, 1991, through May 31, 1998, as having AILD by the French Study Group on Cutaneous Lymphomas were reviewed retrospectively. All patients presented with superficial lymphadenopathies, and 9 had “B symptoms,” ie, fever, weight loss, and sweats. Diagnosis was not made initially in 5 patients, and the mean delay from the onset of the disease to the time of diagnosis in these was 7 months. Arthralgia was observed in 2 patients; pulmonary involvement, in 2. The main biological abnormalities of the 10 patients are summarized in Table 1. A lymph node biopsy was performed in all patients, and examination results showed a typical pattern of AILD in all.

Cutaneous involvement not considered, 1 patient was classified at the time of diagnosis as having stage I disease; 5 patients, stage III; and 4 patients, stage IV according to the Ann Arbor classification of lymphomas.15

IMMUNOHISTOLOGICAL ANALYSIS OF SKIN SPECIMENS

Cutaneous biopsy specimens were studied using conventional microscopic analysis of formalin-fixed, paraffin-embedded samples. For immunohistochemistry, paraffin and cryostat sections of biopsy specimens were incubated with the following monoclonal antibodies: anti-CD2, anti-CD3, anti-CD4, anti-CD5, anti-CD8, anti-CD20, and anti-CD30 (Dako, Glostrup, Denmark).

TCRG GENE REARRANGEMENT ANALYSIS

Genomic DNA analysis of lymphocytes was performed from lymph nodes, blood, and skin samples by the use of the PCR-DGGE technique, as previously described.19 The DNA was prepared using standard proteinase K digestion and phenol/chloroform extraction. The V, D, and J genes were amplified in a multiplex PCR reaction using 4 V and J consensus primers that permitted amplification of all VJ genes segments (Table 2). A 50-µL PCR reaction containing 250 ng of genomic DNA, 40 pmol of each primer, 1.5 U of Taq polymerase, 1 U of uracil DNA glycosylase (UDG) (GIBCO BRL, Gaithersburg, Md), 5 µL of 10× reaction buffer, 2.5-mol/L of magnesium chloride, 200-µmol/L of each deoxynucleotide triphosphate, and 400-µmol/L of deoxyuracil triphosphate was performed in a thermal cycler (model 480; Perkin Elmer Cetus, Norwalk, Conn). To prevent any contamination, a first step was performed at 50°C for 10 minutes to allow UDG to destroy any deoxuryracil triphosphate-containing amplified products from previous reactions. The UDG was inactivated by a 10-minute step at 94°C before cycling. Forty cycles was performed containing a denaturation step (94°C for 1 minute), an annealing step (56°C for 1 minute), and an elongation step (72°C for 2 minutes). Residual UDG was inactivated by addition of 50 µL of chloroform. Thirty microliters of PCR product was loaded onto a 6.5% polyacrylamide gel containing a linearly increasing denaturing gradient (10% to 60%). Gel was allowed to run at 150 V in Tris acetate–EDTA buffer maintained at 60°C for 5 hours, then stained with ethidium bromide and photographed under UV illumination. This technique allows detection of T-cell clones representing less than 0.2% of the whole cellular infiltrate.

EPSTEIN-BARR VIRUS DETECTION IN SKIN AND LYMPH NODE SPECIMENS

Detection of EBV on paraffin-embedded cutaneous and lymph node sections was performed using in situ hybridization. Biotinylated double-stranded oligonucleotide probes of 30 base pair, Epstein-Barr encoded RNAs types 1 and 2 (ARGENE Kreatech, Varilhes, France) were used for detection of these viral abundantly expressed transcripts in latent infections.20 Hybridization was performed overnight after heat denaturation of the probes and proteinase K digestion of deparaffinized tissue sections. Alkaline phosphatase-conjugated streptavidine was used for the detection of heterodimers by addition of nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (ARGENE Kreatech).

STATISTICAL ANALYSIS

Statistical analysis was performed in December 1998. The mean follow-up of patients was 32.5 months. Survival was calculated from the time of diagnosis. Complete remission was defined as the disappearance of all symptoms, including cutaneous eruption. Disease-free survival was only considered for patients who experienced complete remission. It was calculated from the initial complete remission to the first relapse or last contact.

RESULTS

CLINICAL AND IMMUNOHISTOLOGICAL FINDINGS

A cutaneous eruption was present initially in 7 patients (70%) and occurred secondarily during the course of the disease in 3 (30%). In 6 patients, the eruption recurred 2 to 3 times during the follow-up period, sometimes with various clinical and histological features in a same patient. A maculopapular morbilliform rash predominantly involving the chest and trunk was observed in 9 patients (Table 3). Other aspects encountered were infiltrated purpura (n = 2), infiltrated plaques of the trunk and legs (n = 2), pruritic papulosvesicular prurigo-like lesions (n = 1), and urticarial eruption (n = 1). The eruption occurred 2 to 9 days after ingestion of fenoxane in 1 patient and antibiotics in 3 patients (amoxicillin given for dental cares and erythromycin ethylsuccinate for pneumonia in 1 patient, spiramycin [Rovamycin] for throat infection in another patient, and a combination of metronidazole and spiramycin given for dental infection in the last patient). The eruption was initially misdiag-
nosed as a drug-induced rash in 4 patients, and as a viral exanthema in 1 patient.

Fourteen cutaneous biopsy specimens were recovered from the 10 patients. Histological findings are summarized in Table 3. Various histological pictures were observed on biopsy specimens obtained simultaneously in different areas or during the follow-up of the same patient. Four types of histological pictures were observed. The first, observed in 2 specimens, corresponded to a nonspecific pattern of mild perivascular infiltrates of eosinophils and lymphocytes with no atypia in the supraperi- dermal pattern (4 specimens) showed a dense pleomorphic infiltrate composed of atypical lymphocytes in the superficial and deep dermis, associated with capillary hyperplasia. The second pattern, seen in 6 specimens, even if not totally specific, could lead to the diagnosis. It consisted of sparse superficial perivascular infiltrates associated with vascular hyperplasia. Endothelial cells protuded into the lumen rising above a multilamellar lamina stained with periodic acid-Schiff stain. Lymphoid cells were often atypical, with pleomorphic lymphocytes and large kidney-shaped nuclei. In one of these patients, Sternberg-like cells were observed. The third pattern (4 specimens) showed a dense pleomorphic infiltrate composed of atypical lymphocytes in the superficial and deep dermis, suggestive of cutaneous lymphoma. The infiltrates were associated with vascular hyperplasia. The
last 2 specimens showed a histological picture of vasculitis with no cellular atypia. Results of immunophenotypic analysis of skin and lymph nodes are given in Table 4. Proliferating cells showed a mature T-cell phenotype with a predominance of CD4+ lymphocytes.

A lymph node biopsy was performed in all cases. Obliteration of nodal architecture with prominent post-capillary venules and polymorphous cellular infiltrate was present in all cases. The infiltrate was composed of small lymphocytes, medium and large clear cells, immunoblasts, plasmocytes, and eosinophils. Lymphocytes, clear cells, and some immunoblasts expressed T-cell markers with a predominance of CD4+ cells in all but 1 case. Numerous reticular dendritic cells were intermingled with T cells. A loss of the CD5 or CD7 pan–T-cell antigens was noted in 4 cases.

MOLECULAR STUDIES

TCRG Gene Rearrangement

The TCRG gene rearrangement was studied in 11 skin biopsy specimens from 10 patients. A clonal TCRG gene rearrangement was detected in 7 of 11 samples, corresponding to erythematous plaques with a histological picture of T-cell lymphoma in 1 sample and transient morbilliform eruptions with a histological pattern of mild dermal lymphoid infiltrate in 6 samples (Table 3). The TCRG gene rearrangement was also studied in lymph nodes from 8 patients. A clonal pattern was noticed in 6 samples and a polyclonal pattern in 2. A comigration of bands corresponding to cutaneous and nodal samples was noticed in 5 of 5 specimens in which a clonal rearrangement was evidenced in skin and lymph nodes (patients 5 and 7-10), indicating that the same clone was present in the different tissue specimens. Blood samples from 3 patients were also investigated: a clonal rearrangement of the TCRG gene was detected in 1 sample (patient 9) in which an identical clone was present in skin, lymph node, and blood specimens.

EBV Detection

The EBV RNA was detected by in situ hybridization in only 1 of 10 skin samples. Five of the 8 nodal samples tested were also positive for EBV RNA, with a particular pattern showing an intense labeling of a few cells in the proliferating areas.

EVOLUTION AND PROGNOSIS

At the end point of the study, 5 patients were still alive, with a mean follow-up of 31.8 months, and 5 patients had died, with a mean survival of 33.2 months. Causes of mortality were infections (3 patients), progression of the lymphoma (1 patient), and treatment adverse effects (1 patient). Seven patients experienced a complete remission after initial treatment consisting of systemic corticosteroids alone in 2 and polychemotherapy in 5. A relapse occurred in all but 1 patient. The median overall survival was 25.5 months, and the median disease-free survival of patients who experienced a complete remission was 28 months. Four of 5 patients in whom a common T-cell clone was evidenced in skin and lymph node samples died, with a mean survival of 26 months. The fifth patient is still alive at the end of the study with a 6-month follow-up (Table 5).

Cutaneous features are frequently observed during the course of AILD and are usually considered not to be directly related to the lymphoma because of the polymorphous and nonspecific clinical and histological patterns of skin lesions. Indeed, only 2 of 10 patients presented with infiltrated plaques that were clinically suggestive of a cutaneous T-cell lymphoma. On the contrary, 8 patients presented with nonspecific cutaneous features such as maculopapular morbilliform eruptions and urticarial or purpuric lesions. In 4 patients, these eruptions occurred after drug administration. Moreover, the eruptions often regressed spontaneously or after corticosteroid treatment, making the clinical diagnosis of cutaneous location of a T-cell lymphoma difficult, and leading to a misdiagnosis of viral or toxic eruption in some patients. Histological examination of skin biopsy specimens showed a dense infiltrate of atypical lymphoid cells in only 4 of the 14 samples studied, whereas the most common histological pattern was a sparse dermal infiltrate of lymphocytes in which cytologic atypia

<table>
<thead>
<tr>
<th>Table 4. Immunophenotypic Analysis of Skin and Lymph Node Samples From 10 Patients With AILD*</th>
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<tr>
<td><strong>Patient No.</strong></td>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
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<td>3</td>
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<td>4</td>
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<td>9</td>
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<td>10</td>
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</table>

* AILD indicates angioimmunoblastic lymphadenopathy with dysproteinemia; ND, not determined; plus/minus sign, staining of only a few cells; plus sign, positive finding; and minus sign, negative finding.
could evoke the diagnosis. In 4 patients, the histological pictures of leukocytoclastic vasculitis or urticarial infiltrate were nonspecific. Results of immunophenotypic studies of skin lymphoid infiltrates were poorly informative, as inflammatory and/or proliferating T lymphocytes exhibited a mature T-cell phenotype in all cases, as previously demonstrated.

To further analyze the biological significance of skin lesions in patients with AILD, molecular analysis of the TCRG chain rearrangement was performed. To our knowledge, this is the first report in the literature of molecular analysis of skin biopsy specimens from a series of patients with AILD. A monoclonal TCRG gene rearrangement was detected in 7 of the 11 skin samples studied. Furthermore, a clonal TCRG rearrangement could be detected in 6 biopsy specimens, corresponding to a poorly specific histological pattern of mild dermal lymphoid infiltrate. These results may be explained by the sensitivity of the PCR-DGGE technique that allows the detection of T-cell clones representing less than 5% of whole cells. Nevertheless, these results strongly argue for a specific involvement of the skin by a T-cell clone in some patients with AILD, even when the clinical and immunohistological patterns seem to be nonspecific. It is unlikely that TCRG gene rearrangement detected in skin samples may be the consequence of a contamination of skin biopsy specimens by a circulating T-cell clone. Indeed, a monoclonal skin lymphoid infiltrate was detected in 2 patients without detectable T-cell clone in peripheral blood lymphocytes. On the other hand, patients with Sézary syndrome whose peripheral blood lymphocytes consistently exhibit a monoclonal rearrangement of the TCR genes may have polyclonal cutaneous lesions when using the same technique.

The significance of detecting a clonal TCR gene rearrangement in skin and lymph nodes from some patients with AILD remains unclear. Indeed, such a clonal rearrangement has been described in lymph nodes from patients with AILD in whom a malignant lymphoma did not subsequently develop. In addition, different clones have been evidenced in lymph nodes from patients with AILD studied sequentially across several years. This indicates that some clones may regress, whereas others may develop during the evolution of AILD. This observation appears consistent with the regression of cutaneous eruptions observed in most patients of this series, even when a T-cell clone was detected in skin lesions.

On the other hand, we observed a comigration of bands between PCR-DGGE products derived from cutaneous and nodal samples of 5 patients of this series. Wood et al previously demonstrated, by sequencing PCR products eluted from comigrating bands of patients with lymphomatoid papulosis, that this comigration pattern indicates the presence of a common T-cell clone in the different samples studied. Therefore, our results strongly argue for a clonal relationship between cutaneous and nodal proliferative T cells in 5 of the 10 patients with AILD in our study. Accordingly, 4 of these 5 patients died of their lymphoma, which suggests that the detection of a common T-cell clone in skin and lymph nodes from patients with AILD might be a factor of poor prognosis.

Angioimmunoblastic lymphadenopathy with dysproteinemia is often considered to be a primary polyclonal proliferation, sometimes triggered by drugs or virus, that secondarily evolves to a monoclonal T-cell proliferation. Epstein-Barr virus has been detected previously in lymph nodes from some patients with AILD. Because cutaneous lymphoid infiltrates were located predominantly around dermal vessels, we investigated for the presence of EBV in these patients’ skin samples. Using the same technique previously described to detect the presence of EBV in cutaneous angiocentric lymphomas, we demonstrated the expression of this virus in only 1 of 10 skin biopsy specimens from patients with AILD.

Overall, the demonstration of a clonal relationship between skin and lymph node infiltrate from some patients with AILD indicates that skin involvement is specific and appears consistent with a worse prognosis for these patients. Further studies of patients with AILD, including a molecular study of skin lesions, are required to clearly determine the prognosis of patients with a clonal cutaneous eruption.

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Table 5. Follow-up Data of 10 Patients With AILD and Cutaneous Involvement*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Treatment</th>
<th>Complete Remission</th>
<th>Relapse</th>
<th>Current Status (Cause of Death)</th>
<th>Disease-Free Survival, mo</th>
<th>Overall Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prednimustine</td>
<td>Yes</td>
<td>Yes</td>
<td>Died (cerebral involvement)</td>
<td>42</td>
<td>61 mo</td>
</tr>
<tr>
<td>2</td>
<td>Prednisolone, VIP, ABVD, interferon alfa, chlorambial</td>
<td>No</td>
<td>. . .</td>
<td>Alive</td>
<td>. . .</td>
<td>13 mo</td>
</tr>
<tr>
<td>3</td>
<td>VIP with dexamethasone phosphate, ABVD</td>
<td>Yes</td>
<td>Yes</td>
<td>Alive</td>
<td>9</td>
<td>23 mo</td>
</tr>
<tr>
<td>4</td>
<td>VD, DHAP, MIME</td>
<td>Yes</td>
<td>Yes</td>
<td>Alive</td>
<td>48</td>
<td>96 mo</td>
</tr>
<tr>
<td>5</td>
<td>Prednisolone</td>
<td>Yes</td>
<td>Yes</td>
<td>Died (cytomegalovirus infection)</td>
<td>31</td>
<td>47 mo</td>
</tr>
<tr>
<td>6</td>
<td>Prednisolone</td>
<td>Yes</td>
<td>Yes</td>
<td>Alive</td>
<td>13</td>
<td>21 mo</td>
</tr>
<tr>
<td>7</td>
<td>ACVPB, methotrexate (intrathecal)</td>
<td>Yes</td>
<td>No</td>
<td>Died (toxic leukoencephalopathy due to methotrexate)</td>
<td>28</td>
<td>28 mo</td>
</tr>
<tr>
<td>8</td>
<td>Cyclophosphamide (after ACVPB)</td>
<td>Yes</td>
<td>Yes</td>
<td>Died (staphylococcal sepsisemia)</td>
<td>22</td>
<td>29 mo</td>
</tr>
<tr>
<td>9</td>
<td>CHOP</td>
<td>No</td>
<td>. . .</td>
<td>Alive</td>
<td>. . .</td>
<td>6 mo</td>
</tr>
<tr>
<td>10</td>
<td>No treatment</td>
<td>No</td>
<td>No</td>
<td>Died (disseminated intravascular coagulation)</td>
<td>. . .</td>
<td>26 d</td>
</tr>
</tbody>
</table>

*VIP indicates ifosfamide, etoposide, cisplatin, and dexamethasone; ABVD, doxorubicin hydrochloride (Adriamycin), bleomycin sulfate, vincaeleucoblastin, and dacarbazine; VD, vincristine sulfate and dexamethasone; DHAP, dexamethasone, cytarabine hydrochloride, and cisplatin; MIME, methyl glycosaminoglycan, ifosfamide, etoposide phosphate, and methotrexate; ACVPB, doxorubicin, cyclophosphamide, vincristine sulfate, carmustine (BCNU), and prednisone; and CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisolone.
REFERENCES


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