Differences in Epstein-Barr Virus Expression Between Primary and Secondary Cutaneous Angiocentric Lymphomas

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Background: Epstein-Barr virus (EBV) has been demonstrated in angiocentric immunoproliferative lesions, suggesting that it could be a causative factor. We investigated for the presence of EBV in 12 primary and 2 secondary cutaneous angiocentric lymphomas (CALs).

Observations: In the 2 secondary CALs, strong reactivity for EBV RNAs and latent membrane protein 1 were detected on paraffin-embedded sections. In contrast, 10 of 12 primary CALs were completely negative for EBV RNAs and latent membrane protein 1. In 2 primary CALs, EBV RNAs and latent membrane protein 1 were detected in few tumor cells. In the group of primary CALs, 8 of 12 were still alive at last follow-up, 3 died of systemic lymphoma, and 1 died of another cause, whereas both patients with secondary CALs died of disease within 1 year.

Conclusion: Differences in the presence of EBV and clinical behavior between primary and secondary CALs suggest that different mechanisms are operative in the pathogenesis of these conditions, and indicate that the 2 groups should be considered separately.

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RESULTS

HISTOLOGICAL, IMMUNOHISTOCHEMICAL, AND GENOTYPIC FINDINGS

Main findings are summarized in Table 2. All the cases were classified as pleomorphic medium and large cell lymphomas according to the updated Kiel classification. In most patients (13 of 14), the neoplastic cells involved dermis and subcutaneous tissue, sparing the overlying epidermis (Figure 2). Histological examination of cutaneous lesions showed infiltration with destruction of vessel walls by the pleomorphic cell population (Figure 3). In patient 2, in which lesions were limited to the deep dermis, only small vessels (capillaries and venules) were infiltrated. In all the other patients, small and medium vessels (capillaries, veins, and arteries) were involved as well. Thromboses were constantly found and, consequently, extensive necrosis was observed in the subcutaneous fat. In the spectrum of the angiocentric immunoproliferative lesions according to Lipford et al,3 all patients belonged to the angiocentric lymphoma group. Histological examination of specimens from tongue (patient 2) and from lung obtained at autopsy (patient 14) showed a pleomorphic lymphoproliferation similar to that in skin. Except patient 13 (Table 2), all patients were CD56 negative; the atypical cells clearly had a T-cell phenotype, and the infiltrates did not contain B cells. A detectable monoclonal TCRγ gene rearrangement was evident in all 5 investigated patients (patients 1, 11, 12, 13, and 14). CD30 antigen
PATIENTS, MATERIALS, AND METHODS

PATIENTS

The main clinical and follow-up data are summarized in Table 1. Fourteen patients with a diagnosis of CAL registered in 5 centers (Hôpital Henri-Mondor, Créteil, France; Hôpital Charles-Nicolle, Rouen, France; Hôpital Saint-Louis, Paris, France; and Centre Hospitals-Universtitaire de Clermont-Ferrand, Clermont-Ferrand, France; and Free University of Amsterdam, the Netherlands) between January 1, 1989, and December 31, 1993, were selected for this study. All patients had a distinctive histological appearance characterized by a pleomorphic lymphoproliferation that exhibited a T-cell phenotype. To be included in this study, a patient was required to have a prominent invasion of small to large vessels by lymphoma cells with destruction of vessel walls, thromboses, and extensive tumor necrosis. Other types of cutaneous T-cell lymphoma showing angiocentricity of minor importance were excluded. The group contained 10 women and 4 men. The median age at diagnosis was 61 years (range, 23-91 years).

All patients were white, and no patient was immunocompromised. Eleven of 14 patients had only skin lesions at the time of initial examination and no evidence of extracutaneous involvement within 6 months after diagnosis, and were therefore considered to have primary CAL. Patient 2 had generalized skin lesions with a nodule on the tongue. In these 12 patients, extensive staging procedures including physical examination, thoracoabdominal computed tomography, and bone marrow biopsy had failed to demonstrate systemic disease. Skin lesions (nodules, plaques, or tumors) were solitary in 2 patients (Figure 1), regional in 5, and generalized in 3. Interestingly, in 5 of 12 patients, partial or even complete spontaneous resolution of skin lesions had been noted. In particular, the lesion of the tongue (patient 2), which measured 2 cm in diameter, completely regressed without any treatment, as did the skin lesions. The patient was free of lesions 60 months after the onset of the disease. Follow-up data indicate that in primary CAL, only 3 patients developed extracutaneous disease after 10, 11, and 58 months of follow-up after the diagnosis. All 3 died of systemic lymphoma. Eight patients were still alive with (n=3) or without (n=5) ongoing disease at last follow-up. Patient 3 died of unrelated disease.

Six patients with primary cutaneous lymphomas other than CAL, including 2 patients with classic mycosis fungoides and 4 patients with a pleomorphic medium-sized/large cell lymphoma without angiotropism, were selected as controls.

METHODS

Punch skin biopsies were performed in all patients. In addition to 14 skin biopsy specimens, tissue fragments from the nasal cavity (patient 13), tongue (patient 2), and postmortem viscera (patient 14) were also available for study. Fresh tissue samples were obtained from the skin in 12 of 14 patients with CAL and in all 6 controls. Fresh tissue samples from the nasal cavity (patient 13) and from the tongue (patient 2) were available as well. In all patients, material was paraffin embedded after fixation in Bouin liquid or formalin and processed for routine hematoxylin-eosin sections. Lymphomas were classified according to the criteria of the updated Kiel classification.

Immunophenotyping was performed by means of an indirect immunoperoxidase technique (alkaline phosphatase APAAP) method.17 Rabbit antimitomouse immunoglobulins and APAAP complexes were obtained (Dako SA, Glostrup, Denmark). In the 18 patients with available frozen material, cryostat sections were evaluated for the presence of B-cell antigens (CD 19/4, immunoglobulin light chains from Coulter, Hialeah, Fla) and T-cell antigens (CD2/Leu 5 CD3/Leu4, CD5/Leu1, CD4/Leu2, and CD8/Leu2 from Becton Dickinson, Mountain View, Calif). In addition, patients 9 and 13 were tested for the presence of NKH1/CD56/Leu 19 (Coulter), T-cell receptor (TCR)–β chain/β F1 and TCRy chain/TCRy 1 (T-cell Sciences, Cambridge, Mass). In the remaining 2 patients, fixed, paraffin-embedded sections were assessed with anti-B antigen L26/ CD20, anti-T antigens UCHL1/CD45RO, and CD3 (Dako SA) by means of the same APAAP method. Furthermore, all patients were also tested on paraffin-embedded tissues for the presence of CD30 antigen and LMP by means of BerH2 and anti-LMP-1 monoclonal antibodies, respectively (Dako SA). As positive controls for LMP expression, the Raji cell line and 1 case of infectious mononucleosis were used.

In situ hybridization, 2 fluorescent-conjugated EBV (EBER 1 and 2) oligonucleotide probes (Dako SA) were used on paraffinized sections as previously described.18 Briefly, deparaffinized sections were rehydrated and pretreated with proteinase K, then dehydrated, air dried, and hybridized with the fluorescein-conjugated EBER oligonucleotides. Color solution was prepared with bromochloroindophosphate and nitroblue tetrazolium (Dako SA). After washing, the slides were briefly counterstained with 1% methyl green and mounted. As a positive control, Raji cell line was used. As negative controls, the hybridizing mixture was used (1) without EBV oligonucleotide probes and (2) with EBER probes after RNAse treatment. All controls for each case were run in parallel in every experiment. In all EBV-negative specimens, slides were also tested for RNA viability by means of poly d(T) probe.19

DNA extraction from paraffin-embedded tissue sections was performed as previously described.20 To see whether suitable DNA was present, β-globulin polymerase chain reaction was performed with the use of specific primers. In 4 of 12 patients with CAL and 3 of 6 other cutaneous T-cell lymphomas studied by polymerase chain reaction, no β-globulin–specific band appeared after DNA amplification, suggesting that the DNA became unsuitable for the polymerase chain reaction during routine processing. All these specimens were fixed in Bouin liquid.

In 5 of 14 patients with CAL, frozen specimens were used to detect TCRy rearrangement by the polymerase chain reaction method as previously described.21

RESULTS OF EBV STUDIES

The results of EBV studies are summarized in Table 2. Altogether, evidence of EBV by in situ hybridization and im-

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munochemistry was found in 4 of 14 CALs (patients 11 through 14). In the 2 secondary CALs (patients 13 and 14), approximately 70% of the tumor cells showed a strong nuclear reactivity for EBV RNAs (Figure 4). The LMP-1 protein was found in numerous tumor cells (Figure 5). Similar positivity was observed in the primary nasal lymphoma of patient 13. In contrast, 10 of 12 primary CALs were completely negative for EBERs and LMP-1. In the other 2 primary CALs (patients 11 and 12), EBERs were detected in about 10% of the neoplastic cells (Figure 6), whereas only a few scattered tumor cells stained for LMP-1 protein (Figure 7). None of the 6 cutaneous T-cell lymphomas other than CAL showed EBV RNAs or protein reactivity. Poly d(T) in situ hybridization controls were all positive.

Polymerase chain reaction showed EBV DNA in only 2 of 8 evaluable patients with CAL, including patient 14, which was also strongly positive by in situ hybridization and immunohistochemistry. In the 3 evaluable cutaneous T-cell lymphomas other than CAL, no EBV DNA could be detected.

**COMMENT**

Angiocentric lymphoma has been described as the malignant form of a spectrum of T-cell disease termed angiocentric immunoproliferative lesions as defined by Lipford et al. This spectrum includes lymphogranulomatosis first described by Liebow et al, midline malignant granuloma, and angiocentric T-cell lymphoma. These lymphomas may have a CD4+, CD8+, or CD56+ phenotype, and thus represent a heterogeneous group. A relationship between these lymphoproliferations and EBV is now well documented, as EBV DNA or RNA has been identified in most cases of angiocentric immunoproliferative lesions involving lung or nasopharynx, even in nonimmunocompromised patients. Skin can be the only site or one of the prominent sites of initial involvement.

In the present study, the presence of EBV was investigated in a series of CALs, including 12 primary and 2 secondary CALs. In both secondary CALs, EBV was strongly detected by in situ hybridization and immunohistochemistry. In the group of primary CALs, EBV RNAs and LMP-1 were detected in only 2 of 12 patients. Moreover, the pattern was different: only 10% of the tumor cells were positive, suggesting that few cells contained

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**Table 1. Clinical Findings in 14 Patients With CAL**

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Skin Lesion Type</th>
<th>Skin Lesion Extent</th>
<th>Extracutaneous Disease</th>
<th>Self-regression</th>
<th>Follow-up After DG Evolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/25</td>
<td>Tumors</td>
<td>Generalized</td>
<td>Lungs, 11 mo after skin</td>
<td>N</td>
<td>13 mo, DOD</td>
</tr>
<tr>
<td>2/F/75</td>
<td>Nodules</td>
<td>Generalized</td>
<td>Tongue (concurrent)</td>
<td>C</td>
<td>60 mo, A−</td>
</tr>
<tr>
<td>3/M/75</td>
<td>Tumors</td>
<td>Regional</td>
<td>None</td>
<td>N</td>
<td>11 mo, DOD</td>
</tr>
<tr>
<td>4/M/56</td>
<td>Nodules</td>
<td>Regional</td>
<td>None</td>
<td>N</td>
<td>62 mo, A+</td>
</tr>
<tr>
<td>5/F/63</td>
<td>Ulcerating plaques</td>
<td>Regional</td>
<td>None</td>
<td>N</td>
<td>73 mo, A+</td>
</tr>
<tr>
<td>6/F/59</td>
<td>Ulcerating plaques</td>
<td>Generalized</td>
<td>CNS, 58 mo after skin</td>
<td>N</td>
<td>64 mo, DOD</td>
</tr>
<tr>
<td>7/M/55</td>
<td>Nodule</td>
<td>Solitary</td>
<td>None</td>
<td>C</td>
<td>75 mo, A−</td>
</tr>
<tr>
<td>8/F/76</td>
<td>Infiltrated plaques</td>
<td>Generalized</td>
<td>Soft-tissue, abdomen, 10 mo after skin</td>
<td>N</td>
<td>11 mo, DOD</td>
</tr>
<tr>
<td>9/F/33</td>
<td>Nodules</td>
<td>Regional</td>
<td>None</td>
<td>C</td>
<td>90 mo, A−</td>
</tr>
<tr>
<td>10/F/60</td>
<td>Nodule</td>
<td>Generalized</td>
<td>None</td>
<td>C</td>
<td>36 mo, A+</td>
</tr>
<tr>
<td>11/M/91</td>
<td>Nodule</td>
<td>Solitary</td>
<td>None</td>
<td>N</td>
<td>58 mo, A−</td>
</tr>
<tr>
<td>12/F/82</td>
<td>Nodules</td>
<td>Regional</td>
<td>None</td>
<td>P</td>
<td>54 mo, A−</td>
</tr>
<tr>
<td>13/F/44</td>
<td>Tumors</td>
<td>Generalized</td>
<td>Palate, nose, 24 mo before skin</td>
<td>N</td>
<td>28 mo, DOD</td>
</tr>
<tr>
<td>14/F/75</td>
<td>Tumors</td>
<td>Generalized</td>
<td>Lungs (concurrent)</td>
<td>N</td>
<td>27 mo, DOD</td>
</tr>
</tbody>
</table>

*CAL indicates cutaneous angiocentric lymphoma; DG, diagnostic; N, no regression; DOD, dead of disease; DOC, dead of other cause; A+, alive with disease; A−, alive without disease; P, partial regression; C, complete regression; and CNS, central nervous system.*
EBV. The negativity for EBV RNAs in most of the primary CALs (10 of 12) does not appear to result from an inefficient technique, because the EBER RNA probe was tested on positive EBV-infected cells and the viability of RNA was controlled by using poly d(T) probe. A cutoff point of 6 months is arbitrary to divide between primary and secondary, in particular when patients develop extracutaneous disease shortly after this deadline. Two of these negative primary CALs (patients 1 and 8) developed lung involvement (patient 1) and soft-tissue mass (patient 8) 11 months and 10 months, respectively, after diagnosis. Although it cannot be excluded that these patients had already occult extracutaneous lesions not yet detectable by routine staging procedures at time of diagnosis, it is of interest that EBERs and LMP could not be detected in the skin lesions. In those patients, extracutaneous lesions were not studied by biopsy or autopsy.

In other respects, the present study supports the correlation between the presence of EBV and CD30 expression previously reported by Kanavaros et al.12 CD30 antigen was expressed by neoplastic cells in 4 of 4 EBV-positive CALs. In contrast, CD30 expression was noted in only 3 of 10 EBV-negative CALs. This is not unexpected, since CD30 antigen is a marker that can be induced in normal B and T lymphocytes in vitro by exposure to EBV.12

The observation that EBV is not expressed in primary CAL has already been found by Angel et al.16 Study-
ing 7 cases of primary cutaneous lymphogranulomatosis, these authors failed to demonstrate the presence of EBER RNA except in 1 case in which EBV staining was homogeneous and diffuse. Furthermore, the same group recently studied a series of 28 primary cutaneous T-cell lymphomas. They did not detect EBER in any of them. Park and Ko, in a retrospective study of 12 cutaneous T-cell lymphomas (6 primary and 6 secondary), concluded that secondary cutaneous T-cell lymphomas had a higher correlation with EBV than did primary ones. In 1992, Tsai et al demonstrated the presence of EBV by DNA hybridization in 2 cases of CAL associated with a nasal T-cell lymphoma. Later, they investigated a series of 10 EBV-associated cutaneous lymphomas: 6 were miscellaneous nonangiocentric lymphomas and 4 cases belonged to the angiocentric group (grade III AIL). 3 of 4 had systemic disease, with rapid nasal cavity involvement in 2 of them; the last of these 4 patients was unavailable for follow-up. This report suggests that EBV expression in the angiocentric group is correlated with respiratory tract involvement. Kobashi et al demonstrated the presence of EBV in 3 of 9 cases of angiocentric lymphoma of a possible natural killer cell occurring in sites other than the upper and lower respiratory tract. Seven patients had cutaneous or subcutaneous lesions during their course. These authors concluded that primary site seems to be correlated with the presence or the absence of EBV. Among the 12 angiocentric immunoproliferative lesions studied by Medeiros et al, EBV detection was positive in 8 of 10 cases involving the respiratory tract (lung or nasopharynx) and negative in 2 of 2 cases localized in skin or subcutis. The authors concluded that EBV positivity was directly correlated with increasing grade of angioimmunoproliferative lesions (grades I-III), but their results could also suggest that EBV positivity may be site related. Myers et al suggested that only lymphogranulomatosis of B-cell origin could be related to EBV infection. This is not confirmed by the present study, in which all the lymphomas had a T-cell phenotype, especially those with a strong EBV expression. Taken together, most reports in the literature and the present study demonstrate that EBV status seems to be related to the site of the primary lesion and the prognosis. Primary CALs are apparently not or rarely associated with EBV. The 2 primary cases with EBV showed only a few positive cells, contrasting with diffuse and homogeneous EBV positivity in the secondary cases. The 2 former patients did not develop any extracutaneous lesions, whereas the pa-
tient with secondary CAL died of systemic disease. This issue suggests that only large numbers of EBV-infected cells may have prognostic relevance.

Another finding to be pointed out is that only 1 of the 2 strongly EBV-positive cases in this study expressed CD56 antigen. Thus, it can be stipulated that EBV positivity does not appear to be exclusively related to natural killer origin.

In conclusion, the finding of strong EBER-RNAs and LMP-1 expression in a patient with apparent skin-limited lesions suggests that there is a serious probability of systemic disease. Thus, such a finding should be an important indicator of poor prognosis. Indeed, the results of the present study also indicate differences in the clinical behavior between primary and secondary CALs. Previous studies have suggested that CALs represent an aggressive type of cutaneous T-cell lymphoma. However, in these studies, no distinction was made between CAL limited to the skin and CAL with previous or concurrent extracutaneous disease. The results of the present study suggest that most patients with a primary CAL have a more favorable prognosis. Furthermore, in 5 of 12 cases, either partial or complete spontaneous remission of skin lesions was observed, suggesting an effective host immune response. Moreover, follow-up data indicate that 9 of 12 patients were still alive after a median follow-up of 63 months. Similar findings were reported by De Berker et al.26 The differences in clinical behavior and association with EBV between primary and secondary CALs suggest that different mechanisms may be operative in the pathogenesis of these conditions, and indicate that the 2 groups should be considered separately.

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REFERENCES